**Journal of Neonatal Surgery**
***ISSN(Online): 2226-0439***
***Vol. 14, Issue 1s (2025)***
*https://www.jneonatalsurg.com*

OPEN ACCESS

# A prospective study of metformin effectiveness as a mammalian target of rapamycin inhibitor and immunoregulator in women with polycystic ovarian syndrome.

**Asmaa Moslim Allawi*[1], Nisreen Waleed Mustafa[2], Ali Mohammed Hadi[2]**

[1]Al-Mawanee Teaching Hospital, Basra Health directorate, ministry of health, Iraq.

[2]Clinical Pharmacy Department, College of Pharmacy, University of Basra, Iraq.

***Corresponding Author:**

Al-Mawanee Teaching Hospital, Basra Health Directorate, Ministry of Health, Iraq,

Email ID: asmaamoslim924@gmail.com

Cite this paper as: Asmaa Moslim Allawi, Nisreen Waleed Mustafa, Ali Mohammed Hadi, (2025) A prospective study of metformin effectiveness as a mammalian target of rapamycin inhibitor and immunoregulator in women with polycystic ovarian syndrome. *Journal of Neonatal Surgery*, 14 (1s), 608-617.

## ABSTRACT

### BACKGROUND

Polycystic ovarian syndrome (PCOS) is a common disorder affecting ovary function. it's thought that an autoimmune regulatory mechanism could mediate its onset. Metformin is an effective medication for PCOS treatment and is proposed to act as an immunoregulatory drug.

### OBJECTIVE

The present work aimed to study the effectiveness of metformin as a mammalian target of rapamycin (mTOR) inhibitor and immunoregulator in PCOS women.

### SUBJECTS AND METHODS

A total of 80 blood samples were collected, 30 of them from newly diagnosed PCOS women. Once more, blood samples were taken from them following 500 mg of metformin treatment period. In addition, 20 healthy women enrolled as a control group. The levels of mTOR, IL6, and TGFβ1 were measured using the ELISA technique.

### RESULTS

The present work showed that after metformin treatment, levels of mTOR and TGFβ1 dropped significantly, but IL6 levels remained constant. Patients on longer-term metformin treatment experienced a significant decrease in mTOR and TGFβ1 levels, but TGFβ1 remained higher than in healthy controls. No significant differences were found in IL6 levels between treatment duration groups and the body mass index (BMI) groups, according to Spearman correlation, there were no correlation found between mTOR and other studied marker neither pre nor post treatment groups.

### CONCLUSION

This study concluded that metformin has pleiotropic effects on immune reprogramming due to its role as an mTOR inhibitor and immune modulation of regulatory cytokines.

***Keywords:*** *PCOS, metformin, immunomodulation, mTOR, TGFβ1, IL6.*

## 1. INTRODUCTION

PCOS is a known illness of the endocrine system, It primarily affects 4%–20% of women who are of reproductive age (1) causing oligo-ovulation, hyperandrogenism, and polycystic ovary morphology. While the precise cause of this illness is still unknown, several factors, such as endocrine, metabolic, and genetic abnormalities, are important in its development (2). The primary feature of PCOS is hyperandrogenism, which results in a variety of physiological dysfunctions. Moreover, PCOS is significantly influenced by the immune system. Chronic inflammation results from hyperandrogenism suppressing certain immune cells and stimulating others, upsetting the immune system's delicate balance(3). Although PCOS's immunopathogenesis has not been thoroughly investigated, it is thought that Immune system abnormalities are a potential major contributing factor. Inflammation has been linked to changes in ovarian follicular dynamics and ovulation, this implies

that low-grade chronic systemic inflammation and PCOS are closely related. Consequently, the pathophysiology and development of PCOS, which results in infertility, are thought to be significantly influenced by chronic low-grade inflammation(2). An elevation of several inflammatory markers, including interleukin 18, C-reactive protein, monocyte chemoattractant protein-1, and count of white blood cells, along with elevated oxidative stress, is indicative of low-grade systemic inflammation in PCOS (4). An effective ovulation process depends on the immune system operating properly. The ovary's immune cells release cytokine mediators that aid in follicle development, oocyte maturation, timely follicle rupture, angiogenesis, corpus luteum formation, and luteal demise. Ensuring effective ovarian function is greatly influenced by these changes in cells and cytokines(5).

Metformin has been investigated concerning its appropriateness for PCOS-affected women during the last 20 years (6). Numerous studies demonstrated that metformin effectively stimulated ovulation, improved insulin sensitivity, significantly decreased serum androgen levels, and restored menstrual cyclicity when used to treat PCOS. Metformin may therefore help treat infertility brought on by PCOS (7). Several recent studies have examined the effect of metformin on different aspects of PCOS patients, as this treatment is of great importance in controlling this syndrome. In a study conducted in 2023, metformin was regarded as an effective supplement to lifestyle changes aimed at lowering lipids and improving insulin resistance in PCOS patients (8). Moreover, metformin is effective as the best first-line medication for ovulatory infertile non-obese PCOS patients. For women with PCOS undergoing in vitro fertilization, metformin is also an effective medication for lowering the risk of ovarian hyperstimulation (9). Metformin has been demonstrated to lessen the effects of aging by improving the control of hyperglycemia and affecting damaged DNA and the apoptotic process .Metformin's anti-cancer effect is thought to be mediated through inhibition of the mTOR signaling pathway Metformin may have an impact on life-threatening hamartomas by inhibiting or reversing their growth due to its effect on the mTOR pathway (10). An ovulatory growth and the formation of cystic structures are related to mTOR signaling. Thus, increased granulosa cell proliferation is a result of enhanced mTOR activation .The mTOR gene mutation produces serine/threonine kinase, a protein found in all cells. It is essential for regulating the growth, metabolism, and proliferation of cells. In vivo, mTOR regulates follicle growth and serves as a novel checkpoint for mitotic survival (11).

The present study aimed to investigate metformin's role as an immunoregulator to improve the immune system in women suffering from PCOS by studying its ability as an mTOR inhibitor in addition to its effect on some immune markers, especially TGFB1 and IL6.

## 2. METHODS

### *2.1 Design and study population*

This study was conducted as a case-control interventional study. The study group consisted of women with PCOS who attended a private maternity clinic in Basra Province-Iraq, from November 2022 through July 2023.

Those patients diagnosed according to Rotterdam criteria, which include two of the following three characteristics should be present to ensure the diagnosis of PCOS: the criteria are either oligo- or anovulation, hyperandrogenism symptoms, either biochemically or clinically, and polycystic ovaries morphology (12).

Thirty newly diagnosed PCOS women who were clinically and radiologically diagnosed were grouped as pre-treatment and started monotherapy with metformin 500 mg orally, twice daily. These women were later referred to as the post-treatment group after a follow-up period ranging from 3 to 9 months. A short flow-up time was chosen due to the limit of time for the present study. The study also included a control group, this group consisted of twenty healthy women who had: no PCOS and any other autoimmune diseases, or smoking and did not take any medication that may affect in immunity system.

The present study inclusion criteria included female patients with PCOS , an age range from 18 – 45 years old, and start taking metformin as a scheduled treatment for at least 3 months. The study exclusion criteria included patients outside the age range of the study, patients with any other immune system disorders, diabetic patients, pregnant women, and not take any medication that may affect in immunity system. Control group include twenty women how are healthy, free of immune disorder and not pregnant.

### *2.2 Sample collection and laboratory measurements*

A total of 80 blood samples from those enrolled in this study were collected in a sterile gel tube. Consequently, serum was separated for the ELISA technique. 3 ml of venous blood was collected from the patients who met the inclusion criteria, then placed in golden vacuum tubes (gel /clot activator) and left to stand for one hour at room temperature for clot formation. The tube was then centrifuged for 10 minutes at 25ºC at 2000 rpm. The serum was aspirated by using a Pasteur pipette dispensed into the sterile tubes and stored at -80 Cº until used. Multiple freezes and thawing were avoided. The sample was tested about 10 months after the first sample collection, and all samples were analyzed at the same time.

The mTOR, IL6, and TGFβ1 concentrations were measured in patients and healthy control groups using the sandwich ELISA technique. The tests were conducted according to the manufacturer's guidelines (CLOUD-CLONE CORP, USA).

**Ethical consideration**: The study was done after outlining the objectives of the study, evaluating patient satisfaction, and

obtaining informed consent from the subjects.

the proposal was approved by the ethics committee of the College of Pharmacy, University of Basra- Iraq, according to the approval code (EC56).

### *2.3 Statistical analysis*

The statistical analysis of the data was conducted using SPSS version 26. Mann-Whitney U test, Wilcoxon test for tow related samples used for the comparison of data, and kruskal-wallis test were utilized to study variance of data. To examine the relationships between the markers under study, the Spearman correlation was employed. The results were presented as a mean ± standard deviation (SD). A statistically significant difference between the groups was identified as $P < 0.05$.

## 3. RESULTS

Among the women with PCOS who participated in the current study, the age groups of 20–26 years old comprised 40% of the total number of patients, followed by 26–32 years old (36%) and those under 20 years old (14%), whereas 10% of patients were older than 32 years old. Based on BMI, the majority of patients were overweight (BMI>25), which represented 57% of the total, and underweight (BMI≤25), comprising 43% of the total, Figure (1).

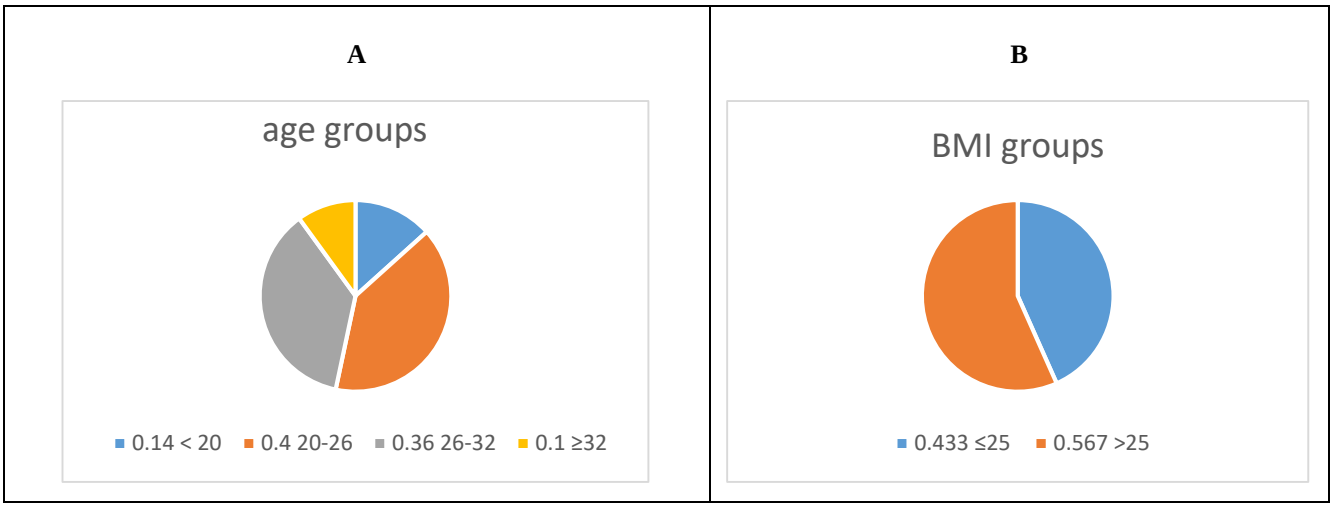



**Figure 1 Basic characteristics of the participants**

The comparisons between the post-metformin treatment group and the control group are shown in Table (1), which indicates that the concentrations of inflammatory markers (TGFβ and IL6) that were investigated in the present study recorded significant elevations among patients who were under treatment compared with the control group. Meanwhile, mTOR concentration dropped significantly as compared with healthy controls.

**Table (1): The Comparison of serum levels of mTOR, TGFβ1, and IL6 between the post-metformin treatment group and the control.**

| | Groups | No. | Mean | SD | P-value |
|---|---|---|---|---|---|
| **mTOR ng/ml** | Post | 30 | 1.420 | 0.811 | 0.035 |
| | control | 20 | 2.509 | 0.773 | |
| **TGFβ1 pg/ml** | Post | 30 | 2.805 | 1.902 | 0.000 |
| | Control | 20 | 0.372 | 0.343 | |
| **IL6 pg/ml** | Post | 30 | 6.611 | 5.370 | 0.000 |
| | Control | 20 | 3.207 | 3.505 | |

Mean ± SD were used for the expression of data. SD: standard deviation. mTOR: mammalian target of rapamycin. TGFB1: Transforming growth factor. IL6: Interleukin 6. level of significance $p<0.05$. Mann-Whitney U test used to analyze data.

While, the comparisons between 30 PCOS women before and after metformin treatment concerning studied markers in the present work are shown in Table (2). The concentrations of mTOR and TGF1β dropped significantly in patients after

receiving the treatment as compared to the same individuals before treatment. Yet, even after treatment, the IL6 concentrations were near their initial values.

**Table (2) The Comparison of Serum Levels of mTOR, TGF1B, and IL6 between pre and post-treatment**

| | Groups | No. | Mean | SD | P-value |
|---|---|---|---|---|---|
| **mTOR ng/ml** | Pre | 30 | 0.421 | 0.328 | 0.040 |
| | Post | 30 | 1.420 | 0.811 | |
| **TGFβ1 pg/ml** | Pre | 30 | 5.383 | 4.335 | 0.008 |
| | Post | 30 | 2.805 | 1.902 | |
| **IL6 pg/ml** | Pre | 30 | 6.814 | 3.675 | 0.934 |
| | Post | 30 | 6.611 | 5.370 | |

Mean ± SD were used for the expression of data. SD: standard deviation. mTOR: mammalian target of rapamycin. TGFB1: Transforming growth factor. IL6: Interleukin 6. level of significance $p<0.05$. Wilcoxon test for tow related samples were used.

In a new finding, the present work result fount that the treatment period had a significant effect on the mTOR, TGFβ1, and IL6 concentrations. As noted in Table (3). The mTOR level decreased significantly after metformin treatment for more than 3 months compared with its level in patients who were treated for only 3 months or less and healthy donors. Compared to TGFβ1 levels in individuals receiving metformin treatment for three months, participants receiving longer-term treatment experienced a substantial drop in mean TGFβ1 levels. However, its level was still higher than that of the healthy control. The study found no significant differences in IL6 levels between treatment duration groups among patients. Moreover, in patients receiving treatment longer than three months and for three months or less, there was a discernible change in IL6 levels as compared to the control group.

**Table (3): The Comparison of serum levels of mTOR, TGFβ1, and IL6 according to treatment period**

| | Groups | No. | Mean | SD | P-value |
|---|---|---|---|---|---|
| **mTOR ng/ml** | ≤3 months | 18 | 2.127 a | 0.705 | 0.000 |
| | >3months | 12 | 0.382 b | 0.174 | |
| | control | 20 | 2.509 a | 0.773 | |
| **TGFβ1 pg/ml** | ≤3 months | 18 | 7.396 a | 2.110 | 0.000 |
| | > 3months | 12 | 3.822 b | 1.910 | |
| | control | 20 | 0.372 c | 0.343 | |
| **IL6 pg/ml** | ≤3 months | 18 | 6.533 a | 3.327 | 0.010 |
| | > 3months | 12 | 6.498a | 3.010 | |
| | control | 20 | 3.208 b | 3.505 | |

Mean ± SD were used for the expression of data. SD: standard deviation. mTOR: mammalian target of rapamycin. TGFB1: Transforming growth factor. IL6: Interleukin 6. level of significance $p<0.05$. Different letters (a, b, and c) refer to significant alterations between the means of groups. kruskal-wallis test used for data analyze.

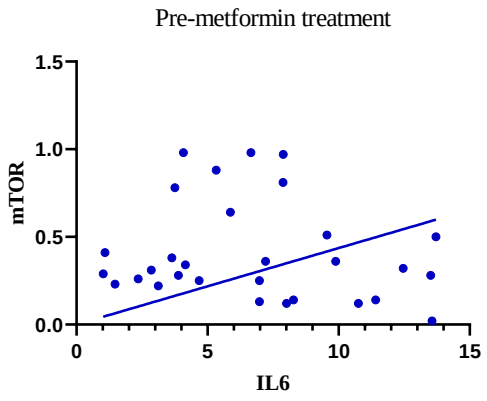
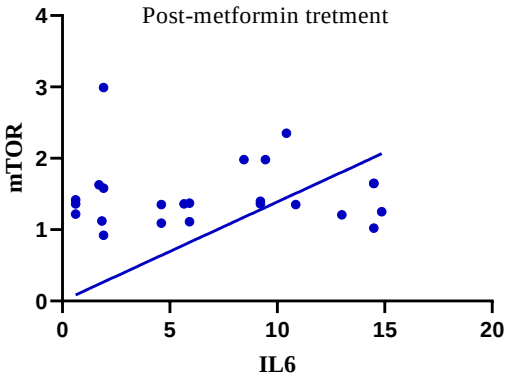
Furthermore, patients were categorized into two groups concerning their BMI: the first group included women whose BMI was ≤ 25, and the second group included women with a BMI < 25. There were no statistically significant changes between the two groups regarding the level of mTOR, TGFβ1, and IL6, table (4).

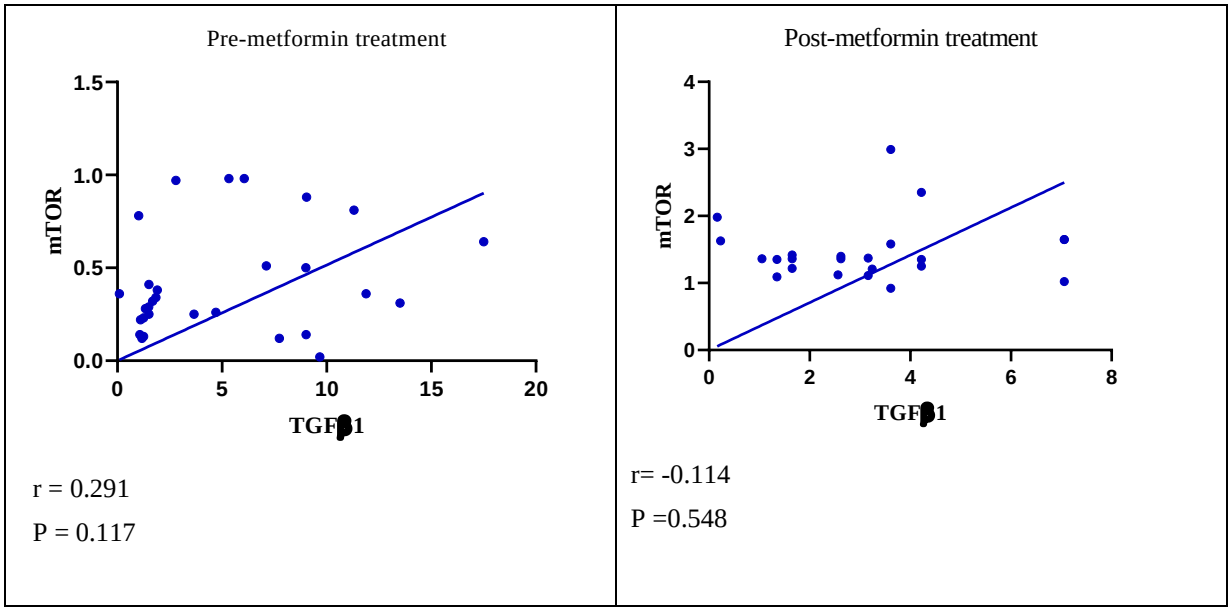
**Table (4): The Comparison of serum levels of mTOR, TGF1B, and IL6 according to BMI**

| | Groups | No. | Mean | SD | P-value |
|---|---|---|---|---|---|
| **mTOR ng/ml** | ≤25 | 13 | 1.158 | 0.951 | 0.346 |
| | >25 | 17 | 1.620 | 0.987 | |
| **TGFβ1 pg/ml** | ≤25 | 13 | 3.469 | 2.298 | 0.252 |
| | >25 | 17 | 2.560 | 2.043 | |
| **IL6 pg/ml** | ≤25 | 13 | 7.536 | 3.219 | 0.124 |
| | >25 | 17 | 5.630 | 4.117 | |

Mean ± SD were used for the expression of data. SD: standard deviation. mTOR: mammalian target of rapamycin. TGFB1: Transforming growth factor. IL6: Interleukin 6. level of significance p<0.05. Mann-Whitney U test used to analyse data.

Finally, the Correlation study that shown in Figure 2-A and B (r = -0.1584, P = 0.4031 and r = 0.2918, P = 0.1177; respectively) and Figure 2-C and D (r = 0.03265, P = 0.8640 and r = -0.1141, P = 0.5482), demonstrated that there was no correlation between mTOR and either IL6 or TGFβ1 in the pre-metformin treatment group as well as in the post-metformin treatment group.

| Pre-metformin treatment | Post-metformin treatment |
|---|---|
| **A**<br>  <br><br>r = -0.158<br>P = 0.403 | **C**<br>  <br><br>r = 0.032<br>P = 0.864 |
| **B** | **D** |




mTOR: mammalian target of rapamycin. TGFβ1: Transforming growth factor. IL6: Interleukin (6). level of significance $p<0.05$

Figure(2): The correlation of levels of mTOR levels with TGFβ1, IL6 in pre and post-treatment patients. Spearman correlation was employed.

## 4. DISCUSSION

The present study was designed to highlight the pleiotropic effects of metformin as an anti-inflammatory and immunoreprogramming agent in women suffering from PCOS.

The present study referred to metformin having an important effect on levels of inflammatory markers regarding the treatment period while the BMI of patients did not reveal any effect on studied markers. An analysis of the impact of the immunomodulatory effect of metformin drug on immune parameters like mTOR, TGFβ1, and IL6 was presented in this study. The obtained result revealed the role of metformin as an mTOR inhibitor via a recorded decrease in its level after treatment for more than 3 months.

The significance of mTOR in cell growth, proliferation, and differentiation has long been known. PCOS and ovarian cancer are among the many diseases that are thought to be related to the over-activation of the mTOR pathway (13). A few studies investigated the effect of metformin on the mTOR pathway among PCOS women. According to a study conducted by Howell et al., metformin depletes cellular energy, which triggers the activation of crucial metabolic regulators like AMP-activated protein kinase (AMPK) and the inhibition of mTOR complex1 (14). Essah et al. studied the effects of metformin on menstrual cyclicity in women with PCOS, their results support the present findings despite not measuring the mTOR level, They came to the conclusion that therapy lasting at least six months improves therapeutic efficacy more than therapy lasting less time. (15). Furthermore, it has been proposed that several medications (like AMPK inhibitors) that affect primordial follicles work through the mTOR pathway (16). Primordial follicles are stimulated by promoting mTOR signaling in the oocytes. Whereas, liver kinase b1 suppresses the mTOR pathway to limit the activation of primordial follicles (17). Inhibiting mTORC1 with medications such as MAPK3/1 inhibitors might help maintain the ovarian reserve(18).

Studies suggested that metformin's therapeutic effect may be through its role as an mTOR inhibitor. Metformin has been shown to inhibit T helper (Th), which may have effects that are dependent on mTOR(19)(20).

Over an extended period of use, metformin has been demonstrated to have anti-inflammatory effects. this could account for the medication's success in clinical trials. Metformin functions by preventing glycolysis, which increases the AMP: ATP ratio and causes AMPK to become activated. AMPK controls a cell's metabolism and energy levels. By preventing cell division during periods of energy stress, it aids in limiting the immune response. Moreover, AMPK inhibits mTORC1, which regulates protein synthesis, cell division, and glucose metabolism(22)

According to Xu et al. study, metformin improves PCOS in a rat model by reducing ovarian granulosa cell excessive autophagy through the PI3K/AKT/mTOR pathway. This study offers proof of the intentional reduction of ovarian granulosa cell excessive autophagy and PCOS improvement(23). In addition, another study conducted by Roa et al. in a mouse model indicated that both mTORC1 and mTORC2 may be important regulators in the PCOS mouse model (11)

Following treatment in the present study, the levels of mTOR, TGF1, and IL6 were not different based on BMI groups,

despite the fact that metformin treatment significantly reduced BMI in obese patients. The current study found that among PCOS women with a BMI over 25, there was a marginally non-significant increase in mTOR levels. This is the first study that investigates the effect of BMI in PCOS women on the mTOR level. Few previous investigations have discussed how fatness affects the mTOR level. Body fat percentage and mTOR pathway activation were found to be positively correlated in a tumor-based study (24). Activation of the mTOR pathway is associated with multiple hallmarks of cancer, including angiogenesis and cell proliferation (25). An overabundance of androgens is associated with adipocyte proliferation, cytokine overproduction, and signal pathway dysregulation. Insulin resistance is another aspect of PCOS that results in abnormal metabolism of fatty acids and carbohydrates (3). A study conducted by Alvarez-Blasco found that there was no association between the higher prevalence of PCOS in overweight and obese women with metabolic syndrome or any associated syndrome even though obese or overweight women had a higher prevalence of PCOS (26). Other researchers concluded that there were no effects of duration and dose of metformin treatment in adiposity in diabetic individuals (27) . Women with PCOS may have altered body fat distribution as a result of long-term exposure to elevated testosterone levels(28). It has been shown by Roa et al. that PCOS women who have disorders of lipid metabolism are known to gain excessive weight. These results in PCOS point to a direct connection between mTOR signaling and cellular energy metabolism (11) .In addition, Li et al. findings of a murine model study revealed that a high-fat diet prevents autophagy by blocking AMPK phosphorylation and encouraging mTOR to transition to phosphorylation (29).

The present study focused on the importance of two regulatory cytokines (IL6 and TGFβ1) in controlling the inflammatory condition among PCOS women. TGFβ1 demonstrated a significant decline in its level even shortly after receiving metformin therapy whereas, IL6 showed a notable decrease following more than three months of metformin treatment. This finding would suggest that additional treatment time is necessary for the IL6 level to drop sufficiently, as in healthy individuals.

In women with PCOS, studies have shown changes in immune cells and elevated levels of proinflammatory cytokine markers. This implies that ovulatory dysfunction may be influenced by immune factors. The ovarian microenvironment's immune cells and cytokines are essential for controlling regular ovulation. Consequently, the endocrine and metabolic disorders linked to PCOS may negatively impact ovulation and implantation (30) .It is uncertain if metformin directly interacts with IL6, but it is known that metformin influences IL6 function through two mechanisms: either via inhibition of this marker expression or by its downstream signaling (31). According to novel findings obtained by Mishra et al. found that metformin selectively reduces the expression of the IL-6 receptors which is mediated through AMPK, and mTOR (32).

Numerous research works have examined the cytokine levels in females diagnosed with PCOS. A study conducted by Lin et al. demonstrated that women with PCOS who were treated with metformin showed a significant reduction in levels of IL-6(33). In another study ,during the follow-up of metformin therapy for PCOS women, plasma IL-6 levels were unchanged (34).

Tumu et al suggested that IL-6 may be a major modulator of low-grade chronic inflammation in PCOS (35) .Lin et al showed that IL-6 might be an early low-grade chronic inflammatory marker in PCOS (33). An investigation with obese PCOS women revealed that six months of metformin treatment was not enough to lower IL-6 levels (36). Nevertheless, other research has demonstrated that regardless of BMI, IL-6 levels are increased in PCOS women (37)

At this point, the precise mechanism of inflammation in PCOS is unknown. Since obesity is frequently present in women with PCOS, its significance as a pro-inflammatory factor has received the greatest research attention. Chronic low-grade inflammation caused by obesity is explained by adipocyte hypertrophy and hyperplasia, which release free fatty acids and raise the amount of pro-inflammatory cytokines in macrophages(38). Almusawy et al investigated the level of IL6, IL18, and TGF-alpha in PCOS women elevated with BMI ≤25 and others with BMI >25, their results support the present finding as both studies did not record sig differences between the two groups (39). In addition, a meta-analysis investigation by Peng et al. found no connection between IL6 level and baseline PCOS features such as androgen status, BMI, and IR (40). Nevertheless, other research has demonstrated that regardless of BMI, IL-6 levels are elevated in women with PCOS (37), This also agrees with present work.

Concerning TGFβ1, the present result showed a significant reduction in its serum levels during the pre-post-study and treatment period. A previous study done by Xiao et al. determined that metformin interacted with the TGF-β1 ligand to inhibit TGF-β1 signaling, which prevented TGF-β1 from attaching to its receptor and decreased signaling downstream, this study has revealed that metformin to be a new type of TGF-β1 suppressor, which is responsible for the drug's pleiotropic effects. The therapeutic utility of metformin as a therapy for many disorders other than diabetes, where TGF-β1 signaling defects are suggested (41). In the same line with the present study, Ramamoorthy and Bhuvaneswari have demonstrated that the treatment period and values of inflammatory markers have an inversely proportional relation (42). Farhangi et al reported that the level of TGF-β in PCOS patients sera did not significantly differ between the (22)obese and those that were not(43), whereas another study indicated there was an increase in TGFB1 in obese and non-obese PCOS patients which means it was not weight-related[15, 30]. Another study did not agree with the current finding, it was improved that BMI and fat mass showed significant associations with TGF-β1 levels, indicating that increased TGF-β1 levels are linked to a poor metabolic profile in humans, these findings demonstrate a strong positive correlation between human obesity and TGF-β1(45).

This study effectively combines the investigation of metformin's role as an mTOR inhibitor and its immunomodulatory effects, providing a holistic view of its therapeutic potential for women with PCOS. This dual focus enriches the understanding of metformin's multifaceted benefits beyond its common use.Metformin pleiotropic effect as an immunoregulatory drug in PCOS studies as an mTOR a cytokines inhibitor in present study, other studies not include human study in PCOS or include studying other markers in humans in this disorder.This study included the effect of BMI in triggering inflammation in this disorder and documented no significant differences observed**.**

The present study's limitations, which led to the small sample size, include difficulty in obtaining samples due to the need for follow-up of patients after treatment, as well as challenges in obtaining patients who received monotherapy, a dearth of patients meeting the inclusion criteria and, a lack of prior research studies on the topic.

We recommended to Increasing the sample size involved in the study, using an interventional longitude study of metformin in PCOS patients to give a clear insight into its effect on inflammation and immunoregulation effect, studying another marker to ensure drugs' anti-inflammatory effect, studying other diseases including inflammatory or immunity aspect or studying other drugs that have immunoregulatory effect similar to metformin.

## 5. CONCLUSIONS

The present study concluded that metformin is effective as an mTOR inhibitor and has immunomodulatory effects on regulatory cytokines in women with PCOS, especially when used for a treatment period exceeding 3 months. This effectiveness may play a crucial role in helping PCOS patients recuperate. In addition, its immunomodulatory effect, especially on the studied markers that contribute to many other important diseases, makes it a targeted drug for various immune disturbance disorders soon. The therapeutic effects of metformin may be due to its inhibitory effect on mTOR, so monitoring its long-term impact on other disorders associated with elevated mTOR pathway activity is important to determine the best course of treatment

**Acknowledgement** : The authors are privileged to convey their profound gratitude and admiration to Dr. Zahra Attalla and her clinic staff for their invaluable assistance in the collecting of the samples.

## REFERENCES

[1] Deswal R, Narwal V, Dang A, Pundir CS. The Prevalence of Polycystic Ovary Syndrome: A Brief Systematic Review. Journal of Human Reproductive Sciences. 2020;13(4):261–71.

[2] Shamsi M, Ghazavi A, Saeedifar AM, Mosayebi G, Pour SK, Ganji A. The immune system's role in PCOS. Molecular biology reports. 2022 Nov;49(11):10689–702.

[3] Shabbir S, Khurram E, Moorthi VS, Eissa YTH, Kamal MA, Butler AE. The interplay between androgens and the immune response in polycystic ovary syndrome. Journal of Translational Medicine [Internet]. 2023;21(1):1–26. Available from: https://doi.org/10.1186/s12967-023-04116-4

[4] Duleba AJ, Dokras A. Is PCOS an inflammatory process? Fertility and Sterility. 2012;97(1):7–12.

[5] Adamczak R, Ukleja-Sokołowska N, Lis K, Dubiel M. Function of follicular cytokines: Roles played during maturation, development and implantation of embryo. Medicina (Lithuania). 2021;57(11):1–10.

[6] Johnson NP. Metformin use in women with polycystic ovary syndrome. Annals of Translational Medicine. 2014;2(6):1–6.

[7] Attia GM, Almouteri MM, Alnakhli FT. Role of Metformin in Polycystic Ovary Syndrome (PCOS): Related Infertility. Cureus. 2023;15(8).

[8] Melin J, Forslund M, Alesi S, Piltonen T, Romualdi D, Spritzer PM, et al. The impact of metformin with or without lifestyle modification versus placebo on polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. European Journal of Endocrinology [Internet]. 2023;189(2):S38–64. Available from: https://doi.org/10.1093/ejendo/lvad098

[9] Attia GM, Almouteri MM, Alnakhli FT. Role of Metformin in Polycystic Ovary Syndrome (PCOS)-Related Infertility. Cureus. 2023 Aug;15(8):e44493.

[10] Amin S, Lux A, O'Callaghan F. The journey of metformin from glycaemic control to mTOR inhibition and the suppression of tumour growth. British Journal of Clinical Pharmacology. 2019;85(1):37–46.

[11] Roa J, Garcia-Galiano D, Varela L, Sánchez-Garrido MA, Pineda R, Castellano JM, et al. The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. Endocrinology. 2009;150(11):5016–26.

[12] Azziz R. Diagnosis of Polycystic Ovarian Syndrome: The Rotterdam Criteria Are Premature. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2006;91(3):781–5. Available from: https://doi.org/10.1210/jc.2005-2153

[13] Liu AL, Liao HQ, Li ZL, Liu J, Zhou CL, Guo ZF, et al. New insights into mTOR signal pathways in ovarian-related diseases: Polycystic ovary syndrome and ovarian cancer. Asian Pacific Journal of Cancer Prevention. 2016;17(12):5087–94.

[14] Howell JJ, Hellberg K, Turner M, Talbott G, Kolar MJ, Ross DS, et al. Metformin Inhibits Hepatic mTORC1 Signaling via Dose-Dependent Mechanisms Involving AMPK and the TSC Complex. Cell Metabolism [Internet]. 2017;25(2):463–71. Available from: http://dx.doi.org/10.1016/j.cmet.2016.12.009

[15] Essah PA, Apridonidze T, Iuorno MJ, Nestler JE. Effects of short-term and long-term metformin treatment on menstrual cyclicity in women with polycystic ovary syndrome. Fertility and Sterility. 2006;86(1):230–2.

[16] Lu X, Guo S, Cheng Y, Kim J-H, Feng Y, Feng Y. Stimulation of ovarian follicle growth after AMPK inhibition. Reproduction (Cambridge, England). 2017 May;153(5):683–94.

[17] Jiang Z-Z, Hu M-W, Ma X-S, Schatten H, Fan H-Y, Wang Z-B, et al. LKB1 acts as a critical gatekeeper of ovarian primordial follicle pool. Oncotarget. 2016 Feb;7(5):5738–53.

[18] Zhao Y, Zhang Y, Li J, Zheng N, Xu X, Yang J, et al. MAPK3/1 participates in the activation of primordial follicles through mTORC1-KITL signaling. Journal of cellular physiology. 2018 Jan;233(1):226–37.

[19] Ren W, Yin J, Duan J, Liu G, Tan B, Yang G, et al. mTORC1 signaling and IL-17 expression: Defining pathways and possible therapeutic targets. European Journal of Immunology. 2016;46(2):291–9.

[20] Nagai S, Kurebayashi Y, Koyasu S. Role of PI3K/Akt and mTOR complexes in Th17 cell differentiation. Annals of the New York Academy of Sciences. 2013;1280(1):30–4.

[21] Wisam A, Fadhil A, Nori W. Unveiling Systemic Immune Inflammation Index Correlations in Women with Polycystic Ovary Syndrome: A Cross-sectional Study. Al-Rafidain Journal of Medical Sciences ( ISSN 2789-3219 ) [Internet]. 2024;6(2):156–161. Available from: https://ajms.iq/index.php/ALRAFIDAIN/article/view/934

[22] Saravia J, Raynor JL, Chapman NM, Lim SA, Chi H. Signaling networks in immunometabolism. Cell Research [Internet]. 2020;30(4):328–42. Available from: http://dx.doi.org/10.1038/s41422-020-0301-1

[23] Xu B, Dai W, Liu L, Han H, Zhang J, Du X, et al. Metformin ameliorates polycystic ovary syndrome in a rat model by decreasing excessive autophagy in ovarian granulosa cells via the PI3K/AKT/mTOR pathway. Endocrine Journal. 2022;69(7):863–75.

[24] Cheng TYD, Omilian AR, Yao S, Sanchez P V., Polk LZ, Zhang W, et al. Body fatness and mTOR pathway activation of breast cancer in the Women's Circle of Health Study. npj Breast Cancer [Internet]. 2020;6(1):1–9. Available from: http://dx.doi.org/10.1038/s41523-020-00187-4

[25] Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. Cell. 2017 Mar;168(6):960–76.

[26] Tsenkova P, Robeva R, Elenkova A, Zacharieva S. Prevalence and Characteristics of the Polycystic Ovarian Syndrome in Overweight and Obese Premenopausal Women. Acta Endocrinologica. 2022;18(4):417–23.

[27] Abdulrahman Z, Atrakji M, Al-Maliky A, Hussein K, Hussain S. The association of metformin dose up-titration and treatment duration with adiposity, lipid profile indicators, and serum leptin levels in T2DM Iraqi patients. Journal of Health Sciences. 2023;

[28] Qamar U, Atkin SL, Sathyapalan T. Obesity and Polycystic Ovary Syndrome. Practical Guide to Obesity Medicine. 2017;3(2):59–70.

[29] Li Y, Cheng Y, Zhou Y, Du H, Zhang C, Zhao Z, et al. High fat diet-induced obesity leads to depressive and anxiety-like behaviors in mice via AMPK/mTOR-mediated autophagy. Experimental neurology. 2022 Feb;348:113949.

[30] Banerjee S, Cooney LG, Stanic AK. Immune Dysfunction in Polycystic Ovary Syndrome. ImmunoHorizons. 2023;7(5):323–32.

[31] Sakata N. The anti-inflammatory effect of metformin: The molecular targets. Genes to Cells [Internet]. 2024;29(3):183–91. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/gtc.13098

[32] Mishra AK, Dingli D. Metformin inhibits IL-6 signaling by decreasing IL-6R expression on multiple myeloma cells. Leukemia. 2019 Nov;33(11):2695–709.

[33] Lin YS, Tsai SJ, Lin MW, Yang CT, Huang MF, Wu MH. Interleukin-6 as an Early Chronic Inflammatory Marker in Polycystic Ovary Syndrome with Insulin Receptor Substrate-2 Polymorphism. American Journal of Reproductive Immunology. 2011;66(6):527–33.

[34] Möhlig M, Spranger J, Osterhoff M, Ristow M, Pfeiffer AFH, Schill T, et al. The polycystic ovary syndrome

per se is not associated with increased chronic inflammation. European Journal of Endocrinology. 2004;150(4):525–32.

[35] Tumu VR, Govatati S, Guruvaiah P, Deenadayal M, Shivaji S, Bhanoori M. An interleukin-6 gene promoter polymorphism is associated with polycystic ovary syndrome in South Indian women. Journal of Assisted Reproduction and Genetics. 2013;30(12):1541–6.

[36] Jakubowska J, Bohdanowicz-Pawlak A, Milewicz A, Szymczak J, Bednarek-Tupikowska G, Demissie M. Plasma cytokines in obese women with polycystic ovary syndrome, before and after metformin treatment. Gynecological Endocrinology. 2008;24(7):378–84.

[37] Vasyukova E, Zaikova E, Kalinina O, Gorelova I, Pyanova I, Bogatyreva E, et al. Inflammatory and Anti-Inflammatory Parameters in PCOS Patients Depending on Body Mass Index: A Case-Control Study. Biomedicines. 2023;11(10):1–13.

[38] Azziz R. Diagnosis of Polycystic Ovarian Syndrome : The Rotterdam Criteria Are Premature. 2006;91(3):781–5.

[39] Al-Musawy SH, Al-Saimary IE, Flaifil MS. Levels of Cytokines Profile in Polycystic Ovary Syndrome. Medical Journal of Babylon [Internet]. 2018;15(2). Available from: https://journals.lww.com/mjby/fulltext/2018/15020/levels_of_cytokines_profile_in_polycystic_ovary.3.aspx

[40] Peng Z, Sun Y, Lv X, Zhang H, Liu C, Dai S. Interleukin-6 Levels in Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis. PloS one. 2016;11(2):e0148531.

[41] Xiao H, Zhang J, Xu Z, Feng Y, Zhang M, Liu J, et al. Metformin is a novel suppressor for transforming growth factor (TGF)-β1. Scientific Reports [Internet]. 2016;6(January):1–9. Available from: http://dx.doi.org/10.1038/srep28597

[42] Ramamoorthy S, Bhuvaneswari K. A Cross Sectional Study on the Status of Inflammatory Markers in Polycystic Ovary Syndrome (PCOS) in Indian Population. Biomedical and Pharmacology Journal [Internet]. 2019 May 11;12:1975+. Available from: https://link.gale.com/apps/doc/A628404734/AONE?u=anon~d8c0180b&sid=googleScholar&xid=3a66e2d7

[43] Farhangi MA, Saboor-Yaraghi AA, Eshraghian M, Ostadrahimi A, Keshavarz SA. Serum transforming growth factor β (TGF-β) is associated with body mass index in healthy women. Acta Endocrinologica. 2013;9(3):361–8.

[44] Liu M, Gao J, Zhang Y, Li P, Wang H, Ren X, et al. Serum levels of TSP-1, NF-κB and TGF-β1 in polycystic ovarian syndrome (PCOS) patients in northern China suggest PCOS is associated with chronic inflammation. Clinical Endocrinology. 2015;83(6):913–22.

[45] Yadav H, Quijano C, Kamaraju AK, Gavrilova O, Malek R, Chen W, et al. Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. Cell Metabolism [Internet]. 2011;14(1):67–79. Available from: http://dx.doi.org/10.1016/j.cmet.2011.04.01