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Original Research Article

Metformin Effects on Blood Levels of Myonectin in Polycystic Ovarian Women

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Abstract: ***Background*:** Polycystic ovary syndrome (PCOS) is a most prevalent endocrine diseases for females of childbearing age, and lead to an ovulatory infertility. It was shown that myonectin may be positively associated with insulin resistance parameters. ***Objectives*:** To evaluate serum myonectin levels and to determine the effects of metformin treatment on myonectin levels in patients with polycystic ovary syndrome. ***Patients and Methods*:** The Cross-Sectional Study carried out in the Department of Obstetrics and Gynecology Salahdeen general hospital in Tikrit city from 1st November 2022-30th January 2023. Patients diagnosed with PCOs depending based on the Rotterdam criteria. Participants were enrolled after all eligibility criteria were confirmed and informed consent completed. Sixty PCOS patients were selected as the PCOS group, while 30 healthy women matched for age with the PCOS patients were selected as the control group. Only 30 patients of them complete the follow up study and they agree to continue on metformin treatment during three months, the duration of the follow up. They take metformin 850 mg twice daily for three months and provide fasting blood samples on the second day of menstruation before and after treatment. The data collection done through: a designed closed and open-ended questionnaire, by using direct interviewing and Ultrasound examination, Laboratory examination. Myonectin, Luteinizing hormone (LH), Follicular Stimulating Hormone (FSH), and insulin were analysed by enzyme-linked immunosorbent assay (ELIZA) technique from Biomeriuex. Data were analyzed using SPSS for Windows 7. ***Results*:** The level of myonectin decreased significantly in PCOS patients compare to control group. The level of blood glucose, Insulin, and HOMA-IR, were increased significantly compared to the control. Serum LH levels were significantly higher, while the level of FSH were lowerin in women with PCOS than in controls. After metformin intake, all patients showed significant in decrease in gremlin concentration at p-values 0.05, but no significant difference (P>0.05) in myonectin level when compared to pre-treatment. Treatment resulted a significant decrease in in body mass index, Blood glucose, Insulin, HOMA-IR, testosterone and LH at p-values 0.05. However, the study found no significant difference ($p > 0.05$) in myonectin level between both groups in treated group with metformin compared with pre-treatment. ***Conclusion*:** Metformin testosterone and insulin resistance but can't induce changes of myonectin level in patients with polycystic ovary syndrome.

Keywords: Polycystic ovary syndrome (PCOS), metformin treatment, Luteinizing hormone (LH), HOMA-IR.

INTRODUCTION

Polycystic ovary syndrome (PCOS), which has been previously known as the Stein-Leventhal syndrome, is one of the most common endocrine/metabolic disorders affecting reproductive age women. PCOS affects 6% to 15% of women at the reproductive age, depending on diagnostic criteria [1]. The Rotterdam criteria (2013) are the most commonly used criteria to diagnose PCOS, and include the following: ovulation disorder, hyperandrogenism diagnosed by biochemical testing and/or clinical aspects, and ovarian volume over 10 ml or 12 or more ovarian cysts. The diagnosis can be established when two of the three conditions are fulfilled. Based on these criteria, four PCOS phenotypes can be detected, namely ovulation disorders, polycystic ovary, and hyperandrogenism, making up the classic phenotype, normal ovarian ultrasonography with hyperandrogenism and ovulation disorder, polycystic ovary ultrasonography image and

hyperandrogenism, with no ovulation abnormalities, and no evidence of hyperandrogenism, but with polycystic ovary ultrasonography image and ovulation disorders [2]. Several endocrinopathies can mimic PCOS, such as Cushing's syndrome, non-classic adrenal hyperplasia, drug-induced androgen excess and androgen-producing tumors. Ovulatory dysfunction can further be found in conditions like hyperprolactinemia or thyroid dysfunction [3-6]. Therefore, in order to proper diagnose PCOS, these disorders need to be excluded.

The skeletal muscle is the largest organ in the body and is essential to maintain vital functions such as determining whole-body insulin sensitivity and metabolic homoeostasis [7, 8].

As an endocrine tissue, skeletal muscle synthesizes various hormones and cytokines, which are termed myokines [9]. In an autocrine, paracrine or endocrine manner, myokines link skeletal muscle to other tissues [10]. Furthermore, myokines take part in regulating inflammatory, metabolic and other physiological functions in non-muscle tissues. As a new myokine, myonectin also plays a role in regulating lipid metabolism [11, 12].

Myonectin, also known as C1q (complement component 1q)/TNF (tumor necrosis factor)-related protein 15/erythroferrone, was discovered in 2011 by Seldin *et al.*, is a member of CTRPs (C1q/ expressed by adipose tissue, myonectin was originally identified as a myokine that is abundantly expressed in skeletal muscle tissue, in particular, type I muscle fibers, whose gene is located on locus 2q37.3 [12, 13].

The protein structure of myonectin resembles that of other CTRPs and contains four domains [12, 14]. In the myonectin protein, the four predicted N-linked glycosylation sites were located in the C1q /TNF-like domain. Moreover, four conserved cysteine residues are also contained in myonectin, which are required for protein folding and multimerization. Myonectin is predominantly expressed in skeletal muscle. Unlike the majority of myokines, myonectin synthesis is not restricted to skeletal muscle [14].

Myonectin plays a vital role in regulating lipid and glucose metabolism. Nutrient intake by skeletal muscle upregulates the expression and secretion of myonectin, resulting in an increased circulating level of the protein. Myonectin induces the expression of CD36, fatty acid transport proteins (FATP), and fatty acid binding proteins (FABP) in hepatocytes and adipocytes, resulting in enhanced fatty acid uptake into hepatocytes and adipocytes [15].

Myonectin mRNA and protein levels were significantly reduced with overnight fasting, and were substantially promoted after refeeding. Serum levels of myonectin are promoted in mice that are gavaged with glucose and lipids. Similarly, *myonectin* expression was markedly increased in mouse myotubes treated with free fatty acids or glucose. In addition, myonectin expression and circulating levels were also increased in mice with a running wheel for two weeks [16, 17].

Myonectin may prevent the increase of insulin resistance by regulating glucose and lipid metabolism.increases AMPK activity and stimulates skeletal muscle glucose transporters and increases glucose uptake in muscle [18]. Studies have shown that exercise training, regardless of its duration, leads to a change in the myonectin quantity and a reduction in insulin resistance [19]. It seems that as a result of weight gain through a high-fat diet, fat tissue increases and deposition of free fatty acids in muscle tissue increases, and as a result, the ability of skeletal muscle to produce myokines, including myonectin decreases [18, 19].

MATERIALS AND METHODS

1- Study Design

This study carried out in in the Department of Obstetrics and Gynecology Salahdeen general hospital in Tikrit city from the 1st November 2022 to the 30th January 2023, This study included 90 subjects: 60 patients with PCOS and 30 controls, only 30 patients of them complete the follow up study and they agree to continue on metformin treatment during three months, the duration of the follow up. The patients who can't complete the follow up, eight of them become pregnant; nine of them can't tolerate the drug due to its side effects, while the other did not communicate.

The diagnosis of PCOS was made according to the Rotterdam criteria. Specifically, patients with anovulation and clinical and/or biochemical hyperandrogenism were enrolled.

2- Treatment

All subjects received metformin (Glucophage, Merck) at a dosage of 850 mg twice daily for 3 months. In addition, standard clinical evaluations and laboratory analyses were performed at baseline and after 3 months of treatment as safety measures after the treatment period, in each patient all of the above parameters were reevaluated as at baseline.

Inclusion Criteria

1. Women newly Diagnosed with PCOS according to modified Rotterdam criteria which include: 1- the presence of clinical and/or biochemical signs of hyperandrogenism; and 2- at least one of the following: oligo- or anovulation and/or polycystic ovaries (1) depending on ultrasound examination, clinical features and laboratory hormonal tests by specialist gynecologist.

The women with PCOS were given metformin drug 850 mg daily during the meal for three months. The changes in clinical and biochemical parameters were measured (before treatment) and then after three months of treatment with above mention drugs. The clinical and biochemical parameters measured including: BMI, acne, menstrual irregularity, serum levels of total testosterone, fasting blood suger, fasting insulin.

Women who had diabetes mellitus, hyperprolactinemia, congenital adrenal hyperplasia, thyroid disorders, Cushing syndrome, androgen secreting tumors, hypertension, and smoking. Women had been treated with any hormone and confounding medications, including oral contraceptive agents, anti lipidemic drugs, and insulin-sensitizing drugs that might affect the ovarian function and /or metabolic criteria; within 3months before enrollment.

Blood Sampling

Venous blood samples were collected after overnight fasting from each patient by using a disposable syringe at about 8:30 to 11 am 5 ml from fresh venous blood were preserved immediately in an anticoagulant containing tube and the remaining were then allowed to clot in a plain tube at room temperature, after which the serum was separated by centrifugation at 3000 rpm for 10 min and kept frozen at -20 °C to be analyzed later on.

Measurement of Insulin Resistance

Measurement of Insulin Resistance: IR status was determined according to IR indices including the homeostatic model assessment (HOMA)-IR. PCOS subjects with HOMA-IR ≥ 2.5 and QUICKI ≤ 0.333 were identified as IR group [20]. The calculation of the above mentioned IR indices was based on these formulas: HOMA= [Fasting insulin (μIU/ml) × Fasting glucose (mg/dl)]/405

RESULTS

The results of this study observed there were a highly significant differences in the mean levels of BMI (kg/m²) between cases and control (29.740 ± 1.332 vs 19.940 ± 0.981) with P-value=0.01). On the other hand the highest mean of BMI (29.75 ±1.49Kg/m^2) was recorded in PCOS women before treatment and decreased significantly (P<0.05) after treatment with metformin (22.420 ± 1.062) as arannged in Figure (1).

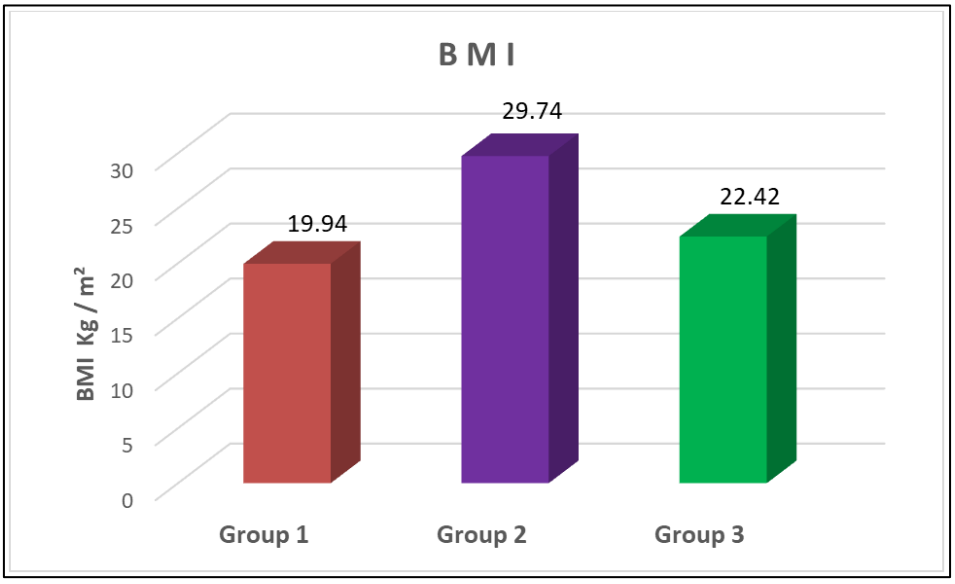

Figure 1: Comparative the mean of BMI (kg/m²) in study groups

Table 1: Comparative the mean of blood glucose in study groups

Test	M±SD		
	Control (n=30)	Patients (n=30)	PCOS + Metformin
Glucose (mg/dl)	92.137±8.61	97.49± 20.42	90.122±18.601

The results of this study observed there were a highly significant differences in the mean levels of glucose (mg/dl)between cases and control(97.49± 20.42 vs 92.137±8.61 mg/dl) with P-value=0.00003. The blood glucose level was reduced significantly (P<0.05) in PCOS women after treatment (90.122±18.601mg/dl) than before treatment (97.49± 20.42mg/dl) as arannged in Table (1).

Level of Insulin in PCOS Women before and after Treatment

The study showed that the highest mean of insulin was recorded in Group 2 (13.975±3.2041 µIU/ml) as compared with group 1(11.139±2.3132 µIU/ml). The difference was significant (p < 0.05), as shown in table (2).

Table 2: Comparative the mean of insulun in study groups

Test	M±SD		
	Control(n=30)	Patients (n=30)	PCOS + Metformin
Insulin(µIU/ml)	11.139±2.3132	13.975±3.2041	13.787± 3.0776

The study showed that there was no significant difference (P>0.05) in insulin level in group2 (13.975±3.2041µIU/ml) and group3 (13.787±3.0776 µIU/ml), as shown in Table 2.

Table 3: Comparative the mean of HOMA-IR in study groups

Test	M±SD		
	Control (n=30)	Patients (n=30)	PCOS + Metformin
HOMA-IR	2.528±0.53877	3.5715±1.15923	3.17±0.87078

P-Value < 0.05

Test result in table (3) shows that there is significant deference's according to HOMA-IR levels between deferent groups is higher in the studied patient groups compared to the control group (3.5715±1.15923 vs 2.528±0.53877) (P-Value < 0.05). After metformin therapy there were a significant decrease in the values of HOMA-IR (3.17±0.87078) as compared with the values before therapy (3.5715±1.15923) at P< 0.05 as arranged in table 3 & figure 2.

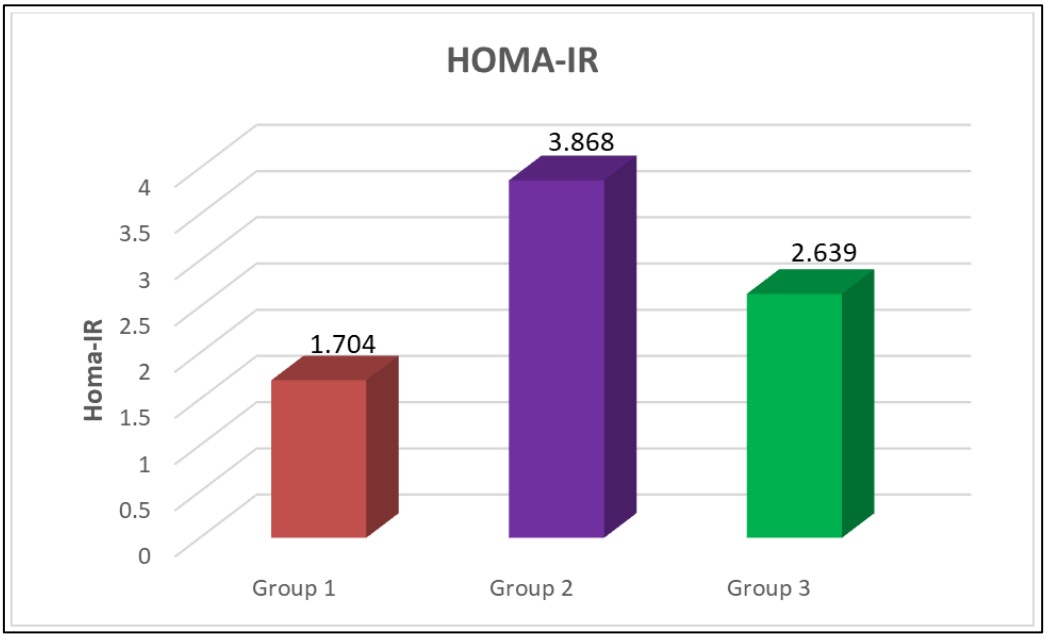

Figure 2: Distribution of PCOS cases according to HOMA-IR

The results of this study observed there were a significant differences in the mean levels of LH (IU/L) between cases and control (12.16±2.6046 vs 10.425±2.2297 IU/L) with P-value=0.0003 as show in table 4.

Table 4: Comparative the mean of LH in study groups

Test	M±SD		
	Control (n=30)	Patients (n=30)	PCOS + Metformin
LH (IU/L)	10.425±2.2297	12.16 ± 2.6046	12.246±2.6499

There was no significant difference (P>0.05) in LH level in PCOS women before treatment (12.16 ± 2.6046 µIU/ml) and after treatment (12.246±2.6499 µIU/ml), as arannged in table 4.

Table 5: Comparative the mean of FSH in study groups

Test	M±SD		
	Control (n=30)	Patients (n=30)	PCOS + Metformin
FSH (MIU/L)	6.918±1.2723	6.152±1.2592	5.560±.9137

FSH levels were lower in PCOS than control groups and there was significant difference (6.152±1.259 *vs* 6.918±1.2723 MIU/L, $p = .0.04$). The PCOS cases after three months of metformin therapy had significantly lower FSH (MIU/L) (5.560±.9137) levels as compared with the non-treated PCOS cases (6.152±1.2592) as shown in Table 5.

Table 6: Comparative the mean of testestrone in study groups

Test	M±SD		
	Control	Patients	PCOS + Metformin
Testestrone (pg/ml)	42.567±13.1428	57.697±11.9885	49.960±8.1856

P ≤ 0.00001

The present study reveals that the mean serum level of testestrone in G:2 57.697±11.9885pg/ml, was significantly higher than that of the G:1 42.567 ± 13.1428 pg/ml. After treatment with metformin testestrone levels were lower compared with PCOS group as evident in the following table 6.

Table 7: Comparative the mean of myonectin in study groups

Test	M±SD		
	Control (n=30)	Patients (n=30)	PCOS + Metformin
Myonectin (ng/mL)	9.510 ±0.573	6.10 ± 0.376	8.000 ± 0.559

P ≤ 0.00001

The mean myonectin levels in the case group were signicantly lower than in the group I (6.100 ± 0.376 vs 9.510 ± 0.573 ng/mL: P-Value ≤ 0.00009). As well as there was significant increase in the level of myonectin group III compared as Group II (8.000 ± 0.559 vs 6.100 ± 0.376 ng/mL: P-Value ≤ 0.00009) as shown in Table 7, & figure 3.

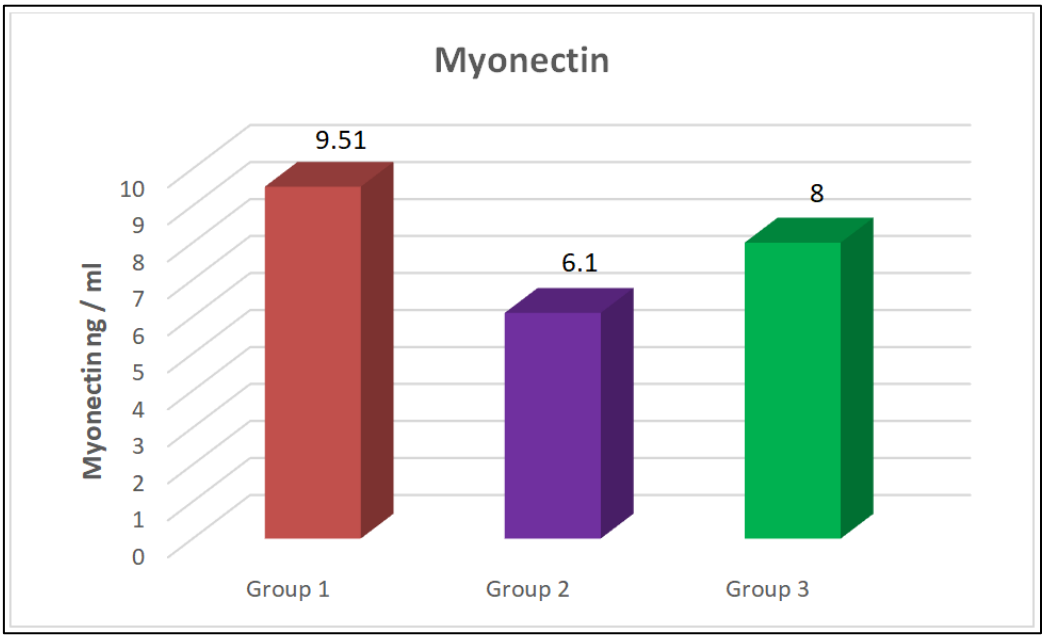

Figure 3: Serum level of Myonectin in study groups

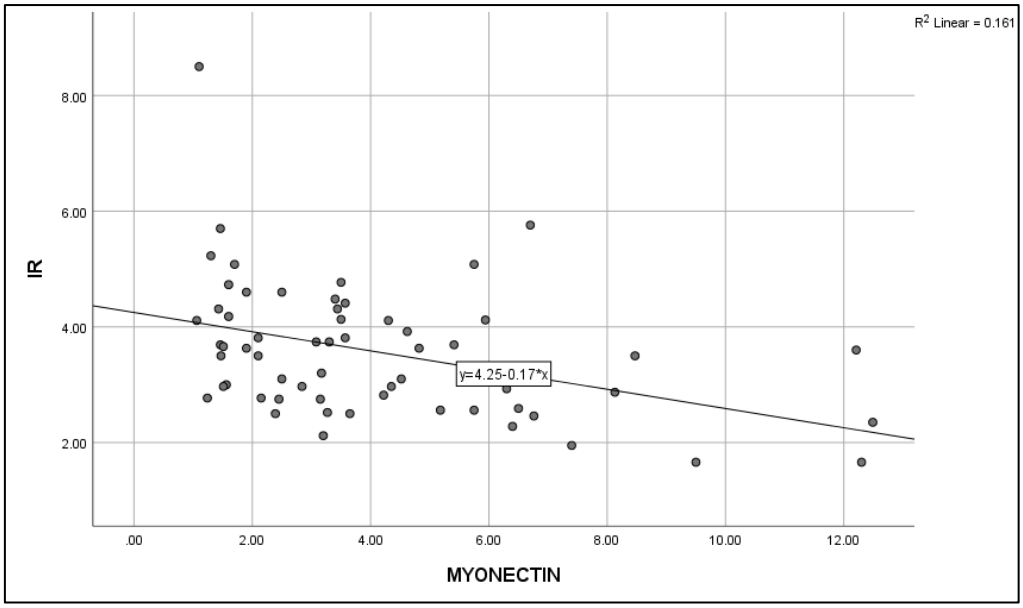

Figure 4: Correlation of myonectin with inslin resistance in PCOS patients

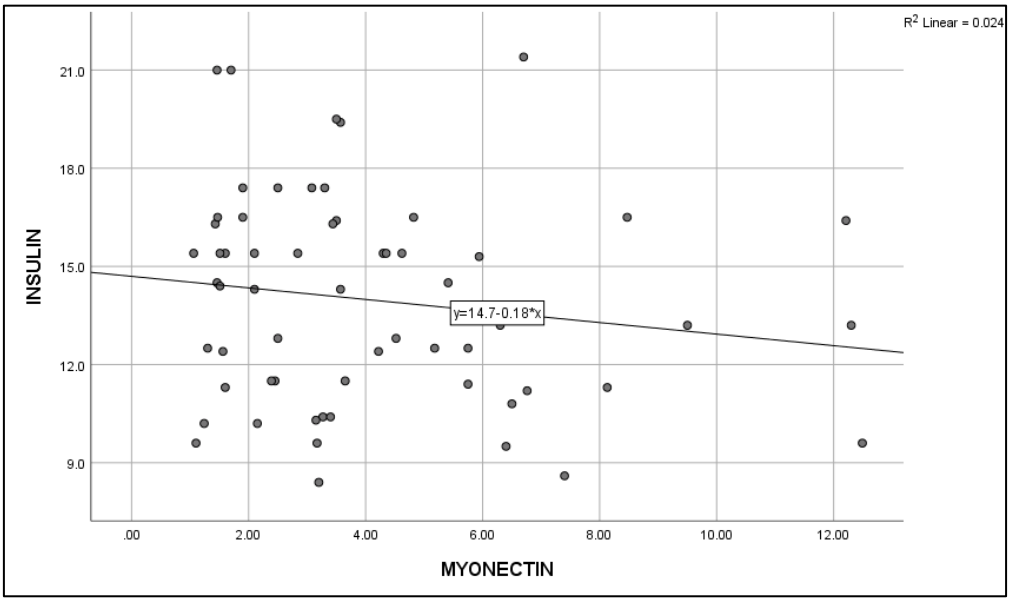

Figure 5: Correlation of myonectin with inslin in PCOS patients

The study showed positive correlation between myonectin and IR in PCOS patients (r = 0.161), the result was highly significant ($p < 0.05$), as shown in figure (4). On the other hand there was negative correlation between myonectin and insulin in PCOSpatients (r = 0.024), the result was significant ($p < 0.01$), as shown in figure (5).

DISCUSSION

The results of this study observed there were a highly significant differences in the mean levels of BMI (kg/m²) between cases and control On the other hand the highest mean of BMI was recorded in PCOS women before treatment and decreased significantly ($P<0.05$) after treatment with metformin.

This study goes with Jamal AF *et al.,* 2019 in Iraq [21] who found that the highest prevalence (43%) of PCO which was also found among participants of high BMI (≥ 30) and 22.5% among overweight women.

Patients with PCOS, especially those of high BMI, require specific therapy to decrease their weight; usually, those patients could not achieve weight loss easily, because of different frustration such as their bad mood, depression, feeling of distress and bad quality of life. Therefore, resolving of emotional problem is very important to encourage those patients to change their lifestyle and hence decrease their weight. It is well known that most common symptoms of PCOS

are daily fatigue, sleep disturbances and changes of appetite, which highlight an importance of changing lifestyle by decreasing high fat diet and high carbohydrate diet with increasing physical activity as well as resolving mental and emotional status by incorporation and encouragement [22, 23, 35].

In the current study, the women with PCOS were found to be significant reductions in body weight and BMI after three months of treatment. Our study is also consistent with those of Fattah *et al.,* revealed that patients who were on Metformin had a mean decrease in BMI of 6.7 ± 3.01 kg/m^2 [23]. Metformin reduces appetite and caloric intake in the gastrointestinal tract. It also alters the adenosine monophosphate-activated kinase in the hypothalamus and mediates anorectic effects, in this way metformin reduces body weight resulting in a reduction of body mass [24].

This result showed that there is significant deference's according to HOMA-IR levels between deferent groups is higher in the studied patient groups compared to the control group. After metformin therapy there were significant decreases in the values of HOMA-IR as compared with the values before therapy.

Insulin resistance in PCOS is caused by impaired insulin action in various target tissues, which is characterized by basal compensatory HI and a reduced insulin response to glucose overload. PCOS affects the majority of organ systems and tissues. Insulin plays different roles in different tissues in balancing the supply and demand of nutrients. HI caused by tissue IR is central to PCOS pathology [25].

HOMAIR is a technique used to asses β-cell function and insulin resistance from the basal glucose and insulin. The normal HOMA- IR is equal to one, 1. The relationship between glucose and insulin in the basal state reflects the balance between hepatic glucose out-put and insulin secretion, which is maintained by feedback loop between liver and β-cell. The HOMA-IR is determined by the equation (fasting insulin multiplied by fasting glucose) divided by 22.5 [26, 27]. Fasting insulin and glucose are easily obtainable, safe, low cost, less invasive test than oral glucose tolerance test [28]. Insulin resistances occur when fasting insulin is over 10Mu/ml and HOMA-IR>2. The metabolic and reproductive groups of PCOS were distinguished based on the HOMA-IR value at cut-off value of 2. The PCOS is reproductive, when HOMA-IR2. It was found that patients with metabolic PCOS usually more obese [29].

Insulin resistance is found to be mild in lean PCOS patients [30], and is significantly increased in higher BMI – PCOS patients [31]. Even normal weight PCOS patients showed certain degree of endocrine dysregulation independently of BMI [32]. Therefore, it is mandatory for women with PCOS to add self-monitoring of blood glucose to their treatment program to achiev fasting and post-prandial normoglycemia. Hyperinsulinemia stimulates IGF-1 receptor in ovarian theca cells, augments androgen production, and inhibits hepatic SHBG production, therefore, results in increase of free testosterone [33, 34]. Moreover, PCOS women revealed an increase level of fasting glucose and decrease level of magnesium, which is important for insulin action, especially that, PCOS women had higher tendency to eat food of high glycemic index [35], with lower fiber content and magnesium [36].

This study reveals that the administration of metformin850 mg twice daily for three months resulted in a significant decrease in HOMA-IR and fasting insulin. According to Liu *et al.,* [37], hyperinsulinemia and hyperandrogenemia are decreased by metformin treatment by improving glycolipid metabolism even in normal insulin resistance PCOS patients. While Sharma *et al.,* [38] showed that metformin is able to reduce insulin levels in both obese and non-obese PCOS patients. Santana *et al.,* [39] suggests that metformin improves insulin resistance and improves hyper-androgenemia via its action on IGF-1 and carrier protein. Metformin can decrease HOMA-IR in PCOS and diabetic patients; this effect is attributed mainly to gut microbiome modulation because it is widely concentrated in the intestine [40].

The present study findings confirmed the results of several studies showed that it is significantly decreased after metformin treatment, and this glucose lowering effect is attributed to: (1)-Suppressing of hepatic glucose production [41]. (2)-Increase glucose uptake by GLUT4. (3)-Increase fatty acid oxidation and inhibition of glucagon [41, 42]. (4)-Metformin increase insulin sensitivity and has direct effects on ovary [41]. Meta-analysis of thirty eight studies in PCOS women revealed that metformin lowers fasting insulin in obese PCOS better than non -obese PCOS [41, 43].

While, Behradmanesh *et al.,* [44] showed no significant changes in fasting glucose, fasting insulin and HOMA-IR after 6 months of metformin administration for forty five PCOS patients in study performed in Iran, Shiraz city.

The difference in response to metformin between different studies may be attributed to different factors such as: 1- Genetic factors. 2- Environmental factors such as chemicals, radiation exposure. 3- Lifestyle factors (diet, drinking, smoking, exercise), and 4- Physiological factors (age and sex) [44-47]. Jayagopal V *et al.,* [48] showed no significant reduction of fasting glucose, fasting insulin and HOMA-IR in PCOS patients, Jayagopal's patients are advised to consume diet consists of 30% fat, 50% carbohydrate and 20% protein with 300 mg cholesterol intake and to increase

metformin stepwise till reach 500mg three times, he attributed his results to the higher diversity of HOMA-IR in PCOS, which can be overcome by increasing sample size [48].

The mean myonectin levels in the case group were signicantly lower than in the group I As well as there was significant increase in the level of myonectin group III compared than Group II.

Myonectin increases the expression of proteins transporting FFA through hepatocytes and adipocyte cell membranes, causing increasing serum FFA uptake.

Myonectin has been implicated in a number of metabolic disorders such as MetS, insulin resistance, T2DM, and CAD [49-51]. Mi *et al*., [51] reported that serum CTRP15 concentration was significantly higher in individuals with MetS and insulin resistance. Toloza *et al*., [52] reported that myonectin level is associated with insulin resistance (IR) in non-diabetic adults. Furthermore, Li *et al*., [53] stated that it could be used as a marker in predicting the development of pre-DM and DM. In the pathogenesis of PCOS, only two studies examined the association of myonectin. Demir and Guler [54], found that myonectin levels were significantly lower in Turkish patients diagnosed with PCOS (6.77 ± 1.96 vs. 9.14 ± 2.87 ng/mL, $P < 0.001$) when compared to healthy controls, predicting PCOS risk. Similarly, Zhang *et al*., [55] had the same results in the Chinese population (6.51 ± 2.13 vs. 9.35 ± 2.64 ng/mL, $P < 0.05$). Vatannejad *et al*., 2022 [49] found that the serum myonectin levels are higher in PCOS patients (91.29 ± 26.42 vs. 54.78 ± 15.45 µg/L, $P < 0.001$) when compared to their healthy counterparts. This discrepancy, along with the elevated serum level, might be due to the different study populations. Inconsistent results on the circulating levels of myonectin have been also observed in other metabolic disorders such as T2DM and obesity [49]. Nevertheless, it highlights the possible role of this myokine in PCOS pathogenesis through its association with the metabolic, hormonal, and inflammatory disturbances observed in this syndrome.

Insulin resistance is one of the main pathogenic pillars exacerbating the metabolic disturbances in PCOS. Eighty-five percent of patients diagnosed with PCOS exhibit hyperinsulinemia. CTRP15 is expressed and secreted by skeletal muscle in response to acute nutritional and metabolic changes, as well as a chronic alteration in the energy state of the animals. Glucose and/or fatty acids directly induce myonectin expression in myotubes. The molecular mechanism of increasing levels of myonectin in patients with PCOS is not clear; however, changes in the regulation of myonectin in response to metabolic disorders such as insulin resistance in PCOS individuals could be suggested as a possible mechanism [49, 56].

Li *et al*., 2018 [53] reported that the, the myonectin levels in circulation are correlated with HOMA-IR, BMI, and triglyceride (TG) contents.

Demir and Guler [54] reported an inverse correlation between serum CTRP15 and free androgen index (FAI) in PCOS patients. Moreover, Zhang *et al.,* found that CTRP15 levels negatively correlated with testosterone and positively correlated with sex hormone-binding globulin (SHBG) in the PCOS group [55]. These differences might be the result of the heterogeneity in the study populations. Nevertheless, CTRP15 levels can be associated with the hormonal profile in PCOS patients.

CONCLUSION

The study demonstrate that there was significant decrease in serum levels of myonectin in PCOS women .While there was no significant difference after 3 months of metformin therapy. The beneficial effect of metformin on the hormonal profile corroborates well with the existing data suggesting that metformin is an effective drug in reversing insulin-resistance and therefore in the management of anovulatory infertility and prevention of long-term consequences So, metformin appears to be a drug with multiple therapeutic effects far beyond its effect on lowering blood glucose in diabetes mellitus and should be integrated in the spectrum of therapeutic options of PCOS.

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