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Original Research Article

Metformin Effects on Plasminogen Activator Inhibitor-1 in Polycystic Ovarian Women

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Abstract: ***Background***: Polycystic ovary syndrome (PCOS) is a widespread complex endocrine disorder of women in the reproductive age group. Plasminogen activator inhibitor-1(PAI-1) has been shown to be associated with the regulation of inflammation and ovulation. ***Objectives*:** The aim of this study was to evaluate serum PAI-1 level and to determine the effects of metformin treatment on serum PAI-1 levels in patients with PCOS. ***Patients and Methods*:** This study was conducted on sixty women with PCOS, 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. ***Study Design*:** A cross sectional study is done in Salah Al-Deen general hospital/gynecology and obstetrics department 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. The body mass index is determined before and after therapy. Homeostatic model assessment for insulin resistance is determined according to the following formula: (fasting insulin multiplied by fasting glucose) divided by 405. Fasting serum PAI-1 was measured by Enzyme-Linked Immunosorbent Assay method. Data were analyzed using SPSS for Windows 7. ***Results*:** Compared with the control group, the levels of PAI1, fasting blood glucose (FBG), Insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) While the levels of follicle stimulating hormone (FSH) were significantly decreased ($P<0.05$). In the PCOS group were significantly increased ($P<0.05$). Serum levels of testosterone in PCOS group was significantly increased ($P<0.05$). In PCOS women treated with metformin for three months, all patients showed significant in decrease in PAI1, and testosterone concentration at p-values 0.05, when compared to pre-treatment. Treatment resulted a significant decrease in in body mass index, Blood glucose, Insulin, HOMA-IR, and LH at p-values 0.05. ***Conclusion*:** Plasminogen activator inhibitor-1 level decreased significantly after 3 months of treatment with 850 mg per day with metformin in women with PCOS.

Keywords: Polycystic ovary syndrome (PCOS), serum PAI-1, metformin, follicle stimulating hormone (FSH).

INTRODUCTION

Polycystic ovary syndrome (PCOS) depending on the term employed is the most common cause of an ovulatory in-fertility and endocrine disorders and affects approximately 8–13% of reproductive age women [1]. The diagnosis and management of PCOS is a challenging endeavor because it is a mysterious condition with major symptoms that vary with age, and the treatment should be tailored to meet the specific requirements of each patient. The application of the Rotterdam criteria for the diagnosis of adult women with PCOS was approved by international evidence-based guidelines. The diagnosis requires fulfillment of a mini-mum of two of the following three conditions: oligo-ovulation or anovulation, clinical or biochemical hyperandrogenism, and detection of the radiographic features of polycysticovaries by means of ultrasonography [2, 3]. The main symptoms of the syndrome include infertility attributable to anovulation, irregular menstrual cycles, and symptoms caused by androgen excess, such as hirsutism. Moreover, the condition can be

associated with concurrent chronic metabolic diseases, such as increased insulin resistance, which necessitates appropriate treatment to prevent complications [3].

Therapeutic approaches for PCOS are varied in their targets and effects and include both pharmacological and non-pharmacological inter-ventions [4]. Metformin (MET) is a biguanide insulin sensitizer which may inhibits hepatic glucose production and increases peripheral glucose uptake and utilization. Metformin can both improve insulin sensitivity in target tissues and directly influence ovarian steroidogenesis, and these effects do not appear to be primarily responsible for the attenuation of ovarian androgen production in women with PCOS. Although metformin benefits patients with diabetes by improving insulin sensitivity, whether it increases insulin secretion, particularly during the first phase of secretion, remains unclear [5-7].

Plasminogen activator inhibitor-1, a member of the serine protease inhibitor (serpin) superfamily, is an adipokine produce in adipose tissue. PAI-1 is a procoagulant, proinflammatory, and profibrotic molecule; it is secreted as an active protein and, by binding to t-PA and u-PA, blocks the activation of plasminogen to plasmin, and therefore the fibrin clot hydrolysis. It was indicated that the production and secretion of PAI-1 could be induced by stimulators like thrombin, endotoxin, cytokines, and chronic inflammation. PAI-1 over-expression is associated with atherosclerosis in humans, particularly in individuals with the metabolic syndrome [8-10].

AIM OF THE STUDY

The aim of this study was to study the effects of metformin therapy on serum plasminogen activator inhibitor-1 levels in women with polycystic ovarian syndrome and to correlate plasminogen activator inhibitor-1 levels with insulin resistance parameters and with other parameters.

MATERIALS AND METHODS

Study Design

This cross-sectional study, used for investigation of some biochemical marker in patients with PCOS before and after metformin treatment.

From the 1st November 2022 to the 30th January 2023, a total 90 subjects: 60 patients with PCOS and 30 controls, with mean age 30.4±5years) were recruited from the Department of Obstetrics and Gynecology Salahdeen general hospital in Tikrit city, 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, nine of them become pregnant; eleven 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.

After their visiting to the gynecologist physicians and diagnosed according to their symptoms and signs and to the picture of ovarian ultrasound, their weight and height are measured and other related information such as age, duration of infertility, number of children.

All patients underwent metformin treatment, administered as pills taken orally in a daily dose of 850 mg for 3 months.

Women newly Diagnosed with PCOS according to modified Rotterdam criteria which include: 1- the presence of clinical and/or biochemical signs of hyper-androgenism; 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 protocol of this study was approved by the Scientific Committee at Tikrit University – College of Medicine, and the agreement of the attendance to Salah al-Din General for collecting the samples from the patients was approved via the Directorate of Salah al-Din Health. Each patient was educated about the research's purpose of study, filled out questionnaire and signed a consent form to participate in the study.

About 5 ml of blood sample was taken by vein puncture from each subject. The blood sample was transferred into a sterilized plain tube and allowed to clot by standing for (10-15 minutes) at room temperature. Then, the tube was centrifuged at 3000 rounds per minute (rpm) for (10 minutes) to obtain serum sample which was immediately separated, placed into 3 clear dry Eppendorf tubes and labeled with number and stored at (-20 °C) till used for analysis.

Chemicals and Reagents: Specific chemicals utilized in this study are listed as below Chemicals and reagents: Specific chemicals utilized in this study are listed as below with their suppliers. Insulin, and PAI, LH, FSH, and trestestrone ELISA kit (Cusabio Biotech co., China) (Demeditec Diagnostics GmbH, Germany), blood glcose were measured by spectrophotometric.

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 [9]. The calculation of the above mentioned IR indices was based on these formulas: **HOMA**= [Fasting insulin (μIU/ml) × Fasting glucose (mg/dl)]/405

RESULTS

Table 1: Demographic characteristics and biochemical results of the two groups

Characteristic	PCOS		Control	
	Mean	SD	Mean	SD
Age (Years)	30.4	5	29.55	5.45^{NS}
BMI (kg/m²)	33.36	1.89	28.46	0.39 *
FSH (MIU/L)	6.154	1.2591	6.916	1.2722*
LH (IU/L)	12.171	2.605	10.5	2.22*
PAI1 (ng/dl)	605.3474	169.1152	326.371	125.278*
Insulin (mIU/mL)	14.662	3.3798	11.140	2.3132
HOMA-IR	3.5710	1.15889	2.5284	0.53964*
Testestrone (pg/ml)	57.697	11.9886	42.567	13.1429*
Glucose (mg/dl)	100.8	20.7	90.14	18.60*

* P<0.05, NS: non-significance

Regarding the serum levels of fasting blood glucose was significantly increased PCOS group (100.8±20.7 mg/dl) when compared to the healthy group 90.14±18.60 mg/dl. Moreover, PCOS groups after 3 months of metformin treatment revealed significant reduction (93±17.9 mg/dl) in serum blood gluose compared to pre-treatment (98.8±20.6) as shown in table 1.

Table 2: Level of blood glucose (mg/dl) in Women before and after Treatment

PCOS women	Blood glucose (mg/dl)		P. value
	Mean	SD	
Before treatment	98.8	20.6	P<0.05 Sig
After treatment	90.14	18.60	

The present study showed significantly higher serum insulin levels in PCOS patients (14.662±3.3798) compared to healthy control groups (11.140±2.3132).

After metformin treatment, serum insulin levels significantly decreased in PCOS groups (13.200±3.0778) compared to their pre-treatment (16.123±3.0571).The difference was significant ($p < 0.05$), as shown in table (2).

Table 3: Level of insulin in Women before and after Treatment

PCOS women	Insulin	
	Mean	SD
Before treatment	16.123	3.0571
After treatment	13.200	3.0778

$p < 0.05$

The study showed that the lowest mean of HOMA-IR was recorded in PCOS group (3.5710±1.15889) as compared with control group (2.5283±0.53879). The difference was significant ($p < 0.05$). While the administration of a metformin for three months showed a significant reduction in the values of HOMA-IR (3.1710±0.87079) as compared with the values before therapy (3.9727±1.28194) at $P< 0.05$, as shown in table 4.

Table 4: Level of HOMA-IR in Women before and after treatment

PCOS women	HOMA-IR	
	Mean	SD
Before treatment	3.9727	0.28194
After treatment	3.1710	1.87079

Luteinizing hormone level was significantly increased the PCOS-induced (12.171 ± 2.605) vs control ((10.5±2.22)), ($p \leq 0.05$) as shown in table (5).

No significant differences were seen in the metformin-treatment levels of LH (12.164±2.6049) vs pre-treatment group (12.079±2.60) (P>0.05) as shown in table (4).

Table 5: Level of LH in women before and after Treatment

PCOS women	LH		P. value
	Mean	SD	
Before treatment	12.164	2.6049	P<0.05
After treatment	12.079	2.60	Sig

P<0.05

Our results demonstrated significantly elevated FSH (MIU/L) levels in PCOS patients (6.154±1.2591) when compared with controls (6.916±1.2722). At the end of the treatment, in this group, the levels of FSH was decreased (5.561 ± 0.91 36 MIU/L), when compared with pre-treatment (6.16±1.25 (MIU/L). These were statistically significant (p< 0.05) as shown in table (6).

Table 6: Level of FSH in Women before and after Treatment

PCOS women	FSH (MIU/L)	
	Mean	SD
Before treatment	6.18	1.25
After treatment	5.561	50.9136

p< 0.05

The current study showed that compared with the control subjects, the values of PAI1, (326.3719±125.27899) were significantly increased (P < 0.05), in Women with PCOS (605.3474±169.11524) as shown in table (1). The serum concentrations of PAI1 were decreased significantly at 3months of treatment with metformin (*520.23±138.105*) compared ton pre-treatment (690.43 ±155.305) as seen in table 7.

Table 7: Level of PAI1 in women before and after Treatment

PCOS women	PAI1		P. value
	Mean	SD	
Before treatment	690.43	155.305	P<0.05
After treatment	520.23	138.105	Sig

P<0.05

This study reveals that the level of testosterone which was significantly elevated in PCOS patients (57.697±11.9886) was as compared with apparently healthy women (42.567±13.1429) at P < 0.05. Our results are in line with the results of other studies.

Table 8: Level of testosterone in women before and after Treatment

PCOS women	Testosterone		P. value
	Mean	SD	
Before treatment	65.433	10.0779	P<0.05
After treatment	49.960	8.1857	Sig

DISCUSSION

There were no statistically significant differences between the women with PCOS and the control group with regard to age as shown in table (1). It is evident from this study that the majority of women with PCOS were from the age 20 to 30 years, this finding initiate the idea that younger age group are more prone to PCOS, this finding may be related to that the ovaries are more physiologically active in women at the child-bearing age, therefor the ovaries are more liable to undergo cystic changes.

The results noticed by this study were in agreement with the finding of Banu Ucar *et al.,* [10] who found that the mean age group for women with PCOS was (23.53 ± 5.51) years. Jamal AF *et al.,* 2019 in Iraq [11] who found that the highest prevalence (32.7% and 43%) was among the age group 18-27 years.

Alhindawi Zena [12] found that the highest prevalence of PCO among the mean age of women (25.8 ± 5.9 SD (ranging between 18-47 years old). While Ali Al-Gareeb [13] reported that the age of women with PCOS was (27.4 ± 7.5) years.

The current study reveals that the majority of women with PCOS were from the age 20 to 30 years, this finding initiate the idea that younger age group are more prone to PCOS, this finding may be related to that the ovaries are more physiologically active in women at the child-bearing age, therefor the ovaries are more liable to undergo cystic changes.

These data are similar to those reported by Miller, 2013 [14]; Diamanti-Kandarakis 2010 [15], Tokubuchi *et al*., 2017 [16]. These conflicting data on glucose lowering effect specially in PCOS women may be explained by the accumulation of metformin in the liver is important for the suppression of hepatic glucose production, which involves the inhibition of fructose-1-6-bisphosphatase10 and mitochondrial glycerol-3-phosphate6, and for the activation of AMP-activated protein kinase (AMPK), which improves insulin sensitivity through the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC). Metformin may also lower blood glucose by acting in the gastrointestinal tract, where it alters the gut microbiome and stimulates glucagon-like peptide-1 (GLP-1) release; however, increases in GLP-1 are not required for metformin-induced glucose lowering [17].

In contrast, Behradmanesh *et al.,* [18] did not confirm decreased 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.

Insulin resistance (IR) is described as a defective physiological response to insulin stimulation of targeted cells, primarily these in the liver, muscles, and various types of adipose tissue. Insulin resistance impairs glucose release, leading to a compensatory increase in β-cell insulin secretion that, in turn, leads to a hyperinsulinemia state within the whole body. The metabolic impact of IR includes the resulting hyperglycemia, elevated blood pressure, dyslipidemia, visceral adiposity, innate and chronic inflammatory responses, endothelial layer function impairment, and dysregulation of the hemostasis balance [19, 20].

The homeostasis model assessment of insulin resistance (HOMA-IR) provides a quick and affordable way to estimate insulin resistance, as well as the assessment results have been demonstrated to be on par with the gold standard approach [21].

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 [22].

Sex-hormone-binding globulin and luteinizing hormone are the top two clusters, representing two common mechanisms of IR increasing PCOS free testosterone level: First, insulin receptors in the pituitary gland are triggered to release luteinizing hormone, and second, the synthesis of sex hormone-binding globulin (SHBG) in the liver is inhibited. Increased androgen can also promote the decomposition of adipose tissue, increase the production of free fatty acids and inflammatory factors, and further aggravate IR, causing a vicious cycle [23, 24].

Metformin is well-known for its effectiveness in increasing the sensitivity of insulin in the peripheral tissues; reducing glucose absorption and hepatic glucose synthesis through an adenosine monophosphate activated protein kinase (AMPK) and increase insulin sensitivity by increasing peripheral glucose uptake with no significant direct effects on pancreatic insulin production. Insulin resistance in PCOS may arise as a result of defects in insulin signaling or receptor activity, decreased insulin clearance due to the inhibitory effects of high serum testosterone levels, and elevated adipose tissue, free fatty acids or cytokine production [2, 25]. Furthermore, the mechanisms of insulin resistance in PCOS and metformin's actions in improving the action of insulin are still largely unknown.

Similar results were obtained in a study done by Sharma (2019) [26] where insulin level was decreased to 23.6–20.2 μU/mol. In another study by Agarwal *et al.,* [27] (2003) a significant reduction in insulin level was also detected from 20.45 to 12.59 μU/mol.

Liu *et al.,* (2019) [28] hyperinsulinemia and hyperandrogenemia are decreased by metformin treatment by improving glycolipid metabolism even in normal insulin resistance PCOS patients. Santana *et al.,* (2004) [29] study

suggests that metformin improves insulin resistance and improves hyperandrogenemia via its action on IGF-1 and carrier protein.

Metformin decrease HOMA-IR in PCOS patients, this effect is attributed mainly to gut microbiome modulation because it is widely concentrated in the intestine [30].

This result agrees with the study of Manal Ibrahim, 2021, who showed that metformin can significantly decrease HOMA-IR only in insulin-resistant PCOS patients [31].

Goldenberg *et al*., [32] divided their PCOS population into quintiles according to HOMA-IR as a parameter for IR and compared the bottom and top quintile after 1 year of intervention with metformin and diet. They demonstrated an improvement of menstrual cyclicity in the bottom quintile that did not differ from that of the top quintile. Nawrocka and Starczewski study reported that metformin can significantly decrease HOMA-IR only in insulin resistant PCOS patients [33].

The current study revealed increased Levels of LH were high in patients with PCOS. This agrees with the study of Haifaa S; Ajaj *et al.,* [34]. (2020), Deliwala K J., *et al.,* [35]. (2020), Al-Assadi AF *et al.,* [1, 36]. (2019), Nath CK *et al.,* [1, 37]. (2019), who found raised serum LH concentrations in most of PCOS patient. This may be explained by the fact that angiogenesis plays an important role in both the follicular and luteal phases of an ovarian cycle.

Deliwala *et al.,* [35] reported that hypersecretion of LH during the follicular phase of the menstrual cycle occurs in PCOS and is associated with hyperplasia of the ovarian theca and stromal cells. Elevated LH levels may be responsible for increased stromal vascularization by influencing neoangiogenesis, catechol-aminergic stimulation and leukocyte and cytokine activation [35] and that the elevated LH level is due to the fact that androgens are the main source of hyperandrogenemia in PCOS. Hyperandrogenemia has both a direct effect on the ovarian alterations and, an increasing effect on pituitary LH pulse frequency and amplitude with relative low FSH secretion. Further, adrenal androgens contribute to PCOS androgen excess. Insulin resistance with compensatory hyper-insulinemia enhances ovarian androgen production as well as, decreases production of SHBG in the liver, and both increase the pool of bioavailable androgens. PCOS is also associated with increased muscle sympathetic nerve activity that is related to high testosterone, insulin resistance, and obesity [35].

In usual menstruation, the elevation of plasma-FSH through the (luteal-follicular change) is crucial for develop follicle and consequently ovulation. In hyperandrogenemic female who destined to improve PCOS, different from natural early pupery, decrease levels of FSH is probably due to the fact that the nocturnal elevate in the ovarian steroid hormones may be not sufficient to repress the GnRH pulse generator, that leads to a continuous increases rapid pulse frequency of LH, and decrease FSH manufacture which causes insufficient follicular growth, and this is due to a reduce GnRH pulse generator sensitivity to repress of steroid hormone especially progesterone. There is an indication for a disorder of early follicular growth in PCOS. But, it stays to be decided whether this situation is the cause of or may the impact of elevating the exposure to the androgens within the ovary [36]. Another cause of decrease FSH levels is that in obese women with PCOS peripheral manufacture (fat cells) of estrogen can cause a negative feedback on pituitary to repress the liberating of FSH [37].

Plasminogen activator inhibitor-1 (PAI-1), plays an important role in follicular development and rupture. High PAI-1 levels can delay maturation and hence can develop ovarian maturation [38]. PAI-1is a critical regulator of endogenous fibrinolysis. Due to a disruption in the hemostatic or fibrinolytic system, PCOS patients have a prothrombotic propensity and have higher levels of PAI-1, which is linked to higher BMI, visceral obesity, thrombosis, and cardiovascular disease (CVD) [39].

However, the precise mechanism between the blood level of PAI-1 and the development of PCOS is unclear. The overproduction of PAI-1, which could result from the polymorphism, may lead to disorders in the ovarian plasminogen-plasmin pathway and anovulation in women with PCOS [40].

The finding of elevated PAI-1 in women with PCOS in this study is consistent with several other studies like Elci *et al.,* in 2016 [38] who stated that cardiovascular risk marker PAI-1 was significantly elevated in nonobese women (BMI < 30 kg/m2) with PCOS. In 2004, Orio *et al.,* [41] also suggested significantly higher levels of PAI-1 in normal-weight PCOS women as compared with controls.

Insulin is known to increase hepatic PAI-1 production. Therefore, PCOS, a state of hyperinsulinemia may have the involvement of PAI-1 as a primary event in anovulation [42].

Higher (PAI-1) level is an essential feature of insulin resistance. Meena, 2021[43] reported that resistance of insulin impairs fibrinolysis through enhancing PAI-1 secretion. Increasing the concentration of PAI-1 in the circulation hinders fibrinolysis by impairing the action of t-PA [43].

This study reveals that the level of testosterone which was significantly elevated in PCOS patients was as compared with apparently healthy womenat $P < 0.05$. Our results are in line with the results of other studies. Mohemeed *et al.,* in 2019 [44] and AL-Tikriti *et al.,* in 2019 [45] since they concluded that mean values of patients serum-T were significantly higher than those of controls.

Hyperandrogenism is one of the important indicators of PCOS that refer to increased blood levels of androgens, increased serum testosterone may be due to insulin who has an inhibitory influence on hepatic production of (SHBG) resulting in increasing of free testosterone levels, moreover its stimulatory influence on the ovarian androgen production that augments the hyperandrogenic state [46]. Another causes of high manufacture of serum-T comprise hypersecretion of LH mediated GnRH, also an intrinsic dysregulation of theca-cell androgen synthesis in ovary, due to essential irregularity of P450c17α [47].

In hyperandrogenemic female who destined to improve PCOS, different from natural early pupery, decrease levels of FSH is probably due to the fact that the nocturnal elevate in the ovarian steroid hormones may be not sufficient to repress the GnRH pulse generator, that leads to a continuous increases rapid pulse frequency of LH, and decrease FSH manufacture which causes insufficient follicular growth, and this is due to a reduce GnRH pulse generator sensitivity to repress of steroid hormone especially progesterone. There is an indication for a disorder of early follicular growth in PCOS. But, it stays to be decided whether this situation is the cause of or may the impact of elevating the exposure to the androgens within the ovary. Another cause of decrease FSH levels is that in obese women with PCOS peripheral manufacture (fat cells) of estrogen can cause a negative feedback on pituitary to repress the liberating of FSH [48].

Overall, 3 months of metformin therapy significantly decreased serum level of testosterone compared to that before treatment at ($P < 0.05$) as seen in table 4.11., this finding reveals an improvement in testosterone secretion in women with PCOS which could be related to an improvement in ovarian function, because the metfomin act to decrease the serum lipid profile by decreasing the synthesis of cholesterol in tissues, and since that testosterone is a one of the steroidal hormone.

This study agrees with studies of Jensterle, (2020) [48] and Findakly 2021 [49] whom found a decline in serum testosterone by reducing hyperinsulinemia. Also, Shahebrahimi *et al.,* [50] found no change in testosterone and BMI after metformin treatment with a significant decrease in fasting insulin, the stimulatory effect of insulin on ovarian androgen production is affected by some genetic predisposition, which may explain differences in testosterone response after metformin treatment [51].

CONCLUSION

The present study demonstrated that circulating PAI1 levels were higher in patients with PCOS than in healthy controls and that metformin treatment significantly reduced circulating PAI1 concentrations after 3 months.

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