



IMPACT OF METFORMIN TREATMENT ON SERUM ADIPONECTIN LEVELS IN OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME IN RELATION TO INSULIN RESISTANCE

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ABSTRACT

Twenty-five obese (BMI ≥ 30) women with PCOS on metformin (3-6 months) therapy (MT- PCOS) and 25 newly diagnosed PCOS (ND- PCOS) matching for age and body mass index (BMI), were enrolled along with a control group of 25 (non-obese, BMI< 30) apparently healthy women. We measured serum total adiponectin, hormonal (testosterone, LH, FSH & prolactin) and metabolic parameters (FSG, FI, lipid profile) for each participant. Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was used to estimate insulin resistance. The ND- PCOS group displayed significantly lower levels of serum adiponectin ($P < 0.05$) and metformin treatment did not affect its level after adjustment for age, and BMI. Waist to hip ratio (WHR), testosterone (T), free testosterone (fT), fasting serum glucose (FSG), fasting insulin (FI), HOMA-IR, triglycerides, and atherogenic index (TC/HDL) were significantly higher ($P < 0.05$) in ND- PCOS compared to controls. However, metformin treatment was able to significantly decrease both of FSG, HOMA-IR values; and improves atherogenic lipid profile, despite the higher WHR values. Furthermore, serum adiponectin levels were significantly correlated with HDL-C in MT-PCOS group. We report a decreased serum adiponectin level in obese PCOS patients in comparison to controls. Although metformin improves some metabolic disturbances associated with PCOS to some degree, but it doesn't exert a significant alterations in serum adiponectin levels, despite its lowering effect on WHR, a major determinant of adiponectin level. Further studies are needed to clarify the role of adiponectin in non obese PCOS.

KEYWORDS: Polycystic Ovary Syndrome; Obesity; Adiponectin; Insulin Resistance; Metformin.

INTRODUCTION

The polycystic ovary syndrome affects about 7 to 8% of women and may be the most common cause of female infertility, an ovulation, early pregnancy loss, and later pregnancy complications all have been implicated in the low fecundity of women with this disorder ⁽¹⁾. PCOS is clinically and biochemically heterogeneous condition characterized by dermatologic, reproductive and metabolic manifestations ⁽²⁾. PCOS can be diagnosed when two of the three following criteria are present: 1) chronic oligo-anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries provided other causes of these abnormalities have been excluded ⁽³⁾. Because adipose tissue is an active endocrine organ, by releasing a variety of bioactive peptides and adipokines, modulate body's metabolism at local and systemic levels ⁽⁴⁾. One specific adipokine, adiponectin, is found to be positively correlated with age and female sex and inversely correlated to insulin resistance, abdominal fat mass, and fasting serum insulin and glucose levels ⁽⁵⁾. However, the association between adiponectin levels and PCOS remains controversial and it is unclear so far whether adiponectin is a primary defect or occurred secondary to, or parallel with insulin resistance ⁽⁶⁾. Metformin has been increasingly used in the treatment of PCOS. It has been found to improve the reproductive abnormalities in women with PCOS, restoring ovulation and improving fertility ⁽⁷⁾, because of the central role of excess adipose tissue in insulin resistance and the well

recognized capacity of adipose tissue to produce adipokines, which is involved in regulating energy balance through affecting insulin action, this study was designed to estimate serum adiponectin levels in obese women with PCOS, compared to those of healthy (non-obese) women, and to evaluate the effect of metformin treatment in those of age- and body mass index (BMI)-matched PCOS women. Additionally, we investigated the metabolic changes including: insulin resistance (measured as HOMA-IR) in women with and without PCOS, in addition to the effects of treatment with metformin on those variables in obese PCOS patients.

Subjects and Methods

The study was performed at Kammal Al-Sammarei Hospital for Infertility and *In Vitro* Fertilization, Baghdad, Iraq, from January/ 2012 to June/ 2012. The study included 75 women were allocated into three groups: A) 25 newly diagnosed obese females (BMI ≥ 30) ⁽⁸⁾ with PCOS [age range 18-37 years], B) 25 obese females (BMI ≥ 30) with PCOS on metformin (1.0 to 1.7 g/day in 2-3 divided doses) for 3-6 months [age range 20-38 years], and C) 25 apparently healthy (BMI<30) control women [age range 20-34 years]. The descriptive and biochemical characteristics of newly diagnosed, metformin treated PCOS group and the control group, are summarized in (Table- 1). PCOS was diagnosed according to the Rotterdam diagnostic criteria of PCOS ⁽³⁾ by a specialist gynecologist. Exclusion criteria for selecting patients were hyperprolactinemia, a known cardiovascular disease,

diabetes mellitus, impaired thyroid; renal; or hepatic functions, hormonal treatment, pregnancy, and lactation. The study was approved by The Local Research Ethics Committee and all subjects were given a written informed consent to participate in this study. Venous blood samples were taken during the early follicular phase (day 2-5) of menstrual cycle for hormonal, lipid profile, glucose, insulin, and adiponectin assays. Fasting serum glucose (FSG) ⁽⁹⁾, total cholesterol (TC) ⁽¹⁰⁾, high density lipoprotein-cholesterol (HDL-C) ⁽¹¹⁾, and triglycerides (TG) ⁽¹²⁾ were determined by using commercial kits (Linear, Spain); testosterone (T) ⁽¹³⁾ was determined by ELFA kit (Biomerieux Vidas, France); fasting serum insulin (FI) ⁽¹⁴⁾, and free testosterone (fT) ⁽¹⁵⁾ were

determined by using ELISA kits (DRG, Germany); and adiponectin was determined by ELISA kit (Demeditec Diagnostic, Germany) ⁽¹⁶⁾. Whereas, low density lipoprotein-cholesterol (LDL-C) was calculated according to Friedwald's equation ⁽¹⁷⁾: $LDL-C = TC - (HDL + TG/5)$. The atherogenic index (AI) ⁽¹⁸⁾ was calculated according to the formula: $AI = TC / HDL - C$. Insulin resistance was assessed by calculating the Homeostasis Model Assessment for Insulin Resistance ⁽¹⁹⁾ (HOMA-IR) according to: $HOMA-IR = I (U/L) \times G (mmol/L) / 22.5$. Unpaired student t-test was used to examine the quantitative differences of mean for tested parameters. The results were expressed as mean \pm SEM and $P \leq 0.05$ was considered significant.

TABLE -1: Descriptive Characteristics of PCOS Groups and Controls

Variables	Controls	ND- PCOS	p ^a	MT- PCOS	p ^b
Number	25	25	-	25	-
Age (years)	26.48 \pm 0.82	27.65 \pm 0.99	0.42	26.81 \pm 1.23	0.56
Body mass index(BMI) (kg/m ²)	28.22 \pm 0.67	31.88 \pm 1.06*	0.008	31.42 \pm 0.99	0.72
Waist to hip ratio(WHR)	0.84 \pm 0.007	0.92 \pm 0.008*	0.0001	0.89 \pm 0.01 [†]	0.006
Testosterone (T) (nmol/L)	2.16 \pm 0.14	3.04 \pm 0.33*	0.01	2.48 \pm 0.29	0.09
Free testosterone (Ft) (pmol/L)	9.45 \pm 1.32	13.22 \pm 1.69*	0.04	10.99 \pm 1.86	0.52
FastingSerumGlucose(FSG) (mmol/L)	4.22 \pm 0.11	4.58 \pm 0.10*	0.02	4.20 \pm 0.12 [†]	0.01

ND- PCOS: newly diagnosed PCOS; MT PCOS: metformin treated PCOS; ^a between control and ND PCOS; ^b Between ND- PCOS and MT- PCOS; * Significant difference from controls; [†] Significant difference from ND- PCOS. Data are presented as mean \pm SEM and $P \leq 0.05$ was considered significant.

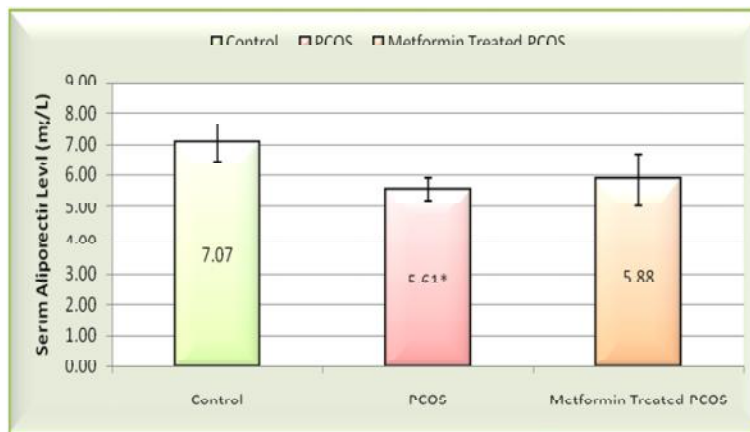
RESULTS

Newly Diagnosed PCOS Patients

Serum total adiponectin (Figure- 1) and HDL-C levels (Figure- 2) were significantly lowered in ND- PCOS group relative to controls. Furthermore T, and fT (Figure- 3); FSG, FI, and HOMA-IR (Figure -4); and TG and AI (Figure-2) were significantly elevated in ND- PCOS group as compared to control group.

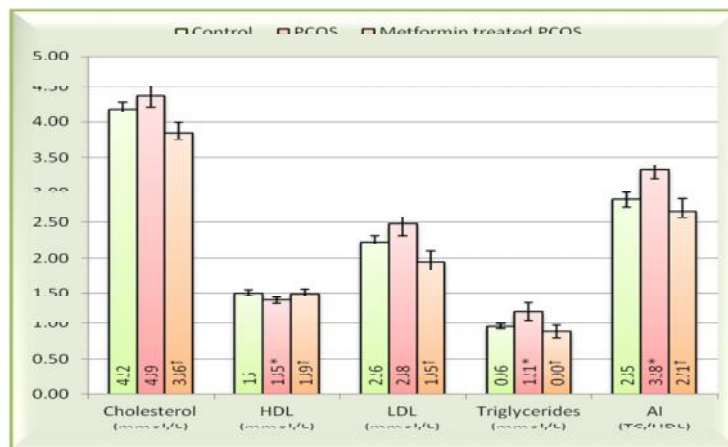
Effect of Metformin Treatment

TC, LDL-C, TG, and AI (Figure-2) and FSG, and HOMA-IR (Figure-4); were significantly lowered in MT- PCOS group compared to ND- PCOS group. However, HDL-C (Figure- 4) was significantly elevated in MT- PCOS group than in ND- PCOS group, while there was no significant difference between MT PCOS and ND PCOS groups in terms of adiponectin (Figure- 1); and T and fT (Figure-3) and Fasting insulin (Figure-4) .



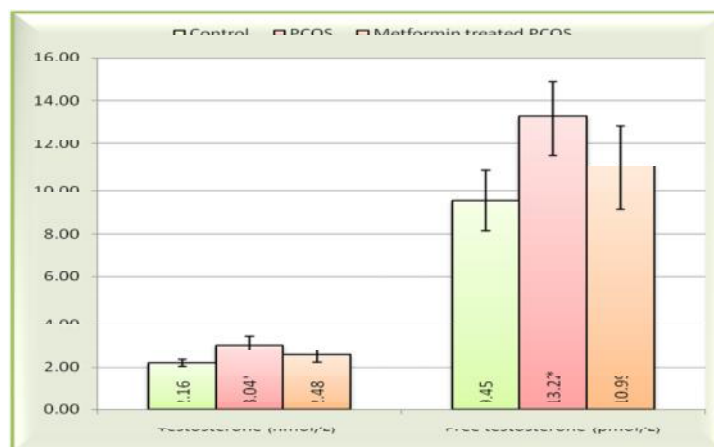
* Significant difference from controls.

FIGURE 1: Mean Serum Adiponectin Levels among The Studied Groups.



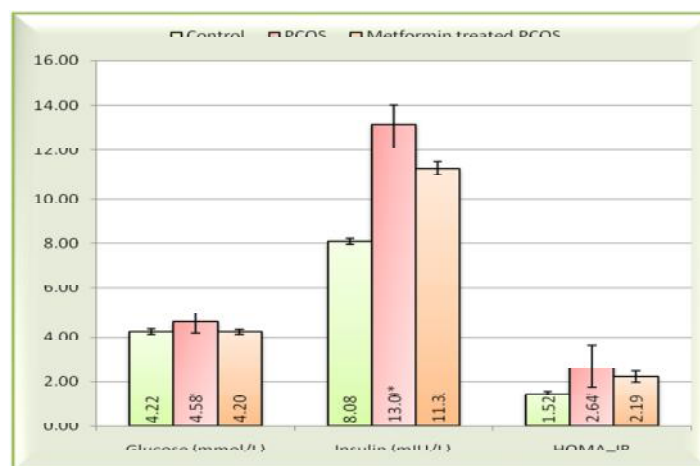
* Significant difference from controls; † Significant difference from PCOS, LDL=low density lipoprotein, HDL=high density lipoprotein, AI= atherogenic index .

FIGURE 2: Mean Lipid Profiles among the Studied Groups.



* Significant difference from controls; † Significant difference from PCOS.

FIGURE 3: Mean Serum Total and Free Testosterone Levels among the Studied Groups.



* Significant difference from controls; † Significant difference from PCOS, FSG=fasting serum glucose,FI=fasting insulin, HOMA-IR=Homeostasis model assessment for insulin resistance

FIGURE 4: Mean Values of Glycemic Indices among the Studied Groups.

Correlation Studies

Correlations among studied variables was assessed by Pearson's bi- variants correlation coefficient test. The significant correlation between the measured parameters, r values, and p values are illustrated in table- 2. Although

adiponectin did 'nt any significant association with HOMA-IR in the PCOS patients ; but it was significantly correlated with HDL-C in MT-PCOS group. Meanwhile, visceral obesity presented as WHR with both FI and FSG.

TABLE 2: The Significant Correlations of Parameters for Controls and PCOS groups

Controls	r	P	ND- PCOS	r	P	MT- PCOS	r	P
AI & FI	0.47	0.009	AI & T	0.77	0.0001	LDL & HOMA-IR	0.43	0.02
AI & HOMA-IR	0.38	0.04	FI & HOMA-IR	0.93	0.001	AI & HOMA-IR	0.39	0.04
FI & HOMA-IR	0.92	0.0001	FI & HDL	0.42	0.02	FI & HOMA-IR	0.92	0.0001
FI & HDL	-0.39	0.03				Adiponectin & HDL	0.53	0.004
HOMA-IR & FSG	0.50	0.005				HOMA-IR & FSG	0.60	0.0008
WHR & BMI	0.65	0.0004				WHR & FI	0.48	0.01
						WHR & TC	0.42	0.02
						WHR & FSG	0.44	0.02

FI=fasting insulin ,AI=atherogenic index,HOMA-IR= Homeostasis model assessment for insulin resistance, WHR= waist to hip ratio,BMI=body mass index ,HDL=low density lip[roprotein ,HDL-high density lipoprotein,TC= total Cholesterol.

DISCUSSION

In the present study we found that serum adiponectin levels were lowered among PCOS females compared to those non-PCOS group (figure- 1). Other studies concerning the relationship between serum adiponectin and PCOS has been controversial, with some groups documenting decreased serum levels of adiponectin in PCOS women compared with weight- and BMI-matched controls^{(20) (21) (22)}, but other studies displayed no difference after controlling for obesity^{(23) (24)}. The variation among these studies may due to heterogeneity of PCOS, different BMI ranges and small sample size of studied subjects. Although the pathogenesis of PCOS is not clear, but it is conceivably due to immature ovarian cysts and dysregulation of insulin signaling in theca cells resulting from genetic defects, extrinsic exposures, or both⁽²⁵⁾. In other hand the metabolic involvement of adiponectin is not fully understood, but regulation of glucose and lipid metabolism via stimulation of fatty acid oxidation, suppression of hepatic glucose output and increased insulin sensitivity in liver and skeletal muscles are known to represent key roles of adiponectin⁽²⁶⁾. It exhibits both , anti-inflammatory and insulin-sensitizing effects and its serum levels are decreased in abdominal adiposity, in obesity and in disorders of glucose tolerance⁽²⁷⁾. Because regulation of fatty acid metabolism and obesity is closely associated with insulin resistance⁽²⁸⁾ and the type of obesity is predominantly of abdominal distribution in PCOS patients^{(29) (30)}, thus adiponectin might contribute to both insulin resistance and metabolic abnormalities in PCOS through dysregulated lipid metabolism and obesity, although the exact pathway is unclear. Meanwhile, present study found that metformin treatment did not increase adiponectin levels significantly when compared to age- and BMI- matched untreated PCOS patients, despite the significantly (p=0.006) lower WHR values (Table-1) , i.e. lowered abdominal adiposity of MT- PCOS compared to ND- PCOS. Moran et al also reported a similar result in overweight PCOS patients⁽³¹⁾ and several other studies^{(32) (33)} agree with this result regardless of obesity classification. But another study revealed that metformin therapy could increase serum adiponectin levels in obese⁽³⁴⁾ and overweight^{(35) (36)} PCOS patents. In contrast to the above mentioned studies, the present study compares subjects with same BMI values for metformin treatment, regardless to its effects decreasing BMI values, knowing that adiponectin levels

are negatively correlated to BMI⁽³⁷⁾, and BMI appears to be one of the predictors of metformin efficacy. Furthermore, our results presents that PCOS group has higher testosterone than the non-PCOS group which is related to one of the diagnostic criteria of PCOS⁽³⁾, which was associated with significant correlation between serum total testosterone and AI (table-2).Metformin exerts a decreasing effect on serum T, and fT (but statistically non significant) as shown in figure-3 . The ND- PCOS group has higher FSG, FI, and HOMA-IR values than the non-PCOS group, which could be related to metabolic disorders in PCOS regarding glucose metabolism as result of insulin resistance as presented by increased HOMA-IR values among the PCOS patients (figure-4). However, metformin treatment was able to decrease serum FSG and HOMA-IR significantly, but non-significantly in respect of FI, which could be due to its effect on glucose disposal although FI exert non significant alterations during this period of treatment with metformin (figure -4).Whilst, lipid profile of PCOS patients were characterized by lowered HDL-C, elevated TG and AI over that of controls , but metformin treatment increased HDL-C, decreased TC, LDL-C, TG, and AI (Figure -2), i.e. it produces preferable protective effects considering dyslipidemia and related vascular complications due to its effect on glucose-lipid metabolism⁽²⁷⁾. As indicated by the significant association of WHR , a major determinant of serum adiponectin ,with serum FI, TC and FSG. Meanwhile, serum adiponectin exerts a significant association with HDL-C in the MT-PCOS group (table-2) .Because the cross-sectional nature of our study; we could not distinguish whether adiponectin was a cause or a consequence of PCOS pathogenesis, despite excluding related factors as the age and BMI.In conclusion, lowered adiponectin levels in obese PCOS group were observed, and adiponectin was not affected by metformin treatment (for 3-6 months) other than its effect of decreasing BMI, improving glycemic control (FSG, HOMA-IR). Further studies are needed to clarify the role of adiponectin in PCOS in relation to adipose tissue distribution.

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