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Serum Ghrelin and Adiponectin Level With Insulin Resistance Parameters in Obese Patients with Polycystic Ovary Syndrome Treated By Metformin

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Original Research Article

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Abstract: To evaluate the effect of metformin therapy for more than three months on serum ghrelin and adiponectin level and to assess the relationship between them with insulin resistance parameters in obese patients with polycystic ovary syndrome (PCOS). A case-control study design was adopted in the Fertility and In Vitro Fertilization (IVF) Center at AL. Batool Teaching Hospital Mosul City/ Iraq, in the period between 1st of November 2013 and 1st of May 2014. This study included a group of 41 obese women with PCOS of reproductive age who used metformin for more than three months (metformin users) with another age-and body mass index (BMI)- matched group of 44 obese women with PCOS who did not use metformin (metformin non-users and served as control). A 10 ml of fasting venous blood sample was taken from each PCOS woman of the two groups. The sera were used to measure serum ghrelin, adiponectin, insulin and fasting serum glucose (FSG) level by using commercially specific kits, whereas; BMI and insulin resistance represented by Homeostasis Model Assessment (HOMA) was calculated by using especial equations. The results of this study revealed that there were no significant differences in the mean BMI, waist to hip ratio (WHR), serum ghrelin, FSG and fasting serum insulin (FSI) and HOMA-IR between the metformin users in comparison with metformin non-users obese PCOS patients groups. This study found that there was a significant higher mean serum adiponectin level of the obese metformin users in comparison with the obese metformin non-users PCOS patients. There was a significant positive correlation between BMI and insulin level, BMI and HOMA-IR and between FSG level and HOMA-IR. Also there were a very high significant positive correlation between insulin level and HOMA-IR. In conclusions, metformin therapy for more than three months in obese PCOS patients was associated with a significant higher mean serum adiponectin level than in metformin non-users group. There were non-significant changes in the mean FSG, serum ghrelin level and insulin resistance parameters. Also there were no significant correlation between neither mean serum ghrelin nor adiponectin with the insulin resistance and anthropometric parameters.

Keywords: polycystic ovary syndrome, metformin, insulin resistance, ghrelin, adiponectin.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder of reproductive aged women and affects approximately 5 to 10 percent in general population studies. It is characterized by oligoovulation or anovulation, signs of androgen excess, and multiple small ovarian cysts [1].

Since its original description in 1935 by Stein and Leventhal, obesity has been recognized as a common feature of the PCOS [2]. Some studies report that obesity is encountered in 30–70% of PCOS-affected women [3, 4].

Adipose tissue represents an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a large number of bioactive mediators (adipokines) [5]. Adipokines signal to organs of metabolic importance including brain, liver, skeletal muscle, and the immune system thereby modulating hemostasis, blood pressure, lipid and glucose metabolism, inflammation, and atherosclerosis [6].

There are two theories in support of a contributory effect of obesity on development of PCOS. One suggests that the adipose tissue behave like a gland that secret several hormones known collectively as adipokines. The aberrant adipokine secretion may cause

the development of PCOS [6]. The other theory is that when adipose tissue mass increases, there comes a stage when it can no longer store more fat subcutaneously, and it cause a state of lipotoxicity. The fat then accumulate around tissues and it starts to deposit in other tissues such as the liver, muscles and pancreas, i.e., ectopic fat deposition. This state of lipotoxicity causes insulin resistance and subsequently hyperandrogenism commonly in PCOS women [7].

Hyperinsulinemia induced by insulin resistance (IR) occurs in roughly 80% of PCOS obese women, as in 30–40% of PCOS lean women suggesting that IR is independent but also exacerbated by obesity, this latter considered an enhancing factor that positively correlates with the multifactorial syndrome [8].

Metformin acts on a number of tissues that have relevance to the metabolic and reproductive abnormalities in PCOS, namely the liver, skeletal muscle, adipose tissue and the ovary. Reduction in hepatic glucose output is the principal action of metformin although its mechanism(s) have not been clearly identified [9].

A full explanation of the mechanism of action of the Biguanides remains elusive, but their primary effect is to activate the enzyme adenosine monophosphate-activated protein kinase (AMPK) and reduce hepatic glucose production. Metformin has been shown to act via both AMP activated protein kinase (AMPK)-dependent and AMPK independent mechanisms; by inhibition of mitochondrial respiration but also perhaps by inhibition of mitochondrial glycerophosphate dehydrogenase, and a mechanism involving the lysosome [10].

Some metabolic hormones such as adiponectin and ghrelin may in part act through AMPK signaling and these hormones are also involved in the control of the reproductive function at the hypothalamic-pituitary gonadal axis level in both men and women; thus, AMPK could be one of the interactions between energy balance and reproduction [11].

Ghrelin is a multifunctional peptide hormone secreted principally in the stomach. Ghrelin has initially been reported to induce growth hormone (GH) secretion through the growth hormone secretagogue receptor (GHSR). It also stimulates several biological functions including food intake, glucose release (hyperglycemic effect), lowering effect on insulin secretion in humans, cell proliferation and reproduction [12]. Low ghrelin levels were found during conditions of positive energy balance such as obesity [11]. Ghrelin levels are decreased in PCOS women and are highly correlated to the degree of insulin resistance. This suggests that ghrelin could be linked to insulin resistance in PCOS women [11, 13].

Insulin-resistant PCOS patients treated with metformin showed improved insulin sensitivity and increased serum ghrelin levels [14]. This suggests a link between insulin sensitivity and ghrelin concentrations and shows that the dysregulated ghrelin system in insulin- resistant PCOS women could be normalized by metformin therapy [15]. Also ghrelin and adiponectin decreased significantly in obese women and obese female PCOS patients than the lean controls and increased significantly after metformin treatment [11].

Adiponectin is a hormone secreted by adipocytes that regulates energy homeostasis and glucose and lipid metabolism [16]. Adiponectin increases insulin sensitivity by increasing tissue fat oxidation, resulting in reduced circulating fatty acid levels and reduced intracellular TG contents in liver and muscle [17]. Adiponectin is the most abundant adipocytokine and is mainly secreted from visceral fat cells. Adiponectin is the only protein expressed in adipose tissue that is down regulated in obesity and insulin resistance, and that an increase in adiponectin may reverse insulin resistance [17]. Obese PCOS patients had lower adiponectin levels than weight-matched controls suggesting a very high risk for the metabolic syndrome [11].

In obese PCOS patients, adiponectin were decreased and reverted to normal after correction of insulin resistance by metformin [11]. So metformin therapy causes increase adiponectin level in obese patient with PCOS [11, 18]. While other study found that in PCOS, adiponectin levels are closely linked to insulin resistance and abdominal adiposity and unaffected by metformin [19].

Both ghrelin and adiponectin regulate food intake. There was a positive correlation between ghrelin and adiponectin, also adiponectin was an important predictor of ghrelin and *vice* versa [11].

The aim of this study was to evaluate the effects of metformin on, serum ghrelin and adiponectin level. Also to assess the relationship between the mean serum ghrelin and adiponectin with insulin resistance and anthropometric parameters in obese patients with PCOS treated by metformin for more than three months.

METHODS

This case-control study was conducted in Fertility and *In vitro* Fertilization (IVF) Center in AL. Batool Teaching Hospital that belongs to Mosul College of Medicine, both are located at the right bank of the river Tigris in Mosul City/ Iraq, from the 1st of November 2013 to 1st May 2014. This study included eighty five women at child-bearing age, who were diagnosed with PCOS according to the Rotterdam 2003

criteria[20]. These participants were divided into two groups, the metformin users included 41 women with PCOS (age ranged from 17-36 years) on traditional therapy with metformin therapy (Piophage® tablet provided by Pioneer Com. Iraqi) of doses (ranged between 1000 to 1700 mg daily) for durations ranged between3 to 18 months. Metformin non-users group consisted of 44 women with PCOS (age ranged from 17-36 years), who had similar criteria as the metformin users except that they did not take metformin. Patients who had diabetes mellitus, hypertension, congenital adrenal hyperplasia, thyroid disorders, Cushing's syndrome were excluded from the study.

Anthropometric measures (blood pressure (mmHg), body weight (Kg), height (cm), Waist and hip circumference (cm)) were taken. To determine W/H ratio (WHR), the waist and hip diameters were measured while the subject was standing and breathing normally**.** The BMI was calculated as weight in kilograms divided by the square of height in meters**.**

Ten milliliters (ml) of venous blood were withdrawn from PCOS patients after 12-hour fasting, the serum was separated and kept frozen at -20 °C to be analyzed for determination of FSG level by enzymatic colorimetric method using kit supplied by (Randox Laboratories Ltd., UK). Serum insulin was measured by chemiluminescent immunoassays (CLIA) technique, using insulin Liaison kits supplied by Diasorin, Saluggia (VC) (Italy.) While insulin resistance represented by HOMA-IR value was measured using the following equation: (fasting serum insulin (µIU/ml) × fasting plasma glucose (mg/dl) /405) [21]. Serum ghrelin level was measured by enzyme linked immunosorbent assay (ELISA) technique, using MyBioSource ELISA kit. Serum adiponectin level was measured by enzyme linked immunosorbent assay (ELISA) technique, using Elabscience ELISA kit (Elabscience Biotechnology Co. Ltd).

STATISTICAL ANALYSIS

Data categorization and coding performed via Microsoft Excel-2007. Descriptive and analytic statistics was carried out by using Minitab version 16.2 software statistical program. The descriptive statistics include mean ± Standard Deviation (SD) for quantitative personal and biochemical variables. Independent t-test of two means (unpaired) was used for comparison between each two quantitative biochemical parameters (cases vs. control). Pearson`s Correlation coefficient (r) was measured between different biochemical parameters, (simple linear correlation). P-values ≤ 0.05 were considered statistically significant throughout data analysis.

RESULTS

The approval of the study protocol by an ethic committee has been obtained from the local health committee of Ministry of Health and College of Medicine -University of Mosul – Iraq.

A total number of 41 women with PCOS who used metformin for more than three months were included in this study, (with mean age ± SD of 25.20 ± 4.83), those have been considered to represent the (metformin users) group. Another 44 women with PCOS (with mean age ± SD of 24.67 ± 5.54), who did not use metformin were considered to represent the control group (metformin non-users). There were no significant differences in the mean weight, BMI, and WHR of the metformin users and non-users but there were a significant higher SBP and DBP level of metformin non-users in comparison with metformin users groups as shown in table (1).

Table-1: General characteristics of the two PCOS groups

Parameters	Mean ± SD		P-value
	metformin users (n = 41)	metformin non-users (n = 44)	
Age (years)	25.20 ± 4.83	24.67 ± 5.54	NS
BMI (Kg/m^2)	32.58 ± 4.28	34.03 ± 3.35	NS
WHR	0.85 ± 0.04	0.86 ± 0.05	NS
SBP(mmHg)	123.8 ± 9.38	127.9 ± 8.69	0.039
DBP (mmHg)	82.0 ± 6.53	84.9 ± 5.54	0.030

Table (2), shows that there were no significant differences of FSG, and although there were lower mean FSI, and HOMA value of the metformin users in comparison with the obese metformin non-users PCOS women.

Table-2: Comparison of the insulin resistance parameters between metformin users and metformin non-users

Parameters	Mean ± SD		P-value
	metformin users (n = 41)	metformin non-users (n = 44)	
FSG (mg/dL)	94.38 ± 23.1	94.72 ± 20.9	NS
Insulin (µIU/mL)	16.49 ± 7.87	18.62 ± 14.80	NS

HOMA-IR	3.82 ± 2.26	4.57 ± 4.22	NS

Table (3) shows a non-significant higher mean serum level of ghrelin in the obese metformin users in comparison with the obese metformin non-users PCOS patients and illustrates that administration of metformin for more than three months caused a significant higher mean serum level of adiponectin of the obese PCOS patients in comparison with the obese metformin non-users PCOS patients.

Table-3: Comparison of mean serum ghrelin and adiponectin levels between metformin users and metformin non-users

Parameters	Mean ± SD		P-value*
	metformin users (n = 41)	metformin non-users (n = 44)	
Ghrelin (ng/ml)	0.99 ± 0.80	0.79 ± 0.70	NS
Adiponectin (µg/ml)	33.28 ± 12.53	27.15 ± 11.7	0.022

Table (4), illustrated that there were non-significant negative correlation between mean serum ghrelin with BMI, WHR, FSG, insulin and HOMA-IR. And non-significant positive correlation between adiponectin with ghrelin. While found a significant positive correlation between insulin with BMI, HOMA-IR with BMI, HOMA-IR with FSG and HOMA-IR with insulin.

Table-4: Correlations between biochemical parameters of metformin PCOS users

Parameters	Correlation	
	r	p-value
Ghrelin: BMI	-0.071	0.661 (NS)
Ghrelin: WHR	-0.056	0.726 (NS)
Ghrelin: FSG	-0.151	0.347 (NS)
Ghrelin: Insulin	-0.070	0.662 (NS)
Ghrelin: HOMA-IR	-0.144	0.371 (NS)
Adiponectin: Ghrelin	0.057	0.724 (NS)
Insulin: BMI	0.380	0.014 (S)
HOMA-IR: BMI	0.392	0.011 (S)
HOMA-IR: FSG	0.383	0.014 (S)
Insulin : HOMA-IR	0.869	0.001 (S)

DISCUSSION

This study showed a significant reduction in both SBP and DBP in metformin users compared with metformin non-users which is in agreement with some studies [22,23], but in contrast to other studies [24-26] which they found no significant differences in SBP and DBP of the metformin users and non-users. The mechanism underlying the effect of metformin on blood pressure has not been fully understood yet. One of the possibilities is that metformin could effectively improve insulin resistance [27]. In the present study, the result of comparison in insulin resistance parameters between metformin users and non-users displayed in table (2), showed a non-significant difference between the two obese PCOS groups. The non-significant differences in mean FSG level between the same two groups support the idea which tells that metformin has a little effect on blood glucose in non-hyperglycemic subjects [28] and is in agreement with the other studies [25, 26, 29]. But in contrast to few studies which found that metformin treatment of PCOS causes a significant reduction of the FSG [19, 30, 31].

The mean fasting serum insulin (FSI) level in this studied PCOS patients (with and without metformin therapy) found to be within normal range (2-20 µIU/ ml) [32], beside that the present study, observed a non-significant lower level of FSI and HOMA-IR in obese patients with PCOS who use metformin for more than three months as compared to age- and BMI-matched group of PCOS women without metformin therapy, due to the fact that all PCOS patients in this study were not markedly hyperinsulinaemic [33].

These results were in agreement with the study of Diamanti-Kandarakis *et al.* [34] which showed that an insignificant (10%) fall in insulin levels, both at the basal state and after a 75 g glucose load, supports the view that metformin increases insulin sensitivity rather than affect secretion which is also in agreement with the other studies [35-36]. Açbay and Gündoğdu [35] suggested that the cellular mechanism of insulin resistance in PCOS is different from other common insulin-resistant states such as noninsulin-dependent diabetes mellitus and obesity.

While other studies observed a significant lower level of FSI and HOMA-IR in with obese PCOS patients who use metformin for more than three months as compared to age- and BMI-matched group of PCOS women without metformin therapy [19, 24, 26, 29, 30].

There was a significant positive correlation between mean serum insulin level and BMI and a significant positive correlation between HOMA-IR and BMI as shown in table (4). This is in agreement with other studies in Mosul City [24, 26], who found a significant positive correlation between mean serum insulin level and BMI, HOMA-IR and BMI in PCOS patients. This is logical as hyperinsulinemia is derived from either over production or decreased clearance of insulin and in obesity insulin production was increased with the reduction of clearance rate [37]. There was a significant positive correlation between HOMA-IR and FSG level. And there was a very high significant positive correlation between insulin level and HOMA-IR. This result similar to correlation of insulin and HOMA-IR in other studies, Two of them was in Mosul City [24, 26, 38].

This study shows a non-significant higher mean serum ghrelin level of the obese metformin users in comparison with non-users PCOS patients and there is no relationship between circulating ghrelin and other parameters, which is in agreement with Orio *et al*. [39] who found that there is no relationship between circulating ghrelin and the abnormal hormonal pattern of the PCOS but in contrast to the study of Shaker *et al*. [15] which found that serum ghrelin level significantly increased in PCOS women treated with metformin for three months. While El-Nahas *et al*. [11] found that in obese PCOS patients, ghrelin were decreased, which suggests metabolic disorders, and reverted to normal after correction of insulin resistance by metformin.

In this study, administration of metformin for more than three months caused a significant higher mean serum level of adiponectin of the obese in comparison with the obese metformin non-users PCOS patients as illustrated in table (3). This result was compatible with the results of studies [11, 18], but in contrast with Trolle *et al*. [19] which found that metformin had no effect on adiponectin in spite of significant decreases in weight, fasting glucose, and insulin resistance.

This study illustrated that there were non-significant negative correlation between mean serum ghrelin with BMI, WHR, FSG, FSI and HOMA-IR. Which are consistent with Pagotto *et al*. [40] who found that ghrelin is inversely correlated with parameters of insulin resistance in obese PCOS. Also Tschöp *et al*. [41] found that ghrelin plasma concentrations are significantly lower in Pima Indians than in Caucasians. These data seem to indicate that ghrelin is down regulated in human obesity. This down regulation may be a consequence of elevated insulin, because fasting plasma ghrelin levels are negatively correlated with fasting plasma levels of insulin [41].

CONCLUSIONS

Metformin therapy for more than three months in obese PCOS patients was associated with a significant higher mean serum adiponectin level in comparison with the obese metformin non-users PCOS patients. Also caused a significant reduction in SBP and DBP. While found that there were no significant changes in the mean serum ghrelin, FSG levels, and the insulin resistance parameters, There is a controversy in the relationship between the mean serum ghrelin and adiponectin level with the insulin resistance parameters in obese patients with PCOS.

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