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Serum adiponectin and ghrelin in polycystic ovarian syndrome and its correlation with insulin resistance

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***Abstract*---**Background: Polycystic ovarian syndrome (PCOS) considered one of the main complex and heterogeneous endocrine syndromes of females in the pregnancy age , it is commonly accompanied by insulin resistance (IR). Current study aimed to compare adiponectin and ghrelin levels between females with PCOS and healthy females, also inspect the association between ghrelin and adiponectin levels in addition to predict their use as diagnostic factors for the disease, finally to study the connection between adiponectin and ghrelin with lipid profiles and IR in PCOS females . Subjects and Methods: The present case-control study involved 120 females; 60 with PCOS and 60 age-matched healthy controls. Fasting serum levels of glucose, lipid profiles, insulin, adiponectin, ghrelin, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and sex hormone-binding globulin (SHBG), were assessed. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR) and body mass index (BMI) also estimated. The statistical analysis of the data were done by using SPSS 22 . Results: The findings revealed that serum adiponectin level elevated significantly in females with PCOS as compared with control, while ghrelin level decreased significantly in females with PCOS in compared to control. Furthermore, results showed that adiponectin levels were positively correlated with BMI, fasting glucose (FG), low density lipoprotein-cholesterol (LDL-C), triglycerides (TG), total cholesterol (TC), insulin

resistance (HOMA-IR), insulin, LH, FSH, and SHBG in PCOS females; while it was negatively correlated with high density lipoprotein-cholesterol (HDL) and ghrelin. On the other hand, ghrelin levels were negatively correlated with BMI, TC, TG, LDL, LH, FSH and adiponectin; but it was positively correlated with FG, HDL, insulin, HOMA-IR, and SHBG. Conclusion: Patients with PCOS have increased levels of adiponectin and decreased levels of ghrelin than that of control group. Also adiponectin was positively correlated with lipid profile and BMI; while ghrelin negatively correlated with them. For this, adiponectin and ghrelin levels may be beneficial in assessing metabolic abnormalities in PCOS.

***Keywords*---**Polycystic ovarian syndrome; Adiponectin; Ghrelin; Insulin resistance.

Introduction

Polycystic ovarian syndrome (PCOS) is a common health problem that influence teenage girls and young females. Although no one actually knows the causes of POCS, it is seem to be related to an imbalance in hormones. It is a complex and heterogeneous endocrine disorder associates with female in the reproductive age, it`s prevalence about 4 to 12% (1). It is characterized by menstrual irregularities, clinical and /or biological hyperandrogenism, in addition to numerous small ovarian subcapsular cystic follicles on ultrasonography with approximately 60% to 80% of the affected cases being obese. PCOS is often connected with insulin resistance (IR) escorted via the compensatory hyperinsulineamia (2). PCOS links with an elevated risk of metabolic complications counting type II diabetes mellitus (T2DM), impaired glucose tolerance (IGT), dyslipidaemia, hypertension and atherosclerosis. On the other hand, the mechanism of PCOS association to the metabolic abnormalities is not completely understood. Strategies that designed to attenuate IR in POCS might be important in diminishing the risk of developing cardiovascular disease (CVD) and T2DM (3). IR is described as inappropriate biological response to insulin “the inferior biological response could be due to the inability of plasma insulin to bind to its receptor or the presence of a post-receptor binding defect” (4). IR associates with many of diseases for example diabetes mellitus type II , obesity, polycystic ovarian syndrome, metabolic syndrome and chronic infection. Insulin resistance varies in different tissues, it minimizes glucose uptake in muscle and fat cells, while in liver cells, it consequences in reduced synthesis and storage of glycogen in addition to a failure to suppress glucose production and release into blood(5). Compensatory hyperinsulinaemia allow maintenance of usual glucose tolerance ,but glucose intolerance and finally diabetes mellitus developed when degree of insulin resistance deteriorates (3). One of the important endocrine organ which produce as well as secret many bioactive molecules like peptides and proteins is adipose tissue. These bioactive substances, collectively called adipokines counting resistin, adiponectin (AND), interleukine-6 (1L-6), tumor necrosis factor (TNF-α), omentin, chemerin, visfatin and others (6). The action of adipokines occur at both systemic (endocrine) and local (autocrine/paracrine) level leading to interlock in between organs and adipose tissue. It allow fatty tissue playing an essential

function in the regulation of metabolism and inflammation (7). Cytokines production and liberation conform a cascading order with one cytokine enhancing the production of additional cytokines from target cell. Cytokines act either synergistically or antagonistically (8).

Cytokine that synthesized then secreted from adipose tissue is adiponectin . It has anti-inflammatory, cardioprotective, insulin-sensitizing and finally anti-atherogenic action (9,10). It is inversely related with IR in T2DM, obesity, dyslipidemia, hypertension, and cardiovascular disease. There is a controversy whether adiponectin is a primary defect or arisen secondary to or at the same time with that of IR in POCS, because the association between adiponectin levels and POCS still vague (11,12).

Ghrelin is a hormone that has an important role in energy balance, food intake and regulation of weight (13). Many studies shown a vital effect of ghrelin on blood glucose homeostasis and insulin level (14). Gherlin has the ability to opposite the down regulation effect of insulin upon phosphoenol pyruvate carboxy kinase, an enzyme used in gluconeogenesis as a rate limiting enzyme (13). So, this study aims to prove the association between ghrelin and adiponectin levels in females with PCOS in comparison with those without PCOS, in addition to study the connection between adiponectin and ghrelin with lipid profiles and IR in female with PCOS.

Materials and Methods

The study involved 60 female with PCOS went to Fertility Center in Al-Sader Medical City in Al-Najaf province and 60 healthy female as a control group through the period from September 2020 to February 2021. Diagnosis of PCOS achieved on the basis of the Rotterdam Criteria (14). Females with diseases other than PCOS were excluded from the study. Five ml of venous fasting blood were collected from the PCOS and healthy control groups in plain tubes without anticoagulant (the samples were collected through the follicular phase of the menstrual cycle 2^{rd}-3^{th}day). Serum lipid profiles and glucose were measured by spectrophotometric method by enzymatic reactions kits (Biolabo, France). Serum SHBG levels were measured by electrochemiluminescence (ECL) technique (Cobas e411 analyzer, Roche Company, Germany). LH, FSH and testosterone levels were measured by immune fluorescence technique/ ELFA (Minividas, Biomerieux, France). Serum insulin has been assessed by ELISA using DRG kit, Germany. Body mass index (BMI) calculated with unit Kg/m^2. Homeostatic model assessment used for calculation of insulin resistance:

HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (mIU/L)] /22.5, cutoff value is >2.5 (15).

Statistical analysis

Statistical analysis has been accomplished by SPSS version 22, Inc, Chicago, IL, USA. T-test was applied for comparing between the studied groups. The results were stated as (mean ± SD). Pearson's correlation coefficients (r) were estimated to

evaluate the relationship between parameters. The statistical difference between groups was at $p<0.05$.

Results

Sociodemographic data and clinical characteristics

Clinical and sociodemographic data for participants displayed in Table 1. Results indicate that the two groups were approximately well matched in age and no significant difference between the PCOS patients and the control group in the terms of age, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP) . Significant elevations ($P<0.01$) in FG, TC, TG, LDL-C, insulin, HOMA-IR, FSH, LH, free testosterone, and total testosterone concentrations and a significant decrease in HDL-C and SHBG showed in the PCOS females in comparison with control. Serum adiponectin concentration was established to be higher ($P <0.01$) in PCOS group when compared with control females. While, serum ghrelin was decreased in females with PCOS as compared with those of non-PCOS.

Table 1: Socio-demographic, clinical and biomarker data in PCOS patients as compared with controls.

Parameters	Control (n=60) Mean±SD	PCOS patients (n=60) Mean±SD	P-Value
Age (years)	30.04±6.18	28.05±5.11	0.056
SBP (mm/Hg)	132.7±22.6	131.0±14.7	0.901
DBP (mm/Hg)	77.1±13.9	81.0±10.4	0.232
BMI (Kg/m^2)	27.22±2.87	24.85±2.19	0.189
Fasting glucose(mmol/L)	5.30±1.28	6.56±0.52	0.010
TC (mg/dL)	150.48±30.16	172.55±20.61	0.001
TG (mg/dL)	113.04±11.65	125.22±18.70	0.001
HDL-C (mg/dL)	43.43±2.45	33.91±2.36	0.001
LDL-C (mg/dL)	60.22±4.93	108.23±20.54	0.001
Insulin (IU/L)	5.70±3.88	13.31±2.21	0.001
HOMA-IR	1.12±0.38	2.56±0.67	0.001
FSH (IU/L)	5.36±1.36	6.77±0.65	0.010
LH (IU/L)	5.82±0.81	11.95±3.73	0.001
Free Testosterone (pM)	3.08±2.41	12.14±3.63	0.001
Total Testosterone(nM)	1.49±0.50	3.85±1.98	0.001
SHBG (nmol/L)	128.00±13.58	24.82±6.59	0.000
Adiponectines(µg/mL)	4.44±0.79	19.64±1.75	0.019
Ghrelin(ng/mL)	21.64±3.32	16.54±4.45	0.000

-Results expressed as mean±SD .
-p-values were carried out by using pooled T-test.
-HOMA-IR index> 2.5, P-value≤ 0.05= significant

Abbreviations: SBP : Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, HOMA-IR: Homeostatic model assessment of insulin resistance , FSH: follicle stimulating hormone, LH: luteinzing hormone, SHBG: Sex hormone-binding globulin.

Correlation among parameters in PCOS patients

The results of correlation study among parameters in PCOS patients was illustrated in table 2. The linear regression analysis revealed significant positive correlations of BMI, insulin, FG, HOMA-IR, TC, LDL-C, TG levels; while ghrelin and HDL-C where negatively correlated with adiponectin levels in PCOS group.

Table 2: Correlation of Adiponectin and ghrelin levels with clinical and biochemical parameters in PCOS patients.

Parameters	Adiponectin (µg/mL)	Ghrelin (ng/mL)
BMI (Kg/m²)	0.82**	-0.45**
FG (mmol/L)	0.32*	0.64**
TC (mg/dL)	0.85**	-0.48**
TG (mg/dL)	0.84**	-0.66**
HDL-C (mg/dL)	-0.75**	0.55**
LDL-C (mg/dL)	0.63**	-0.28*
Insulin (IU/L)	0.3*	0.32*
HOMA-IR	0.45**	0.48*
Free testosterone (nM)	0.07	-0.04
Total testosterone (pM)	0.08	-0.06
LH (IU/L)	0.06	-0.33*
FSH (IU/L)	0.08	-0.25*
SHBG (nmol/L)	0.04	0.74**
Ghrelin (ng/mL)	-0.41**	-

*= P <0.05 **= p<0.001

The data analysis pointed out significant positive correlations of fasting glucose, HOMA-IR, insulin, HDL-C and SHBG concentrations with ghrelin levels in females with PCOS . In addition, BMI, TC, TG, LDL-C, LH, FSH and adiponectin were significantly negatively correlated with ghrelin levels in polycystic ovary patients.

Discussion

The PCOS definition has been debated and unclear because of the syndromes hetero-trophic nature. PCOS is linked to short term reproductive and long term hormonal abnormalities, ovulatory dysfunction and polycystic ovaries (16). Hyper-androgenaemia is connected to clinical manifestations like hirsutin, infertility, irregular menses, male-pattern baldness and acne. “Ovulatory dysfunction may include chronic an ovulation and is linked with menstrual disturbances and

infertility. PCOS is characterized by an elevated number of small antral follicles withhold development and a hypertrophied theca cell layer" (1,2).
Current study revealed that there was an elevation in HOMA-IR, insulin, testosterone, FSH, and LH levels and lower level of SHBG in the females with PCOS.

A number of mechanisms are thought to direct the development of PCOS, such as insulin resistance and hyperinsulinaemia, hyperandrogenaemia and genetic factors (17). The cause of the elevated levels of insulin in PCOS patients is unclear but it may be the causes for the development of the other pathophysiological changes. Hyperinsulinaemia is a condition of elevation in insulin level abnormally ,it needs to begin a cellular response which associated with insulin resistance. It is usually met clinical disorder in non-obese as well as obese patients with PCOS (18).

The abnormal gonadotrophin secretion in PCOS patients, like high level of LH and low level of FSH, may be caused by enhancement of pituitary sensitivity to gonadotrophin-releasing hormone (GnRH) stimulus or to increase secretion of GnRH. change in GnRH secretion which lead to change in gonadotrophin secretion may result in an insensitivity of the GnRH producer to the negative feedback effects of progesterone and estrogens (19).

Biochemical hyperandrogenism is assessed with the evaluation of sex hormone-binding globulin (SHBG) and total testosterone (20). Results of this study show an elevation of in the level of total testosterone in females with PCOS while SHBG levels was depressed. These results are in agreement with Hoeger *et al.* who found decreased in SHBG but increased in testosterone levels ,caused a more androgenic profile in PCOS patients (21). Cupisti *et al.* reported that "SHBG concentrations were significantly decreases in females with PCOS that had a BMI greater or equal to 25 Kg/m^2 further females with PCOS represent more severe hyperandrogenism when compared to lean females with PCOS" (22).

Adiponectin is a (135) amino acids polypeptide (30 KDa) synthesized then secreted from adipose tissue, it has a reverse relation with body weight, dysmetabolic syndrome and IR (9,23). There is no adequit informations about how adiponectin metabolically act, but there is a suggested key roles of adiponectin which refers to the regulation of lipid metabolism and glucose by enhanced oxidation of fatty acids, inhibit generation of glucose from liver , finally enhancement of sensitivity to insulin in skeletal muscle and liver (24).

The present study indicates that there was a significant elevation of adiponectin levels in females with PCOS in comparison with healthy females. In addition, there was a positive correlation of adiponectin with BMI, FG, insulin, TC, HOMA-IR, LDL and TG, and a negative correlation with HDL and ghrelin. Adiponectin is a cytokine that commonly produced by adipose tissue, with a vital insulin sensitizing effect accompanying with antiatherogenetic properties (25). Adiponectin (a hormone derived from adipocyte) with anti-inflammatory action, this effect mediates by TNF-α activity modulating. Many studies reported the role of TNF-α in suppression of gene expression for adiponectin in *vitro* (26,27) and adiponectin inhibitory effect on the nuclear factor kβ (28). During obesity and

dysregulated lipid metabolism, adiponectin cause metabolic abnormalities and insulin resistance in PCOS (even though the precise mechanism yet unclear) , due to that insulin resistance is closely linked with obesity and fatty acid metabolism (5). This result disagree with some studies which show low adiponectin level in PCOS females as compared with control group (29,30).

In the current study, there was a significant decreased in ghrelin levels in females with PCOS as compared with control. In addition, it was correlated negatively with BMI, TC, TG, LDL, LH, FSH and adiponectin and has a positive correlation with FG, HDL, insulin, HOMA-IR, and SHBG. Glinborg *et al.* (31) and Kamal *et al.* (32) established that serum ghrelin levels low in females with PCOS when compared with control. Mitkov *et al.* (33) proved that serum ghrelin level was lower in PCOS females than in control, but another study done by Wasko *et al.* (34) demonstrated that increased concentration of serum ghrelin in females with PCOS in comparison with control (non-PCOS). This difference of results may be due to disturbing factors, such as BMI, age, hormonal status, fat mass, and severity of disease.

Other study by Daghestani *et al.* recognized an opposite association between BMI and ghrelin in PCOS patients (35). Receptors of ghrelin present in the ovary in addition to central nervous system, so this explain its reproductive function (31). Other study documented ghrelin ability to change stimulated secretion of testosterone in vitro (36). Many studies reports that there was no significant relationship and a reverse relationship between ghrelin and testosterone level (37,38).

Limitations of the Study

The study has some limitations, one is the relatively low sample size, the others are fasting measurement of ghrelin and adiponectin while they should be also measured in a postprandial state in association to other parameters , finally narrow range of BMI. Strength points of this study is matching participants for age.

Conclusions

Higher adiponectin level and lower ghrelin level in the PCOS group suggest an important role of ghrelin and adiponectin in PCOS development. Thus, adiponectin and ghrelin levels may be beneficial in assessing metabolic abnormalities in PCOS. In addition to the relation of adiponectin and ghrelin with insulin resistance, lipid metabolism, and BMI in PCOS. Additional large sample size studies needed to elucidate the exact mechanism of adiponectin and ghrelin in PCOS.

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