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Copeptin and Obestatin Levels in Polycystic Ovary Women and their Relation to Obesity, Insulin Metabolism and Cardiovascular Diseases

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ABSTRACT

Background: Is to investigate the correlations between the serum levels of Obestatin and Copeptin, carotid artery intima-media thickness (CIMT), and brachial artery flow mediated dilatation (FMD) in obese and non-obese women with PCOS.

Design: Randomized prospective study.

Aim of the work: The present study was, therefore, undertaken to investigate the correlations between both serum levels of Obestatin, Copeptin, in PCOS women and to evaluate their relationship with obesity, insulin resistance as well as cardiovascular disease.

Patients and methods: We analyzed 54 patients with PCOS and 20 normal women as controls. PCOS patients were divided into two groups based on body mass index (BMI): obese group (BMI > 30 kg/m2, n = 28) and non-obese group (BMI < 30 kg/m2, n = 26). Serum Copeptin and Obestatin levels, Insulin Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), CIMT and brachial artery FMD were determined and compared among both groups.

Results: Serum Obestatin levels were significantly lower in obese PCOS group than non-obese and control. While Serum Copeptin levels were significantly higher in obese PCOS group than non-obese and control. Brachial artery FMD was lower in the PCOS groups than control. Obestatin was positively correlated with cardiovascular risk factor (FMD), whereas Copeptin was negatively correlated with FMD

Conclusion: Obestatin and Copeptin may provide useful information regarding future cardiovascular risk in PCOS patients as Obestatin was negatively correlated and Copeptin was positively correlated with cardiovascular risk factor (FMD).

Keywords*: Cardiovascular Risk; Copeptin, Insulin resistance; Obesity; Obestatin; Polycystic ovary.*

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INTRODUCTION

Polycystic ovary syndrome is defined by any two out of the following criteria: infrequent or absent menstruation, indicating anovulation; hyperandrogenism; and polycystic ovaries diagnosed by ultrasound after the exclusion of other aetiologies of menstrual disturbance and hyperandrogenism.[1] At least 40% of women with PCOS are obese, [2] and they are more insulin resistant than weight matched women with normal ovaries. Increasing abdominal obesity is correlated with reduced menstrual frequency and fertility, together with greater insulin resistance factors. [3]

Obestatin is peptide hormone secreted by the cells of the stomach and small intestine of diffrent mammals including humans. Although Obestatin and ghrelin are both encoded by the same gene and derived from the precursor protein Proghrelin, Obestatin behaves as a physiological opponent to ghrelin in inhibiting food intake, body weight gain, and gastric emptying.[4]

Arginine vasopressin, which is also named antidiuretic hormone, is released from the posterior pituitary gland in conditions of chronic psychosocial stress via inducing the hypothalamic–pituitary–adrenal (HPA)Axis along with corticotropin-releasinghormone. [5]

Bjorntorp and Rosmond (1999) suggest that stress-mediated activation of the HPA axis may have a role in the pathogenesis of insulin metabolism and metabolic syndrome. [6]

Copeptin is C-terminal portion of the precursor of Arginine vasopressin (AVP). Copeptin is considered to be a reliable and clinically useful surrogate marker for AVP. In healthy populations and in patients with diffrent cardiovascular diseases, there is a significant positive association between copeptin and AVP levels. [7,8]

However, the association between copeptin, obestatin levels and women with PCOS remains unknown.

The present study was, therefore, undertaken to investigate the correlations between both serum levels of Obestatin, Copeptin, in PCOS women and to evaluate their relationship with obesity, insulin resistance as well as cardiovascular disease.

PATIENTS AND METHODS

In this prospective study, we analyzed 54 women with PCOS and 20 healthy women as controls. Women with PCOS were divided into two groups based on body mass index (BMI): obese group (BMI > 30 kg/m2, n = 28) or non-obese group (BMI < 30 kg/m2, n = 26). Women were recruited from the outpatient clinics of Obstetrics and Gynecology department , El-Hussein and Sayed Galal university Hospitals, Al-Azhar University, in the period between January and August 2017.

The study protocol was in accordance with the Helsinki Committee requirements and was approved by the Ethics Committee of Faculty of Pharmacy (Girls), Al-Azhar University. All patients gave informed written consent before the work. The diagnosis of PCOS was made based on the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) criteria. [9]

A pelvic ultrasound examination was performed on the same day as blood sampling. All women were examined by the same physician.

Inclusion criteria

Patient age 20 -30 yrs and all women obese and non-obese.

Exclusion criteria

Patients who had taken COC, antilipidemic or antihypertensive drugs, glucocorticoids, antiandrogens, insulin sensitizers, anticoagulants, or antiplatelet agents at least 3 months before the study.

The body mass index (BMI) was calculated as weight (in kilograms)/height squared (meters squared).

Laboratory Investigations

Sampling

A sample of 10 ml venous blood was collected from each women after an overnight fasting. The venous blood sample was divided into two test tubes. 1ml was added to a mixture of potassium oxalate and sodium fluoride (for plasma fasting glucose estimation (FBG) by oxidase/ peroxidase kit) (10) and the remaining 9 ml were allowed to clot at room temperature then centrifuged at 1000 rpm for 15 minutes. Serum was separated and divided into aliquots then frozen at -20 C till the time of assay. The serum samples were used to estimate the following parameters:

Specific laboratory tests

Determination of serum obestatin was measured by a solid phase enzyme linked immunosorbent assay (ELISA) technique. The kit supplied by ALPCO DIAGNOSTICS, Catalog Number: 48-OBEHU-E01, inc:.[11]

Determination of serum copeptin was determined with a sandwich ELISA technique using Phoenix Pharmaceuticals, Inc: USA. [12]

Determination of Total testosterone was measured by the solid phase enzyme immunoassay (ELISA Kit). [13]

Determination of serum CRP was measured using the Monobind Inc:USA. [14]

Determination of serum Insulin: by a solid phase enzyme linked immunosorbent assay (ELISA) Kit [15]

Routine laboratory investigations

They include estimation of serum Triglycerides: by enzymatic colorimetric kit (16). Total cholesterol: by enzymatic colorimetric kit (17). HDL-cholesterol: by phosphotungstate precipitation kit. [18]

Insulin resistance was estimated by Homeostasis Model Assessment. HOMA-IR = [fasting insulin (μU/ml) X fasting glucose (mmol/L)]/22.5

Measurement of carotid artery intima-media thickness and brachial artery flow-mediated vasodilation.

The determination of endothelial dysfunction was performed 16 Measurements were made by a single observer using an ultrasound (Medison) with a 12-MHz probe.

The maximum flow-mediated dilatation (FMD) diameters were calculated as the average of the 3 consecutive maximum diameter measurements. The FMD was then calculated as the percent change in diameter compared with baseline resting diameters. All patients were blindly examined by 1 experienced operator. [19]

Carotid intima-media thickness (CIMT) is measured at 1 cm proximal to the bifurcation on each side as previously described. Carotid atherosclerosis is described as having a CIMT greater than 0.8 mm and/or a carotid plaque with protrusion into the vascular lumen 1-1.3 mm. [20]

Statistical Method

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. A one-way analysis of variance (ANOVA) when comparing between more than two means. Post Hoc test was used for multiple comparisons between different variables. Chi-square (X2) test of significance was used in order to compare proportions between two qualitative parameters. Pearson's correlation coefficient (r) test was used for correlating data. Probability (P-value) <0.05 was considered significant.

RESULTS

The distribution frequency % of history of diabetes mellitus(DM) and CVD in PCOS patients showed no difference between obese and non-obese ones.

SBP, DBP, total testosterone and CRP were higher in the PCOS patients especially obese group than in the control group.

Regarding lipid profile, TG was significantly increased in obese PCOS patients when compared to both control and non-obese ones. While HDL-c was significantly decreased in obese PCOS patients when compared to both control and non-obese ones (Table 1).

In relation to insulin resistance assessment, it was found that FBG, serum insulin and HOMA-IR were significantly increased in obese PCOS patients when compared to both control and non-obese ones (Table 1).

	Obese		Non obese		Control		LSD		
	Mean	±SD	Mean	±SD	Mean	±SD	I vs. II	I vs. III	II vs. III
Age years	24.03	2.61	23.23	2.45	23.75	2.77	0.235	0.706	0.491
DM	5 (16.67%)		4 (13.33%)		0 (0.0%)		0.485	0.097	0.092
CVD	7 (23.33%)		5 (16.67%)		0 (0.0%)		0.522	0.063	0.070
BMI Kg/m2	37.25	1.16	23.69	1.18	23.54	1.34	<0.001	<0.001	0.670
SBP mmHg	132.67	17.99	122.67	12.85	99.50	9.99	0.009	<0.001	<0.001
DBP mmHg	81.00	10.94	73.33	8.84	63.50	8.75	0.003	<0.001	<0.001
Total Testosterone ng/dl	1.25	0.94	1.12	0.90	0.12	0.05	0.042	<0.001	<0.001
Total Chol. mg/dl	168.44	18.70	164.70	13.03	159.44	15.81	0.369	0.056	0.260
LDL mg/dl	84.86	11.62	83.05	11.18	79.73	12.46	0.550	0.132	0.329
HDL mg/dl	35.33	3.91	49.79	4.20	47.72	5.39	<0.001	<0.001	0.109
TG mg/dl	241.17	40.97	159.12	4.72	158.77	4.25	<0.001	<0.001	0.962
CRP	6.47	3.64	4.40	2.70	1.46	0.99	0.027	<0.001	0.003
FBG mmol/L	4.58	0.77	3.21	1.27	2.91	0.74	<0.001	<0.001	0.298
Insulin IU/ml	8.55	0.88	6.91	0.96	6.54	0.91	<0.001	<0.001	0.171
HOMA-IR	1.72	0.32	0.97	0.43	0.86	0.21	<0.001	<0.001	0.245

Table 1: Clinical characteristics and biochemical data of all studied groups.

Serum obestatin levels were significantly decreased in obese PCOS patients when compared to both control and non-obese ones. While serum copeptin levels were significantly increased in obese PCOS patients when compared to both control and non-obese. FMD% was significantly decreased in PCOS patients when compared to controls. CIMT showed no difference between all groups (Table 2).

	Obese		Non-obese		Control		LSD		
	Mean	±SD	Mean	±SD	Mean	±SD	I vs. II	I vs. III	II vs. III
CIMT mm	0.30	0.01	0.30	0.01	0.30	0.01	0.468	0.132	0.387
Obestatin Pg/ml	2.25	1.03	4.74	4.38	6.97	5.31	0.014	<0.001	0.047
Copeptinn g/ml	6.65	2.29	5.22	2.12	3.35	1.31	0.016	<0.001	0.002
FMD %	15.13	0.95	14.86	0.91	18.03	3.61	0.601	<0.001	<0.001

Table 2: Comparison between all studied groups according to serum obestatin and copeptin levels, CIMT, and FMD.

Obestatin was negatively correlated with BMI, WHR, total cholesterol, CRP, serum insulin and HOMA-IR in PCOS patients.

On the other hand, there was a significant positive correlation between Obestatin levels and FMD in PCOS patients.

Regarding Copeptin, there was a significant positive correlation between Copeptin and total cholesterol, triglycerides, LDL, blood pressure, BMI, WHR, hirsutism score, total testosterone, CRP and HOMA-IR in PCOS patients (Table 3).

Additionally, there was a significant negative correlation between Copeptin levels and cardiovascular risk marker (FMD)and HDL-c in PCOS patients (Table 3).

	CIMT mm		Obestatin Pg/ml		Copeptinn g/ml		FMD %	
	R	p-value	R	p-value	R	p-value	R	p-value
CIMT mm			-0.057	0.766	-0.064	0.738	-0.011	0.954
Obestatin Pg/ml	-0.057	0.766			-0.073	0.702	0.033	0.864
Copeptinn g/ml	-0.064	0.738	-0.073	0.702			0.072	0.706
FMD %	-0.011	0.954	0.517	0.014	-0.463	0.025		
Age years	0.285	0.127	-0.317	0.088	-0.009	0.963	-0.241	0.200
BMI Kg/m2	0.181	0.338	-0.368	0.004	0.472	0.020	0.024	0.899
WHR	-0.135	0.478	-0.334	0.036	0.336	0.042	0.055	0.772
SBP mmHg	0.001	0.995	-0.217	0.250	0.268	0.047	-0.128	0.501
DBP mmHg	0.026	0.892	-0.287	0.124	0.332	0.028	-0.119	0.532
Total Testosterone ng/dl	-0.094	0.622	0.257	0.170	0.617	0.013	-0.219	0.246
Total Chol. mg/dl	0.064	0.738	-0.488	0.025	0.650	<0.001	0.079	0.677
LDL mg/dl	0.097	0.609	0.156	0.410	0.500	0.005	0.030	0.876
HDL mg/dl	0.052	0.785	0.124	0.516	-0.806	<0.001	-0.081	0.671
TG mg/dl	-0.017	0.927	0.307	0.099	0.390	0.033	0.176	0.353
CRP	-0.024	0.899	-0.432	0.021	0.543	0.007	0.388	0.034
FBG mmol/L	-0.036	0.851	-0.252	0.052	0.526	0.003	-0.218	0.246
Insulin IU/ml	0.047	0.805	0.381-	0.003	0.294	0.115	0.016	0.932
HOMA-IR	0.018	0.924	-0.321	0.012	0.661	<0.001	-0.159	0.401

Table 3: Correlation between copeptin, obestatin, CIMT, FMD and all studied parameters in PCOS patients.

DISCUSSION

Polycystic ovaries (PCO) are the morphological ovarian phenotype in women with the polycystic ovary syndrome (PCOS).

Several studies performed to attempt to determine the prevalence of PCO as detected by ultrasound alone in the general population, and have found prevalence rates in the order of 17–33%. [21]

The current study, we correlates the serum levels of both Obestatin and Copeptin with obesity, insulin resistance and cardiovascular disease in polycystic ovary, through the estimation of the serum levels of Obestatin and Copeptin, carotid artery intima-media thickness, brachial artery flow mediated dilatation and other metabolic and hormonal parameters in obese and non-obese women with PCOS and healthy controls.

In the present study, the obese women with PCOS have a significantly higher level of triglycerides, HOMA-IR, total testosterone, , CRP, blood pressure and WHR values and lower LDH levels when compared to control and non-obese ones.

In accordance with these results, Giallauria et al. (2008) [22] reported that PCOS women represent an intriguing biological model illustrating the relationship between hormonal pattern and cardiovascular risk profile, presenting a cluster of cardiovascular features, such as obesity, insulin resistance, hypertension, impaired cardiopulmonary functional capacity, auto-nomic dysfunction and low-grade chronic inflammation Obestatin was first described as a bioactive peptide encoded by the same gene as ghrelin, playing a role in reducing food intake, body weight gain, and gastric emptying and suppressing intestinal motility and regulation of hormone secretion (Tang et al., 2008).[23]

In the current study, the serum levels of Obestatin were significantly lower in PCOS group, especially in obese ones than control group. This finding was in accordance with other studies in humans have shown that plasma Obestatin is significantly lower in obese subjects as compared to lean controls, indicating a role for Obestatin in long-term body weight regulation (Ren et al., 2009). [24] Additionally, concluded that Obestatin and ghrelin in normal weight groups were significantly higher than they were in obese groups. All these findings potentiate the hypothesis that the increased obesity rates in PCOS may be attributed to low Obestatin levels. Therefore, low Obestatin levels may predict the underlying factor of obesity in PCOS patients.

Regarding the possibility of using Obestatin for cardiovascular risk assessment in PCOS, the present study showed that there was a significant positive correlation between Obestatin levels and cardiovascular risk markers (FMD) in PCOS patients. This in accordance with Taskin et al. (2015). [25] who found that Obestatin is correlated with FMD and may be used for cardiovascular risk assessment in PCOS.

In the current study, Obestatin was also negatively correlated with CRP in PCOS patients. This result came in agreement with Taskin et al. (2015). [25] who suggested that low Obestatin levels may reflect low grade chronic inflammation in PCOS.

PCOS is associated with oxidative stress, namely increased production of free radicals followed by decreased serum antioxidant levels and antioxidant enzyme activity. It is thought that metabolic dysfunction like obesity, hyperinsulinemia, and dyslipidemia might be responsible for PCOS-associated oxidative stress (Macut et al., 2013). [26]

Since antioxidant effects of Obestatin have been recently approved (Koc et al., 2014). [27] Therefore, we would expect an association between Obestatin and dyslipidemia and insulin resistance. In our study, obestatin was also negatively correlated with total cholesterol and HOMA-IR in PCOS patients. This finding potentiates the hypothesis that decreased Obestatin levels in PCOS, as an antioxidant, may contribute to increased oxidative stress in PCOS patients (Taskin et al., 2015). [25]

Copeptin is a marker of vasopressin level that reflects the individual stress level because of its hemodynamic osmoregulatory effects. In the present study, the serum levels of Copeptin were significantly higher in PCOS group, especially in obese ones than control group. This finding was in accordance with Karbek et al. (2014). [28] and Taskin et al. (2015). [25]

In relation to the association between Copeptin levels, insulin resistance and metabolic syndrome, the present study demonstrated that there was a significant positive correlation between Copeptin and total cholesterol, triglycerides, LDL, blood pressure, BMI and HOMA-IR in PCOS patients. These findings came in agreement with several studies which assumed that stress mediated hypothalamic pituitary adrenal axis activation, regulated by Copeptin, was found to have a role in the pathophysiology of insulin resistance and metabolic syndrome (Enhornling et al., 2010). [29] Additionally, Tenderenda-Banasiuk et al. (2014). [30] reported that higher serum Copeptin levels are associated with systolic and diastolic blood pressure and several components of metabolic syndrome including obesity, elevated triglycerides.

Since Copeptin is a neurohormon (NH) of the Arginine vasopressin AVP system (Voors et al., 2009), [29] we would expect the possibility of using Copeptin for cardiovascular risk assessment in PCOS, the present study showed that there was a significant negative correlation between Copeptin levels and cardiovascular risk markers (FMD) in PCOS patients. This in accordance with recent studies showed that Copeptin was elevated in acute myocardial infarction (AMI) and resulted in better diagnostic performance when assessed in combination with cardiac troponin, particularly during the first hour after onset of symptoms and Taskin et al. (2015) [25] observed PCOS patients had higher copeptin levels and these elevated Copeptin levels are associated with increased cardiovascular risk in PCOS These previous studies revealed that Copeptin is not only a marker of cardiovascular diseases, but of other conditions as well. Potential links of Copeptin with DM, metabolic syndrome (MetS) and microalbuminuria have drawn particular interest in the recent years. The AVP system has also been suggested to contribute to insulin resistance and DM potentially through a variety of mechanisms including stimulation of glucagon and ACTH secretion and glycogenolysis (Enhörning et al., 2010). [29] Therefore, Copeptin, as a surrogate marker of this system, might also be associated with disrupted glucose homeostasis: a recent study demonstrated that increased Copeptin levels were found to be associated with insulin resistance ($p < 0.001$) in a large population of 4742 subjects (cross-sectionally) (Enhörning et al., 2013).[31] Consistent with this, Copeptin was also reported to have a cross-sectional association with metabolic syndrome in a large population of subjects (Enhörning et al., 2011). [32]

CONCLUSION

Obestatin and Copeptin may be regarded as promising markers of cardiometabolic risk as well as additional guide in the early identification of PCOS patients at risk for cardiovascular disease. The present study concluded also that insulin resistance and obesity are associated with both serum Obestatin and Copeptin levels, hence they appeared to have an important role in metabolic response and subsequent development of atherosclerosis in insulin resistant, obese, hyperandrogenemic PCOS patients.

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