Original Article

Serum and salivary obestatin concentrations in the diagnosis of polycystic ovary syndrome

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Abstract

Aim: : To measure obestatin levels in the blood and saliva samples of normal-weight patients with polycystic ovary syndrome (PCOS) in comparison with normal-weight healthy controls, and to determine whether there were relationships between blood and/or salivary obestatin levels and other measured parameters.

Materials and Methods: Fifteen healthy women and 15 patients with PCOS, all of which had normal weight, were included in the study. Participants' age, height, weight, menstrual characteristics, hormone levels, body mass index, waist/hip ratio, and modified Ferriman-Gallwey (FG) scores were recorded. Obestatin levels were measured in both fasting blood and saliva samples. Homeostasis Model Assessment (HOMA-IR) was used to predict insulin resistance.

Results: In the PCOS group, menstrual cycle duration and FG scores were significantly higher (P-value, <0.001, <0.001, respectively). The levels of luteinizing hormone, total testosterone and androstenedione were significantly higher in the PCOS group than in the control group (P-value, 0.001, 0.009, 0.004, respectively). In the PCOS group, blood obestatin level was 1265.2 ± 221.9 pg/ml, salivary obestatin level was 3095.33 ± 310.2 pg/ml; whereas the control group demonstrated lower levels of 939.66 ± 72.3 pg/ml and 2611.20 ± 217.1 pg/mL, respectively. However, no statistically significant difference was found between the PCOS and control groups when comparing obestatin levels in blood or saliva (P-value, 0.218, 0.369, respectively). No correlation was found between blood and salivary obestatin levels in either group. Finally, obestatin levels were not associated with any of the other measured parameters.

Conclusion: Our results suggest that increased levels of obestatin, albeit in a small amount that would not be statistically significant in normal weight patients with PCOS, may have significant effects on weight control in these patients. In addition, our findings show that saliva sample can be used as an alternative to blood sample in the measurement of obestatin level in patients with PCOS.

Keywords: Ferriman-Gallwey; normal weight; obestatin; polycystic ovary syndrome; saliva

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among reproductive-age women, with a prevalence of 6% to 10% worldwide (1). Clinical symptoms of PCOS may include menstrual irregularity, symptoms of hyperandrogenism, and obesity (2). PCOS is often associated with abdominal adiposity, insulin resistance, obesity, metabolic disorders, and cardiovascular risk factors (3). One of the striking puzzles about PCOS is the common denominator behind its relationship with ovarian hyperandrogenism, obesity, and insulin resistance (4).

Obestatin is an intestinal hormone derived from pre-pro-ghrelin, which was discovered in 2005 by Zhang et al. (5). It is a 23 amino acid peptide encoded by 3 exons in the ghrelin gene localized on chromosome 3p25-26. Original studies focused on the functions of obestatin reported that it inhibits food intake, reduces body weight (6), supports pancreatic β-cell survival (7), and increases insulin secretion (8). Additionally, as an in vitro effect, it was reported that both non-acetylated ghrelin and obestatin were able to significantly reduce luteal steroidogenesis in human luteal cells by reducing progesterone release (9).

Conflicting results have been reported in studies comparing fasting serum obestatin levels between normal-weight PCOS patients and healthy women. Studies have reported low obestatin levels in normal-weight women with PCOS (10,11), while others have not found any difference, when compared with normal weight healthy subjects (12); furthermore, elevated levels have been described in normal-weight adolescents with PCOS (13). On the other hand, correlations between salivary and serum levels of obestatin have been suggested (14).

This study was designed primarily to measure obestatin levels in the blood and saliva samples of normal-weight patients with PCOS and healthy controls in order to compare obestatin levels in these two groups. We also aimed to determine whether there existed a relationship between blood and salivary obestatin levels in patients or controls, and to identify any other relationships between measured parameters and the levels of obestatin in the blood or saliva.

MATERIALS and METHODS

Study Design and Ethics
In this cross-sectional study, we enrolled 15 normal-weight patients with PCOS and 15 normal-weight healthy women as controls. Participants were selected between February 2008 and May 2008 among the patients examined at Fırat University Medical Faculty (FUMF) Gynecology and Obstetrics outpatient clinic. The study protocol was prepared according to the ethical principles put forth by the Helsinki Declaration, which was approved by the FUMF Ethics Committee. Before participating in the study, all women were informed in detail about the study, and those accepting to participate signed written informed consent forms.

Patient Selection and Follow-up
The gynecological and general medical history of all participants and demographic characteristics were recorded. The length of the menstrual cycle was identified based on anamnesis. All participants underwent a complete physical and gynecological examination by the same doctor on the third to fifth days of menstruation, and were evaluated by transvaginal and/or abdominal pelvic ultrasonography following the examination. Heights (cm), weight (kg), waist circumference (cm), hip circumference (cm), blood pressure (mm/Hg) were measured, and hirsutism was assessed using the Ferriman-Gallwey (FG) scoring (15). Body Mass Index (BMI), waist/hip circumference ratio (WHR), and ovarian volume were calculated. Age and BMI criteria were applied for the selection of the participants; individuals with a BMI of 18.5-24.9 kg/m² between the ages of 18–35 years were included in the study. Diagnosis of PCOS was made according to the criteria of the European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine (ESHRE/ASRM) (16).

The control group consisted of 15 healthy volunteers who had applied for preconception counseling, given that they had no history of chronic disease or substance use disorders, did not engage in active sports, were not adhering to any diet, and did not have any acute illness in their routine examinations.

Participants with a history of thyroid dysfunction, hyperprolactinemia, diabetes mellitus (DM), smoking, alcohol or any substance abuse, medication use, chronic disease, gastric or intestinal surgery history, gestational diabetes mellitus, engagement in active sports and dietary restrictions were excluded, as well as those with any findings beyond PCOS diagnostic criteria in pelvic ultrasonography or systemic examination.

Collection of Blood and Saliva Samples
All participants were advised not to smoke, eat, or drink liquids other than water during the night before the samples were collected. Three ml of saliva and 5 ml of fasting venous blood samples were obtained in the early follicular phase of the natural menstrual cycle (day 2-5), between 8:00 and 9:00 AM after fasting overnight. Immediately after blood collection into serum separator tubes, 20-30 μl aprotinin (a protease inhibitor) per 1 ml of blood was added to samples in order to prevent degradation of peptides in cells. After clotting, blood samples were centrifuged at 3000×g for 5 min to separate the serum. All specimens were kept at -20 °C until analysis.

Hormonal and Biochemical Measurements
Serum and salivary obestatin levels were measured using a Human Obestatin ELISA kit [Lot No: A01016, limit determination 0–25 nanogram (ng/mL)] of BACHEM trademark (Peninsula Laboratories Inc., a member of the BACHEM group, California, USA) according to the recommendations of the manufacturing company. Fasting plasma glucose (FPG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglycerides (TG), urea, and creatinine measurements were analyzed from venous blood samples using the Olympus AU2700 (Optical Co., Ltd., Tokyo-Japan) chemistry analyzer. Insulin was measured by the chemiluminescent assay method in a Beckman Coulter DXI device. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), free thyroxine (FT4), total testosterone (T), androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), 17-hydroxy-progesterone (17-OHP), sex hormone-binding globulin (SHBG), cortisol, progesterone, and prolactin were measured with the Immulite2000 device (IEMA; Diagnostic Products Corporation, Los Angeles, USA). The Homeostatic Model Assessment Insulin Resistance (HOMA-IR), which reflects insulin resistance, was calculated with the formula following formula: fasting insulin (U/ml) x fasting plasma glucose (mg/dL) / 405 (17).

Statistical Analysis
The Statistical Package for the Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The normality of distribution of continuous variables was checked with the Shapiro Wilk test. Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed as frequency (n) and percentage values. In group comparisons, the independent samples t-test was used for the comparison of continuous variables, and Chi-square tests were used to compare categorical variables. P-values of <0.05 were considered statistically significant.

RESULTS

Data for a total of 30 participants were analyzed. The clinical characteristics and anthropometric measurements of the participants are shown in Table 1. There was no statistically significant difference between PCOS and control groups with regard to mean age, gravidity, parity, BMI, and WHR. However, there was a significant difference between the groups concerning the duration of menstrual cycle and FG score. In the PCOS group, the length of the menstrual cycle was longer and the FG score was higher than controls (P-value, <0.001, <0.001, respectively).

Table 1. Clinical characteristics and anthropometric measurements of the study population			
Variable	Control (n= 15)	PCOS (n=15)	P-Value
Age (years)	25.4 ± 5.8	26.5 ± 5.1	0.546
Gravidity	0.80 ± 1.32	0.86 ± 1.24	0.773
Parity	0.60 ± 0.98	0.66 ± 0.97	0.772
Cycle Length (days)	28.60 ± 1.72	45.86 ± 16.03	<0.001
FG score	0.26 ± 0.45	4.33 ± 2.63	<0.001
BMI (kg/m2)	22.50 ± 2.29	22.41 ± 2.15	0.693
Waist/hip ratio	0.73 ± 0.92	0.77 ± 0.70	0.176
Data presented as mean ± SD			

There were no statistically significant differences between the two groups in terms of plasma glucose, insulin, HDL, LDL, TG and total cholesterol levels in blood, and HOMA-IR value (Table 2).

Table 2. Clinical characteristics and anthropometric measurements of the study population			
Variable	Control (n= 15)	PCOS (n= 15)	p Value
Fasting glucose (mg/dL)	91 ± 8.74	88.86 ± 6.55	0.602
Fasting Insulin (µIU/mL)	8.80 ± 6.23	5.77 ± 3.19	0.206
HOMA-IR	2.07 ± 1.74	1.46 ± 0.87	0.468
Total cholesterol (mg/dL)	167.60 ± 28.48	183.53 ± 27.70	0.141
HDL cholesterol (mg/dL)	56.80 ± 27.55	60.33 ± 14.91	0.110
LDL cholesterol (mg/dL)	106.66 ± 21.44	108.33 ± 32.58	0.418
TG (mg/dL)	96.86 ± 37.21	82.20 ± 22.36	0.280
Data presented as mean ± SD			

We found that the LH, T, and AS levels in the blood were significantly higher in the PCOS group than in the control group. However, there was no statistically significant difference between the two groups with respect to FSH, E2, progesterone, prolactin, DHEAS and SHBG levels (Table 3).

In the PCOS group, blood obestatin level was 1265.2 ± 221.9 pg/ml, salivary obestatin level was 3095.33 ± 310.2 pg/ml; whereas the control group demonstrated lower levels of 939.66 ± 72.3 pg/ml and 2611.20 ± 217.1 pg/mL, respectively. However, no statistically significant

difference was found between the PCOS and control groups when comparing obestatin levels in blood or saliva (P-value, 0.218, 0.369, respectively) (Table 3, Figure 1). Furthermore, no correlation was found between blood and salivary obestatin levels in either group. Finally, no association was found between blood or salivary obestatin levels and other measured parameters in either group.

| Table 3. Hormonal features of the study population			
Variable	Control (n= 15)	PCOS (n= 15)	p Value
FSH (mIU/mL)	5.56±2.81	6.98±1.94	0.097
LH (mIU/mL)	3.91±1.56	9.17±4.74	0.001
E2 (pg/mL)	66.57±38.59	44.86±12.95	0.130
Progesterone (ng/mL)	1.02±0.9	0.58±0.30	0.896
Prolactin (ng/mL)	17.02±13.92	14.58±9.12	0.950
Total testosterone (ng/dL)	35.88±16.97	55.20±21.31	0.009
Androstenedione (ng/mL)	4.45±1.99	14.86±7.90	0.004
DHEA-S (µg/dL)	174.74±69.36	236.71±137.38	0.254
SHBG (nmol/L)	44.27±22.26	71.05±14.09	0.604
Blood obestatin level (pg/ml)	939.66 ± 72.3	1265.2 ± 221.9	0.218
Salivary obestatin level (pg/ml)	2611.20 ± 217.1	3095.33 ± 310.2	0.369
Data presented as mean ± SD			
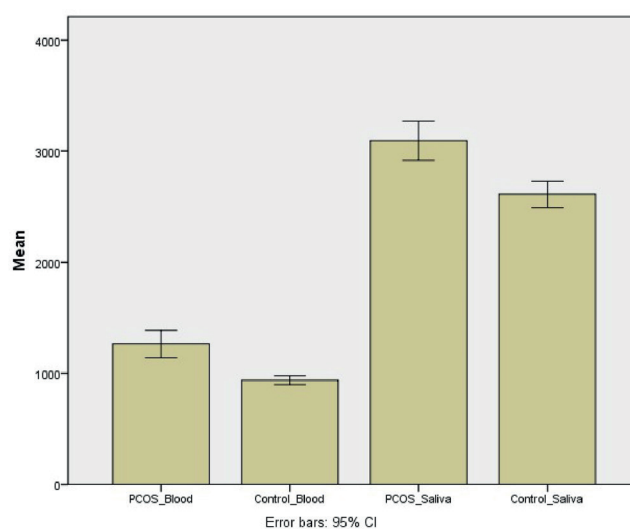
Figure 1. Obestatin levels (pg/ml) in fasting blood and saliva samples of the study population

DISCUSSION

Results of studies comparing fasting obestatin levels of healthy individuals and normal-weight patients with PCOS are contradictory. In a recent study by Varli B. et al. (18), significantly lower serum obestatin levels were reported in patients with PCOS compared to controls with similar characteristics and BMI levels. Since there was no study evaluating salivary obestatin levels in patients with normal-weight PCOS in the literature, we could not compare our results in this regard. However, concerning obestatin levels in the blood samples, our results were similar to those of Rahmani E. et al. (12) but different to those of Askin M. et al. (10) and Abd El-Fattah A. et al. (11). Because obestatin is one of the metabolic hormones that contributes to energy regulation, it may be plausible to suggest that the partial increase in obestatin levels may be directly associated with PCOS, or that it could be a metabolic response. Besides low sample size, our results may be affected by various factors, such as, age, body weight, fat mass, hormonal status and disease severity (particularly with respect to insulin resistance), as these parameters are known to significantly affect metabolism.

Weight gain and central obesity are common features of PCOS (19). Weight gain between the ages of 14–46 years was significantly higher in women with PCOS in the presence of type-2 diabetes mellitus compared to women with PCOS and normal glucose tolerance (20). Besides, it is well established that women with PCOS are more likely to have visceral adiposity and demonstrate higher levels of visceral adiposity indices than their non-PCOS counterparts (21). These abnormalities in body fat distribution may cause insulin resistance, hyperinsulinemia and abnormal glucose tolerance (22). For instance, when overweight/obese (BMI ≥ 25.0 kg/m2) PCOS patients were compared with overweight/obese controls, it was reported that PCOS independently increased the risk of type-2 diabetes mellitus; whereas there was no such increase in the risk of pre-diabetic findings or type-2 diabetes mellitus in normal-weight women with PCOS (20,23). In addition, many studies have shown that obestatin plays a role in regulating energy homeostasis (24,25) and that obestatin levels are low in overweight/obese patients (26-28). Current evidence suggests that obestatin has significant effects on adipose tissue, and may even act as an autocrine/paracrine mediator (29,30). Although there is some debate about the significance of obestatin, an increasing number of studies show the ability of obestatin to stimulate insulin secretion (7,8,24). All these effects suggest that there may be a direct or indirect link between the pathophysiology of PCOS and the levels of obestatin. Rahmani E. et al. (12) reported that serum obestatin levels had a significant inverse correlation with insulin and HOMA-IR in PCOS patients with normal BMI. However, in our study, no association was found between blood and saliva obestatin levels and any of the measured parameters in either group. Although mean salivary obestatin levels were 2.78-times higher than mean serum obestatin levels, and despite the report of Ozbay et al.

(14) on the correlation between salivary and serum levels of obestatin, we did not find any significant relationship in our study. However, measurement of obestatin levels in the saliva may be an important alternative to blood measurements. In fact, saliva samples may be preferable to serum for obestatin measurements because saliva samples are easy to obtain and the procedure is non-invasive.

LIMITATIONS

The most important limitation of our study is the small sample size. Also, we performed our hormone measurements only once in the early follicular phase of the menstrual cycle. Therefore, we were unable to determine changes in obestatin levels throughout the day and at different periods of the menstrual cycle.

CONCLUSION

Even though there were some limitations as mentioned above and the fact that statistically significant differences were absent in the current study, our results support the literature in terms of showing higher obestatin levels in the blood of normal-weight PCOS patients, while, to our knowledge, this is the first study to demonstrate that saliva samples may be utilized to determine obestatin levels in patients with PCOS. Our results suggest that increased levels of obestatin, albeit in a small amount that would not be statistically significant in normal weight patients with PCOS, may have significant effects on weight control in these patients. Further studies are required to understand the nature of the relationship between PCOS and obestatin levels.

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