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Original Study

Characteristics of Polycystic Ovarian Syndrome and Relationship with Ghrelin in Adolescents

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Abstract. *Objective:* Some points of pathogenesis in polycystic ovarian syndrome (PCOS) are still unknown. In this study we evaluated the characteristics of this disease and its relationship with ghrelin in adolescence.

Design: A prospective case control study was designed. Four groups: obese PCOS (n = 13), lean PCOS (n = 13), obese control (n = 10) and lean control (n = 10) were formed. Oral glucose tolerance tests (OGTT) were performed on all subjects. Laboratory and clinical features of groups were compared.

Setting: University pediatric endocrinology clinic.

Participants: Adolescents with PCOS.

Interventions: None.

Main Outcome Measures: Insulin resistance, ghrelin, delta ghrelin (difference of ghrelin between basal and 120th minute after OGTT), androgens

Results: Insulin resistance ratios were 93.3%, 46.6%, 50% in obese PCOS, lean PCOS and obese controls respectively. Ghrelin levels were lower in obese PCOS group but statistically different only between obese and lean PCOS groups. Ghrelin was correlated negatively with HOMA-IR ($P < 0.001$), 17 OH progesterone ($P = 0.05$), total ($P = 0.015$) and free testosterone ($P = 0.013$). Ghrelin suppression was blunted in PCOS groups. Ghrelin suppression ratios after glucose load were 24.4%, 28.7%, 36%, 35% obese PCOS, lean PCOS, obese control and lean control groups respectively.

Conclusion: Low ghrelin levels in obese PCOS patients, correlations between insulin resistance, androgens and ghrelin, blunted suppression of ghrelin after glucose load in PCOS have been considered as evidences of ghrelin role in pathogenesis of this syndrome.

Key Words. Polycystic ovarian syndrome—Adolescence—Insulin resistance—Ghrelin

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Introduction

Polycystic ovary syndrome (PCOS) is one of the frequent endocrinopathies in adult women.[1] Insulin resistance and neuroendocrine dysfunctions play a big role in pathogenesis of the disease.[2–4] Some points on pathogenesis of PCOS are still unclear. Obesity is common in girls with PCOS.[5] Previously, low ghrelin levels have been shown in obese population, especially in PCOS.[6] Ghrelin stimulates growth hormone secretion, also it has different functions such as the regulations of glucose metabolism, appetite, body weight, endocrine pancreatic, and ovarian functions.[7,8] Existence of ghrelin receptor and ghrelin itself on ovaries, its capability of suppression of luteinizing hormone, low ghrelin levels in woman with PCOS, and its relation with obesity and insulin resistance are some evidence demonstrating the role of ghrelin on reproductive functions.[6,9,10] There are few studies demonstrating PCOS and ghrelin relation in adolescence. In this study, we investigated the characteristics of patients, ghrelin status, and ghrelin response to oral glucose load in adolescents with PCOS.

Material and Methods

Adolescents with PCOS admitted to Ondokuz Mayis University Medical Faculty Division of Pediatric Endocrinology and age matched control subjects were enrolled to the study. There were 26 girls (13 obese, 13 non obese) at mean age 15.24 ± 1.06 in PCOS group and 20 girls (10 obese, 10 non obese) at mean age 15.15 ± 1.26 in control group. Finally, four groups were formed: obese PCOS, lean PCOS, obese control, and lean control.

Adolescents were diagnosed as PCOS with four findings out of the five criteria listed below.[11]

- Oligomenorrhea or amenorrhea existence after two years of menarche

- Clinical findings of hyperandrogenism (acne, hirsutism)
- Biologic hyperandrogenism (plasma testosterone $>$ 50 ng/dl, increased LH/FSH ratio $>$ 2)
- Insulin resistance, hyperinsulinism
- Microcysts in ovaries or increased ovarian size ($>$10 ml) in ultrasonography

Physical examination was performed by the same physician. Systolic and diastolic blood pressure, existence of acanthosis nigricans, and hirsutism score were recorded. Patients who were under the treatment of metformin or oral contraceptive and who had endocrinologic diseases such as hypothyroidism, hyperprolactinemia, late onset congenital adrenal hyperplasia, or Cushing syndrome were excluded from the study.

After overnight fasting, oral glucose tolerance test (OGTT) was performed for all adolescents within the same time interval (9:00−12:00 AM) on the third day of spontaneous or progesterone induced menstruation. Fasting serum samples were obtained for luteinizing hormone (LH) follicle stimulating hormone (FSH), estradiol (E2), prolactin (PRL), total testosterone, free testosterone, 17 OH progesterone, total cholesterol, triglyceride, and high density lipoprotein. The venous blood glucose and insulin levels were measured on the 30th, 60th, 90th, and 120th minute of OGTT. Blood samples for ghrelin were obtained on the beginning and 120th minutes of OGTT. Blood samples were immediately centrifuged and stored at −80 °C until analysis. The decrease of ghrelin from basal to 120th minute of OGTT is defined as delta ghrelin. Also, we calculated the decrease ratio of ghrelin in groups.

Subjects who had a body mass index (BMI) above the 95th percentile for age and sex or BMI above standard deviation score (SD) +2.0 were classified as obese.[12] Homeostasis model assessment of insulin resistance (HOMA-IR) index (fasting insulin × fasting glucose/22.5) was used for determining insulin resistance.[13] Insulin resistance criterion was HOMA-IR $>$ 4.0 for adolescents.[14] Evaluation of hirsutism was performed by using the Ferriman Gallwey scoring system.[15]

All participants were examined for PCOS via transabdominal pelvic ultrasonography with a conventional full bladder by the same physician. Both ovarian volumes were calculated as: width × length × height × 0.523.[16]

Assays

Serum glucose and insulin were measured by the enzymatic spectrophotometric glucose oxidase method (Roche Diagnostics, Mannheim, Germany) and the commercial kit of DPC (Diagnostic Products Corporation, CA) with immune chemiluminescence assay (ICMA) respectively. LH (mIU/ml), FSH (mIU/ml), PRL (ng/ml), E2 (pg/ml), and total testosterone (ng/dl) were measured by ICMA with the Architect analyzer (Architect Reagent Kit, IL). Free testosterone (pg/ml) and 17-OH-progesterone (μg/dl) were analyzed with Diagnostic Systems Laboratories (DSL, Lot. No 06087, and 03167, respectively) kit using radioimmunoassay. Cortisol (mg/dl), ACTH (pg/ml), and DHEAS (mg/dl) were measured by ICMA with the Immulite 2500 analyzer (DPC). Total T4 (μg/dl), free T4 (ng/dl), and TSH (mIU/ml) were analyzed with commercial kits (Roche Diagnostic GmbH, Mannheim, Germany) by ICMA with the E170 Hitachi analyzer (Tokyo, Japan). Lipid parameters were determined by enzymatic methods using commercial kits. Total ghrelin levels were measured with commercial RIA kit (Linco RIA kit).

The local ethics committee approved the study. Written informed consents were obtained from the parents of patients and control children.

Statistics

All statistical analysis were done using SPSS 10.0 software program. All values are given as mean ± SD. The Mann-Whitney U test was applied for the comparison of two groups. Data involving more than two groups were assessed by one-way ANOVA test with Tamhane's test for post hoc analysis. Spearman's test was used for the assessment of correlations between ghrelin and other parameters.

Results

Clinical and laboratory features of groups are given in Table 1.

In the comparison of the groups, obese PCOS and obese control: BMI, systolic and diastolic blood pressure were not statistically different. The ratios of adolescents who had acanthosis nigricans were 73.3% and 30.0% in obese PCOS and obese control groups respectively ($P = 0.032$). In obese PCOS group, free testosterone levels, LH/FSH ratio, HOMA-IR and ovarian volumes were higher than those of the obese control group. Serum ghrelin levels were not statistically different between these groups. Delta ghrelin was lower in obese PCOS group (217.6 ± 144.9 vs. 371.8 ± 187.8, $P = 0.03$). Ghrelin decrease ratios after glucose load were 24.4% and 36.1% in obese PCOS and obese control groups respectively (Table 2).

In the comparison of the lean PCOS and lean control groups: BMI, systolic and diastolic blood pressure were not statistically different. Serum LH/FSH ratio and ovarian volumes were higher in lean PCOS group ($P < 0.001$ for both parameters). In lean PCOS group insulin resistance was observed in 46.6% of adolescents; however, in lean control group there were no

Table 1. Characteristics of Groups

	Obese PCOS	Lean PCOS	Obese Control	Lean Control	*P*
Age	14.90 ± 0.90	15.45 ± 1.26	14.98 ± 1..55	15.37 ± 0.96	0.381
BMI (kg/m^2)	32.6 ± 4.9	20.4 ± 2.2	31.6 ± 4.7	20.4 ± 1.5	**0.001**
SBP (mmHg)	127.50 ± 17.25	113.75 ± 9.16	125.56 ± 16.67	110.71 ± 9.32	**0.001**
DBP (mmHg)	78.33 ± 11.14	70.00 ± 7.55	80.55 ± 8.81	71.42 ± 6.90	**0.005**
Hirsutism score	13.58 ± 9.71	11.50 ± 7.84	1.89 ± 2.85	0.00 ± 0.00	**0.001**
Acanthosis nigricans	73.33%	6.66%	30.00%	0.00%	**0.001**
LH/FSH ratio	2.96 ± 2.01	3.11 ± 0.92	0.87 ± 0.44	1.15 ± 0.79	**0.001**
Total testosterone (ng/ml)	0.91 ± 0.45	0.88 ± 0.79	0.68 ± 0.46	0.54 ± 0.15	0.686
Free testosteron (pg/ml)	4.24 ± 2.21	1.84 ± 0.85	1.34 ± 0.61	1.56 ± 0.53	**0.002**
DHEA-S (μg/dl)	183.03 ± 104.71	233.44 ± 65.77	115.71 ± 24.68	91.92 ± 91.28	0.069
17OH progesterone (ng/ml)	3.26 ± 1.89	2.91 ± 1.37	1.71 ± 1.22	1.83 ± 0.28	0.104
TT4 (μg/dl)	8.40 ± 1.58	8.47 ± 0.73	9.69 ± 1.15	8.40 ± 1.42	**0.017**
TSH (μIU/ml)	2.53 ± 1.84	1.61 ± 0.94	2.53 ± 0.67	2.09 ± 1.29	0.325
HDL(mg/dl)	44.88 ± 11.94	52.01 ± 13.19	35.61 ± 10.88	43.92 ± 8.84	**0.015**
LDL(mg/dl)	97.96 ± 40.02	80.34 ± 17.09	75.58 ± 38.88	60.37 ± 15.84	**0.050**
Total cholesterol (mg/dl)	165.66 ± 31.03	150.44 ± 13.11	129.66 ± 43.68	121.25 ± 26.96	0.058
Triglycerides (mg/dl)	115.50 ± 64.28	86.22 ± 45.81	91.83 ± 22.83	84.75 ± 35.53	0.067
Right ovarian volume(ml)	12.85 ± 2.62	11.15 ± 5.13	4.76 ± 0.79	3.95 ± 0.90	**0.001**
Left ovarian volume (ml)	11.20 ± 1.69	8.16 ± 2.82	5.01 ± 1.03	5.27 ± 0.67	**0.001**
Ratio of insulin resistance	93.3%	46.6%	50.0%	0.0%	**0.001**
HOMA-IR	5.97 ± 3.09	2.25 ± 0.78	3.51 ± 3.03	1.94 ± 0.80	**0.001**
Ghrelin (pg/ml)	888.7 ± 330.4	1244.5 ± 289.4	1020.4 ± 226.0	1172.9 ± 393.6	**0.047**

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LH, luteinizing hormone; FSH, follicle stimulating hormone; DHEA-S, Dehydroepiandrosterone sulphate; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, Homeostasis model assessment of insulin resistance.
Values are given as mean ± SD.

insulin resistant adolescents. Serum ghrelin and delta ghrelin levels were not statistically different between these groups ($P = 0.606$), Table 2.

In the comparison of the obese PCOS and lean PCOS groups: Systolic and diastolic blood pressure, existence of acanthosis nigricans, and ratio of adolescent who had insulin resistance were higher in obese PCOS group. Serum free testosterone level was higher in obese group ($P = 0.009$). Serum ghrelin levels and delta ghrelin were lower in obese PCOS group ($P = 0.028$ and $P = 0.036$ respectively). The ratios of ghrelin decrease after glucose load were 24.4% and 28.7% in obese PCOS and lean PCOS groups respectively.

Ghrelin was negatively correlated with BMI, systolic blood pressure, HOMA-IR, 17OH progesterone and testosterone levels (Table 3).

Discussion

Polycystic ovary syndrome is a heterogeneous syndrome characterized by anovulation, hirsutism, and infertility in women. Diamanti-Kandarakis has stated that adolescents display important differences, considering the function of hypothalamic-pituitary-ovarian axis compared with adult women, and that the diagnostic approach of PCOS in this age group should be in pace with the 'endocrine identity' of puberty.[17] There are a lot of studies on PCOS in adults but there are a few studies which evaluate lean and obese PCOS groups in adolescence.

Insulin causes hyperandrogenism with different mechanisms.[18] One of the mechanisms is that insulin acts like IGF1 which increases LH effect on ovaries via cross reaction with IGF1 receptors.[19] The other ones are that it may increase free IGF1 levels,[20] stimulate cytochrome p450c17 enzyme both in ovaries and in adrenal gland,[21,22] and decrease sex hormone binding globulin levels.[23] Hyperandrogenism plays a key role in occurring clinical appearance. The increase of free testosterone and DHEA-S in obese PCOS group was also one of the important findings of this study.

Table 2. Ghrelin Response to Glucose Load

	Obese PCOS	Lean PCOS	Obese Control	Lean Control	*P*
Basal ghrelin	888.7 ± 330.4	1244.5 ± 289.4	1020.4 ± 226.0	1172.9 ± 393.6	0.047
120th minute ghrelin	671.1 ± 185.5	886.6 ± 143.7	648.6 ± 38.2	751.2 ± 180.4	0.008
Delta ghrelin	217.6 ± 144.9	357.9 ± 146.2	371.8 ± 187.8	421.7 ± 113.2	0.048
Decrease ratio (%)	24.4	28.7	36.0	35.0	0.040

Values are given as mean ± SD.

Table 3. Correlations between Clinical and Laboratory Parameters and Ghrelin

	r	*P*		r	*P*
Age	0.233	0.241	Total testosterone	−0.461	0.015
BMI	−0.508	0.007	Free testosterone	−0.512	0.013
SBP	−0.549	0.003	DHEA-S	−0.240	0.237
DBP	−0.251	0.207	HDL	0.143	0.515
Hirsutism score	−0.303	0.124	LDL	−0.212	0.331
LH/FSH ratio	0.077	0.493	Total cholesterol	−0.086	0.690
Estradiol	0.251	0.227	Triglycerides	−0.135	0.528
HOMA−IR	−0.652	<0.001	17 OH progesterone	−0.396	0.050

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LH, luteinizing hormone; FSH, follicle stimulating hormone; HOMA-IR, Homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein.

Insulin resistance was higher in lean and obese PCOS groups than in BMI matched control groups in our study but insulin resistance in lean PCOS patients was not as high as in obese PCOS group. In adults, it has been demonstrated that obese women with PCOS had higher insulin resistance frequency than control.[24] However, in lean patients, the relation between PCOS and insulin resistance is still controversial today. There are some studies which demonstrate insulin resistance or hyperinsulinism in lean PCOS patients.[25,26] Nevertheless, it has been reported that status of insulin resistance was not different in lean PCOS patients compared with BMI matched controls.[27,28] Bhattacharya et al have reported that insulin resistance frequency in adolescent PCOS patients was 69.4% and BMI was not statistically different in patients who had insulin resistance compared with those who did not.[29]

Previously in adults, low ghrelin levels have been reported in obese PCOS patients and these ghrelin levels were correlated with insulin resistance and androgens.[6,30] Panidis et al have reported that, in PCOS, circulating ghrelin and androgens are inversely related and they supposed that this peptide may have been involved in steroidal synthesis and/or action.[31] In adolescents, Bideci et al have reported that ghrelin levels were not statistically different in PCOS compared with BMI matched controls. They have reported that ghrelin levels were correlated with LH/FSH ratio.[32] In our study, ghrelin levels tend to be lower in obese PCOS group than other groups but statistically difference has only existed between obese and lean PCOS group. We have demonstrated that ghrelin was correlated with HOMA-IR and testosterone levels but no correlation between LH/FSH ratio and ghrelin was observed.

Moran et al have reported that postprandial ghrelin response has been impaired. They have also reported that weight loss improves postprandial ghrelin response.[33] Barber et al provide strong evidence about dysregulation of ghrelin secretion in PCOS patients. They have demonstrated that ghrelin suppression after OGTT in PCOS patient has been blunted.[4] They have supposed that this ghrelin dysregulation may contribute to the development of obesity in women with PCOS via reduction in the postprandial satiating effect of acute food intake, and a tendency towards overeating during meals caused by blunted postprandial ghrelin suppression.[34] In the current study, we have demonstrated that in adolescence, in both lean and obese PCOS patients, ghrelin suppression after glucose load was lower than the controls.

In conclusion, low ghrelin levels in obese PCOS patients, correlations between insulin resistance, androgens, and ghrelin, as well as blunted suppression of ghrelin after glucose load in PCOS have been considered as the evidences of ghrelin role in pathogenesis of this syndrome

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