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Evaluation of Apelin and Insulin Resistance in Patients with PCOS and Therapeutic Effect of Drospirenone-Ethinylestradiol Plus Metformin

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Background: The aim of this study was to determine the relevance of apelin and insulin resistance (IR) with polycystic ovary syndrome (PCOS) and to assess the possible therapeutic effect of the combined therapy of drospirenone-ethinylestradiol (DRSP-EE) combined with metformin.

Material/Methods: Sixty-three PCOS patients and 40 non-PCOS infertile patients were recruited. The fasting serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), prolactin (PRL), estradiol (E_2), glucose (FBG), insulin (FINS), and apelin at the early follicular phase were measured. To further investigate the relation between apelin and IR, we treated the PCOS patients with DRSP-EE (1 tablet daily, 21 d/month) plus metformin (500 mg tid) for 3 months. All of the above indices were measured again after treatment.

Results: 1) Levels of apelin, LH, LH/FSH, T, and FINS, as well as homeostatic model assessment of IR (HOMA-IR) in PCOS patients, were significantly higher than in the control group before treatment. 2) These indices significantly decreased after treatment with DRSP-EE plus metformin. 3) Correlation analysis showed that apelin level was positively correlated with body mass index (BMI), FINS level, and HOMA-IR.

Conclusions: Apelin level significantly increased in PCOS patients. The combined therapy of DRSP-EE plus metformin not only decreases IR, but also improves apelin level. This combination is a superior approach for PCOS treatment.

MeSH Keywords: Body Mass Index • Insulin Resistance • Polycystic Ovary Syndrome

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Background

Polycystic ovary syndrome (PCOS) is a common endocrine/metabolic disorder [1] among women of reproductive age. PCOS is characterized by chronic anovulation (oligomenorrhea or amenorrhea) and hyperandrogenemia (hirsutism, acne, and increased androgen hormone plasma level, or a combination of these conditions) [2]. Many studies indicate that PCOS is associated with metabolic disorders [3,4] that lead to cardiovascular events, dyslipidemia, and insulin resistance (IR) [5,6]. IR with hyperinsulinemia plays an important role in the development of hyperandrogenism through the enhancement of androgen hormone biosynthesis in the ovaries In addition, IR and the resultant hyperinsulinemia raise the risk of long-term metabolic disorders, such as impaired glucose tolerance, type 2 diabetes, and cardiovascular diseases.

Apelin is a bioactive peptide originally identified from bovine stomach extracts as the endogenous ligand of the G protein-coupled receptor APJ [7]. Apelin has been recently identified as a new adipokine expressed and secreted by mature adipocytes in both humans and mice [8,9]. The apelinergic system has been demonstrated to be involved in the pathogenesis of many conditions, such as hypertension, heart failure, glucose intolerance, and diabetes mellitus [10–12]. Apelin may be a key regulator in glucose and lipid metabolism and may be associated with IR. PCOS is associated with the occurrence of IR and other metabolic disorders, such as dyslipidemia, hypertension, and atherosclerosis. Based on these facts, we conducted this study to determine whether serum apelin levels are different between PCOS women and healthy women. We also evaluated the therapeutic effects of drospirenone-ethinylestradiol (DRSP-EE) plus metformin combination on PCOS.

Material and Methods

PCOS patients

We recruited 63 PCOS women from the Outpatient Center of Reproductive Medicine at the Affiliated Hospital of Taishan Medical University between March 2014 and January 2015. None of the patients had used hormonal preparations, including oral contraceptives (OC). PCOS was diagnosed according to the 2003 Rotterdam Criteria with at least 2 of the following features: oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasound. Patients with oligomenorrhea or hyperandrogenism caused by any other clinical conditions were excluded, such as non-classical 21-hydroxylase deficiency, congenital adrenal hyperplasia, hypothyroidism, Cushing's syndrome, or significant elevation in serum prolactin (PRL) [13,14].

Control group

Forty infertile women with regular menstrual periods (26 days ≤ menstrual period <35 days) were recruited as a control group at the same period. All of the controls were carefully evaluated to avoid any selection bias. None of them had any hirsutism or other manifestation of hyperandrogenism, sonographic evidence of PCOS, sign of galactorrhea, thyroid dysfunction, or diabetes, nor had any received hormonal therapy (including oral contraceptives) or any drug therapy in the last 3 months.

This study was approved by the Institutional Review Board of Taishan Medical University. Written informed consent was obtained from all participants.

PCOS patients were divided into subgroups A (BMI <25 kg/m^2) and B (BMI ≥25 kg/m^2) by body mass index (BMI). Similarly, the controls were divided into subgroups C (BMI <25 kg/m^2) and D (BMI ≥25 kg/m^2).

Clinical and biochemical measurements

Serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E_2), testosterone (T) and PRL were measured during the early follicular phase (days 2 to 5 of the menstrual cycle). Serum samples were collected after fasting for 12 hours to test apelin, glucose (FBG), and insulin (FINS), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels. In PCOS patients, the last menstrual period was either spontaneous or induced by the administration of dydrogesterone (10 mg/d for 7 days). FBG was detected by the oxidase method with an AU640 biochemical auto-analyzer (Olympus Company, Hamburg, Germany). FSH, LH, T, PRL, E_2, T, and FINS were detected by chemiluminescence immunization. Blood lipid levels were measured using an Ft-7060 precipitation and enzymatic device (Beckman Coulter Inc., Galway, Ireland). Apelin was determined by platinum enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, North America).

PCOS patients were treated with drospirenone-ethinylestradiol (DRSP-EE, 3 mg DRSP – 30 ug EE, 21d/month) plus metformin (500 mg tid) for 3 months. The above parameters were recorded at day 0 and at 3 months after treatment.

IR expressed as the homeostatic model assessment of IR (HOMA-IR) was calculated as fasting glucose × fasting insulin/22.5. BMI was calculated as the weight (kg) divided by the square of body height (m^2).

Statistical methods

Descriptive characteristics are reported as mean ± standard deviation (X±SD). The differences in clinical, hormonal, and

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Table 1. Comparison of hormonal characteristics among four groups ($\bar{X}$±SD).

	A (n=40)	B (n=23)	C (n=20)	D (n=20)
FSH (IU/L)	5.78±1.77	5.56±1.66	7.16±1.46[a,b]	6.76±1.45[a,b]
LH (IU/L)	10.65±5.17	9.80±4.63	5.19±1.89[a,b]	4.55±1.35[a,b]
LH/FSH	1.85±0.81	1.90±1.02	0.74±0.30[a,b]	0.69±0.20[a,b]
T (ng/dl)	48.48±19.53	48.21±21.19	21.66±10.21[a,b]	28.41±10.69[a,b]
PRL (ng/ml)	17.91±12.86	20.13±10.19	18.51±6.58	18.51±8.01
E_2 (pg/ml)	45.75±19.50	52.71±20.12	43.12±19.25	48.85±21.35

[a] Compared with A, P<0.05; [b] compared with B, P<0.05.

Table 2. Comparison of clinical, metabolic characteristics among four groups ($\bar{X}$±SD).

	A (n=40)	B (n=23)	C (n=20)	D (n=20)
Age	27.45±3.61	28.04±3.47	26.70±4.40	28.20±4.20
Weight (kg)	57.74±5.18	76.08±6.92*	53.30±5.86[a,b]	72.20±4.34[a]
Height (cm)	162.53±4.66	160.52±5.32	161.75±4.68	162.30±4.32
BMI (kg/m^2)	21.80±1.89	29.47±3.93*	20.39±2.17[a,b]	27.43±1.76[a,b,c]
FBG (mmol/L)	5.31±0.45	5.46±0.46	5.49±0.49	5.46±0.44
FINS (uIU/L)	15.97±10.43	16.46±9.15	7.63±3.42[a,b]	9.96±4.67[a,b]
HOMA-IR	3.79±0.73	3.99±0.76	1.86±0.41[a,b]	2.42±0.50[a,b]
TC (mmol/L)	4.86±0.88	5.07±0.83	4.18±0.71[a,b]	4.48±0.74[b]
TG (mmol/L)	1.17±0.55	1.15±0.38	1.04±0.76	1.21±0.87
HDL(mmol/L)	1.25±0.31	1.23±0.27	1.44±0.36[a,b]	1.33±0.31
LDL (mmol/L)	3.00± 0.71	3.04 ± 0.60	2.58 ± 0.66[a,b]	2.92± 0.51
Apelin (ng/ml)	2.09±0.70	3.02±0.86*	0.24±0.08[a,b]	0.48±0.13[a,b]

[a] Compared with A, P<0.05; [b] compared with B, P<0.05; [c] compared C, P<0.05

biochemical variables among subgroups were evaluated by 1-way analysis of variance (ANOVA) on SPSS 15.0 (Chicago, IL, USA). The modifications linked to the treatment of PCOS were evaluated with the *t* test for paired data. Correlation was analyzed by Pearson's test and multiple stepwise regression analysis. P<0.05 was considered as significant.

Results

Comparison of clinical, hormonal, and metabolic parameters among the 4 subgroups before treatment

PCOS patients and the controls had similar E_2 and PRL levels. However, PCOS women had significantly higher levels of LH, LH/FSH, and T than the controls (Table 1).

The PCOS group was not significantly different in age, height, FBG level, or TG level from the control group, but had higher apelin level, FINS level, and HOMA-IR (Table 2).

Comparisons before and after combined treatment in PCOS women

After the 3-month combined treatment with DRSP-EE plus metformin, the levels of apelin, T, LH, LH/FSH, and FINS, as well as HOMA-IR, were decreased significantly (P<0.05). The levels of FBG, TC, TG, HDL, LDL, and FSH did not change significantly (P>0.05) (Tables 3, 4).

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Table 3. Comparison of hormonal characteristics before and after treatment ($\bar{X}$±SD).

	Non-obese PCOS group		Obese PCOS group	
	Before treatment	After treatment	Before treatment	After treatment
FSH (IU/L)	5.78±1.77	6.06±0.95	5.56±1.66	6.08±1.57
LH (IU/L)	10.65±5.17	6.18±1.33[a]	9.80±4.63	5.90±0.84[a]
LH/FSH	1.85±0.81	1.12±0.33[a]	1.90±1.02	1.01±0.31[a]
T (ng/dl)	48.48±19.53	33.68±8.06[a]	48.21±21.19	32.22±8.15[a]
PRL (ng/ml)	17.91±12.86	16.88±4.67	20.13±10.19	18.66±5.48
E_2 (pg/ml)	45.75±19.50	47.49±9.75	52.71±20.12	49.37±13.74

[a] Before and after treatment, P<0.05.

Table 4. Comparison of clinical, metabolic characteristics before and after treatment ($\bar{X}$±SD).

	Non-obese PCOS group		Obese PCOS group	
	Before treatment	After treatment	Before treatment	After treatment
BMI	21.80±1.89	20.25±2.34	29.47±3.93	27.67±4.55
FBG (mmol/L)	5.31±0.45	4.99±0.59	5.46±0.46	5.08±0.57
FINS (uIU/L)	15.97±10.43	11.18±3.53[a]	16.46±9.15	11.90±3.54[a]
HOMA-IR	3.79±0.73	2.47±0.56[a]	3.99±0.76	2.68±0.65[a]
TC (mmol/L)	4.86±0.88	4.71±0.81	5.07±0.83	4.87±0.98
TG (mmol/L)	1.17±0.55	1.08±0.33	1.15±0.38	1.05±0.31
HDL(mmol/L)	1.25±0.31	1.45±0.34	1.23±0.27	1.42±0.32
LDL (mmol/L)	3.00± 0.71	2.85±0.57	3.04 ± 0.60	2.66±0.64
TC (mmol/L)	4.86±0.88	4.53±1.08	5.07±0.83	4.94±1.27
Apelin (ng/ml)	2.09±0.70	1.38±0.46a	3.02±0.86	1.69±0.58[a]

[a] Before and after treatment, P<0.05.

Table 5. correlation analysis of serum Apelin and the variables.

	FSH	LH	LH/FSH	T	PRL	E_2
r	0.063	0.162	0.137	0.130	0.038	–0.071
p	0.607	0.187	0.199	0.208	0.709	0.569
	Weight	**BMI**	**FBG**	**FINS**	**HOMA-IR**	**TC**	**TG**	**HDL**	**LDL**
r	0.055	0.383[a]	–0.095	0.33[5a]	0.343[a]	0.127	0.061	–0.195	0.034
p	0.580	<0.001	0.337	0.001	<0.001	0.209	0.650	0.054	0.740

[a] P<0.01.

Correlations

Apelin level is positively correlated with BMI, FINS level, and HOMA-IR (Table 5). Multiple stepwise regression analysis showed that BMI and HOMA-IR were the independent factors related to the level of serum apelin.

Discussion

PCOS is the most common endocrine cause of menstrual irregularities, hirsutism and acne, but its pathogenesis remains unclear. IR and its compensatory hyperinsulinemia play an important role in the pathogenesis of PCOS. This study shows that

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FINS level and HOMA-IR as parameters of IR in PCOS women are significantly different from the control group. Also, serum T levels are significantly different in PCOS women with and without obesity compared with the control group. This result suggests that IR and hypotestosteronemia are actively involved in the pathogenesis of PCOS regardless of obesity.

The increase of LH level probably plays an important role in the pathological mechanism of the higher androgens production in the ovaries, which can interfere with the maturation of the oocyte. In the present study, the serum LH levels are significantly higher, while FSH level is lower in PCOS women compared with controls. These results further confirm that high LH level and relative insufficiency of FSH are the characteristics of PCOS.

Apelin is a recently discovered peptide that is designated as an endogenous receptor ligand and found in several organs such as heart, brain, kidneys, and lungs [15]. Apelin level is related with the occurrence of obesity and IR [9]. Recently, varying expression levels of apelin and its receptor (APJ) have been observed in different stages of cattle ovarian follicles [16,17]. There is little data in the literature regarding changes in apelin level or its relation to PCOS, and even the existing published results are inconsistent. One published research study reported lower apelin level in normal-weight PCOS women than in control subjects [18]. Choi et al. published a similar finding of significantly lower serum apelin levels in PCOS women [19]. These 2 studies also suggest that serum apelin level is positively correlated with apolipoprotein A level, but is negatively correlated with total testosterone level and free androgen index (FAI) independent of IR. Moreover, serum apelin levels are lower in PCOS women than in controls [20]. In contrast to this finding, Cekmez et al. [21] reported that the mean level of apelin is higher in PCOS adolescents than in controls, and the apelin level is positively correlated with BMI and HOMA-IR [21]. However, similar apelin levels between women with and without PCOS were also reported in other clinical research studies [22]. In our present study, however, serum apelin levels are significantly higher in PCOS women compared with controls. Moreover, we also observed that apelin level is significantly and positively correlated with BMI, HOMA-IR, and FINS level. These results are consistent with a previous study (Cekmez et al.). Discrepant findings among the published studies may be attributed to the differences in ethnicity, age, study design, sample size, genetic characteristics of populations, and assessment methodology. The limitation of our study is that the size of both groups was small, which perhaps resulted in inconsistent findings; therefore, further studies are required in larger cohorts with different genetic backgrounds. Pannaciulli et al. [23] found the IMT-CCA was significantly higher in young adult normal-weight, overweight, and obese glucose-tolerant first-degree relatives of type 2 diabetic patients compared with control subjects with no family history of diabetes. Considering the metabolic features of PCOS, such as T2DM, obesity, and insulin resistance, family-based studies are needed to further investigate the association between Apelin and PCOS.

After the 3-month treatment with DRSP-EE plus metformin, PCOS patients show significantly decreased levels of apelin, FINS, LH, LH/FSH, and T, as well as HOMA-IR. A multivariate regression analysis found that, in addition to IR, the use of the combined treatment also reduced apelin level, LH level, and other parameters, which indicates that apelin level is related to IR. Thus, we speculate that apelin level reduction can improve IR and reduce the risk of cardiovascular events, dyslipidemia, and IR associated with PCOS. The combination of metformin and DRSP-EE might be a recommendation for treatment of PCOS.

Conclusions

Chinese PCOS women exhibit higher apelin levels than controls. Serum apelin level is positively correlated with BMI and HOMA-IR. The treatment with metformin plus drospirenone-ethinylestradiol is effective for the reduction of insulin resistance, apelin level, and the improvement of other parameters linked with a higher risk of type 2 diabetes mellitus and cardiovascular diseases. Early recognition, proper intervention, and long-term monitoring are therefore necessary, and apelin is a candidate target for treatment of PCOS and follow-up.

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Conflict of interest

The authors have no conflicts of interest.

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