Research Article

Apelin-36 in polycystic ovary syndrome

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Abstract
Objective: To evaluate serum concentrations of apelin-36 in relation to the hormonal and metabolic profiles in patients with and without polycystic ovary syndrome (PCOS). **Study design**: A total of 50 women with PCOS and 30 healthy women forming the control group were eligible for the study. Each group was subdivided according to BMI into 2 subgroups (1^{st} with BMI < 25 and the 2^{nd} BMI ≥ 25). Lipid profile, fasting insulin level, Homeostasis Model Assessment of insulin resistance (HOMA-IR), FSH, LH, Prolactin, fT_4, TSH, free testosterone (fT) and serum apelin-36 (AP-36) levels were done to all subjects. **Results**: women with PCOS exhibited higher serum concentrations of apelin-36 than controls (38.7±13.59 nmol/l versus 31.43±6.97 nmol/l, P= 0.002). Apelin levels significantly correlated positively with body mass index (BMI) in patients with PCOS. There were no significant difference between apelin levels when normal weight (BMI<25) women with PCOS were compared to BMI-matched controls (P=0.341), but there were significant increased AP-36 levels when obese women with PCOS compared to BMI matched controls. Moreover obese PCOS exhibit increased AP-36 levels than non-obese PCOS (44.25±15.61nmol/l versus 32.17±6.43nmol/l, P= 0.001). **Conclusion:** The data suggest that serum AP-36 level is positively correlated with BMI in patients with PCOS. AP-36 is not directly implicated in the pathogenesis of PCOS, but it might be involved as an adipokine that is affected by BMI.
Keywords: Polycystic ovary syndrome (PCOS), Apelin-36 (AP-36), insulin resistance (IR), Body mass index (BMI).

Introduction
Polycystic ovary syndrome (PCOS) is a functional disorder of unclear etiology and as such is a diagnosis of exclusion with other androgen excess and ovulatory disorders of clearly defined etiologies excluded. Androgen excess disorders to be excluded are 21-hydroxylase deficient non classic adrenal hyperplasia, adrenal or ovarian androgen-secreting tumors, disorders of generalized adrenocortical dysfunction (e.g., Cushing's disease) and use or abuse of androgenic or anabolic drugs[1].

Obesity may impact the risk of PCOS via insulin resistance (IR) and compensatory hyperinsulinemia (HI), which augments ovarian/adrenal androgen production and suppresses sex hormone binding globulin (SHBG), thereby increasing androgen bioavailability. Altered LH secretion plays an important role in the pathophysiology of PCOS and although obesity is generally associated with relative reductions of LH, higher LH appears to be the best predictor of increased free testosterone (fT) among peripubertal girls with obesity. Other potential mechanisms of obesity-associated hyperandrogenemia include enhanced androgen production in an expanded fat mass and potential effect of abnormal adipokine/cytokine levels[2].

Insulin resistance is considered the main pathogenic factor in the background of increased metabolic disturbances in women with PCOS menstrual irregularity and other metabolic manifestations seen in this disease but it is not a diagnostic criterion for PCOS[3].

Adipose tissue acts as an enormous endocrine organ secreting a variety of

signaling molecules that regulate feeding behavior, energy spending, metabolism, reproduction and endocrine and immune function[4]. Adipocyte hypertrophy in PCOS may be a consequence of variations in storage and/or adipocyte lipolytic capacity. Thus, obesity in women with PCOS is mainly characterized by an increase in fat cell size (hypertrophic obesity) rather than an increase in fat cell number (hyperplastic obesity)[5].

Apelin (AP) is an endogenous cytokine synthesized and secreted by adipocytes[6]. AP seems to be a key regulator in glucose and lipid metabolism and may be associated with IR and PCOS is known to be associated with increased IR[7].

The aim of this work is to study the role of apelin-36 in the pathogenesis of PCOS and their correlation with other hormonal and metabolic parameters associated.

Subjects and Methods

This study was carried out in El-Minia University Hospital, from the period of March to September 2015. Fifty patients with PCOS (group I) (their ages ranging from 19 to 34 years, subdivided according to BMI into Subgroup **Ia**: BMI <25 kg/m^2 (n=23) and Subgroup **Ib**: BMI ≥25 kg/m^2 (n=27) and thirty apparently healthy women (group II) (their ages ranged from 19 to 32 years subdivided according to BMI into Subgroup **IIa**: BMI <25 kg/m^2 (n=12) and Subgroup **IIb**: BMI ≥25 kg/m^2 (n=18) were recruited for this study after their approval. The **PCOS** women were diagnosed according to the 2003 Rotterdam criteria[8] with at least 2 of the following features: oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasound. Exclusion criteria including: Non-classic congenital adrenal hyperplasia due to21-α hydroxylase deficiency, hyperprolactinemia, 1ry hypothyroidism, acromegaly, premature ovarian failure, virilizing ovarian neoplasia, drug, metabolic, hepatic, or cardiovascular related condition or other concurrent medical illness (e.g diabetes mellitus, renal insufficiency, or malabsorption disorders),women who are intending to start a diet or a specific program of physical activity. Body Mass Index (BMI) was calculated using the following formula: weight in kilograms /height in meters squared (kg/m^2).

Laboratory methods

In the early follicular phase (day 3–5 of the menstrual cycle); 5mls of venous blood were withdrawn from each subject by sterile venipuncture into plain tube and left to be clotted then centrifuged at 3000 rpm for 15 minutes, the expressed serum was used for the assay of blood glucose and lipogram using fully automated chemistry analyzer (Konelab 20i, Thermo Electron Incorporation, Finland), hormonal profile was done using miniVIDAS analyzer (BIOMERIEUX FRANCE 5.P.A), the remaining serum was kept frozen at -20 °C for determination of insulin by ELISA (IMMUNOSPECT CORPORATION, Canoga Park, CA), apelin by ELISA (GENASIA BIOTECH CO., LTD, Shanghai China).

Statistical analysis

Data entry and analysis were done using software SPSS version 19. Graphics were done using Excel. Quantitative data were presented by mean and standard deviation, while qualitative data were presented by frequency distribution. Student t test and Chi-square test was used to compare qualitative variables between groups. The probability of less than 0.05 was used as a cut off point for all significant tests.

Results

All obtained results of different groups were summarized in tables 1-2 and figures (1).

Table (1): Demographic, metabolic and hormonal characteristics of women with PCOS and controls regarding BMI

variables	PCOS			Control			I-II	Ia-Ib	Ia-IIa	Ib-IIb
	I	Ia	Ib	II	IIa	IIb				
Age (years)	24.6±3.73	23.43±3.15	25.16±4.62	25.8±4.24	25.16±4.62	26.2±4.0	0.20	**0.041***	0.199	0.50
BMI (kg/m^2)	27.24±4.91	22.66±1.73	23.58±1.13	27.3±3.51	23.58±1.13	29.77±1.98	0.958	**<0.001***	0.109	0.088
FBG (mg/dl)	90.62±12.51	87.65±13.42	89.41±16.16	91.6±12.79	89.41±16.16	93.05±10.21	0.738	0.123	0.733	0.978
FI (µIU/ml)	13.47±10.66	8.51±5.98	6.02±2.51	6.73±3.26	6.02±2.51	7.21±3.66	**0.043***	**0.002***	0.986	**0.005***
HOMA-IR	3.55±2.46	2.79±2.24	1.31±0.54	1.52±0.77	1.31±0.54	1.66±0.88	**0.016***	**0.028***	0.498	**0.006***
TG (mg/dl)	117.18±50.69	114.61±43.24	119.37±46.43	100.13±40.26	78.58±35.12	114. 5±36.44	0.101	0.744	**0.030***	0.747
TC (mg/dl)	174.02±40.43	164.47±40.75	182.14±39.06	159±31.24	151 ± 32.17	164.33±30.32	0.067	0.195	0.265	0.119
HDL (mg/dl)	39.78±4.42	39.21 ± 4.23	40.25 ± 4.6	41.23±4.76	42.08 ± 4.88	40.66 ± 4.74	0.171	0.412	0.080	0.775
LDL (mg/dl)	110.8±38.11	102.33±37.51	118.01±37.81	98.34±30.29	93.2±31.75	101.76±29.7	0.111	0.21	0.42	0.14
FSH(µIU/ml)	5.21±2.27	4.54±1.67	5.78 ± 2.58	7.05±0.92	6.96 ± 0.93	7.11±0.94	**<0.001***	0.054	**<0.001***	**0.043***
LH(µIU/ml)	6.87±3.27	6.18±2.96	7.46±3.46	5.1±0.96	5.19±1.05	5.04±0.93	**0.001***	0.172	0.159	**0.002***
LH/FSH	1.57±1.09	1.51±0.74	1.63±1.33	0.71±0.06	0.74±0.06	0.71±0.06	**<0.001***	0.697	**0.001***	**<0.001***
fT (pg/ml)	5.06±3.2	4.54±2.87	5.5±3.44	1.92±0.91	1.73±0.94	2.05±1.04	**<0.001***	0.392	**0.006***	**0.002***
PRL. (ng/ml)	14.81±6.81	14.9±6.15	14.74±7.44	14.69±6.88	14.86±7.64	14.58±6.55	0.941	0.934	0.989	0.942
fT4 (pmol/l)	13.75±2.6	13.66±2.8	13.82±2.38	14.4±3.4	14.28±4.73	14.56±2.31	0.34	0.828	0.636	0.313
TSH(µIU/ml)	1.91±1.28	1.89±1.47	1.92±1.12	1.8±0.7	2.0±0.51	1.66±0.73	0.611	0.477	0.122	0.618
AP-36 (nmol/l)	38.7±13.59	32.17±6.43	44.25±15.61	31.43±6.97	30.08±5.26	32.33±7.93	**0.002***	**0.001***	0.341	**0.002***

BMI, body mass index; FBG, fasting blood glucose; FI, fasting insulin; HOMA-IR, Homeostasis Model Assessment-Insulin resistance; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; fT, free testosterone; fT4, free thyroxine levels; TSH, thyroid stimulating hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; Bold characters define statistically significant results.

A total of 50 women with PCOS and 30 controls participated in this study. Demographic, biochemical and hormonal characteristics of women with PCOS and controls regarding BMI are summarized in (Table-1). Fasting insulin, HOMA-IR, LH/FSH ratio, LH levels and fT were significantly higher, whereas FSH level was significantly lower in the PCOS group compared to controls, as expected ($p < 0.05$). Apelin levels were also found to be higher in women with PCOS than controls (38.7±13.59 nmol/ml versus 31.43±6.97 nmol/ml, $p = 0.002$).

Table (2): correlation between apelin-36 and some variables

variables		I	Ia	Ib
BMI (kg/m²)	r	**0.529**	**0.536**	0.276
	P	**<0.001***	**0.008***	0.164
FBG (mg/dl)	r	-0.035	0.021	-0.251
	p	0.811	0.923	0.207
FI (µIU/ml)	r	-0.094	-0.039	-0.364
	p	0.514	0.860	0.062
HOMA-IR	r	-0.084	0.334	-0.384
	p	0.563	0.120	0.068
TG (mg/dl)	r	0.081	0.039	0.090
	P	0.578	0.861	0.655
TC (mg/dl)	r	0.175	0.143	0.078
	P	0.224	0.515	0.700
HDL (mg/dl)	r	0.095	0.019	0.059
	P	0.514	0.933	0.768
LDL (mg/dl)	r	0.153	0.142	0.051
	P	0.288	0.518	0.800
FSH(µIU/ml)	r	0.088	-0.002	-0.050
	P	0.544	0.994	0.806
LH(µIU/ml)	r	0.159	0.111	0.078
	P	0.270	0.615	0.700
LH/FSH	r	0.253	0.099	0.286
	P	0.076	0.654	0.147
fT (pg/ml)	r	0.062	0.094	-0.035
	P	0.670	0.671	0.862

Apelin-36 was positively correlated with BMI in group I and subgroup Ia (Table-2).

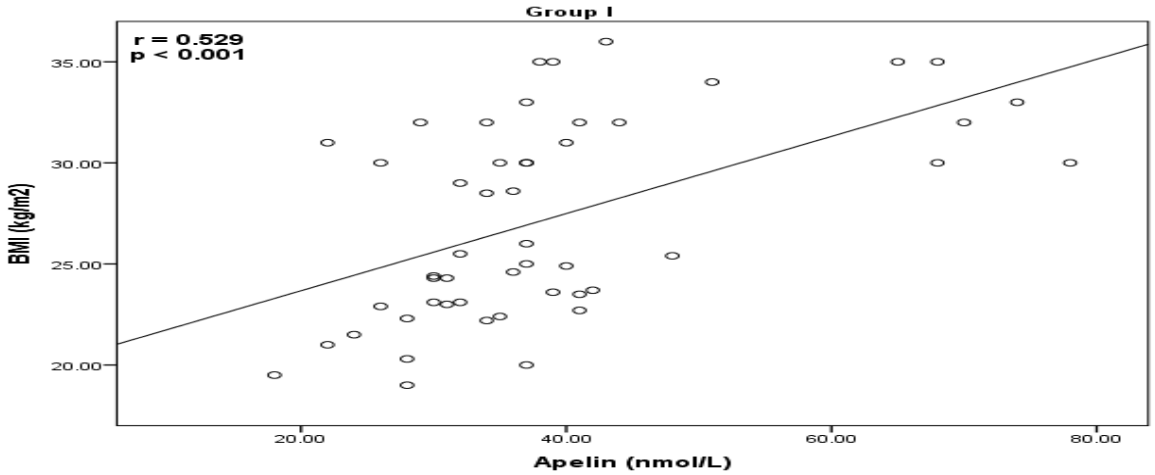

Figure (1): Significant positive correlation between AP-36 and BMI in group I

Discussion

Polycystic ovary syndrome (PCOS) is a well-recognized endocrine and metabolic disturbance affecting 6% to 20% of women in fertile age [9].

Apelin is one of the new described adipokines secreted by mature adipocytes in human[10] which plays a key role in the regulation of normal glucose and lipid metabolism and associated with IR[11].

In the present study, apelin-36 levels were increased in patients with PCOS when compared to control group. There were increased AP-36 levels in obese PCOS patients when compared with obese control while when comparing non-obese PCOS

and non-obese control there was non-significant difference.

These results were in agreement with Sun et al.,[12] who reported higher AP-36 level in obese PCOS when compared to non-obese PCOS and higher AP-36 level in obese PCOS when compared to obese control.

In the current study, we observed that AP-36 levels were positively correlated with BMI in PCOS group and in non-obese PCOS. There were non-significant correlation between AP-36 and FBG, fasting insulin, HOMA-IR, TG, TC, HDL, LDL, FSH, LH, LH/FSH ratio and fT in PCOS group, obese PCOS and non obese PCOS.

Our results were in agreement with Chang et al.,[11] and Choi et al.,[13] 12 who declared no association between AP-36 level and HOMA-IR. This may be due to HOMA-IR based on fasting glucose and insulin levels primarily and AP improves in vivo glucose metabolism by increasing glucose utilization in insulin sensitive tissues, most likely in an insulin-independent manner rather than through inhibition of hepatic glucose output[13]. These facts might be behind the lack of correlation between AP levels and HOMA-IR.

Discrepant findings among the published studies may be attributed to the differences in ethnicity, age, study design, genetic characteristics of populations and assessment methodology. Therefore further studies are required in larger cohorts with different genetic backgrounds.

Conclusion

The data of the present study showed Increased AP-36, fasting insulin level and HOMA-IR in obese PCOS patients than non-obese PCOS. According to our data, AP-36 is not directly implicated in the pathogenesis of PCOS, but they may be involved as an adipokines affected by BMI.

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