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Research Article

Levels of Apelin, Endoglin, and Transforming Growth Factor Beta 1 in Iraqi Women with Polycystic Ovary Syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common causes of infertility in women of reproductive age. The aim of the study was to determine the level of apelin, insulin resistance (IR), transforming growth factor beta 1 (TGF-β1), and endoglin in women with polycystic ovary syndrome. Fifty PCOS patients and 40 non-PCOS infertile patients were recruited. The fasting serum levels of folliclestimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), prolactin, fasting blood glucose, insulin, and apelin at the early follicular phase were measured. Levels of apelin, LH, LH/FSH, T, and fasting insulin, as well as homeostatic model assessment of IR (HOMA-IR) in PCOS patients, were significantly higher than in the control group. Correlation analysis showed that apelin level was positively correlated with body mass index and HOMA-IR. Apelin levels and TGF-β1 were significantly increased in PCOS patients while show decrease levels of endoglin.

Keywords: Apelin, endoglin, transforming growth factor beta

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disease affecting women of reproductive age. The prevalence of PCOS varies according to the diagnostic criteria used, with estimates ranging from 9% in women of reproductive age, according to NIH criteria, up to 18%, with the Rotterdam criteria.[1]

It is primarily characterized by ovulatory dysfunction and hyperandrogenism,[2] but the clinical presentation is heterogeneous and patients may present some of various signs and symptoms. This heterogeneity seems to be modulated by multiple factors such as prenatal androgen exposure, nutritional status in the uterus, genetic factors, and ethnicity, insulin resistance (IR) of puberty and/or exaggerated adrenarche and changes in body weight.[3] Environmental factors, such as obesity, appear to exacerbate the underlying genetic predisposition. Concerning ethnicity, the presence of hirsutism is less frequent in Asian patients (around 10%), compared to Caucasian ones (70%).[4]

Obesity is a prevalent characteristic of PCOS, ranging from 12.5% to 100%, with a pooled estimated prevalence of 49%, as shown by a recent meta-analysis.[5] The presence of obesity may exacerbate the metabolic and reproductive disorders associated with the syndrome, including IR, dyslipidemia, and metabolic syndrome.[6] A meta-analysis[7] has shown that women with PCOS have higher levels of triglycerides (TG), low-density lipoprotein (LDL)-cholesterol and total cholesterol (TC), and lower high-density lipoprotein (HDL)-cholesterol levels compared with control women, regardless of body mass index (BMI). In addition, PCOS women present higher risk for type 2 diabetes. PCOS is also associated with a clustering of cardiovascular risk factors.[8] However, there is no definitive evidence for increased cardiovascular events, nor data showing that PCOS alone leads to increased cardiovascular risk independent of associated risk factors. In fact, more rigorous cohort studies of long-term cardiovascular outcomes and clinical trials of risk factor modification are required for women with PCOS.

In addition, evidence suggests clinical phenotypes are related with different metabolic risks. In this sense, IR seems to be a specific feature of the classic phenotype and, to a lesser extent, of the ovulatory phenotype. Non-hyperandrogenic phenotype behaves as a separate group that is metabolically similar to non-PCOS women.[7]

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Adipose tissue is considered not only a storage tissue but also aproper endocrine organ, metabolically active.[6] IR is considered the main pathogenic factor in the background of increased metabolic disturbances in women with PCOS which can explain hyperandrogenism, menstrual irregularity, and other metabolic manifestations seen in this disease.[7] However, it is not a diagnostic criterion for PCOS.[8]

Obesity alters the expression, circulating levels, and signaling mechanisms of adipose-secreted factors and these changes have been linked to the development of IR, type 2 diabetes, dyslipidemia, atherosclerosis, cancer, and other diseases.[9]

Apelin is an endogenous cytokine,[10] an adipokine,[11] a regulatory peptide,[12] and a neuropeptide[13] that has been linked to obesity and IR.

Apelin is a 36-amino acid peptide (AP-36) generated from a larger precursor the 77-amino acid proapelin. The human preproapelin gene is located on chromosome X at locus Xq25-q26.1.[10]

Collected data from both the clinical and basic settings show that AP (1) correlates with states of IR and obesity, (2) stimulates glucose utilization, (3) decreases insulin secretion, and (4) negatively regulates catecholamine-mediated lipolysis. AP seems to be a key regulator in glucose and lipid metabolism and may be associated with IR and possibly implicated in PCOS which is known to be associated with increased IR[14] and from hence the possible link to apelin levels.

Endoglin (sEng)/CD105 is a transmembrane auxiliary receptor for transforming growth factor beta (TGF-b) it works as a non-signaling coreceptor of the TGF-b modulating its responses. There are a number of reports suggesting that soluble endoglin may be regarded as a biomarker of endothelial dysfunction, for instance, atherosclerosis, hypercholesterolemia,[15] Type 2 diabetes mellitus (T2DM), and hypertension.[16]

TGF-b1 is a multifunctional cytokine that is produced by a variety of cells and its level dysregulated in women with PCOS, which might play a role in the pathophysiology of this syndrome. Substantial evidences implicate that TGF-b1 activation is closely associated with endothelial dysfunction and hypertension.[17] There is scattered evidence for the association between TGF-b1, visceral adiposity, metabolic syndrome, nonalcoholic fatty liver disease, and T2DM.[15]

METHODS

This case–control study was conducted at the obstetrics and gynecology department in medical city in Baghdad. It was conducted on ninety women during the period from March 2018 to September 2018. These were divided into two groups: Group I: Fifty overweight and obese women with PCOS (PCOS group) and Group II: Forty overweight and obese women without PCOS (non-PCOS group). The recruited women were counseled and written informed consent was obtained from each woman before her participation in the study.

Diagnosis of PCOS was made according to the Rotterdam criteria[18] in the presence of at least two of the following: (a) Oligomenorrhea and/or anovulation, (b) biochemical and/or clinical hyperandrogenism, and (c) ultrasound appearance of polycystic ovaries (multiple cysts >12 in number of 2–9 mm size),[3] BMI P 25 kg/m^2.

Samples were obtained between days 2 and 5 of normal spontaneous or withdrawn menstrual cycle. Blood allowed to clot for 10–20 min at room temperature and centrifuged at (2000–3000 RPM) for approximately 20 min to separate the serum from the cells.

2 ml serum was stored at _20_c until the time of AP-36 and hormonal profile assay. Hormonal evaluation included a baseline plasma determination of luteinizing hormone (LH), follicle-stimulating hormone (FSH), dehydroepiandrosterone sulfate, TSH, E2, and free testosterone. All hormones were measured by radioimmunoassay methods using commercial kits (DSL Inc., Webster, Texas, USA). The fasting insulin was measured by radioimmunoassay methods using commercial kits (the intra and inter-assay coefficients of variation were 1.98% and 0.84%, respectively) (Immunospect Corporation, Owensmouth Ave, Canoga Park, CA). Fasting glucose was measured by the glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH, USA). TC, TG, and HDL were assayed using fully automated clinical chemistry autoanalyzer system Konelab 20i LDL was calculated according to Friedewald formula: LDL = TC−(TG/5+HDL).[19] IR was calculated by homeostatic model assessment (HOMA) using the following formula: HOMA-IR = ([FG in mg/dL_0.05551]_ FI in mU/ml)/22.5. A diagnosis of IR established for HOMA-IR values >2.71 nmol_mU/l[20] equal (FG in mg/dL_FI in mU/ml)/405. IR was diagnosed according to the FG to FI ratio. If a participant's FG/FI was 4.5 or less, she would be classified as being IR and if her FG/FI was >4.5, she would be classified as being non-IR.[21]

RESULTS

The results obtained are shown in Table 1 and Table 2.

Table 1: The mean and SD of biochemical and immune markers level in the patients group and healthy control group

The group	Mean±SD		*P*-value
	PCOS	Control	
Age (year)	28.28±5.11	33.27±6018	0.00033*
BMI (kg/m^2)	32.16±2.28	29.07±1.40	0.00001*
Hirsutism score	13.79±1.2	5.33±0.75	0.00001*
TGF-β1 (ng/ml)	19.34±2.73	12.29±1.19	0.00001*

(Contd...)

Table 1: ***(Continued)***

The group	Mean±SD		*P*-value
	PCOS	Control	
s-endoglin ng/ml	3.37±1.25	5.02±1.34	0.00001*
Apelin (pg/ml)	220.92±16.87	367.05±37.87	0.00001*
Fasting plasma glucose (mg/dl)	94.76±20.71	90.0±14.7	0.00001*
Insulin (IU/ml)	18.76±4.33	12.0±6.5	0.00001*
HOMA	2.44±1.16	1.72±1.01	0.001375*
Prolactin (pg/ml)	13.71±1.25	13.14±1.56	0.02867*
FSH (mIU/ml)	7.04±1.13	6.03±1.32	0.000099*
LH (mIU/ml)	12.13±1.77	5.88±1.2	0.00001*
SHBG (nmol/L)	30.25±2.51	54.12±4.3	0.00001*
DHEA-s (mg/ml)	2.21±0.59	0.95±0.3	0.00001*
Total testosterone (ng/ml)	0.91±0.41	0.59±0.22	0.000017*
Free testosterone (pg/ml)	2.65±0.66	1.064±0.26	0.00001*
h-CRP (μg/ml)	5.75±1.34	2.3±0.7	0.00001*
Cholesterol mg/dl	172.6±49.9	166.0±27.3	0.254423 NS
Triglyceride (mg/dl)	155.86±34.69	103.0±32.2	0.00001*
HDL (mg/dl)	39.14±10.09	39.92±7.15	0.347843 NS
LDL (mg/dl)	103.42±36.31	101.9±24.2	0.413274 NS

*($P<0.05$), **($P<0.01$), NS: Non-significant. PCOS: Polycystic ovary syndrome, BMI: Body mass index, TGF: Transforming growth factor, HOMA: Homeostatic model assessment, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, SHBG: Sex hormone-binding globulin, DHEA: Dehydroepiandrosterone, h-CRP: High sensitivity C-reactive protein, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Table 2: Correlation between apelin, transforming growth factor beta, and endoglin with other parameters in the PCOS disease patients

The group	Correlation coefficient (r)		
	TGF-β1 (ng/ml)	s-endoglin ng/ml	Apelin (pg/ml)
Age (year)	0.0917	−0.0948	−0.1572
BMI (kg/m^2)	0.3333	0.0379	0.0353
Hirsutism score	−0.2653	0.0955	−0.1134
TGF-β1 (ng/ml)	-	0.2007	0.1903
s-endoglin ng/ml	0.2007	-	−0.0502
Apelin (pg/ml)	0.1903	−0.0502	-
Fasting plasma glucose (mg/dl)	0.1447	−0.0654	−0.0531
Insulin (IU/ml)	−0.0978	−0.0906	0.0132
HOMA	0.1547	−0.1586	0.1233
Prolactin (pg/ml)	−0.1503	0.3305	−0.223
FSH (mIU/ml)	−0.0841	−0.0611	−0.1269
LH (mIU/ml)	−0.0413	0.0798	0.2071
SHBG (nmol/L)	0.2081	−0.189	0.198
DHEA-s (mg/ml)	−0.1289	−0.0002	−0.1991
Total testosterone (ng/ml)	0.0887	−0.1985	−0.0872
Free testosterone (pg/ml)	0.1543	−0.4447	0.0568
h-CRP (μg/ml)	0.0484	−0.1559	−0.0966
Cholesterol mg/dl	−0.1114	0.0285	0.323
Triglyceride (mg/dl)	0.354	0.1584	0.0839
HDL (mg/dl)	−0.1063	0.1887	−0.0182
LDL (mg/dl)	−0.1063	−0.1289	0.43

*($P<0.05$), **($P<0.01$), NS: Non-significant. PCOS: Polycystic ovary syndrome, BMI: Body mass index, TGF: Transforming growth factor, HOMA: Homeostatic model assessment, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, SHBG: Sex hormone-binding globulin, DHEA: Dehydroepiandrosterone, h-CRP: High sensitivity C-reactive protein, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

DISCUSSION

PCOS is one of the most common causes of infertility in women of reproductive age. IR is a main pathophysiologic feature in these patients.[2] Apelin (APLN) is a recently discovered adipokine involved in the regulation of various metabolic functions. Its receptor, APLNR, is expressed in reproductive tissues; however, its role in human ovarian cells is unknown. PCOS patients are more vulnerable to develop diabetes, cardiovascular diseases, and metabolic syndrome.[3] IR is prevalent in women with PCOS independently of obesity and is critically involved in reproductive and metabolic complications of the syndrome.[10]

Several tests have been developed to measure IR, some very reliable but complex like the hyperinsulinemic euglycemic glucose clamp and others less precise but easier and less invasive like HOMA-IR.[22] New markers are needed to reach a more reliable assessment of IR.

To date, several surrogate markers have been proposed in literature to facilitate and improve the determination of IR. Many new proteins are strongly involved with PCOS physiopathology and IR such as some adipocytokines (adiponectin, visfatin, vaspin, and apelin), copeptin, irisin, PAI-1, and zonulin. Many other proteins have been proposed as potential new markers of IR in PCOS such as resistin, leptin, retinol-binding protein 4 (RBP4), kisspeptin, and ghrelin, but their role is still controversial.[14]

Adipose tissue is implicated in the secretion of several hormones such as adiponectin, resistin, leptin, visfatin, apelin, and RBP4 called adipocytokines that are involved in energy homeostasis and metabolism.[11]

Apelin is a peptide isolated from bovine stomachs, but it is expressed in several other organs and also in visceral and subcutaneous tissues.[4] 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 and HOMA-IR. Apelin was found to be higher in PCOS patients by Gören *et al*.[23] but without a significant correlation with HOMA-IR. Olszanecka-Glinianowicz *et al*.[18] reported an inverse association between apelin and glucose, insulin, and HOMA-IR values, supporting the role of apelin in the regulation of insulin sensitivity. Apelin levels were higher in non-obese PCOS patients, suggesting a compensatory mechanism for metabolic consequences of IR. Lower serum concentrations of apelin were found in PCOS subjects by Altinkaya *et al*.[19] with positive correlation with BMI, insulin, HOMA-IR, TG, and free testosterone, speculating that apelin can be used as a marker for insulin sensitivity. Conversely, Sun *et al*.[20] observed significantly enhanced apelin concentration in PCOS patients with positive association with BMI and HOMA-IR; treatment with drospirenone-ethinylestradiol plus metformin improved IR and apelin levels. 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 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.[21] 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.

In line with our results, Tal *et al*. found increased TGF-b1 and decreased sEng in PCOS. These data suggest that increased TGF-b1 bioavailability may contribute to the pathogenesis of PCOS and its increased risk for ovarian hyperstimulation.[17] By contrast, the study conducted by Irani *et al*. found that sENG protein concentration was not different between PCOS and non-PCOS control group at baseline.[15] Women with PCOS have abnormal increase in TGF-b1 bioavailability (TGF-b1/sENG) due to an increase in serum TGF-b1 and decrease in sENG levels.[17]

CONCLUSION

Polycystic ovary syndrome (PCOS) is a condition that affects a woman's hormone levels.

Levels of TGF β1, LH, LH/FSH, T, and, as well as homeostatic model assessment of IR (HOMA-IR) in PCOS patients, were significantly higher than in the control group, while show decrease levels of Apelin and endoglin levels.

Therefore the correlation analysis showed that apelin level was negatively correlated with body mass index (BMI) and HOMA-IR.

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