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Study of Some Cytokines and Hormones in a Sample of Iraqi women with Polycystic ovarian syndrome and Their Relation to Obesity.

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Abstract:

The present study aimed to measure serum vaspin and apelin levels in women with PCOS to show their rule in the pathogenesis of PCOS. Ninety eight women with PCOS, 51 non-obese [body mass index (BMI) less than 25 kg/m2] and 47 obese (BMI >25 kg/m2) were enrolled in the study. Each group is compared to apparently healthy women as a control group matched for age and BMI. Clinical history, anthropometric measurements and biochemical and hormonal analysis were determined. The mean serum level of vaspin and apelin showed statistically significant difference between PCOS patients (non-obese and obese) compared to control women (non-obese and obese) respectively. Also, the levels of both parameters (vaspin, apelin)showed significant differences between PCOS obese patients and non-obese ones. It is concluded that serum vaspin and apelin level increased in PCOS women particularly the obese. These data suggest their involvement in the pathogenesis of PCOS

Key word: *Polycystic ovary syndrome, vaspin, apelin.*

الخلاصة:

تهدف الدراسة الحالية لقياس مستويات الفاسبين والابلين في مصل الدم لدى النساء المصابات بمتلازمة تكيس المبيض وعرض دورهم في التسبب بـمتلازمة تكيس المبيض. تضمنت هذه الدراسة(98) مريضة مصابات بمتلازمة تكيس المبيض, 51 مريضة محتوى الجسم من الكتلة لهم اقل من 25 كغم/م^2 و47 مريضة محتوى الجسم من الكتله لهم اعلى من 25 كغم/م^2. قورنت كل مجموعة سيطرة مطابقة لهذه المجاميع من حيث العمر وBMI. التاريخ ألسريري وقياسات الانثروبومترية وبعض الفحوصات الكيموحيوية و الهرمونية قيست. وفي كلا مجموعتي المريضات (البدينات و النحيفات) وجد ان هناك فرق معنويا ملحوظا في مستويات الفاسبين والابلين عند مقارنتهم بمجموعتي (البدينات والنحيفات) على التوالي من مجموعة السيطرة. كذلك كلا العاملين اظهرا اختلاف معنوي عند المقارنة بين مجموعة المريضات البدينات مع مجموعة المريضات النحيفات.نستنتج مما سبق ان الزيادة في مستويات الفاسبين والابلين في مريضات تكيس المبيض بالتحديد البدينات منهم وهذه النتائج تفترض مشاركتهم كمسبب للـPCOS.

Introduction:

Polycystic ovary syndrome (PCOS) is a common hormonal disorder among women of reproductive age with a prevalence of 6.6–6.8%[1]. They commonly display a clustering of metabolic abnormalities, including impaired glucose tolerance, insulin resistance, dyslipidemia, increased prevalence of obesity, low-grade chronic inflammation and increased oxidative stress[2]. Obesity is present in approximately 44% of women with PCOS and it is characterized by central distribution of fat [3].

Insulin resistance (IR) is the most important pathophysiological factor in PCOS [4]. It has been demonstrated in both obese and non-obese women with PCOS[5]. The cellular and molecular mechanisms of insulin resistance in PCOS have not yet been elucidated, but they are considered to be distinct from those of other diseases associated with insulin resistance. Therefore, they have an increased prevalence of hypertension, diabetes and cardiovascular disease[6].

Apelin is a novel bioactive peptide, secreted by adipose tissue. Its gene expression in adipocytokines is directly regulated by insulin, and through this pathway upregulated by obesity and hyperinsulinemia in both humans and mice

[7]. Furthermore, higher levels of Apelin have been found in patients suffering from type 2 diabetes mellitus (DM2). Apelin was found to be related to obesity and insulin resistance [8]. It has been shown that this adipokine had effects on water intake and hypothalamo-hypophyseal axis [9].
Apelin has also been found to affect cardiovascular system in terms of hypotension[10], positive inotropy and angiogenesis [11]. It has also been reported that apelin and apelinergic system were effective on mammalian ovarian development, follicular atresia and thecal tissue angiogenesis [12, 13].

Recently Hida et al., 2005 characterized vaspin as an interesting novel adipokine with insulin sensitizing effects. Vaspin (visceral adipose tissuederived serine protease inhibitor) belongs to the serine protease inhibitor (serpine) super family and is produced in the visceral adipose tissue depot of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of obesity with type 2 diabetes mellites (T2DM) [14,15]. It is demonstrated convincingly in the initial report that administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity[16]. Furthermore, dysregulated expression of insulin sensitivity-modulating genes in adipose tissue including adiponectin and leptin was reversed after vaspin treatment [17]. Moreover, vaspin production was down-regulated with worsening of T2DM in OLETF rats [18]. In addition, it has recently shown that induction of vaspin mRNA expression in human adipose tissue is regulated in a fat depot-specific manner and could be associated with parameters of obesity, insulin resistance, and glucose metabolism[19].

The aim of the present study was to measure serum vaspin and apelin levels in women with PCOS and asses possible correlations between each of them and clinical, biochemical and hormonal parameters of the syndrome as serum levels of vaspin and apelin may show possible involvement in the pathogenesis of PCOS.

Subjects and Methods:

Subjects:

Ninety one women with PCOS, 51 non-obese [body mass index (BMI) less than 25 kg/m^2] and 47 overweight-obese (BMI >25 kg/m^2) were enrolled in the study obtained from Kamal Al- samarae'e Hospital. The diagnosis of PCOS was made according to European society of human reproduction and embryology and American society for reproductive medicine criteria: PCOS is diagnosed if there are any two of the following:1. Presence of polycystic ovary on ultrasound examination.2. Clinical or biochemical hyperandrogenemia. 3. Menstrual dysfunction with an ovulation. And excluding any other endocrine disorder, patients with hormonal therapy or any medication known to interfere with follicular development or hormonal levels under the study for last 4 months of sample aspiration, diabetic patients and patients with oligomenorrhea, amenorrhea due to other than PCOS causes, Pregnant women and women with a menstrual cycle less than 26 days or more than 30 days. Fourty healthy women (employees from the staff of Kamal Al-samarae,e Hospital) matched for age, 20 non-obese (BMI <25 kg/m^2) and obese (BMI > 25 kg/m^2) participate in this study as controls.. All subjects were studied during the early follicular phase (second to fifth day) of the menstrual cycle. 10 ml of venous blood were withdrawn after an overnight fasting from all subjects and allowed to clot in a plain sterile tube and then centrifuged. The separated serum was stored into aliquots at -20C for biochemical and hormonal determinations.

Protocol:

Clinical and anthropo metrical variables, including clinical blood pressure, BMI, (calculated as kg/m2) and waist-to-hip ratio (WHR) were determined in all the subjects. Vaspin, Apelin (Ray biotech, USA), insulin (Demedetec Com-

pany, Germany) were determined by ELISA methods. Total testosterone (TT) were measured by an automated quantitative enzyme immunoassay on the VIDAS instrument, BioMerieux, France using the Enzyme Linked Fluorescent Assay (ELFA).Serum fasting plasma glucose level was measured by enzymatic method supplied by Bio Labo Diagnostics, France.

Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (uIU/ml) × glucose (mmol/l) / 22.5[20]. Measurement of glucose level by mg/dl was multiplied by 0.555 to get result by mmol/l to calculate HOMA-IR.

Statistical analysis:

Statistical analysis performed by using SPSS version 15.0 for Windows. The significant difference between mean values was estimated by the Student t-test. The point of statistical significance was noted when probability was $p<0.05$, and no statistical significance was noted when $p>0.05$. Correlation analysis was used to test the linear relationship between parameters.

Results:

Table-1 showed the clinical characteristics of PCOS groups and control groups, there is a significant ($P \leq 0.05$) increased in the duration of infertility in obese patients as compared to lean patients (39.57 vs. 30.63 month).

Table-1: The host information of all studied groups and healthy individuals (control).

Mean±SD (Range)	Obese patients	Obese control	Lean patients	Lean control	P value
BMI (Kg/m2)	30.44±3.11 (25.90-35.73)	30.03±3.02 (25.43-35.60)	23.16±1.63 (18.70-24.80)	23.18±1.13 (20.75-24.66)	0.0001*
WHR	0.89±0.05 (0.82-0.98)	0.86±0.04 (0.80-0.95)	0.81±0.04 (0.73-0.89)	0.78±0.02 (0.74-0.82)	0.0001*
Duration of infertility (month)	39.57±19.04 (12-120)	-- --	30.63±15.89 (12-60)	-- --	0.013*

In both PCOS non-obese and obese patients groups as compared to the non-obese and obese control groups, the mean serum level of vaspin showed a statistically significant increase ($P<0.05$) in both PCOS groups and the mean serum level of apelin also showed a statistically significant increase($P<0.05$) in the same PCOS groups, table -2.

Table-2: Serum vaspin and apelin levels in PCOS patients and controls according to BMI.

Mean±SD (Range)	Obese patients	Obese control	P value	Lean patients	Lean control	P value
Apelin	265.53±103.5 (104-808)	222.2±39.28 (184-312)	0.048*	247.48±64.25 (184-392)	204.25±48.71 (89-288)	0.001*
Vaspin	2.50±1.63 (0.15-7.89)	1.50±.75 (0.23-2.98)	0.012*	2.00±1.18 (0.14-5.89)	1.28±0.72 (0.20-3.31)	0.014*

The results showed that the mean serum levels of LH/FSH, testosterone, 17.OHP, insulin, cholesterol, TG, HDL-C, and HOMA-IR showed statistically significant increase between PCOS patients(non-obese and obese) when compared to control women (non-obese and obese) respectively, while FBS not significantly differed, table-3.

Table- 3: Clinical and biochemical features of PCOS patients and controls according to BMI.

Mean±SD (Rang)	Obese patients	Obese control	P value	Lean patients	Lean control	P value
LH/FSH	2.04±0.81 (0.58-4.50)	0.87±0.13 (0.64-1.10)	0.0001*	1.92±0.77 (0.49-4.51)	0.76±0.17 (0.50-1.10)	0.0001*
Testosteron (nmol/L)	1.81±1.07 (0.40-5.70)	0.71±0.08 (0.54-0.82)	0.0001*	1.70±1.43 (0.47-11.10)	0.67±.12 (0.50-0.90)	0.002*
17.OHP (ng/ml)	1.81±0.62 (0.80-2.90)	1.36±0.68 (0.40-2.84)	0.011*	1.74±0.75 (0.60-2.80)	1.13±0.49 (0.45-1.98)	0.001*
FPG(mmol/L)	4.79±0.82 (3.06-7.06)	4.76±0.37 (4.00-5.44)	NS	4.50±0.74 (3.11-6.50)	4.51±0.53 (3.28-5.33)	NS
Triglycerid (mg/dl)	144.45±20.07 (113-198)	118.70±17.41 (89-156)	0.0001*	126.65±22.51 (78-177)	109.55±19.42 (79-142)	0.004*
HDL (mg/dl)	58.15±10.91 (35-89)	58.35±13.24 (31-77)	NS	57.24±8.11 (32-78)	65.25±9.17 (52-83)	0.001*
Insulin (MU/ml)	26.12±13.43 (5.56-79.00)	14.55±3.36 (8.26-21.28)	0.0001*	24.96±10.29 (12.08-77.00)	12.60±4.83 (5.08-25.64)	0.0001*
HOMA	5.57±3.16 (1.26-19.51)	3.08±0.73 (1.65-4.41)	0.001*	4.90±1.76 (1.76-13.31)	2.53±1.05 (1.17-5.38)	0.0001*

Table-4 displayed significant positive correlation in lean patient between vaspin and both FBG and HOMA. In control groups, a significant correlation between vaspin and age, BMI, and highly significant correlation between vaspin and 17.OHP, HOMA in obese group while in lean vaspin significantly correlated with BMI and HOMA.

Table-4: Baseline Pearson correlations coefficients of vaspin levels with various metabolic and hormonal parameters in patients with PCOS.

		Vaspin (ng/ml)			
		Obese patients	Lean Patients	Obese Controls	Lean Controls
Age (years)	**r**	0.034	0.042	0.472*	0.338
	P	0.821	0.769	0.036	0.146
BMI (Kg/m2)	**r**	0.069	0.241	0.555*	0.452*
	P	0.647	0.088	0.011	0.045
LH/FSH	**r**	0.062	0.297*	0.002	0.229
	P	0.680	0.034	0.995	0.332
Testosteron (nmol/L)	**r**	0.182	0.283*	0.245	0.348
	P	0.220	0.044	0.299	0.132
17.OHP (ng/ml)	**r**	0.093	0.116	0.606**	0.045
	P	0.535	0.417	0.005	0.849
Fasting blood glucose (mmol/L)	**r**	0.024	0.310*	0.070	0.193
	P	0.872	0.027	0.768	0.415
Insulin (MU/ml)	**r**	0.821**	0.849**	0.575**	0.815**
	P	0.000	0.000	0.008	0.000
HOMA	**r**	0.776**	0.752**	0.562**	0.851**
	P	0.000	0.000	0.010	0.000

Serum apelin level was positivly correlated with Testosteron , 17.OHP , HDL and FAI as shown in table-5.

Table-5: Baseline Pearson correlations coefficients of apelin levels with various metabolic and hormonal parameters in patients with PCOS.

		Apelin (pg/ml)			
		Obese patients	**Lean Patients**	**Obese Control**	**Lean Controls**
Testosteron (nmol/L)	**r**	0.050	0.092	0.347	0.569**
	P	0.736	0.522	0.133	0.009
17.OHP (ng/ml)	**r**	0.143	0.155	0.281	0.476*
	P	0.337	0.277	0.230	0.034
HDL (mg/dl)	**r**	0.104	0.380**	0.199	0.210
	P	0.486	0.006	0.400	0.375

Discussion:

In the current study, the mean serum VA spin level showed a statistically signif icant increase (P<0.05) in PCOS obese women when compared to PCOS nonobese women. In addition, significantly higher mean serum vaspin levels were detected in both same previous groups [PCOS (obese & non-obese)] when compared to the control women (obese & non-obese) respectively, table-2, such finding is in agreement with Soha et al. (2011)[21] and Erman et al. (2011)[22]. Furthermore, a statistically signifi cant positive correlation was observed between serum vaspin level and insulin and insuin resestance parameters in all study groups and also a significant positive correlation was observed between serum vaspin level and HOMA-IR in obese patients groups. Vaspin also significantly correlated with both LH/FSH, testosterone, FBG and TNF-alpha.

In control groups, a significant correlation between vaspin and age, BMI, and highly significant correlation between vaspin and 17.OHP, HOMA obese group while in lean vaspin significantly correlated with BMI. Many patients with polycystic ovary syndrome (PCOS) have insulin resistance, obesity (mostly visceral), glucose intolerance and abnormallities in the secretion of steroid hormones from the ovaries and the adrenal gland, conditions associated with abnormalities in the production of vaspin. Accordingly, recent studies evaluated vaspin levels in women with PCOS. Serum vaspin levels were evaluated and vaspin gene (mRNA) expression was determined in both subcutaneous and omental adipose tissue in vitro. Additionally, the effects of glucose, insulin and steroid hormone administration on vaspin gene expression and on vaspin levels in the adipose tissue were examined [23].

In the present study, apelin level were found to be significantly higher in obese-overweight patients with PCOS compared to the obese controls, apelin level also significantly elevated in lean PCOS women when compared to control with same weight, this could be due to that apelin increases glucose uptake and Akt phosphorylation in differen tiated tissue also apelin affects insulin sensi tivity by secondarily influencing the syste mic environment of insulin resistance (e.g., altering hormone secretion, lipolysis, infla mmation, etc.)[24].

In PCOS , higher levels of plasma apelin might be related to insulin resistance and androgenic obesity, increased waist to hip ratio, increased adiposity, impairment in LH/FSH interaction, hypo thalamohypophyseal axis effects and local paracrine and endocrine-logical attitudes deriving from the nature of the polycystic ovaries and also the compensatory mech anisms due to the metabolic changes in PCOS[25]. It is difficult to evaluate, apelin levels in patients with PCOS due to lack of sufficient studies, and other study showed that TNF-α increased apelin levels in human adipose tissue [26].

There are also studies emphasizing visfatin, which is an adipokine that has similar properties like apelin, increased in patients with PCOS[27]. Other study showed that apelin and other adipokines (visfatin and adiponectin) can be used as specific markers for insulin sensitivity, and these adipocytokines might play a part in the pathogenesis of PCOS[28].

In conclusion, serum vaspin and apelin level increased in PCOS women in the same manner particularly the obese. These data suggest their involvement in the pathogenesis of PCOS. Further studies are needed to explain the pathophysiological roles of the increased serum vaspin and apelin observed in PCOS.

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