Serum resistin levels, and other hormonal and biochemical parameters in patients with polycystic ovary syndrome (PCOS)

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Summary:

Background: Polycystic ovarian syndrome (PCOS) is one of the most common cause of anovulation during reproductive life.Resistin can increase ovarian androgen production by directly stimulating ovarian theca cell or indirectly by augmenting pancreatic – B cell production of insulin.

Patients and Methods: Sixty patients with PCOS who were non diabetic and not taking any medicine for the last three months were involved in the study .Thirty normal fertile female serves as control group. Fasting blood samples were aspirated from all individuals from 3rd - 6th day of the menstrual cycle to measure resistin, insulin, glucose, LH, FSH, TT3, TT4, Prolactin , Total Testosterone and lipid profile, by ELISA and routine methods.

Results: mean serum resistin concentration was increased in women with PCOS compared with the control group (Mean ±SD) (19.83 ± 6.101 vs 9.36 ± 2.17) ng/ml. Serum resistin concentration correlated positively with BMI, which is divided into two subgroups. The first with BMI < 25 kg/m^2 and the second with BMI ≥25kg/m^2 in both control and patient groups. In BMI < 25kg/m^2 serum resistin concentration for the control group was (8.90 ± 1.76) and (14.66 ± 2.09) for patients group ,while BMI ≥ 25 kg/m^2 serum resistin concentration for the control group was (10.62 ± 1.76) and (21.55 ± 5.40) ng/ml for patients group. Resistin also correlated positively with Insulin, LH, LH/FSH ratio and total Testosteron in women with PCOS but not in control. Fasting insulin level was higher in PCOS group compared with the control group (Mean ±SD) (27.45 ± 4.47 vs 13.27 ± 3.80) mIU/ml.The Fasting serum glucose was also higher in PCOS group compared with the control group (Mean ±SD) (125.27 ± 28.63 vs 92.63 ± 13.99) mg/dl. Total Testosterone level was elevated in the PCOS group compared with the control group (1.04 ± 0.37 vs 0.52 ± 0.25) ng/ml.Total Testosterone correlated positively with BMI, Resistin, Insulin, LH, and LH/FSH ratio.

Conclusion: PCOS women with BMI >25 kg/m² were found to have a marked increase level of Resistin ,Insulin , Glucose ,LH ,and Total Testosterone .and a decrease level in their insulin sensitivity i.e increased insulin resistance.These data indicate that abnormal resistin secretion in obese PCOS women may play a role in causing ovarian hyperandrogenism and hyperinsulinemia. Therefore fasting serum resistin level could be helpful in diagnosing PCOS patient.

Keywords: resistin , polycystic ovary syndrome

Introduction:

Polycystic ovarian syndrome:-PCOS) is a heterogeneous syndrome characterized by oligomenorrhea or amenorrhea, hyperandrogenism, dyslipidemia and multiple small subcapsular cystic follicles in the ovary on ultrasonography (1).It affects nearly 5-10% of women of reproductive age (2) and nearly 16-80% of the affected women are obese(3) .The syndrome is frequently associated with insulin resistance accompanied by a compensatory hyperinsulinemia and obesity(4), which have been thought to play an important role in the etiology of PCOS(5).The studies had shown that, in women with PCOS, the sensitivity of insulin to glucose metabolism is subnormal and that modest hyperinsulinemia prevails (6). The administration of insulin sensitizing agent as metformin, may increase insulin sensitivity and thus induce ovulation (7) . Several studies have investigated the possible cellular mechanism underlying insulin resistance in PCOS employing the major insulin target, the adipocyte. Decreased numbers of insulin receptors and severe impairment of insulin action in adipocytes from amenorrheic women with PCOS had been reported (8) Insulin resistance is known to be developed by the important risk factor obesity. Adipose tissue is an endocrine organ that secretes a variety of hormones among them is resistin (9) . It is a cytokine that can antagonize insulin action induced during adipocyte differentiation and down regulated by insulin sensitizing agents thiazolidinedione (TZD) compounds, which are a new class of antidiabetic drugs with an insulin-sensitizing action(10).Resistin hormone is a novel 12500 cystine-rich protein, secreted by adipocytes. It is a signaling molecule isolated in mice and has been suggested to be the putative hormone thought to link obesity with type 2 diabetes (11), which can impair

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both glucose tolerance and insulin action. this syndrome.

Subjects and methods:

Sixty patients with confirmed PCOS were involved in this study. Age range (13-45) with a mean (29.3 ± 8.2 years) and a mean BMI (27.3±2.81 kg/m^2). They were subdivided according to their BMI into two subgroups: BMI< 25 kg/m^2 (15 patients) with a mean BMI of (24.60 ± 0.5) , and BMI ≥ 25 kg/m^2 (45 patients) with a mean BMI of (28.20 ± 2.68). All were recruited from the Infertility Clinic of Baghdad Teaching hospital and were in a good health and had not taken any drug of oral contraceptives for the last three months.Thirty healthy fertile women with an age range (14 – 45) and with a Mean ± SD (29.2 ± 1.07) years and a mean BMI (23.73 ± 2.37) kg/m^2 . Also they were subdivided according to their BMI into two subgroups: BMI< 25 kg/m^2 (10 patients) with a mean BMI of (22.68 ± 1.71) and BMI ≥ 25 kg/m^2 (20 patients) with a mean BMI of (26.62 ± 1.06). None of them were hirsute and all had a normal regular menstrual period (every 27-35 days) with a normal level of serum androgen concentration.After an overnight fasting, venous blood samples (10ml) were aspirated at 09.00 – 10.00 am during the 3rd-6th day of the menstrual cycle (early follicular phase) for those with normal cycle. For patients with an ovulation or oligomenorrha blood samples were collected regardless of the duration of the cycle. The blood samples were collected into plain tubes and the blood was centrifuged within 30 minutes of collection at 300 – 400rpm, for 15 min. The serum then removed immediately and glucose measured using the enzyme colorimetric method. The rest of the serum then stored at (- 20 C°) until needed for the assay of resistin insulin LH , FSH , PRL , T3 , T4, total testosterone and lipid profile.Enzyme linked immunosorbent assay was used to measure resistin, insulin and total testosterone**.** The kits for quantitative determination in human serum were supplied by the Chemicon International Inc. and DRG international – Inc USA respectively.Other parameters included in this study (cholesterol, HDL-C, triglycerides) were also measured colorimetricaly.Analysis of insulin resistance was estimated using quantitative insulin sensitivity check index (QUICKI). Using the QUICKI formula=

$$\frac{1}{\text{Log } (I_o) + \text{Log } (G_o)}$$

Where (I_O) is the fasting insulin level measured by mlU/ml and (G_o) is the fasting glucose level measured by mg/dl .To compare the significance of difference in mean values of any two groups, patient and controls, the SPSS (social process statistical system) and student's t – test were applied. ($P < 0.05$) was considered statistically significant, and the correlation coefficient [r] test is used to describe the association between the different parameters studied and ($P< 0.05$) was considered statistically significant.

Results and Discussion:

Polycystic ovary syndrome is a clinical condition that has brought the attention of many specialties; gynecologists, endocrinologists, cardiologists, pediatricians, and dermatologists. Insulin resistance, is a common feature of PCOS and is more marked in obese women, suggesting that PCOS and obesity have a synergistic effect on the magnitude of the insulin disorders (12).Hyperinsulinemia associated with insulin resistance has been causally linked to all features of the syndrome, such as hyperandergenuism, reproductive disorders, acne, hersuitism, and metabolic disturbances. Several insulin lowering agents have been tested in the management of PCOS in particular metformin (glucofage) which is the only drug currently in wide spread clinical use for the treatment of PCOS. In addition modification in diet and life style had been suggested to obese subjects. The newly discovered hormone resistin has shown to be increased in women with PCOS (13). It is adepokine belonging to a recently described family of small cystien rich secreted proteins that can be induced during adipocyte differentiation, and down-regulated by insulin sensitizing agent. It has a putative prodiabetogenic properties. Like other hormones secreted by adipose tissues, resistin is being investigated as a possible link between excessive adiposity and insulin resistance, and there is growing evidence that circulating levels of this adipokine are proportional to the degree of adiposity.

The clinical characteristics for all subjects involved in the study are shown in table1.

Table (1): Age, menstrual regularity, BMI, hirsutism and duration of infertility in PCOS and control groups.

Characteristic	PCOS(Mean±SD)	Control (Mean±SD)
Number	60	30
Age(years)	29.70 ± 8.89	29.23 ± 8.43
Menstrual cycle(days)	Irregular	Regular 27 ± 6(days)
BMI <25 kg/m^2	24.60 ± 0.50	22.68 ± 1.71
BMI ≥25 kg/m^2	28.2 ± 2.68	26.62 ± 1.06
Hirsutism	All of them	None
Duration of infertility (years)	3.2 ± 1.5	None

Table 2 shows the mean values of all hormonal and biochemical parameters measured for patients and control groups.

Table(2) (Mean ± SD) values of all hormonal and biochemical parameters measured for patients and control groups

Characteristic	Normal Values	PCOS group	Control group	P value
Age (year)	None	29.70 ± 8.892	29.23 ± 8.431	N.S
BMI (kg/m^2)	None	27.30 ± 2.812	23.73 ± 2.377	< 0.001
Resistine (ng/dl)	3-12 ng/ml	19.83 ± 6.101	9.36 ± 2.17	< 0.001
Insulin (mIU/ml)	2-25 mIU/ml	27.45 ± 4.470	13.27 ± 3.805	< 0.001
FSG (mg/dl)	80-120 mg/dl	125.27 ± 28.63	92.63 ± 13.991	< 0.001
Quicki	0.3	0.28 ± 0.008	0.32 ± 0.018	< 0.001
LH/FSH Ratio	1/1	2.24 ± 0.832	0.77 ± 0.188	< 0.001
LH (mIU/ml)	0.7-9.0mIU/ml	8.32 ± 3.152	2.97 ± 0.682	< 0.001
FSH (mIU/ml)	0.6-9.5mIU/ml	3.87 ± 1.2	3.93 ± 0.96	N.S
TT3 (mIU/ml)	1-3.3 nmol/L	3.03 ± 0.716	2.68 ± 0.704	N.S
TT4 (mIU/ml)	55-170 nmol/L	134.35 ± 27.383	135.13 ± 26.21	N.S
Prolactine (mIU/ml)	80-500mIU/ml	193.72 ± 60.165	136.70 ± 44.399	< 0.001
Total Testosteronee (ng/ml)	0.1-0.9 ng/ml	1.04 ± 0.379	0.52 ± 0.251	< 0.001
Cholesterol (mg/dl)	150-250 mg/dl	210.2 ± 34.312	188.87 ± 30.842	< 0.005
Triglycerides (mg/dl)	65-180 mg/dl	140.4 ± 16.18	137 ± 17.3	N.S
VLDL (mg/dl)	12-36 mg/dl	28.08 ± 3.243	27.4 ± 3.46	N.S
HDL (mg/dl)	35-65 mg/dl	40.1 ± 5.76	45.87 ± 5.45	< 0.001
LDL (mg/dl)	90-190 mg/dl	137.22 ± 32.59	121.17 ± 26.289	< 0.05

It clearly shows that serum resistin level is higher in patients than in control.

Serum resistin level is positively correlated with BMI (r=0.589, P<0.001), This agrees with the fact that adipose tissues usually function as highly specialized endocrine and paracrine organs producing an array of adpokines that include leptin , TNF- α, and adiponektine (9). This is in conflict with what have been mentioned by Seow et al who found a poor correlation between serum resistin levels and BMI (13). This is because the number of PCOS patients were very small and statistically feasible. Table 2 in the current study shows that PCOS group has elevated serum insulin level indicating that most of the PCOS women (60-70%) were insulin resistance and (30-40%) of the women with PCOS have impaired glucose tolerance or diabetes.Serum resistin levels show a positive correlation with serum Insulin (r=0.271, P=0.036) but negatively correlated with QUICKI (r= -0.328, P=0.011). These results go with the one of the major concerns in obesity that can be manifested peripherally in muscles and adipose tissues or in the liver. The metabolic consequences evident with the development of insulin resistance include increased circulating non-esterified fatty acid via elevated lipolysis of triglycerides in adipose tissues and lipoproteins (rich in triglycerides) and other tissues causing impaired glucose uptake in muscles and in adipose tissues, overproduction of glucose and overproduction of insulin by pancreatic β cells. This is why resistin is considered to be a potential link between obesity and insulin resistance in PCOS (14). In the mean time resistin is negatively correlated with QUICKI because resistin augments insulin production.The results also show a positive correlation between resistin and LH level (r=0.379, P=0.003), and with LH/FSH ratio (r= 3.6) respectively. This indicates that resistin can augment pancreatic β-cell production of insulin which induces pituitary LH pulse generation activity.On the other hand serum insulin level is positively correlated with BMI (r=0.411, P=0.01), this indicates that hyper insulineamia in PCOS women causes central obesity (intra-abdominal fat deposits) due to the termination of fat breakdown at high levels of insulin(15) . Fasting insulin level is also correlated with LH (r=0.673, P=0.001) due to increased pituitary LH pulse frequency and with total testosrone (r=0.32,) P=0.012) because hyperinsulinemia directly increases ovary and adrenal androgen production in PCOS women by inhancing cytochtome P450c 17α enzyme activity in both glands . Secretion of insulin may also stimulate adrenal androgen secretion by disregulation of 17α hydroxylase and 17, 20 hydroxylase activity (16). In this study, serum insulin level is higher in PCOS patients than in controls the difference is statistically significant.(27.45 ± 4.470)(Mean ±SD)for patient group (PCOS) and (13.27 ± 3.805)(Mean ±SD)for control group (p<0.001) .As excepted PCOS

patients showed significantly higher serum LH than control, LH (8.32±3.152) mIU/ml(Mean ±SD) and for control (2.97±0.682)mIU/ml (Mean ±SD) (P<0.001).Table 3 shows the level of resistin in both patients and control group subdivided according to their body mass index (BMI <25 kg/m^2) and (BMI ≥25 kg/m^2)

Table 3: resistin level in both patients and control group subdivided according to their body mass index (BMI <25 kg/m^2) (BMI ≥25 kg/m^2)

	PCOS(Mean±SD) (n=60)		Control (Mean±SD) (n=30)	
	BMI <25 kg/m^2 N=15	BMI ≥25 kg/m^2 N=45	BMI <25 kg/m^2 N=10	BMI ≥25 kg/m^2 N=20
Resistin (ng/dl)	14.66±2.09	21.55±5.4	8.9±1.06	10.62±1.76

The table shows clearly that serum resistin level is significantly higher in obese subjects in both patients and control.To explain the relationship between PCOS , resistin and obesity as shown in table 3, PCOS women with BMI ≥25 kg/m^2 showed high serum resistin. PCOS women with BMI <25 kg/m^2 showed lower serum resistin levels, but slightly higher than he normal value.This is due to the fact that obesity has a high lipolitic activity releasing non-esterified fatty acid into blood circulation. This usually competes with glucose uptake in muscles and fat cells, resulting in increased fatty acid oxidation and impaired insulin mediated glucose utilization in skeletal muscle and acceleration of glycogenesis in the liver (17).

Conclusion:

PCOS women with BMI >25 kg/m² were found to have a marked increase level of Resistin ,Insulin , Glucose ,LH ,and Total Testosterone .and a decrease level in their insulin sensitivity i.e. increased insulin resistance.

These data indicate that abnormal resistin secretion in obese PCOS women may play a role in causing ovarian hyperandrogenism and hyperinsulinemia. Therefore fasting serum resistin level could be helpful in diagnosing PCOS patient. Unfortunately not many data could be obtained in this regards that can be compared with ours.

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