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Investigating the Relationship between Serum Leptin levels and Insulin Resistance in Poly Cystic Ovary Syndrome (PCOS) Patients

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ABSTRACT: *Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 5 -10% of women and is a major cause of anovulatory infertility. Etiology of PCOS remains unknown but hyperandrogenism and chronic anovulation have both been associated with PCOS. Insulin resistance, abdominal obesity have also been frequently associated with PCOS. Abdominal adipose tissue is a source of leptin; however, the role of leptin in the pathogenesis of PCOS is unclear. The current study aimed to investigate the relationships between serum leptin levels with insulin resistance and anthropometric measures in patients diagnosed with PCOS attending Aleppo gynecology university hospital. We analyzed differences between 46 patients with PCOS and 25 healthy control subjects with the same range of age- and body mass index (BMI). Related parameters were measured for both groups: serum glucose, leptin and insulin levels, waist to hip ratio, BMI, Waist circumference, and HOMA-IR. We found that, 67% of PCOS patients have Insulin resistance, with significant correlation between PCOS and Insulin resistance (P<0.05), Serum leptin levels was significantly correlated with basal insulin levels, BMI and HOMA-IR in both groups (P<0.05). The levels of fasting leptin, insulin, waist circumference and HOMA-IR were significantly higher in the PCOS group in comparison with the control group (p<0.05). Increased leptin levels in PCOS patients is independently associated with insulin resistance.*

Keywords: *BMI, Insulin, HOMA-IR, Leptin, PCOS.*

I. INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, characterized by clinical and/or biochemical hyperandrogenism and chronic anovulation, polycystic ovaries in ultrasonography findings, and frequently morbid obesity, which is associated with infertility, menstrual dysfunction, hirsutism and frequent miscarriages [1].

Insulin resistance (IR), as a major abnormality associated with PCOS, represents a disorder with increased risk of type 2 diabetes [2] and is usually associated with an increase in inflammatory markers [3]. IR is now known to be intrinsic to PCOS, present in approximately 50- 70 percent of PCOS women independently of obesity, and contributing in a major way to its pathogenesis [4]. Insulin resistance and hyperinsulinemia promote abnormal ovarian androgen secretion and subsequently abnormal follicular development leading to dysfunctional ovarian and menstrual activity [5].

Skeletal muscle and adipose tissue become insulin resistant, resulting in decreased glucose uptake and increased lipolysis, respectively, whereas the ovary, adrenal, liver, and skin remain insulin sensitive [6]. The cause of insulin resistance in PCOS appears to be a post-binding defect in insulin receptor mediated signal transduction [7]. IR is believed to be associated with chronic inflammatory response, which is characterized, by abnormal cytokine production and the activations of pro-inflammatory signaling pathways [8].

Leptin is a 167 amino acid peptide of tumor necrosis factor family of cytokines [9], secreted from adipocytes in pulsatile fashion [10], and regulates energy homeostasis by regulating food intake and energy balance [11]. Leptin levels fall during weight loss and increase the brain activity in areas involved in the control of food intake while restoration of leptin levels maintained weight loss and reversed the changes in the brain activity [12].

Caro *et al*. reported that leptin and insulin receptors deficient mice showed elevated testosterone, infertility and insulin resistance, which are reminiscent of PCOS in humans [13]. The role of leptin in PCOS is under investigation since the disease involves impairment of reproduction and nutrition [14].

Leptin resistance was introduced in an apparent analogy with that of insulin resistance to explain why hyperleptinemia associated with obesity fails to correct the defect in energy balance and feeding behavior. According to Farooqi *et al.* (2002), Leptin binds to its receptor in hypothalamus and activates JAK-STAT 3 pathway leading to suppression of Neuropeptide Y and Agouti-related protein (peptides which increase food intake) and secretion of Proopiomelanocortin and Corticotropin releasing hormone (peptides which reduce food intake) [15]. In obesity, the transport of Leptin across the blood brain barrier is reduced and levels of SOCS 3, an inhibitor of Leptin signaling is increased in hypothalamus, which leads to Leptin resistance [16].

Therefore, in an attempt to indirectly learn about Leptin, ovarian function and insulin resistance, PCOS has been targeted for many studies, and the relationship between Leptin, Insulin sensitivity and Insulin concentration in derangement of ovarian function, remain elusive and controversial. Our study aims to clarify the role of Leptin in the pathology of PCOS, by investigating the relationship between serum levels of Leptin, insulin resistance, and anthropometric measures in patients with PCOS compared with healthy women.

II. MATERIALS AND METHODS

1.1. Patient's characteristics

This study was performed at Aleppo gynecology university hospital, Aleppo, Syria. 46 PCOS patients was included in our study. The patient inclusion criteria included females aged 18-35 years, Arab population, BMI>25 Kg/m^2, waist circumference> 80 Cm, waist to hip ratio >0.85. The criteria for diagnosis of PCOS according to the 2003 Rotterdam ESHRE/ASRM criteria: 1) oligo- and/or anovulation; 2) clinical and/or biochemical signs of hyperandrogenism (patients presented with hirsute, acne or alopecia, and/or increased circulating levels of testosterone; 3) polycystic ovaries (ovarian morphology was assessed using transvaginal ultrasound), and exclusion of other etiologies [17]. In all participants, BMI, waist circumference, waist to hip ratio, and homeostasis model assessment of insulin resistance (HOMA-IR), serum levels of fasting glucose, insulin, and leptin were assessed.

The exclusion criteria was: patients who received gonadotropins, hormonal contraception, Metformin or thiazolidinediones in the three months prior to the study, patients with hyperprolactinemia (morning plasma prolactin≥ 30 ng/ml) or other endocrine, hepatic, or renal disorders.Twenty-five healthy, fertile non-pregnant females with cross-matched age, BMI, waist circumference, waist to hip ratio were recruited as a control group. BMI was calculated as weight in kilograms divided by height in meters squared for all eligible subjects.

1.2. Laboratory assays

Venous blood samples (10 ml) collected between 8-10 a.m. after overnight fasting were allowed to clot and centrifuged at 3,000 rpm for five minutes. Serum was stored at −20°C for biochemical assays. Blood samples were taken from patients and controls on days 2-5 of their menstrual cycles (early follicular phase), but blood samples were taken randomly for those suffering from severe oligo- or amenorrhea.

Waist Circumference was measured in the standing position and by placing a soft tape measure midway between the lowest rib and the iliac crest. Hip circumference was measured at the level of the major trochanters. Hormonal and biochemical assays were performed at the researches Laboratory of the Faculty of pharmacy, Aleppo University. Glucose level was measured by glucose oxidase/peroxidase method and spectrophotometric quantitation (Biosystems S.A., SPAIN).

Insulin was detected by Enzyme–linked immunosorbent assay (Sandwich-ELISA) kits (DiaMetra Catalog No: DCM076-7, ITALY), its analytical sensitivity was 0.25 µU /ml. Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) by the following formula: HOMA-IR (mg/dl × µU /ml) = fasting blood glucose (mg/dL) × fasting insulin (µU /ml)/ 405(1). Patients were considered as Insulin resistant if HOMA-IR > 3.875 [18] [19]. Leptin was detected by Enzyme–linked immunosorbent assay (Sandwich-ELISA) kits (DIAGNOSTIC AUTOMATION, INC Catalog No: 1742-6, USA), its analytical sensitivity was 0.3 ng/ml.

1.3. Statistical analyses

Data were analyzed using Statistical Package for the Social Science, version 20 (SPSS, Chicago, IL, USA) and was expressed as mean ± SD. Comparison between patients and controls was performed with independent samples "*t* test", one-way ANOVA and the tukey post-hoc test. The degree of correlation between IR and PCOS was assessed using Q- square test. The degree of correlation between Leptin and the variables of interest was assessed using Pearson's correlation coefficient. For all tests, a probability (P-value) <0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

1.4. Study the relationship between PCOS and IR

The PCOS patients were divided into two subgroups: PCOS patients with Insulin Resistance group (PCOS-IR) and PCOS patients without IR or None IR group (PCOS-NIR). Insulin resistance was judged by using the homeostatic model index (HOMA-IR) (calculated by equation (1)), and 3.875 was selected as a cutoff point [18-19]. As shown in Table 1, Thirty-one cases of PCOS had insulin resistance (HOMA-IR ≥3.875), whereas fifteen cases of PCOS without insulin resistance (HOMA-IR < 3.875).

Table 1. : Rates of distribution of insulin resistance among the PCOS and the control groups.

IR or NIR	PCOS N (%)	CONTROL N (%)
Insulin Resistance (IR)	31 (67 %)	9 (36 %)
Non-Insulin Resistance (NIR)	15 (33 %)	16 (64 %)

The Insulin resistance percentage among PCOS patients is (67%) and it is within the range got by many of other studies, Deugarte *et al*, (2005) showed that about 64% of PCOS patients have Insulin resistance. About 50-70% of PCOS patients have a degree of Insulin resistance [20]. Both of obese and lean PCOS patients have a degree of Insulin resistance, but the obese PCOS patients are the highest incidence of Insulin resistance compared to lean PCOS or obese women without PCOS [21]. Therefore, we investigate the effect of BMI in Insulin resistance. We found that from the total 46 PCOS patients, thirty-one PCOS-IR were overweight (BMI 28.5±4.2 kg/m^2) and fifteen PCOS-NIR were also overweight (BMI 27 ±3.0 kg/m^2), so there is no significant differences between them in mean BMI values (*P* value> 0.05). In Addition, there is no significant correlation between BMI and HOMA-IR in PCOS and control groups (R=0.185,*p* value=0.219)(R=0.151. *p* value=0.471) respectively , thus we suggest that the IR could be due to other factors other than BMI.

We applied the Chi-Square test to study the correlation between PCOS and insulin resistance. In agreement with previous studies [22,23], our results showed that PCOS is correlated to Insulin resistance (*P* value = 0.011). This indicates that IR is an independent pathogenic variable linked to PCOS.

This correlation could be explained that increased insulin levels, which is one of Insulin resistance indicators, stimulate directly and indirectly to increase endogenous androgen levels via several mechanisms:

1. Excess insulin enhances androgen production in ovarian theca cells in response to luteinizing hormone (LH) stimulation, resulting in follicular arrest and anovulation [24].
2. Extreme hyperinsulinemia may result in activation of ovarian Insulin-like Growth Factor IGF-1 receptors and thereby stimulate androgen production. In addition, atypical IGF receptor subtypes may be present in the ovary that have extremely high affinity for insulin binding [25].
3. Hyperinsulinemia inhibits synthesis of sex hormone-binding globulin by the liver, which leads to elevated
   Levels of serum-free testosterone [26].
4. Hyperinsulinemia causes a decrease in secretion of IGF binding protein-1 in both the liver and the ovary, which in turn results in increased intraovarian bioavailability of IGF-1 and IGF-2, two important regulators of ovarian follicular maturation and steroidogenesis [27].

1.5. Analysis of results

The measured parameters of PCOS and control groups: Age, body weight, BMI, waist circumference, and waist/hip ratio, hormonal and biochemical levels are shown in Table 2.

Table 2. : Basic characteristics of the total study population

Variables	PCOS patients (n=46)	Controls (n=25)	*P* (value)
Age (years)	24.13 ± 5.48	25.6 ± 5.41	NS
Weight(Kg)	69.72 ± 10.27	73.52 ± 8.29	NS
Length (cm)	157.92 ± 7.07	160.56 ± 8.61	NS
BMI (kg/m2)	28.05 ± 3.87	29.82 ± 3.07	NS
Waist circumference (cm)	90.88 ± 8.67	86.23 ± 6.97	**<0.05**
waist-to-hip ratio	0.88 ± 0.2	0.87 ± 0.2	NS
Fasting Glucose (mg/dl)	103.83 ±17.96	96.95 ± 16.55	NS
Fasting Insulin (µIU/ml)	21.92 ± 6.97	16.07 ± 6.17	**<0.05**
HOMA-IR (mg/dl. µIU/ml)	5.64 ± 2.16	3.82 ± 1.52	**<0.05**
Fasting Leptin (ng/ml)	19.52 ± 7.45	10.7 ± 2.48	**<0.05**

PCOS patients and healthy controls had no significant differences in age, weight, BMI, length, waist-to-hip ratio or fasting glucose levels (*p* value>0.05) .Waist circumference, fasting insulin, fasting Leptin as well as HOMA-IR, were significantly higher in PCOS patients than in healthy controls (*p* value<0.05) Table 2.

In order to study the correlation between Leptin levels with anthropometric and biochemical parameters, without dividing the study groups into insulin resistance (IR) or non-insulin resistance (NIR), we

applied Pearson correlation test; in which R values ranging from -1 to 1 and a probability (*P*-value) <0.05 was considered statistically significant as shown in Table 3.

Table 3. : Baseline Pearson correlations coefficients (R) of Leptin with anthropometric and biochemical parameters in PCOS and control.

Variables	Leptin in PCOS (n=46)		Leptin in control (n=25)	
	R	*P* value	R	*P* value
BMI	**0.335**	**0.023***	**0.414**	**0.039***
Insulin	**0.406**	**0.005***	**0.474**	**0.016***
HOMA-IR	**0.363**	**0.013***	**0.568**	**0.003***

As shown in Table 3, no correlation was found between Leptin levels and weight, length, waist, waist to Hip ratio, glucose in both groups (*P* >0.05), whereas Leptin levels were correlated positively with basal insulin levels, BMI and HOMA-IR in the PCOS and control groups (*P* <0.05).

As we notice in Table 3, the Leptin levels was correlated positively with insulin levels and HOMA-IR in both groups, so in order to study the correlation between Leptin levels and insulin resistance, we divided Patient and control groups by using (HOMA-IR≥3.875) as IR and (HOMA-IR < 3.875) as NIR. The mean differences between the four groups (Patient-IR, Patient-NIR, control-IR, control-NIR) was tested by using one-way ANOVA test as shown in Table 4.

Table 4. : Mean differences between the four groups (Patient-IR, Patient-NIR, control-IR, control-NIR) in insulin and Leptin

Variables	PCOS-IR	PCOS-NIR	Control- IR	Control-NIR	P Value
Insulin (µIU/ml)	25.7±4.7	14.1±3.2	22.3±5.2	12.6±3.1	**0.000**
Leptin (ng/ml)	22.3±7.2	13.8±3.7	12.7±2.2	9.6± 2	**0.000**

Table 4. shows that, there are significant differences between the four groups in Insulin and Leptin (*P*<0.05). These results were then explained by using Tukey-Post hoc Test as shown in Table 5.

Table 5. : Explanation the mean differences between four groups in Insulin and Leptin levels after application Tukey-Post hoc Test

Variables	1st group	2nd group	Mean Difference	*P* Value
Insulin	Patient-IR	Patient NIR	11.644	**0.000***
	Patient-IR	Control IR	3.4	0.151
	Patient-IR	Control NIR	13.159	**0.000***
	Patient-NIR	Control IR	-8.239	**0.000***
	Patient-NIR	Control NIR	1.5	0.748

	Control-IR	Control NIR	9.754	**0.000***
Leptin	Patient-IR	Patient NIR	8.537	**0.000***
	Patient-IR	Control IR	9.665	**0.000***
	Patient-IR	Control NIR	12.703	**0.000***
	Patient-NIR	Control IR	1.12	0.975
	Patient-NIR	Control NIR	4.166	**0.017***
	Control IR	Control NIR	3.239	**0.023***

The results in Table 5, show that, the mean serum levels of insulin were significantly higher in Patient-IR (25.7±4.7) than in Patient-NIR and Control-NIR respectively (14.1±3.2, 12.6±3.1), whereas mean serum levels of insulin were significantly more in Control-IR (22.3±5.2) than in Patient-NIR (14.1±3.2), and mean serum levels of insulin were significantly higher in Control-IR (22.3±5.2) than in Control-NIR (12.6±3.1). The mean serum levels of leptin were significantly higher in Patient-IR (22.3±7.2) than in Patient-NIR, Control-IR and Control-NIR respectively (13.8±3.7 , 12.7 ±2.2 , 9.6±2), and mean serum levels of leptin were significantly higher in Patient-NIR (13.8±3.7) than in Control-NIR (9.6±2). As we notice in Table 3, serum Leptin levels were correlated positively with BMI in Patient and control groups. This result is in agreement with many studies have reported that leptin is correlated to BMI in both groups [28 -30].

The major source of Leptin is the white adipose tissue, and the level of circulating Leptin is proportional to the total amount of fat in the body. In addition to white adipose tissue, Leptin can be also produced by brown adipose tissue, placenta (syncytiotrophoblasts), ovaries, skeletal muscle, stomach (the lower part of the fundic glands),mammary epithelial cells, bone marrow, pituitary, and liver [31]. Considine *et al*, (1996) found that the mean serum Leptin concentrations were in normal-weight subjects lower than in obese subjects. The *ob* mRNA content of adipocytes was twice as high in the obese subjects as in the normal weight subjects [32]. To determine the role of BMI, we divided the samples into overweight and obese each individually. We found that serum Leptin levels were statically higher in Patients (overweight: 18.6±7.4, obese: 23± 6.6) than in control (overweight: 9.9± 1.9, obese: 11.5± 2.9) respectively. This result suggest that the total mass of fatty tissue is more important than BMI factor in the regulation of leptin secretion in the PCOS patients. This suggestion is supported by our result which shows that waist circumference (which reflects visceral lipid mass) was higher in the patients as compared with control who have the same range of the BMI [33]. Tayfun Alper *et al*, (2004) showed that serum Leptin levels correlate positively with BMI in both groups, but when the effects of fat percentage on serum Leptin were eliminated, the levels of Leptin remain higher statistically in PCOS group. These findings support the idea that other factors other than excess of fat mass or fat-free mass might be important in the regulation of serum Leptin levels in PCOS [34]. Similarly, Chakrabarti(2013) showed that serum Leptin levels correlate positively with BMI in both groups, and PCOS patients have higher Leptin levels, regardless of BMI or Insulin resistance [28].

There was a significant difference in waist circumference in both groups which was significantly higher in PCOS patients than controls ($p < 0.05$) as shown in Table 2. Overweight has been linked to PCOS since its very first description by Stein and Leventhal (1935), who noticed the association of obesity, hirsutism, anovulation, and infertility in women. In fact, approximately 50% of PCOS women are overweight or obese [35], and usually the obesity is characterized by a central distribution. Even in lean women matched for body mass index BMI, women with PCOS have a higher percentage of body fat and a larger waist-to-hip ratio than their matched controls [36,37]. However, it has been suggested that the distribution of fat, rather than the mere presence of obesity or increased body mass index, is mainly significant in PCOS [38]. Regardless of ethnicity,

most overweight women with PCOS have central, or android (visceral) obesity, resulting in a waist-to-hip ratio greater than 0.85 [39].

Serum Leptin levels correlated positively to Insulin and HOMA-IR in patient and control groups (Table 3). This result is in agreement with several studies which found that the mean of fasting Insulin, Leptin and HOMA-IR were higher in PCOS patients than healthy control, and there was a significant correlation between Leptin levels with BMI, insulin and HOMA-IR in both groups, these findings are in line with our study[2,28,33] [40-42]. While, our findings conflicted with others studies which did not notice any differences in Leptin levels between patient and healthy groups [43-45] . Our results also contradicted with other studies which found no correlation between the serum levels of insulin and Leptin in PCOS patients [44-46].

Taking in consideration the Insulin Resistance, our results in Table 5, showed that serum Leptin levels were higher in Patient-IR than Patient-NIR, which is in agreement with many studies [23,43]. Therefore, we conclude from the foregoing that Leptin level is related to PCOS and insulin resistance, which in turns suggests a relationship between Leptin and insulin receptors in the pathogenesis of PCOS.

Growing evidence suggests that either impaired or deficient Leptin signaling results in the development of insulin resistance and impaired glucose metabolism [47,48]. Several studies confirmed this deficient in the function of Leptin receptor. In fact there are large numbers of Leptin receptors on ovarian cells [49], and Leptin treatment of these cells in vitro caused significant reduction in steroid output [50]. Hence, the role of Leptin when it links to its receptor, is to reduce the steroidogenesis, probably by antagonizing stimulatory factors, such as insulin like growth factor-1, transforming growth factor-β, insulin, and luteinizing hormone. Since the pathogenesis of PCOS known as high levels of androgens, so this indicates that there is a defect in the Leptin work through lack in response of the ovarian cells to its effects, i.e. there is no connection with its receptor, and this is expressed in the status of similar way the resistance to insulin and this called Leptin resistance [51].

To explain the Leptin resistance, many mechanisms that might be proposed: On one hand, the resistance could be due to the defect in the transferring of Leptin through the blood brain barrier by linking with carrier as CRP. The defect of transferring could limit the entry of Leptin to the central nervous system and it creates a kind of resistance [20]. On other hand, Leptin resistance may be a result of disruption in signal transduction process through Janus-activating kinase2-signal transducer and activator of transcription 3 (JAK2-STAT3) pathway on Leptin receptors by suppressor of cytokine signaling-3 (SOCS-3) and Protein tyrosine phosphatase 1ß (PTP-1ß) [52]. The Mice which deficient the PTP-1ß and SOCS3 have shown high sensitivity to Leptin and were resistant to obesity [53,54]. Deletion of SOCS3 in LepR-expressing cells should release Leptin signaling from the inhibitory influence of SOCS3 [55]. Interestingly, SOCS3 binds to the same residue (phospho-Tyr985) of LepR [56]. A mutation in the Tyr985 of LepR produces a lean phenotype, indicating that the phosphorylation of this residue exerts a predominantly inhibitory effect on Leptin signaling [57]. SOCS3 contributes to both leptin resistance and insulin resistance because of increased ceramide synthesis [58].

The liver and skeletal muscle are key tissues that control glycaemia, and hypothalamic Leptin signaling modulates insulin sensitivity in these tissues [59]. Indeed, it has been previously shown that ICV injection of PI3K inhibitors abolishes the effect of Leptin in the reduction of food intake, inhibits the central actions of Leptin in the increase of peripheral insulin sensitivity, and inhibits the central action of Leptin in the enhancement of hypothalamic insulin sensitivity [60]. On other hand, it has been reported that leptin action in the hypothalamic arcuate nucleus improves hepatic insulin action via a mechanism involving the hepatic vagus nerve [61]. Kim *et al.*, (2006) showed that metformin restores Leptin sensitivity in obese rats with Leptin resistance and metformin treatment increased CSF Leptin concentrations in both standard Chow and high fat–fed obese rats compared with the untreated rats [62]. It is suggested that the increase in CSF leptin level may be the cause of reduced resistance because the defect in Leptin transport through the blood-brain barrier is a

possible mechanism of Leptin resistance [63]. Based on the above it can be said that, when there is resistance to Leptin and defect in the signal transmission of Leptin in the hypothalamus, it will accompanied with a defect in insulin signal in the liver, leading to increased insulin levels.

IV. CONCLUSION

In fact, the pathogenesis of PCOS is quietly complicated, which trigged many scientific researches in attempt to elucidate this pathogenesis. There were many clinical argumentative studies about the role of Leptin in PCOS patients with insulin resistance , so this study comes to clarify the role of Leptin in PCOS pathogenesis by assessment the serum level of Leptin in Syrian PCOS patients and healthy groups, in addition to the others classical related parameters. Our study showed that there was a correlation between PCOS disease and Insulin Resistance (IR), and the percentage of PCOS patients who are IR was 67%, so these findings show the importance of IR in PCOS patients as a research goal that should be more investigated.

Serum Leptin, Insulin and HOMA-IR were higher in PCOS group than matched-healthy control, and there were a significant correlation between serum Leptin levels and Insulin resistance, which manifested in increasing serum Leptin levels in PCOS-IR group more than PCOS-NIR. This find suggests that serum Leptin level could be on one hand, an important predictor for PCOS and on other hand, for Insulin Resistance, according to the impaired or deficient in Leptin signaling which results in the development of Insulin resistance and impaired glucose metabolism. Perhaps further studies with larger sample sizes and long-term follow-up will help to support our results.

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