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Research Article

INVESTIGATING THE RELATIONSHIP BETWEEN SERUM LEPTINL EVELS AND C-REACTIVE PROTEIN IN POLYCYSTIC OVARY SYNDROME PATIENTS

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ABSTRACT

Objective: Despite the using of some biological and clinical criteria for the definition of polycystic ovarian syndrome (PCOS) such as hyperandrogenism and menstrual dysfunction. However, the complex mechanism of this syndrome is still of interest to researchers, and began to investigate new parameters intervene in the pathogenesis of this disease including leptin and C-reactive protein (CRP). However, the role of these parameters is still not clear and under controversy. This study aimed to investigate the relationship between serum leptin levels with CRP in patients diagnosed with PCOS attending Aleppo Gynecology University Hospital.

Methods: The study included 46 patients and 25 healthy control subjects with the same range of age- and body mass index. Related parameters were measured for both groups: Serum glucose, leptin, insulin and CRP levels, and homeostasis model assessment of insulin resistance (HOMA-IR).

Results: Serum leptin levels were significantly correlated with CRP only in PCOS group ($p<0.05$). The levels of CRP and leptin were significantly higher in the PCOS group in comparison with the control group ($p<0.05$). There were a correlation between leptin with CRP only in patients with IR group ($p<0.05$), and there was a correlation between CRP and HOMA-IR only in PCOS group ($p<0.001$), but not in the control group.

Conclusion: Increased leptin, CRP levels in PCOS patients is independently associated with IR and make these parameters more important to take them in consideration.

Keywords: CRP, HOMA-IR, Leptin, PCOS

INTRODUCTION

The polycystic ovary syndrome (PCOS), which is characterized by hyperandrogenism, chronic anovulation and infertility, is one of the most frequent endocrine disorders in women. In addition to the reproductive abnormalities, a significant proportion of PCOS women suffers from obesity, insulin resistance (IR) and features of the metabolic syndrome [1,2].

IR, as a major abnormality associated with PCOS, represents a disorder with increased risk of type 2 diabetes [3] and is usually associated with an increase in inflammatory markers [4].

IR is now known to be intrinsic to PCOS, present in approximately 50–70% of PCOS women independently of obesity, and contributing in a major way to its pathogenesis [5]. IR and hyperinsulinemia promote abnormal ovarian androgen secretion and subsequently abnormal follicular development leading to dysfunctional ovarian and menstrual activity [6].

The cause of IR 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].

In recent years, several studies have demonstrated a high risk for impaired glucose tolerance and type 2 diabetes mellitus in PCOS [9]. It has not yet been clarified whether this increase in risk is related to endocrine abnormalities associated with PCOS, such as hyperandrogenemia, or it is a consequence of the anthropometric or metabolic abnormalities frequently observed in PCOS women.

The adipose tissue-derived hormone leptin is produced in proportion to fat stores. Circulating leptin serves to communicate the state of body energy repletion to the central nervous system in order to suppress food intake and permit energy expenditure. Adequate leptin levels permit energy expenditure in the processes of reproduction and growth and similarly regulate the autonomic nervous system, other elements of the endocrine system and the immune system [10]. Conversely, a lack of leptin signaling due to mutation of leptin (e.g., ob/ob mice) or the leptin receptor (e.g., db/db mice) in rodents and humans results in increased food intake in combination with a reduced energy expenditure phenotype reminiscent of the neuroendocrine starvation response (including hypothyroidism, decreased growth, infertility, and decreased immune function) in spite of their obesity [11].

Caro *et al.* reported that leptin and insulin receptors deficient mice showed elevated testosterone, infertility and IR, which are reminiscent of PCOS in humans [12]. The role of leptin in PCOS is under investigation since the disease involves impairment of reproduction and nutrition [13].

Markers of chronic subclinical inflammation such as C-reactive protein (CRP) or interleukin-6 (IL-6) have been shown to be independent predictors of risk for the development of type 2 diabetes [14-16]. Consistently increased CRP levels have been reported in PCOS patients [17], supporting the hypothesis that PCOS increases diabetes risk by activating chronic inflammation. Circulating CRP and IL-6 concentrations are correlated to obesity as well as to IR [18-20].

Sampson and coworkers showed that increased levels of CRP are associated with increased cardiovascular risk in PCOS [21]. Elevated CRP in association with hyperinsulinemia is a significant risk factor for cardiovascular diseases and that plays a key role in the development of the PCOS [22].

Some studies have investigated the association between CRP with IR and PCOS. They have shown a positive correlation between the increase in CRP with IR and PCOS [4,23]. In another study, plasma leptin levels were found to correlate closely with inflammatory cytokine levels tumor necrosis factor-alpha (TNF-α, IL-6) and with acute phase proteins (CRP, alpha-1-antitrypsin) [24]. It is still not known whether these parameters of chronic inflammation are primary or secondary to obesity and/or IR especially since short-term administration of IL-6 in humans failed to impair insulin sensitivity [25].

The present study aimed to evaluate CRP serum level changes in PCOS comparison with healthy controls matched in age and body mass index (BMI), and to determine in one hand the association between leptin and CRP in PCOS patient, and in other hand determine the correlation between leptin, CRP and IR (according to the homeostasis model assessment [HOMA] in patients with PCOS).

METHODS

This was a cohort study involving 46 PCOS patients who Attended Aleppo Gynecology University Hospital and 25 age and BMI matched healthy controls were recruited.

Patient's characteristics

The patient inclusion criteria included females aged 18–35 years, Arab population, BMI> 25 kg/m^2. The criteria for diagnosis of PCOS are 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 [26]. 25 healthy, fertile nonpregnant females with cross-matched age were recruited as a control group.

In all participants, BMI, HOMA-IR, serum levels of fasting glucose, insulin, leptin, CRP were assessed. BMI was calculated as weight in kilograms divided by height in meters squared for all eligible subjects.

The exclusion criteria were: Patients who received gonadotropins, hormonal contraception, metformin, or thiazolidinediones in the 3 months before the study, the patients with hyperprolactinemia (morning plasma prolactin ≥30 ng/ml) or other endocrine, hepatic, or renal disorders.

Laboratory assays

Venous blood samples (10 ml) collected between 8 and 10 a.m. after overnight fasting and were allowed to clot and centrifuged at 3000 rpm for 5 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. 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 SA, 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. IR was assessed using the HOMA-IR by the following formula: HOMA-IR (mg/dl × μU/ml) = fasting blood glucose (mg/dL) × fasting insulin (μU/ml)/405. The patients were considered as insulin resistant if HOMA-IR >3.875 [27,28]. Leptin was detected by Sandwich-ELISA kits (Diagnostic Automation, INC Catalog No: 1742-6, USA), its analytical sensitivity was 0.3 ng/ml. CRP was measured by immune-turbidimetric methods with commercially available Latex kits (Biosystems SA, Spain).

Statistical analyses

Data were analyzed using Statistical Package for the Social Science, version 20 (SPSS, Chicago, IL, USA) and were expressed as a mean ± standard deviation. 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 leptin and the variables of interest was assessed using Pearson's correlation coefficient.

In addition, multivariate stepwise regression analysis was performed to identify important predictors of leptin. For all tests, a probability (*p<0.05) was considered statistically significant.

RESULTS

The measured parameters of PCOS and control groups: Age, BMI, hormonal, and biochemical levels are shown in Table 1. All parameters were comparable between the two groups, considering p<0.5 if the differences are significant.

PCOS patients and healthy controls had no significant differences in age, BMI (p>0.05). Fasting leptin, CRP as well as HOMA-IR, were significantly higher in PCOS patients than in healthy controls (p<0.05) as shown in Table 1.

To study the correlation between leptin levels with CRP levels, without dividing the study groups into IR or non-IR (NIR), we applied Pearson correlation test; in which *r* values ranging from −1 to 1 and probability (p<0.05) was considered statistically significant as shown in Table 2.

Table 3 showed that there were significant differences between the four groups in CRP and leptin serum levels (p<0.05). These results were

Table 1: Age, anthropometric and biochemical parameters investigated in polycystic ovary syndrome patients and in age, body mass index matched healthy control subjects

Variables	PCOS patients (n=46)	Controls (n=25)	p
Age (years)	24.13±5.48	25.6±5.41	NS
BMI (kg/m^2)	28.05±3.87	29.82±3.07	NS
HOMA-IR (mg/dl μIU/ml)	5.64±2.16	3.82±1.52	0.000*
Fasting leptin (ng/ml)	19.52±7.45	10.7±2.48	0.000*
CRP (mg/l)	12.69±5.51	5.97±2.36	0.000*

*Indicates existence of statistically significant p<0.05 value. NS: Nonsignificant, BMI: Body mass index, CRP: C-reactive protein, PCOS: Polycystic ovary syndrome, HOMA-IR: Homeostasis model assessment of insulin resistance

Table 2: Baseline Pearson correlations coefficients (R) of leptin with C-reactive protein in polycystic ovary syndrome and control groups

Variables	Leptin in PCOS (n=46)		Leptin in control (n=25)	
	R	p	R	p
CRP (mg/l)	0.605	0.000*	0.158	0.448

*Indicates existence of statistically significant *P*<0.05 value. CRP: C-reactive protein, PCOS: Polycystic ovary syndrome

Table 3: Mean differences of leptin and C-reactive protein between the four groups (patient-insulin resistance, patient-noninsulin resistance, control-insulin resistance, and control-noninsulin resistance)

Variables	PCOS-IR	PCOS-NIR	Control-IR	Control-NIR	p
CRP (mg/L)	14.8±5.1	8.4±3.5	7.5±2.1	5.1±2.1	0.000*
Leptin (ng/ml)	22.3±7.2	13.8±3.7	12.7±2.2	9.6±2	0.000*

*Indicates existence of statistically significant p<0.05 value. PCOS-IR: Polycystic ovary syndrome insulin resistance, PCOS-NIR: Polycystic ovary syndrome noninsulin resistance, IR: Insulin resistance, NIR: Noninsulin resistance, CRP: C-reactive protein

explained by using Tukey *post hoc* test, in this, we noticed that the mean serum levels of CRP were significantly higher in patient-IR (14.8 ± 50.1) than in patient-NIR, control-IR and control-NIR, respectively (8.4 ± 3.5, 7.5 ± 2.1, 5.1 ± 2.1), and 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).

We noticed that there was a correlation between leptin with CRP in PCOS-IR ($p<0.05$), but no correlation between them in PCOS-NIR ($p=0.301$) as shown in Table 4. There was a correlation between CRP and HOMA-IR only in PCOS group ($p<0.001$) and no correlation between them in the control group ($p=0.094$) as shown in Tables 4 and 5.

Multivariate stepwise regression analysis was performed for PCOS patients to identify the best predictor factors of leptin levels. Leptin was introduced as a dependent variable and BMI, fasting insulin, CRP and HOMA-IR (variables that have been significantly correlated with leptin, (data not shown) [29], were introduced as independent variables. After adjusting the effects of other variables, only CRP was found to be independent predictor of leptin levels ($\beta = 9.137$, $p=0.000$) and showed that CRP levels determined 36.6% serum leptin concentration, while other variables were below of this percentage. Thus, the proposed model was: Leptin level = 9.137 + 0.818* CRP. This equation was considered because it had the best-adjusted $r^2 = 0.366$. Therefore, serum leptin levels appear to be determined by serum levels of CRP, which seemed to be the most important influencing factor.

Table 4. Baseline Pearson correlations coefficients (R) of leptin with C-reactive protein in polycystic ovary syndrome group after dividing them into patient insulin resistance and patient noninsulin resistance

Variables	Leptin in patient-IR (n=31)		Leptin in patient NIR (n=15)	
	R	p	R	p
CRP (mg/l)	0.464	0.008*	0.285	0.301

*Indicates existence of statistically significant $p<0.05$ value. IR: Insulin resistance, NIR: Noninsulin resistance, CRP: C-reactive protein

Table 5: Baseline Pearson correlations coefficients (R) of C-reactive protein with homeostasis model assessment of insulin resistance in polycystic ovary syndrome and control

Variables	CRP in PCOS (n=46)		CRP in control (n=25)	
	R	p	R	p
HOMA-IR	0.475	0.001*	0.342	0.094

*Indicates existence of statistically significant $p<0.05$ value.
HOMA-IR: Homeostasis model assessment of insulin resistance, CRP: C-reactive protein, PCOS: Polycystic ovary syndrome

DISCUSSION

The increasing mean values of leptin in the PCOS group are in agreement with several studies which attributed this to the presence of the case of resistance to leptin in PCOS patients [24,30].

Taking in consideration the IR, we noticed in Table 3, that leptin was higher in Patient-IR than Patient-NIR, which is in agreement with many studies [31,32], and lead to suggest a relationship between leptin and insulin receptors in the pathogenesis of PCOS [29].

The increasing level of CRP in the PCOS group is correlated to CRP meta-analysis, which showed that circulating CRP was 96% higher in women with PCOS compared to controls. The present meta-analysis of the mean differences in CRP, IL-6 and TNF-α clearly indicates that CRP is a circulating marker of the pro-inflammatory state in PCOS as evidenced by the 2-fold elevation in circulating CRP in women with disorder compared to controls. There is no difference in the levels of IL-6 or TNF-α between both groups [33].

We noticed in Table 2, there was a correlation between serum leptin levels and CRP only in PCOS group, and this could be explained that PCOS has also been described as a low-grade inflammation state characterized by elevated levels of CRP [34]. One of the reasons that makes serum leptin levels high in PCOS is the CRP, which in turn binds with leptin and impairs leptin transport across the blood-brain-barrier and leptin signaling at a cellular level and this is one of the proposed mechanisms that lead to a defect in the function of leptin. Many studies have shown an association between the CRP with leptin, wherein a survey study of the extent of leptin association with a number of serological proteins, showed that the greatest affinity was with the CRP, and these studies also showed that CRP, which binding to leptin, prevented it entering the blood-brain-barrier and thus inhibition of its physiological signaling to cause a feeling of satiety [30].

In addition, we noticed that CRP was higher in PCOS-IR group than PCOS-NIR, Moreover, Table 4 showed a correlation between leptin with CRP in PCOS-IR, and Table 5 showed a correlation between CRP and HOMA-IR only in PCOS group. These findings are consistent with several studies have shown a positive relationship between the increasing CRP with IR and PCOS [4,23,35], so we can suggest that the CRP levels are higher when there is IR in comparing with the absence of resistance. However, our results conflicted with other studies, which showed that there were no differences in serum CRP levels between both groups [36], and there was no correlation between CRP and HOMA-IR [37].

To explain the correlation between CRP, leptin and IR, we take in consideration that adipose tissue-derived cytokine expression (tumor necrosis factor-α, leptin and IL-6) may be an important contributor to low-grade chronic inflammation. In other words, the accumulation of visceral adipose tissue may be a key factor underpinning features of the metabolic syndrome and of low-grade chronic inflammation. These combined observations would also explain the correlation of insulin sensitivity to CRP [19]. It appears that adipose tissue in general; visceral adipose tissue in particular, plays a key role in regulating inflammation. Notably, CRP is primary synthesized in the liver and regulated by the pro-inflammatory cytokine IL-6 and TNF-α in adiposities [38].

The previous studies suggest that the cytokines, arising partly from adipose tissue, could possibly be responsible for the metabolic abnormalities associated with IR. In this respect, many markers are proven associated with IR, metabolic syndrome, and diabetes among which CRP has been the most studied marker. However, the causal association has not been proven yet. One hypothesis is that the inflammatory cytokines that stimulate the hepatic production of acute phase proteins are mainly secreted by the adipose tissue excessively and that such cytokines may result in IR by indirectly causing the phosphorylation and proteosomal degradation of insulin receptor substrates or by indirectly interfering with the insulin receptor substrate interaction [4]. The decrease of serum CRP levels during metformin therapy is in accordance with the known beneficial metabolic effects of this drug and suggests that CRP or other inflammation parameters could be used as markers of treatment efficiency in women with PCOS [39].

There were many clinical argumentative studies about the role of leptin and CRP in PCOS patients with IR, so this study comes to clarify the role of them in PCOS pathogenesis by assessment the serum levels of leptin and CRP in Syrian PCOS patients and healthy groups, in addition to the others classical related parameters. Therefore, the more attention should be paid to leptin and CRP in the treatment of PCOS, and more clinical studies should be done to make sure about our model to calculate leptin levels. Perhaps further studies with larger sample sizes and long-term follow-up will help to support our results.

CONCLUSION

Serum leptin, CRP and HOMA-IR were higher in PCOS group than matched healthy control. Serum leptin levels were significantly correlated with CRP only in PCOS group ($p<0.05$). Increasing serum CRP levels in PCOS-IR group more than PCOS-NIR, suggests the involvement of inflammatory processes in PCOS, and the correlation between CRP and IR, which are the main factors in PCOS women.

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