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The Effect of Leptin, Ghrelin and Estradiol Hormones on Insulin Resistance and Anthropometric Markers in Polycystic Ovary Syndrome Women Patients

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Abstract

Background: Patients with Polycystic ovary syndrome (PCOS) are more prone to abnormal production of some regulatory peptides. The importance of leptin in PCOS-related endocrine and metabolic disorders have been gradually recognized. This study aims to evaluate serum levels of leptin and ghrelin and their correlation with insulin resistance and anthropometric indices in PCOS. **Methods:** This case-control study was conducted on 80 women;65 with PCOS and 15 healthy women whose age ranging from 17 to 37 years old and body mass index (BMI) were matched and who referred to Dr. Shahid Xalid Hospital, koya, Kurdistan. Serum levels of leptin, ghrelin, insulin, fast glucose and estradiol were measured, Homeostatic Model Assessment for Insulin Resistance was calculated. **Results:** The results showed, that serum levels of leptin, insulin and fast glucose and estradiol were significantly higher (p, <0.05) while ghrelin were significantly lower in PCOS patients women compared with healthy. Serum level of leptin showed positive correlation with BMI (r= 0.85, *p*= 0.001), waist circumference (WC) (r= 0.55, *p*= 0.01), insulin resistance (r= 0.67, *p*= 0.01), while ghrelin level showed negative correlation with BMI, IR, WC. **Conclusion:** The results showed increased leptin level while ghrelin level was decreased in PCOS patients. leptin level was positively correlated with BMI, WC, IR, while ghrelin level was negatively correlated with BMI, WC and IR.

Keywords: PCOS, Leptin, Ghrelin, HOMA-IR

Introduction

Polycystic ovary syndrome is considered as one of the most commonly encountered hormonal diseases in women during reproduction age (Sirmans and Pate, 2014). Polycystic ovarian syndrome (PCOS) is a heterogeneous multiplex genetic disorder of multifactorial nature where each of genetic and ambient element participate in the underlying pathophysiological implements. Recent investigations had showed the relation between PCOS and metabolic syndrome, obesity and insulin resistance (Targher *et al*., 2016, Polak *et al*., 2017).

One of the major factor considered in the evolution of PCOS is obesity since 35-80% of the women suffering from PCOS are documented to be overweight subjects (Messinis *et al*., 2015). Many investigations have been related to leptin and ghrelin as attainable factors that have a direct relation to the development of PCOS (Daghestani *et al*., 2018). Leptin, as a catabolic enhancer polypeptide hormone, works as an adipostatin, where it represses food absorption and initiates catabolic pathways correlated with overloaded energy construction (Monteiro and Batterham, 2017). Abnormal levels of leptin can lead to a variety of endocrine and metabolic dysfunctions, and due

to the close relationship between obesity and hyperleptinemia, the case of obese PCOS becomes more complicated (Becker *et al*., 2015). Ghrelin, is an additional peptide hormone, that has an anorexigenic impact. It also energizes growth hormone discharge, regulates glucose metabolism, appetite, body mass, pancreatic endocrine, and ovarian activity (Sato *et al*., 2014, Lugilde *et al*., 2022).

Many research reported variable results on the level of ghrelin in PCOS patients, Furthermore, lower ghrelin level have been documented in some investigations, while others stated that there was no change in ghrelin concentration in PCOS (Garin *et al*., 2017). A number of metabolic and hormonal dysfunctions occur in patients suffering from PCOS, and these cover insulin resistances, glucose intolerance, and dyslipidemia. Hyperinsulinemia is commonly documented in PCOS and may be jumbled in anovulation, leading to premature maturation of granulosa cells, inhibition of granulosa cell maturation, and expansion in the attendance of raised LH levels. In addition, some research has indicated that insulin resistance and neuroendocrine dysfunctions play an appreciable part in the pathogenesis of PCOS (Davinelli *et al*., 2020) .

The current study was conducted on PCOS patients as an attempt to determine leptin and ghrelin, insulin and estradiol levels with calculating the HOMA-IR to investigate the relationship that exists between these hormones and anthropometric and hormonal parameters.

MATERIALS and METHODOLOGY

The case-control study was conducted on 65 PCOS patients whose age ranging from 17 to 37 years old, that were referred to Dr. Shahid Xalid Hospital in Koya, Kurdistan, IRAQ. Also 15 healthy women of same ages , BMI and WC were included. Sampling lasted from November 2021 to February 2022, from each patient and control subject 5ml of venous blood was withdrawn into gel and clot activator tube after overnight fasting and the serum was separated by centrifuge and stored at -80 until chemical analysis. The study protocol was approved by the Ethics Committee of faculty of science and health at koya university , 2021).

The PCOS patients were diagnosed by a gynecologist using Rotterdam criteria of hyperandrogenism, oligomenorrhoea, or amenorrhoea, and polycystic ovaries by ultrasonographic examination.

Medical history, physical and pelvic examination, and complete blood tests were used to determine the healthy status of women in the control group. Exclusion criteria for all subjects included pregnancy, hypothyroidism, hyperprolactinemia, cushing syndrome and congenital adrenal hyperplasia were excluded from the study. Weight and height were measured to calculate the BMI by using the standard formula of weight (kg)\height (m2). Waist circumferences were also measured.

Serum samples were used to evaluate serum leptin, ghrelin, insulin, glucose and E2 levels, and also insulin resistance (IR) HOMA-IR was calculated. The levels of Leptin, Ghrelin, insulin and were measured using a Human ELISA Kit (SUNLONG BIOTECH CO., LTD, CHINA), following the instruction supplied with the kits. The intra-assay CVs of the assay used were, (Leptin)10% and (Ghrelin)10% and (Insulin)10%. The inter-assay CVs of the assay used were, (Leptin)12%, (Ghrelin)12%, (Insulin)12%, respectively and sensitivity of (Leptin) 12pg/ml, (Ghrelin) 26pg/ml and (Insulin) 0.05mU/L. The measurement of serum Estradiol was performed using an enzyme-linked immunosorbent assay ELISA Kit (Monobind Inc., 1oo North pointe Drive, Lake Forest, CA 92630, USA) which had an intra-assay CV of 7.5% and an inter-assay CV of 8.2%, respectively and sensitivity of 8.2pg/ml.

RESULTS and DISCUSSION

The anthropometric data of included PCOS patients and control groups are presented in table (1).

Table 1:Anthropometric characteristics of enrolled patients and controls

Parameters	PCOS group	Normal group	*P* value
Age (years)	22 (20 - 28)	20 (18.75 - 22)	0.0268
Weight (Kg)	68.00 (60 - 75)	64.00 (57 - 69)	0.1595
Height (cm)	159.0 (155 - 161)	163.0(159.8 - 165)	0.0025
BMI (kg/m$^{2)}$	27 (24.75 - 31.00)	18.25 (15.88 - 21.25)	0.001
WC (cm)	95 (85 - 100)	75 (68 - 82.50)	0.001

Data presented as means - interquartile range. BMI: body mass index: WC: Waist circumference

The results of hormonal analysis in the current study showed that in the PCOS group, serum levels of leptin, insulin, estradiol and fast glucose were significantly higher (P<0.05) than in the healthy control group, whereas levels of ghrelin in PCOS patients showed significantly lower (P<0.05) than in normal heathy group as illustrated in table (2).

Table 2. The levels of leptin, ghrelin, insulin estradiol hormones and HOMA-IR in PCOS patients and healthy women

Parameters	PCOS Patients group	Healthy group	P value
Leptin pg/ml	828.1 (732.2 – 941.4)	548.3 (456.7 - 671.3)	0.0012
Ghrelin pg/ml	576.3 (492.8 - 696.7)	912.4 (770.9 - 1235)	0.0001
Estradiol pg/ml	129.9 (107.1 - 146)	117.4 (80.58 - 145.1)	0.0097
Insulin (mU/L)	15.90 (14.09 - 17.13)	12.26 (12.12 - 12.89)	0.001
Fasting glucose (mmol/L)	130 (120 - 138)	89 (85 - 90)	0.001
HOMA-IR	74.68 (60.63 – 90.27)	53 (44 - 56)	0.0002

Data presented as means - interquartile range

The current investigation revealed a higher concentrations of leptin, insulin, IR, and estradiol with lower levels of ghrelin in PCOS women patients. These results are in line with those previously recorded for PCOS patients (Sengupta *et al*., 2019; Pehlivanoy and Mitkov,2009 and Mitkov *et al*., 2008) , that showed increase of leptin, insulin, IR and estradiol and decrease of ghrelin in PCOS patient. Also in a previous study in PCOS women, serum levels of leptin, insulin, HOMA-IR, were significantly higher, while Ghrelin was the same in both groups (Houjeghani *et al*, 2012). Leptin has a binary effect on reproduction, it has a beneficial impact on puberty by the effect on hypothalamic-pituitary axis by stimulating estrogen discharge.

The hyperinsulinemia and insulin resistance and higher estradiol were found in PCOS patients' women in the current study is agree with those of other study since they reported that 50-70% of PCOS patients showed insulin resistance, high insulin level which are associated with hyperandrogenism, (Salehpour *et al*., 2008). It has been confirmed that insulin is capable of stimulating ovarian steroidogenesis, and increasing ovary LH receptors and the reactivity of pituitary gonadotropes to GnRH effort (Rostamtabar *et al*., 2021). All mentioned that ghrelin level was significantly lower; and leptin level were significantly higher in obese PCOS patients in comparison with lean patients, these findings possibly linked to obesity, hyperinsulinemia and insulin resistance (Daghestani *et al*., 2018).

The data analysis of the current results in table (3) and figures (1 and 2), for correlation evaluation showed, that serum level of leptin in PCOS women was significantly correlated with BMI (r= 0.85, p= 0.001), WC (r= 0.55, p= 0.010), HOMA-IR (r= 0.67, p= 0.01). Whereas the level of ghrelin was non significantly correlated with BMI, WC and HOMA-IR.

Table 3: Correlation between (serum leptin and BMI), (Leptin and IR), (Leptin and WC), (Ghrelin and BMI), (Ghrelin and IR), (Ghrelin and WC)) in PCOS women

Parameters	Patients group	
	R	P value
Leptin and BMI	0.85	0.001
Leptin and IR	0.67	0.01
Leptin and WC	0.55	0.01
Ghrelin and BMI	0.007	> 0.05
Ghrelin and IR	0.011	> 0.05
	5	> 0.05

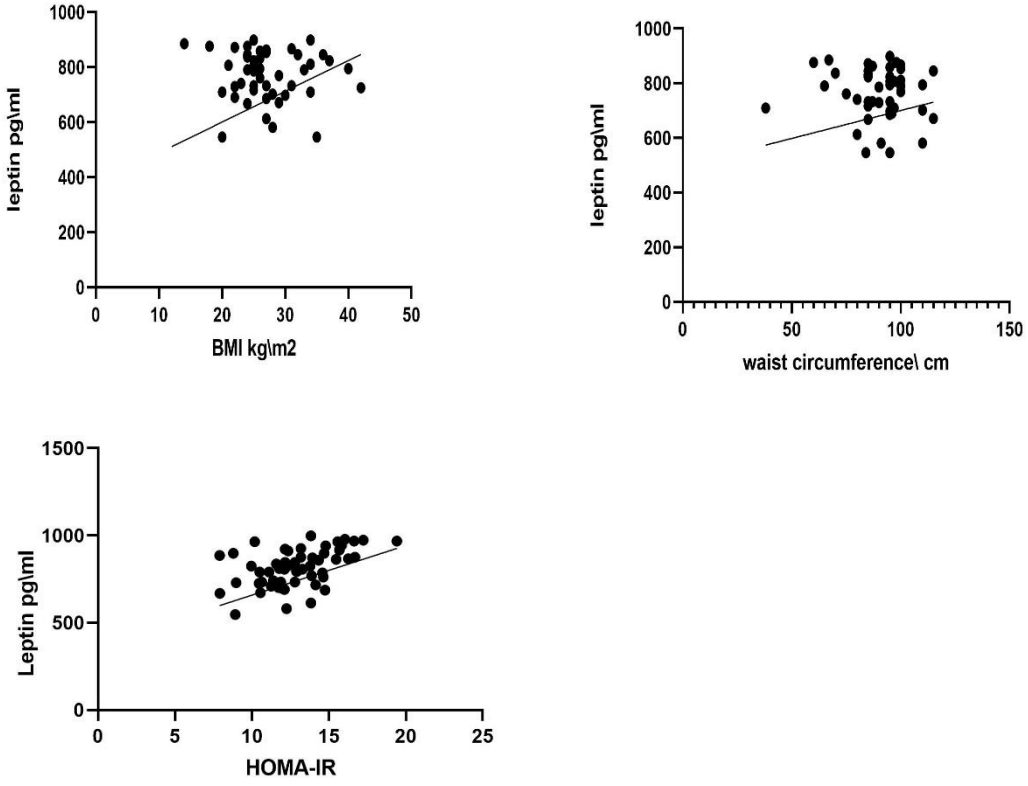

Figure 1: Correlation between serum leptin level with BMI, HOMA-IR, WC

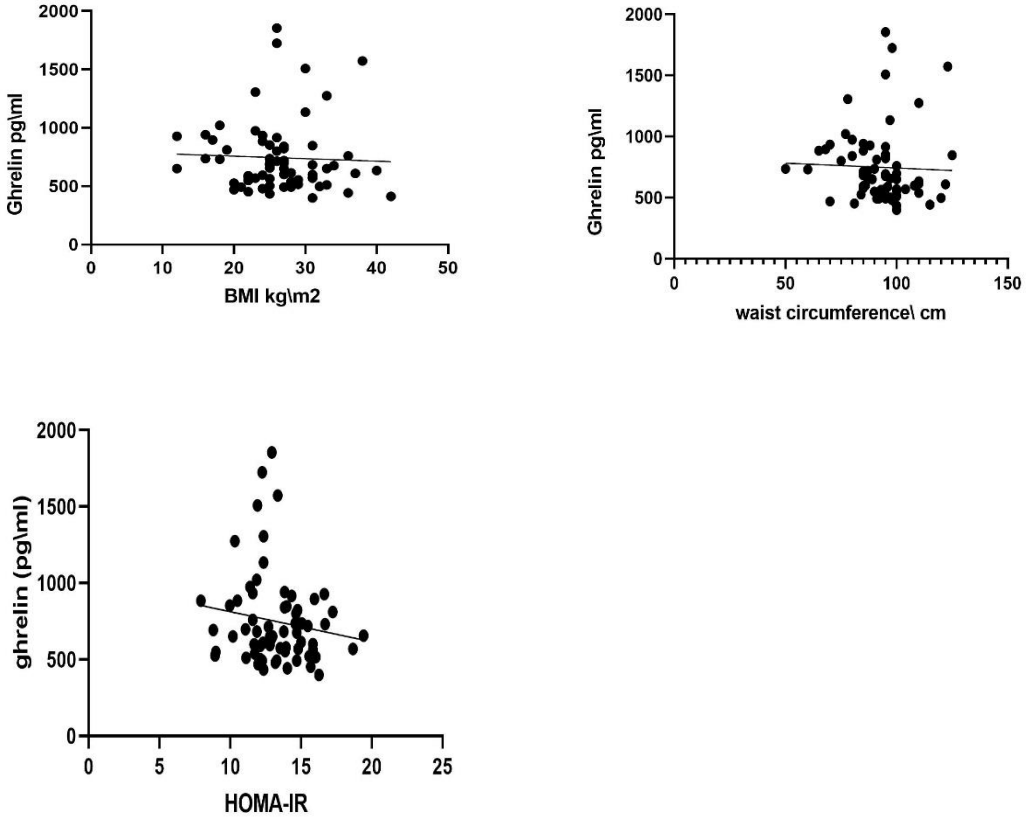

Figure 2: Correlation between serum Ghrelin level with IR, BMI, WC

The correlations coefficient of leptin and ghrelin with BMI, WC and IR in PCOS women, are in accordance with the previous studies which showed a significant correlation between leptin with BMI and WC in PCOS patients, these relationships, possibly are linked to obesity, hyperinsulinemia and insulin resistance (Daghestani *et al*., 2018). This supports the importance of abdominal fat mass in the secretion of leptin and resulting abnormalities which exist in the PCOS. It can be concluded that leptin or ghrelin levels in the serum are associated with insulin metabolism and insulin resistance, this evidence is supported by the data from HOMA-S (Mohamed *et al*., 2014). Current study have shown that among in the PCOS and control group, leptin was directly correlated with insulin secretion and IR. Similarly, Peng *et al.* (2022), found an association between elevated serum leptin level and insulin resistance in PCOS patients. Thus, leptin may serves as an important biochemical indicator for the diagnosis and treatment of PCOS. Moreover, hyperleptinemia may interact with hyperandrogenemia and chronic low-grade inflammation in PCOS, forming a vicious circle in PCOS, indicating that leptin might play a role in the occurrence and development of PCOS (Shorakae *et al*., 2015). In a study Ghrelin revealed, non-significant difference in PCOS patient compared with control (Orio *et al*.,2003), whereas , Daghestani *et al*. 2011; Pektas *et al*., 2020 and Ibrahim *et al*., 2021 , reported lower level of ghrelin in PCOS patients compared to healthy controls .

CONCLUSION

The results of this investigation have suggested that indices of adiposity (BMI and WC) were in charge of uplifted leptin, and insulin resistance in PCOS diseases. The causative effects of leptin and ghrelin in the pathogenesis of PCOS may happen in ways other than the simple levels of these hormones in the circulation system, especially as leptin excretes its endocrine effects mostly via modulating insulin concentration.

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