Serum Leptin, Ghrelin and Insulin Resistance in Iraqi Women with Clomiphene Resistance Polycystic Ovary Syndrome

مستوى الغريلين ، اللبتين ومقاومة الأنسولين في مصل الدم عند النساء الذين يعانون من عدم الاستجابة لدواء الكلوميفين في علاج متلازمة تكيس المبايض في العراق

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الخلاصة
خلفية الدراسة: متلازمة المبيض المتعدد التكيس هو اضطراب الغدد الصماء الأكثر شيوعا في النساء اللواتي في سن الإنجاب. وهناك عدة عوامل قد تسبب هذا المرض. يتميز باضطرابات الحيض، كما يظهر ندرة الطمث أو انقطاع الطمث، وذلك بسبب فرط الأندر وجينية وتكيس المبايض. وقد أثبتت الأدلة الأخيرة الوظيفة المعقدة للأنسجة الدهنية والمعدة كأجهزة أو غدد صماء من خلال إفراز هرمونات في مجرى الدم تشارك في الأنشطة الفسيولوجية للجسم مع الآثار المحتملة في تطوير متلازمة التكيس المبايض. ومن هذه الهرمونات هو هرمونات جريلين، اللبتين والأنسولين التي لها دوراساسي في تكيس المبايض.
الهدف: إن الهدف من الدراسة الحالية لتقييم مستوى هرمونات الغريلين والليبتين وكذلك مستوى المقاومة للأنسولين في النساء الذين يعانون من عدم الاستجابة لدواء الكلوميفين في علاج متلازمة تكيس المبايض وكذلك محاولة إيجاد علاقة بين هذه العوامل.
طرق العمل: خلال سبتمبر 2013 إلى مايو عام 2014،عدد من النساء المشخصات والذين يعانون من العقم وتكيس المبايض تم جمعهم في العيادة الطبية الخاصة للدكتورة الاختصاص أحلام علي ناصرمن خلال الزيارة الدورية للعلاج. كانت هناك 23 امرأة في متوسط العمر 21.3 ± 1.2 مستمرة على علاج الكلوميفين وتعتبر كمجموعة متلازمة تكيس المبايض المقاومة للكلوميفين (CR PCOS)، 25 امرأة في متوسط العمر 23.5±1.4 ويأخذون علاجات أخرى غير عقار كلوميفين لعلاج متلازمة تكيس المبايض تعتبر (OT PCOS) و20 فتاة في متوسط العمر 22 ± 2.4 كمجموعة سيطرة كل هذه المجاميع أدرجت في هذه الدراسة. وتم أخذ عينات الدم بعد 8 ساعات على الأقل من الصيام في جميع الفتيات متلازمة تكيس المبايض والسيطرة، حيث تم قياس مستويات كل من الجلوكوز ، هرمون جريلين وهرمون اللبتين في مصل الدم ثم تم حساب قيمة المقاومة للأنسولين باستخدام نموذج HOMA-IR.
النتائج: لوحظ أعلى مستويات لهرمونات الغريلين والليبتين وكذلك مستوى المقاومة للأنسولين كان في مجموعة CR-PCOS ويعتبر هذا المستوى مرتفع بشكل ملحوظ بالمقارنة مع مجموعة السيطرة ومجموعةOT-PCOS. وقد لوحظ ارتباط إيجابي كبير بين المقاومة للأنسولين ومستوى الجريلين في مجموعة CR PCOS مع عدم وجود أي ارتباط في بقية المجاميع. من ناحية أخرى هناك علاقة سلبية بين الجريلين واللبتين في مجموعة المراقبة لم تلاحظ في بقية المجاميع، وفي الوقت نفسه عدم وجود أي ارتباط بين جريلين واللبتين أو المقاومة للأنسولين في مجموعة OT-PCOS
الاستنتاجات: النساء الذين يعانون من عدم الاستجابة لدواء الكلوميفين في علاج متلازمة تكيس المبايض كانت هناك زيادة كبيرة في مستويات الجريلين ،الليبتين ومقاومة الأنسولين مقارنة بمجموعة السيطرة ومجموعة OT-PCOS كما تشير الدراسة ان هذه الهرمونات تعتبر كعلامات البيوكيميائية جيدة في الكشف عن عدم الاستجابة لدواء الكلوميفين في علاج متلازمة تكيس المبايض. علاوة على ذلك فإننا نتكهن بأن هناك علاقة إيجابية ذات دلالة إحصائية بين جريلين والمقاومة للأنسولين في مجموعة CR PCOS.
التوصيات :تقييم مستويات كل من هرمون جريلين وهرمون اللبتين في النساء اللواتي يتناولن عقاري الميتفورمين و الكلوميفين لعلاج متلازمة تكيس المبايض

Abstract

Background: Polycystic ovary syndrome is the most common endocrine disorder in women with reproductive age. it has a multifactorial etiology characterized by menstrual irregularities, manifesting as oligomenorrhea or amenorrhea, apart from abnormalities of hyperandrogenism and polycystic ovaries. Recent evidence has demonstrated the complex function of adipose tissue and stomach as endocrine organs through release of hormones into the blood stream involved in physiological activities of the body with potential implication in PCOS development. One of the most mportant of these hormones are ghrelin, leptin and insulin that have role in process of PCOS development.
Aims of the study: The aim of present study to evaluate serum ghrelin, leptin and insulin resistance in clomiphene resistance PCOS women and to find correlation between these parameters.
Material and method: During Sep 2013 to May 2014, already diagnosed infertile Iraqi women with PCOS they are recruited in the private medical clinic of Dr. Ahlam ali naser during their periodic visit. There were 23girls at mean age 21.3±1.2 on clomiphene therapy as clomiphene resistant PCOS group(CR-PCOS), 25 PCOS girls at mean age

23.5±1.4 consumed drugs other than clomiphene for PCOS treatment consider as (OT-PCOS) and 20 girls at mean age 22±2.4 as control group included in the present study. Blood samples were taken after at least 8 hours of fasting in all PCOS girls and control, serum glucose, ghrelin and leptin were measure, insulin resistance value were calculated using HOMA-IR model.

Results: The highest Ghrelin, leptin and IR levels were observed in CR-PCOS group and this level considered significantly high when compared with control and OT-PCOS groups. The only significant positive correlation between IR and ghrelin was observed in CR-PCOS group with no significant correlation in all study groups were observed. In other hand when we make correlation between ghrelin and study parameters, significant negative correlations that observed with leptin in control group not observed in CR-PCOS and OT-PCOS, meanwhile no significant correlation between ghrelin and leptin or IR in OT-PCOS group.

Conclusions: Women with clomiphene resistance PCOS exhibit significantly increased serum ghrelin, leptin and insulin resistance levels than control subjects and PCOS women taking therapy other than clomiphene indicating good biochemical markers in detection clomiphene resistance status in PCOS women. Furthermore we speculate significant positive correlation between ghrelin and IR in CR-PCOS group.

Recommendation: Assessment of ghrelin and leptin levels in women taking metformin and clomiphene combination therapy for PCOS treatment

Keywords: PCOS, Ghrelin, Leptin, Clomiphene resistance, Insulin resistance

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the frequent endocrinopathies in adult women; it has a complex and multifactorial etiology, where adipose tissue seems to have an important role in the etiology, development and maintenance of the condition. [1]. It is the most common endocrine disorder in reproductive age, with an incidence of 5 -10% and the most enigmatic diseases, may be due to the multisystem nature of associated disorders, and the confounding variables in diagnosis[2]. PCOS is a heterogeneous disorder of functional androgen excess, detectable either by laboratory analysis or by clinical exam, characterized by menstrual irregularities, manifesting as oligomenorrhea or amenorrhea, apart from abnormalities of hyperandrogenism and polycystic ovaries [3]. Obesity of the central type and insulin resistance (IR) are highly associated with the syndrome, predisposing women to the development of glucose intolerance and type 2 diabetes mellitus[4]. Some points on pathogenesis of PCOS are still unclear but obesity is common, recent evidence has demonstrated the complex function of adipose tissue and stomach as endocrine organs through release of hormones into the blood stream involved in physiological activities of the body with potential implication in PCOS development, One of the most important of these hormones is recently discovered ghrelin which is a 28 amino acid peptide hormone, primarily produced by the stomach [5]. Ghrelin stimulates growth hormone secretion; also it has different functions such as the regulations of glucose metabolism, appetite, body weight, endocrine pancreatic, and ovarian functions [6]. In other hand the implication of the insulin resistance and compensatory hyperinsulinemia accelerate the effect of luteinizing hormone (LH) on ovarian theca cells and androgen synthesis which in turn inhibits ovulation through cessation of the follicular maturity process and PCOS development [7]**.** Leptin, is a single-chain proteohormone with a molecular mass of 16kDa that is thought to works as a mediator in the stomach – hypothalamus pathway and provides information about the body's energy storage in adipocytes in addition, its level is associated with obesity[8]. Classically clomiphene citrate is the first choice to induce ovulation in patients with PCOS, however 70-80% of PCOS women can ovulate and only 40%of them become pregnant [9]. Women who do

not ovulate with increasing doses of clomiphene during the treatment with a total dose of 200 mg of clomiphene for at least four cycles are described as being CR-PCOS and remain a major challenge in gynecologic endocrinology[10]. Clomiphene is a triphenylethylene derivative prevents the action of the endogenous estrogen on the estrogenic receptors at the hypothalamic–pituitary system. This blocks the ovarian negative feedback effect on gonadotrophin secretion, which results in increase in secretion of Follicle Stimulating Hormone (FSH) and LH leading to follicle development[11]. The present study was designed to evaluate and compare serum levels of leptin and ghrelin in clomiphene resistance PCOS women and to study the relationship of these hormones to the insulin resistance and obesity markers.

MATERIAL AND METHODS

During Sep 2013 to May 2014, already diagnosed infertile Iraqi women with PCOS according to the Rotterdam criteria[12] they are recruited in a gynecologist private medical clinic during their periodic visit seeking for medical advice concerning drug prescription for infertility. The protocol was approved by Local Ethical Committee and all participants obtained written consent. Body weight, height and waist circumference were measured and body mass index (BMI) was calculated. There were 23 girls at mean age 21.3±1.2 on clomiphene therapy as clomiphene resistant PCOS group(CR-PCOS), 25 PCOS girls at mean age 23.5±1.4 consumed drugs other than clomiphene for PCOS treatment consider as (OT-PCOS) group and 20 girls at mean age 22±2.4 as control group were included in the present study. All tested groups taking infertility therapy for at least 6 months. The present study excluded subjects with BMI > 25 kg/m^2, patients with chronic disease, pregnant or lactating female and any patient with renal or hepatic impairment or taking other therapy which could interfere with the tested parameters. Blood samples were taken after at least 8 hours of fasting in all PCOS girls and control. Serum glucose was measured using glucose-peroxidase colorimetric enzymatic method. Insulin concentrations were determined by the DRG Insulin ELISA Kit based on the sandwich principle. Regarding Leptin levels were measured by using The DRG Leptin ELISA Kit,a specimen sample containing endogenous leptin is incubated in the coated well with a specific biotinylated monoclonal anti Leptin antibody. Serum ghrelin levels were measured by enzyme Immunoassay using DRG- kit that designed to detect a specific peptide(ghrelin) based on the principle of "competitive" enzyme immunoassay. The immunoplate in this kit is pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or in sample. Insulin resistance (IR) was assessed using the Homeostasis model assessment (HOMA-IR) according to the formula: fasting insulin (μIU/ml) x fasting glucose (mmol/l)/22.5 [13].

Statistical analysis: Values were expressed as mean ± S.D; the values were statistically evaluated using unpaired Student's t-test and one way analysis of variance (ANOVA), supported by Bonferroni's post hoc analysis. Values regarded as significant when $p < 0.05$. Analysis was

performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

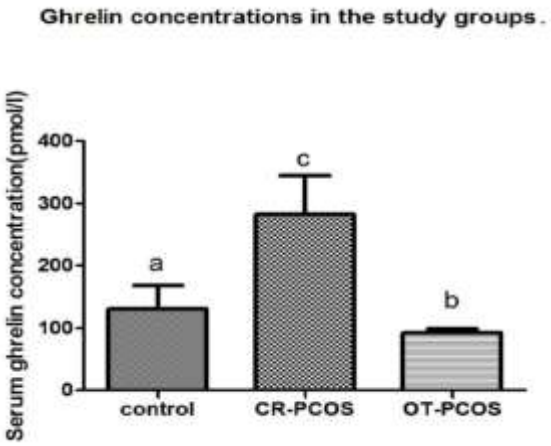
RESULTS

The study group consisted of 68 girls; forty eight girls were diagnosed with PCOS they subdivided according to PCOS therapy to CR-PCOS or those taking other therapy OT-PCOS while 20 girls without PCOS served as controls. The groups were matched on age and BMI as shown on table (1).

	CR-PCOS (n=25)	OT-PCOS (n=23)	Control (n=20)
Age	21.3±1.2	23.5±1.4	22±2.4
BMI (kg/m2)	23.2±1.4	21.4±1.7	22.2±1.1

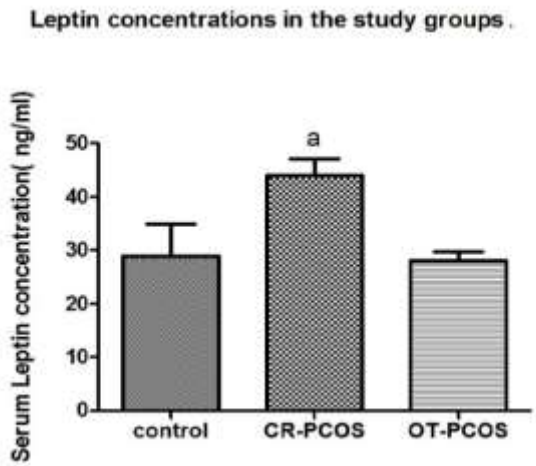
Table(1) Characteristics of Groups regarding age and body weight., value are expressed as Mean±SD.

Ghrelin data are summarized in figure(1),the highest Ghrelin concentrations were observed in CR-PCOS group and this level considered significantly high when compared with control with significant differences was found between the three studies groups for their mean serum ghrelin concentrations while lowest ghrelin level observed in OT-PCOS group.



Figure(1). Ghrelin concentration in Iraqi clomiphene resistance PCOS women(CR-PCOS n=23) and PCOS women taking other therapy (OT-PCOS n=25) as compared with control(n=20). Values are presented as Mean±SD . values with different letters (a,b,c) represent significant differences (P<0.05)

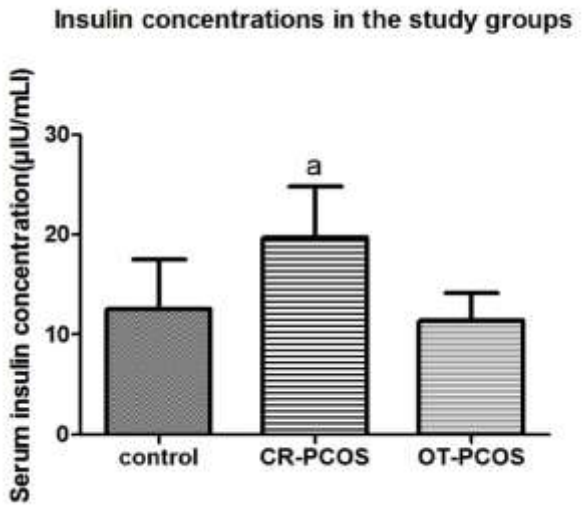
Regarding leptin our finding show the highest leptin concentrations were observed in CR-PCOS and considered significantly high compared to control and OT-PCOS groups all these finding presented in figure(2).



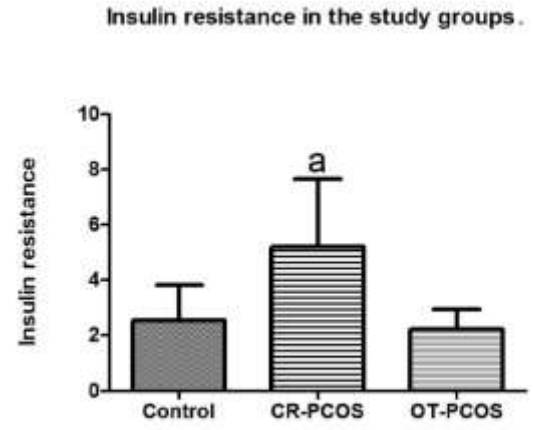
Figure(2) Leptin concentration in(CR-PCOS wamen n=23) and PCOS women taking other therapy compared to control.

Values are presented as Mean±SD. (a) represent significant differences compared with other groups (P<0.05)

In the CR-PCOS group insulin concentrations and IR values were significantly elevated compared to control and OT-PCOS groups as summarized in figures (3&4).

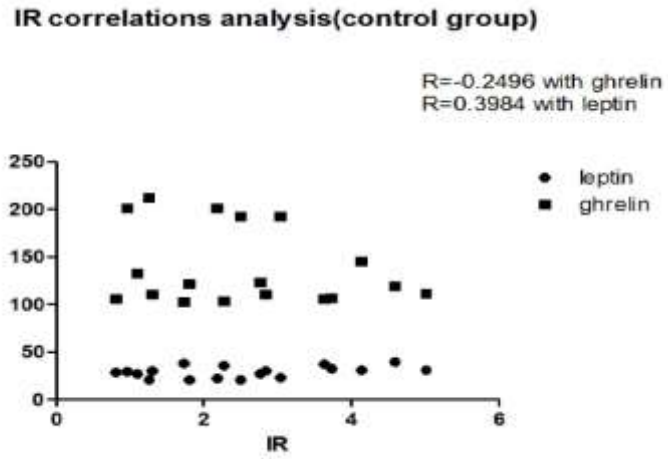


Figure(3) Insulin concentration in (CR-PCOS women n=23) and (OT-PCOS women n=25) as compared with control(n=20).Values are presented as Mean±SD. values with different letters (a) represent significant differences compared with other groups(P<0.05)



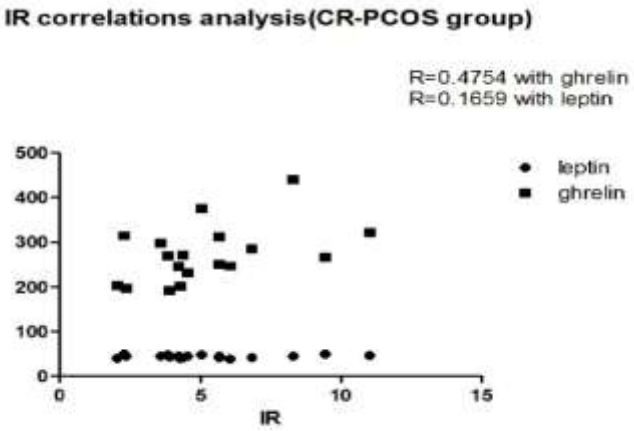
Figures (4) Insulin resistance in (CR-PCOS women n=23) and PCOS women taking other therapy(OT-PCOS women n=25) as compared with control(n=20). Values are presented as Mean±SD. values with different letters (a) represent significant differences compared with other groups(P<0.05)

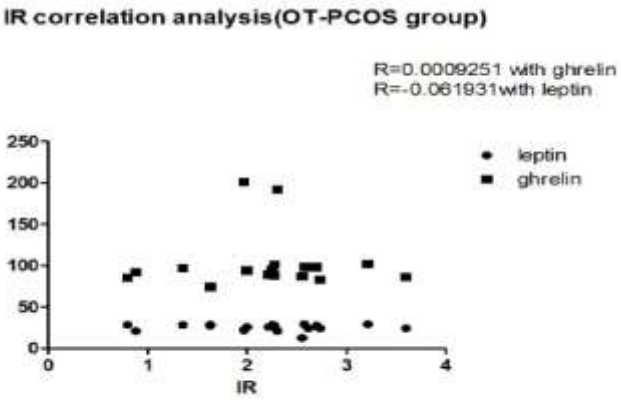
In figures (5,6 & 7) correlations analysis of IR values with ghrelin and leptin levels in study groups had been presented, the only significant positive correlation with ghrelin in CR-PCOS group with no significant correlation in all study groups were observed.



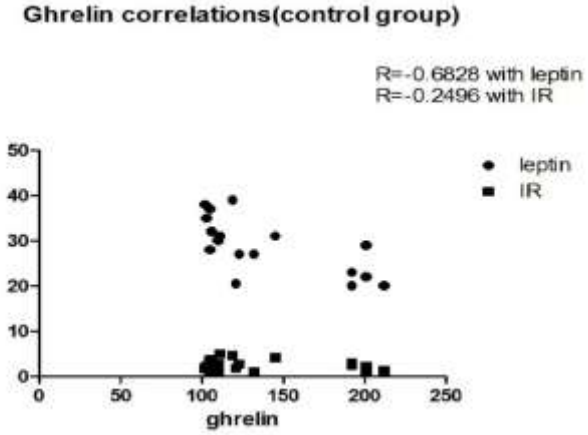
Figure(5) Correlation analysis between insulin resistance versus leptin and ghrelin levels in control group .

Figure(6) Correlation analysis between insulin resistance versus leptin and ghrelin levels in CR-PCOS group were significant positive correlation observed with ghrelin.





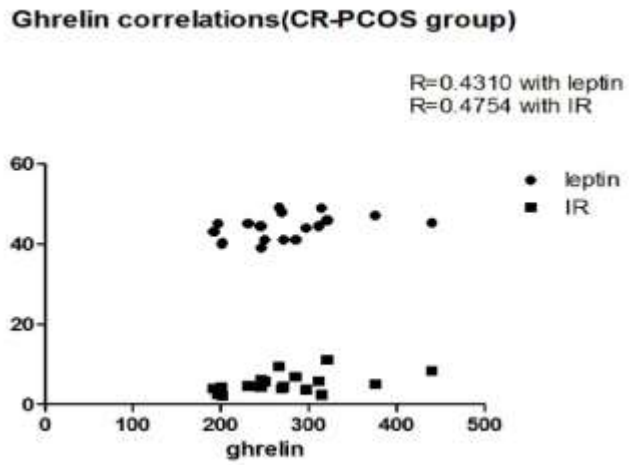
Figure(7) Correlation analysis between insulin resistance versus leptin and ghrelin levels in OT-PCOS group were no significant correlation observed.



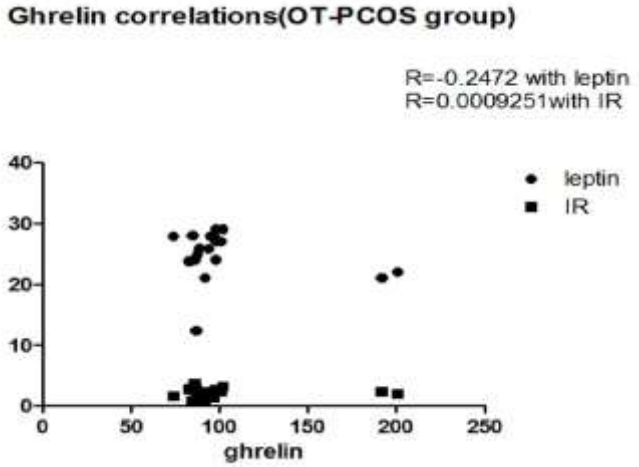
In other hand when we make correlation between ghrelin and study parameters figures (8,9 and10), the significant negative correlations that observed with leptin in control group not observed in CR-PCOS and OT-PCOS, meanwhile no significant

correlation between ghrelin and leptin or IR in OT-PCOS group.

Figure(8) Correlation analysis between ghrelin level versus leptin and insulin resistance levels in control group were significant negative correlation observed with leptin.



Figure(9) Correlation analysis between ghrelin versus leptin and insulin resistance levels in CR-PCOS group were significant positive correlation observed with IR.



Figure(10) Correlation analysis between ghrelin level versus leptin and insulin resistance levels in OT-PCOS group were no significant correlation observed.

DISCUSSION

PCOS is a complex disorder characterized by hyperandrogenism, insulin resistance, an abnormal secretary pattern of GnRH resulting in a high LH/FSH ratio and disturbance of other factors produced in the stomach and adipose tissue that could involved in modulating peripheral metabolic processes[14]. Although there is a large body of literature examining ghrelin and leptin levels in women with PCOS but according to our limited knowledge, there is no study evaluates

such parameters in clomiphene resistance PCOS women. The present study revealed a significant increase in serum ghrelin concentration in CR-PCOS compared with control group while lowest ghrelin concentration observed in OT-PCOS. Explanation of such finding seems to be little bit difficult since there are conflicting reports in this respect. Wasko R. et al. concluded higher ghrelin levels in PCOS than controls[15] such conclusion came in agreement with our study regarding CR-PCOS group. In contrast to our findings Ozgen IT et al. concluded that ghrelin levels were decreased in PCOS women as compared to control [16]. Furthermore Glintborg et al. [17]concluded that ghrelin levels were decreased in hirsute PCOS patients and showed a significant negative correlation with testosterone, independent of body composition on the other hand Altuğ Şen T et al [18] concluded that ghrelin levels were depressed with PCOS in relation to obesity. Meanwhile in adolescents, Bideci et al have reported that ghrelin levels were not statistically different in PCOS compared with BMI matched controls[19]. Our finding reveals a fundamental role for clomiphene in determining serum ghrelin level where highest concentration in clomiphene treated group, It is not clear which mechanism explain increased serum ghrelin level associated with clomiphene therapy but our results support the view that such increment is due to anti estrogenic properties of clomiphene to hypothalamic that results in increase in secretion of LH and FSH and subsequently affect Sex hormones release that may be involved in the regulation of ghrelin secretion [20,21].

Arvat et al found elevated gherlin concentrations might diminish reproductive function by inhibiting luteinizing hormone [22], where this may interfere with ovulation in patients with PCOS. Regarding leptin our finding demonstrated that highest leptin concentrations were observed in CR-PCOS group, the relationship between leptin and reproductive function is complex and incompletely understood but it has a permissive role in the pathogenesis of reproductive dysfunction [23]. Lambrinoudaki IV et al found that raloxifene a new selective estrogen receptor modulator(SERM) increases serum leptin significantly compared to control [21] These results have been confirmed by the present study regarding effect of clomiphene on serum leptin. Many studies revealed no significant difference in serum leptin levels between PCOS women and healthy controls and concluded no role for leptin in the pathophysiology of PCOS [24] such results came in line with our study regarding OT-PCOS group and this support the idea of significant effect of SERM on serum leptin of PCOS women [21]. In other hand there is considerable evidence about the role of leptin in the regulation of the hypothalamic-pituitary-gonadotropin axis by accelerating GnRH pulsatility via indirect mechanisms [14]. Furthermore, it may stimulate LH and FSH release[25]. So leptin may act as the critical link between adipose tissue and the reproductive system[14]. There is evidence that leptin is produced directly in the ovary, because bilateral ovariectomy induced decreased serum leptin levels [26]. The present study reveals highly significant increase in serum insulin concentrations and IR values in CR-PCOS group compared to control and OT-PCOS groups, this result could be attributed to clomiphene administration or sex hormones disturbance. The present results are in line with those of previous studies regarding PCOS. Parsanezhad ME et al demonstrated that serum insulin levels of clomiphene resistance were higher than the normal range[27], such high insulin concentrations cause hyperandrogenism because of increased production of ovarian androgen

and decreased synthesis of sex hormone-binding globulin[28]. Meirow et al reported that PCOS results from both hypothalamic imbalance and insulin resistance [29]. Furthermore Ozgen IT et al reported that Insulin resistance was higher in lean and obese PCOS groups than in BMI matched control groups [16] this came in line of our study. It has been found that Ovarian stromal tissue in clomiphene resistance is increased and such increment is proportional to high insulin levels [30]. In the present study significant positive correlation between IR and ghrelin in CR-PCOS group, this result considered conflicting since there are many studies show negative correlation between two hormones in PCOS women, this could be explained by clomiphene resistance and subsequent hormonal disturbance[27].In contrast to our finding in CR-PCOS group, Orio F Jr et al reported that fasting ghrelin didn't significantly correlate with fasting serum insulin concentration or insulin resistance [31] but this came in line of our study regarding OT-PCOS group**.**

CONCLUSION:

Our data showed that women with clomiphene resistance PCOS exhibit significantly increased serum ghrelin, leptin and insulin resistance levels than control subjects and PCOS women taking therapy other than clomiphene indicating that these parameters as a good biochemical marker in detection clomiphene resistance status in PCOS women. Furthermore we speculate significant positive correlation between ghrelin and IR in CR-PCOS group.

RECOMMENDATION:

Assessment of ghrelin and leptin levels in women taking metformin and clomiphene combination therapy for PCOS treatment

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