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Serum Prolactin, Preptin, CCL 18 and Genetic Polymorphisms in Iraqi Women with Polycystic Ovary Syndrome

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Abstract:

The polycystic ovary syndrome is an endocrine condition. One of the leading causes of female infertility and the most common disorder among women. The work was carried out on 100 Iraqi women (50 cases confirmed with PCOS and 50 controls) between October 2019 and March 2020. Blood samples were collected from the Advanced Institute of Infertility Diagnosis and Assisted Reproductive Technology at AL-Nahrain University and a private laboratory. ELISA was used to evaluate the biochemical parameters of Preptin, and CCL 18 , while FSH, insulin, and LH were measured by using AFIAS-6 in serum samples .The findings of this study indicate that, as opposed to the control group, values of prolactin (ng/ml), insulin (µIU/ml), LH (mIU/ml), Preptin (pg/ml) and CCL 18 (ng/ml) Quite higher in PCOS patients (P <0.001), while the control group showed highly significant value (P <0.05) in testosterone (ng/ml) and FSH (mIU/ml) , finally PRLR gene expression levels in PCOS patients were significantly increased by 3.6 times. In summary, the levels of Preptin and CCL18 can be regarded as PCOS markers.

Keywords: CCL 18, FSH, insulin, LH, polycystic ovary syndrome, Preptin.

Introduction:

One of the most common metabolic diseases is polycystic ovary syndrome (PCOS). Overall, the prevalence of PCOS ranges from 2.2% to 26%. Despite decades of study, the exact etiology of PCOS is still unclear [1]. Changes in ovarian steroid development, hyperinsulinemia, and neuronal endocrine abnormalities are all suspected to be important causes [2]. It is assumed that the neuroendocrine characteristic of PCOS is always the rapid pulsation of LH (GnRH),which is conducive to the LH produced by the pituitary over FSH, and leads to an increase in LH concentration, thereby changing the LH: typical PCOS FSH ratio .Follicular growth is impaired when FSH levels are low, while ovarian androgen development is enhanced when LH levels are high [3].CC chemokine ligand 18 is one of the chemokines that is basically expressed in the lungs and has chemotactic properties. Antigen-presenting cells, such as macrophages and dendritic cells are the most important producers of CCL18 [4]In vitro, certain human cells secrete CCL18 spontaneously, just like monocyte-derived dendritic cells [5]. Preptin hormone is a 34-amino-acid protein that correlates to proinsulin-like growth factor II Asp69-Leu102 and has recently been isolated. Islet b-cell granules contain Preptin, which is secreted in combination with insulin in reaction to glucose [6]. New research has identified a correlation between Preptin and insulin resistance in rats, as well as a possible link between Preptin and human insulin resistance[7]. Prolactin (PRL) is a pituitary hormone that regulates lactation initiation and maintenance [8]. Since the PRL receptor is found in a variety of tissues and cells, including the endometrium, prostate, pancreatic islets, and adipocytes, PRL is involved in a variety of physiological functions, including metabolism [9-11]. This study was aimed to investigate the levels of Preptin and CCL18 as a diagnostic and can be regarded as PCOS markers.

Materials and Methods:

The study was carried out on 100 Iraqi women (50 patients diagnosed with PCOS and 50 control groups) with ages that range from (18-38 year). All topics are arranged according to age. Blood samples were collected from the Advanced Diagnostic Institute of Infertility and Assisted Reproductive Technology/ AL-Nahrain University and a private

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laboratory between October 2019 and March 2020.About 6 ml of venous blood is taken from each patient and healthy woman and divided into two test tubes: 2.5 ml is placed in K2-EDTA containing triazole for genetic analysis, and 3.5 ml in a gel tube, and then centrifuged in a centrifuge at 3000 rpm for 3 minutes for biochemical parameters analysis.

Measurements:

The parameters of biochemistry, Prolactin, FSH, Insulin, LH were measured by (AFIAS-6) AFIAS-automated fluorescent immunoassay system) Preptin, and CCL 18 were assessed from serum samples using kits (MyBioSource, USA, Cat No. MBS456746, MBS704178) respectively by enzyme-linked immunosorbent assay. According to the protocol provided by the manufacturer of the Zymo Quick-RNA Micro-Prep Kit, RNA was extracted from all samples included in this study. A set of primers is used to amplify specific regions within the prolactin receptor gene. The forward primer is TGCCAAGACTTTCCTCCCAC, and the reverse primer is CACCCAACATCAAGGGGTCA (this study). Another set of amplified reference genes GAPDH genes was used the reference genes for calculation. Forward primer AGGTCATCCCTGAGCTGAT and reverse primer CTGCTTCACCACCTTCTTATT (this study). The thermal cycling program is as follows. Enzyme activation is performed at 95 °C for 7 minutes, and then 40 cycles are divided into two steps. The first step is denaturation at 95 °C for 20 seconds, and the second step is annealing and fluorescence screening for 20 seconds (55 °C) and extends for 20 seconds.

Results:

When comparing patients and controls, the findings indicate PCOS patients had significantly higher prolactin (ng/ml), LH (mIU/ml), insulin (µIU/ml), Preptin (pg/ml) and CCL 18 (ng/ml) ($p < 0.001$). In contrast, the control group had substantially higher Testosterone (ng / ml) and FSH (mIU / ml) values ($p<0.05$). As seen in Tab.1

Table 1. The distinction between biochemical parameters between PCOS patients and control groups.

Parameters	Groups	NO.	Mean ± SD	P- value
Prolactin (ng/ ml)	**Control**	**50**	**8.745 ± 1.415**	**0.001**
	Patients	**50**	**26.414 ± 3.405**	
FSH (mIU / ml)	**Control**	**50**	**5.833 ± 0.662**	**0.05**
	Patients	**50**	**4.133 ± 2.877**	
Insulin (µIU / ml)	**Control**	**50**	**11.210 ± 0.747**	**0.05**
	Patients	**50**	**36.118 ± 1.383**	
LH (mIU / ml)	**Control**	**50**	**4.240 ± 1.193**	**0.001**
	Patients	**50**	**15.298 ± 3.881**	
Preptin (pg / ml)	**Control**	**50**	**580.142 ± 114.14**	**0.001**
	Patients	**50**	**626.818 ± 161.5**	
CCL18 (ng / ml)	**Control**	**50**	**13.222 ± 5.455**	**0.001**
	Patients	**50**	**31.466 ± 4.63**	

The primer set is designed to complement the specific sequence of the PRLR isoform 3 genes to amplify the gene. The amplification result is represented by a curve in the real-time PCR instrument in the Fig.1, which illustrates the real-time amplification during each cycle, and illustrates the real-time amplification during each cycle.

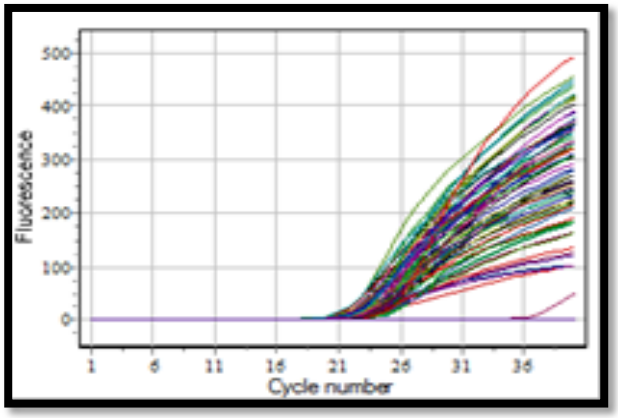

Figure1. Amplification of PRLR and GAPDH genes were submitted to the RT-PCR.

The results of PRLR gene expression are summarized in Fig.2, which shows that the gene expression level of PCOS patients significantly increased by 3.6 times compared with the control.

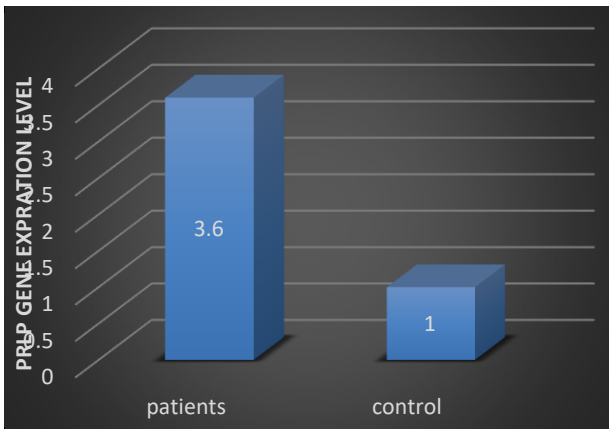

Figure 2. *PRLP* gene expression level of patients and control.

The correlation between prolactin measured by ELISA and prolactin receptor gene expression detected by real-time PCR is shown in Fig.3, and a positive correlation (0.947) between these two parameters is shown.

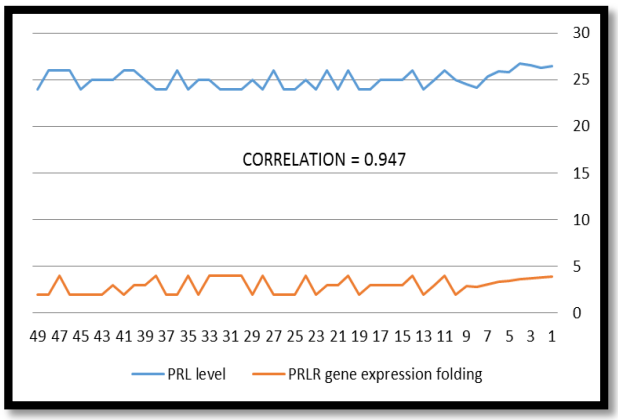

Figure 3. Correlation between the level of PRLR gene expression and the level of PRL.

Discussion:

Given the insights into modern pathogenesis and treatment strategies, PCOS is the subject of ongoing research [12] .A small amount of FSH levels leads to impaired follicular growth, while elevated LH levels lead to increased ovarian androgen production [13] .Al-Salihi *et al.*, found that PCOS patients had soaring LH, but FSH levels were lesser than those of the healthy group [14] .For the same reason Fakhoury *et al.*, also found that the FSH level of PCOS patients in Saudi Arabia was lower than that of the control group [15], this is compatible with previous research [12, 15, 16], and our research. Research shows that Preptin may be related to diabetes. So far, rarely published studies possess focused on the importance of Preptin in humans, and only patient studies have been conducted. Celik *et al.* reported that the serum Preptin level of patients with PCOS is higher than that of patients without PCOS, which indicates that preptin is related to the pathogenesis of PCOS [17], which is consistent with our study. Recently, the role of serum CCL 18 level as a marker of white adipose tissue inflammation has become increasingly important. When reviewing the literature, Justin's work was the first study to investigate the relationship between serum CCL 18 levels and insulin in PCOS. In our study, in women with PCOS, the serum CCL18 level was higher than the control in this protocol [18, 19]. This is the first study to evaluate serum CCL 18 PCOS levels in Iraqi women. Previous studies have confirmed that prolactin levels in PCOS patients are elevated [20], which is consistent with our findings. PCOS is related to an enlarge danger of infection endometrial cancer. There is a lot of confirmation to support the theory of the relationship between prolactin and its receptor (PRLR) in the evolution of cancer cells [21]. Research administered by Paulson *et al.*, [21]has tested the expression of PRLR mRNA, and then detected PRLR on different days of the cycle by immunostaining, and performed this on both tubby and typical-weight women with PCOSItem measurement. The obese people experienced 3 months of lifestyle intervention in the PCOS group. Before the intrusion, the expression level of PRLR gene in the control group and PCOS tubby women was lower than the normal weight women. When they compared the changes within the cycle, there were no significant differences between cycles in obese women with PCOS. In the case of immune staining at the secretion stage of PRLR, it was found to be related to Ki67, which is a protein secreted in cells during cell preparation and can divide into new cells. Based on these results, it was suggested that teeny-interval lifestyle disturbances

can improve ovulation without affecting PRLR expression. In previous studies, both PCOS and non-PCOS prolactin levels changed during the day and the normal weight was higher than overweight and tubby women. Moreover, after the regression test, it was found that the change in the daytime was positively correlated with the coexistence of TSH concentration and PCOS, and was inversely proportional to BMI [22].

Conclusion:

It is possible to consider Preptin and CCL18 levels as a PCOS marker. Finally, PRLR gene expression levels in PCOS patients significantly increased. There is significant positive correlation between serum prolactin and PRLP gene expression, and we were found that a small amount of FSH levels leads to impaired follicular growth, while elevated LH levels lead to increased ovarian androgen production.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' contributions:

I.F., A.SH., and H.I. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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مصل البرولاكتين ، البربتين ، CCL 18 والتعدد الأشكال الجينية لدى النساء العراقيات المصابات بمتلازمة تكيس المبايض

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الخلاصة:
تعتبر متلازمة تكيس المبايض من اضطرابات الغدد الصماء وهي أكثر الاضطرابات انتشارا بين النساء وأحد الأسباب الرئيسية لضعف الخصوبة عند النساء .أجريت الدراسة على 100 امرأة عراقية (50 مريضة تم تشخيصهن بمرض متلازمة تكيس المبايض و 50 عينة من نسوة صحيحات تم الحصول على عينات الدم من المعهد المتقدم لتشخيص العقم والتكنولوجيا الإنجابية / جامعة النهرين ومختبر خاص بين تشرين الاول 2019 واذار 2020. تم تقييم المعلمات البيوكيميائية لـAFIAS-6و Preptin و FSH والأنسولين و LH و CCL 18 في عينات المصل بواسطة التحاليل المناعية المرتبطة (ELISA).أظهرت نتائج الدراسة أن قيم البرولاكتين (نانوغرام / مل, LH)(mlU / مل) ، بريتين (بيكوغرام / مل) و18 CCL(نانوغرام / مل) كانت أعلى بشكل ملحوظ في متلازمة تكيس المبايض مقارنة بمجموعة التحكم p). (0.001>كانت قيم هرمون التستوستيرون (نانوغرام / مل) و FSH (mIU / ml) أعلى بشكل ملحوظ (p <0.05) ، وزادت مستويات التعبير الجيني PRLR في مرضى متلازمة تكيس المبايض بشكل ملحوظ بمقدار 3. 6مرات.نستنتج من ذلك ان من الممكن اعتبار مستويات البريتين و CCL18 كواسم لـمتلازمة تكيس المبايض.

الكلمات المفتاحية : جين CCL 18 ، الهرمون المحفز للجريب (FSH) ، الانسولين ، هرمون اللوتين (LH), متلازمة تكيس المبايض ، هرمون البريتين .