# Assessment Adipokines gene expression and serum level of *Chemerin* in a sample of insulin resistance polycystic ovary Iraqi women

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**Abstract**
The aim of this study is to detect the relation between Chemerin gene and polycystic ovary Iraqi women (PCOs), One hundred blood samples were taken, fifty samples were diagnosed as polycystic ovary cases, and the other fifty cases were healthy women, These samples were divided into two groups: the PCOs as patients group and the healthy people as a control group. Next the biochemical test were done for all samples such as blood glucose levels, insulin level lipid profile, Testosterone, E2, FSH , LH and Measurement of Chemerin level by ELISA. Then RNA extracted and converted to cDNA to detection gene expression for Chemerin gene by real time PCR. The data were then analyzed with Statistical Analysis System- SAS (2012) to detect the effect of difference factors in study parameters, Results. The women with PCOS showed significantly higher expression and level of Serum compared to healthy control women.
Keywords: *Chemerin* gene, gene expression, cDNA, polycystic ovary

## 1. Introduction

Polycystic Ovary Syndrome (PCOS) is the most frequent heterogeneous complicated endocrine condition is . It affects between 6% and 13% of women of reproductive age and is one of the leading causes of female infertility 1. It was first defined as a link between anovulation and clinical or biological hyperandrogenism (1990 National Institutes of Health-Sponsored conference). Polycystic ovaries on ultrasound (equivalent to a follicle number per ovary of _12 and/or an ovarian volume of _10 mL) were established as an additional, not essential, diagnosis in 2003 by the Rotterdam Consensus Conference. 2. Thus As a result, the presence of at least two of the following three criteria is now required for PCOs diagnosis: The morphology of polycystic ovaries (PCOM), oligo/anovulation, and hyperandrogenism3. The patients affected by this syndrome may show an increased prevalence of obesity, insulin resistance, hypertension, dyslipidemia, metabolic syndrome, insulin tolerance, diabetes mellitus, gestational diabete smellitus, hyperandrogensim (hirsutism, acne, and alopecia), oligomenorrhea, amenorrhea, anovulation and infertility,high BMI (weight gain), and high LH:FSH ratio. . Diagnosis is made by physical and medical history and blood tests to check the hormones levels and ultrasound 4. In PCOS, it has been postulated that adipose tissue malfunction, presumably caused by in utero androgen hyper exposure and resulting to large visceral fat depots, may play a major role in regulating both IR and androgen metabolism. 5 White adipose tissue is generally can act as a metabolically active tissue capable to synthesizing and secreting a variety of endocrine chemicals known as adipokines 6. In last years, there has been a surge of interest in the role of these molecules in human fertility. Leptin's role in the interplay between energy metabolism and the reproductive system is nowadays extensively admitted. 7. Several more adipokines have recently been shown to play a role in female reproductive function, mainly ovarian physiology. 8. Chemerin and its receptor Chemerin chemokine-Like Receptor 1 (CMKLR1) Indeed, in vitro investigations demonstrated that 9,are expressed at both mRNA and protein levels in human granulosa cells (GCs). Furthermore, several of these adipokines have been linked to human ovarian follicle activity and steroidgenesis regulation10.

## 2. Materials and Methods

### Samples

The sample collected from several Hospitals and laboratories in the city of Baghdad the total numbers of samples are100 include.
The patients group: 50 patients were diagnostic as (PCOS) according to WHO diagnostic standard, the cases were excluded auto thyroid disorders or other endocrine or chronic disease based on clinical examination and family history
The control group: 50 healthy individuals were randomly selected from the people whose do not have (PCOS) disease and medical history and their age was ranged from (21-38) years and their weight from (60-130) Kg.

### Biochemical test

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Taken the serum of all samples in order to made biochemical tests like such as insulin level, blood glucose levels, lipid profile, E2, Testosterone, FSH , LH.
Measurement of Chemerin level by ELISA
Serum of all samples was Taken in order to Measurement of Chemerin level by ELISA protocol was taken from / sun long/ China Company
Genomic RNA extraction:
RNA was extraction from fresh as well as frozen blood samples that collected in TransZol
tube extraction.
cDNA synthesis form mRNA
Total RNA was reversely transcribed to complementary DNA(c DNA) using Easy Script tow-Step g DNA Removal and c DNA Synthesis Super Mix. protocol was taken from Applied Bio systems Company

The thermal cycler was programmed by using the conditions shown in table (1).

Table (1). Thermal Cycler Steps of Conditions cDNA Reverse Transcription.

| Stage 3 | Stage 2 | Stage 1 | |
|---|---|---|---|
| 85°C | 37°C | 25°C | Temperature |
| 5 min | 120min | 10 min | Time |

## Detection of *Chemerin* gene expression

Primers were synthesized by Macrogen Company as a lyophilized product of different pico moles concentrations. According to the instructions of the manufacturing company, the primers dissolved in a free nuclease water to provide a final concentration of100 pmol/µl

Table (2): Sequences of Primers used for the *Chemerin and B-actin* Expression

| primer | Sequence (5' →3' direction) |
|---|---|
| Chemerin gene expression | |
| Forward | 5'- GCCCTGGAGGAATTTCACAAG -3' |
| Reverse | 5'- GACTTTGCACTCGGGTTTCTT -3' |
| B-actin housekeeping gene (control) | |
| Forward | CCAGAGGCGTACAGGGATAG |
| Reverse | CCAACCGCGAGAAGATGA |

## Real-time PCR run

The expression level of Chemerin gene was evaluate by (qRT-PCR). To verify the expression of the target gene, quantitative real time (qRT-PCR) SYBR Green assay was used The mRNA levels of endogenous control gene B-Actin (reference gene) were amplified and used to normalize the mRNA levels of the Chemerin. B-Actin primers were prepared according to Thiele et al.,( 2012) synthesized by Macrogen and stored lyophilized until use.

QRT-PCR was performed by using the QIAGEN Real-time PCR System with qPCRsoft software (Rotor-Gene Germany). By detecting the threshold cycle (Ct) using the 2xqPCR Master Mix Kits components, the gene expression levels and fold change were estimated.
The cycling protocol was established according to the thermal profile in table (3) shown for Chemerin and B-Actin gene

Table (3): Thermal profile of *Chemerin* , *Omentin* and *B-Actin* gene expression

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial Denaturation | 95°C | 8 min | 1 |
| Denature | 95°C | 20 sec | 40 |
| Anneal/extend | 60°C | 50 sec | |
| Melting curve analysis | 65-95°C | sec/step | 1 |

# 3. Results and Discussion

The relationship of parameters Age and BMI between PCOS patients and apparently healthy control groups
This study found that the age (year) is non-significant in each of patients and control group (31.04 ±0.55vs. 29.64 ±1.03respectively) these result obtained because of the pick of age group

Women with polycystic ovary syndrome have an overabundance of androgens, which can contribute to increased visceral and subcutaneous fat, as well as appetite disorders., so obesity is more common among( PCOS) women. (Baldani et al., 2015).BMI (kg/m2) was analyzed as 30.50 ±0.64for women with PCOS and 24.67 ±0.75 for apparently healthy control with high significant (P< 0.01) Table(4)contains the results.

Table (4): Comparison between patients and control groups in Age and Body Mass Index( BMI)

| Group | Mean ± SE | |
|---|---|---|
| | Age (year) | BMI (kg/m2) |
| Patients | 31.04 ±0.55 | 30.50 ±0.64 |
| Control | 29.64 ±1.03 | 24.67 ±0.75 |
| T-test | 2.130 NS | 2.097 ** |
| P-value | 0.194 | 0.0001 |
| **: Highly Significant (P≤0.01), NS: Non-Significant. | | |

## Biochemical Results

### Insulin level and Fasting glucose

The results of this study showed the women with PCOS compared to healthy control women showed Highly significant increase in Fasting glucose (inPCOs 105.77 ±2.04 vs in control 90.06 ±0.92; P≤0.01) respectively and Insulin level compared to healthy control women (inPCOs 13.41 ±0.12vs. in control 8.38 ±0.30; P≤0.01) results of study shown in table (5) .

| table (5) Comparison between patients and control groups in Insulin level and Fasting glucose | | |
|---|---|---|
| Group | Mean ± SE | |
| | Insulin level (ulu/ml) | FBS: Fasting glucose (mg/dl) |
| Patients | 13.41 ±0.12 | 105.77 ±2.04 |
| Control | 8.38 ±0.30 | 90.06 ±0.92 |
| T-test | 0.558 ** | 5.915 ** |
| P-value | 0.0001 | 0.0001 |
| **: Highly Significant (P≤0.01). | | |

## Lipid profile

The study reveals that the cholesterol in women with PCOS and apparently healthy control were (183.11 ±3.33and 173.22 ±2.69 respectively) with non-significant ,and the results of low-density lipoprotein (VLDL showed that the women with PCOS and apparently healthy control were(20.79±0.78 and19.0 ±1.13mg/ml respectively) with significant (P≤0.05), While the PCOS women have High significant increase in serum Triglyceride (in PCOS 137.50 ±4. vs in control 109.71 ±7.81;P≤0.01) and significant increase low-density lipoproteins (LDL) were(in PCOS 117.35 ±3.31 vs in control 106.01 ±4.23; P<0.05). Table(6)contains the results.

| Table (6): A comparison between PCOS patients and apparently healthy control in lipid profile tests | | | | | |
|---|---|---|---|---|---|
| Group | Mean ± SE (mg\dl) | | | | |
| | Cholesterol | Triglyceride | HDL | LDL | VLDL |
| Patients | 183.11 ±3.33 | 137.50 ±4.38 | 41.42 ±0.67 | 117.35 ±3.31 | 20.79 ±0.78 |
| Control | 173.22 ±2.69 | 109.71 ±7.81 | 46.81 ±1.04 | 106.01 ±4.23 | 19.03 ±1.13 |
| T-test | 10.155 NS | 16.52 ** | 2.382 ** | 11.075 * | 2.714 NS |
| P-value | 0.0561 | 0.0013 | 0.0001 | 0.0450 | 0.203 |
| *: Significant (P≤0.05), **: Highly Significant (P≤0.01), NS: Non-Significant. | | | | | |

## Hormonal Results

The women with PCOS compared to healthy women showed significant increase in LH (7.52 ±0.37vs. 5.21 ±0.32; P≤0.01) respectively and Testosterone level (0.951 ±0.05vs. 0.615 ±0.04; P≤0.01) respectively While showed Non-Significant decreasing in the FSH level (in PCOs 6.33 ±0.19vs in control 6.84 ±0.35) respectively and Non-Significant increase in E2 level (inPCOs 53.55 ±3.81vs in control 49.57 ±4.05) respectively The results shown in table (7).

| Table (7): Comparison between difference groups in Hormones level | | | | |
|---|---|---|---|---|
| Group | Mean ± SE | | | |
| | LH (mlu/ml ) | FSH (mlu/ml ) | Testosterone (ng/ml) | E2 (pg/ml) |
| Patients | 7.52 ±0.37 | 6.33 ±0.19 | 0.951 ±0.05 | 53.55 ±3.81 |
| Control | 5.21 ±0.32 | 6.84 ±0.35 | 0.615 ±0.04 | 49.57 ±4.05 |
| T-test | 1.164 ** | 0.755 NS | 0.153 ** | 12.204 NS |
| P-value | 0.0002 | 0.179 | 0.0001 | 0.517 |
| **: Highly Significant (P≤0.01), NS: Non-Significant. | | | | |

## Measurement of Chemerin level by ELISA

Serum Chemerin levels were significantly higher in PCOS women than in control(inPCOs 1285.56 ±188.54 vs. in control. 366.63 ±18.28; P≤0.01) respectively The results shown in table (8). Figures (1) exhibit the Standard curve of Chemerin level by ELISA.

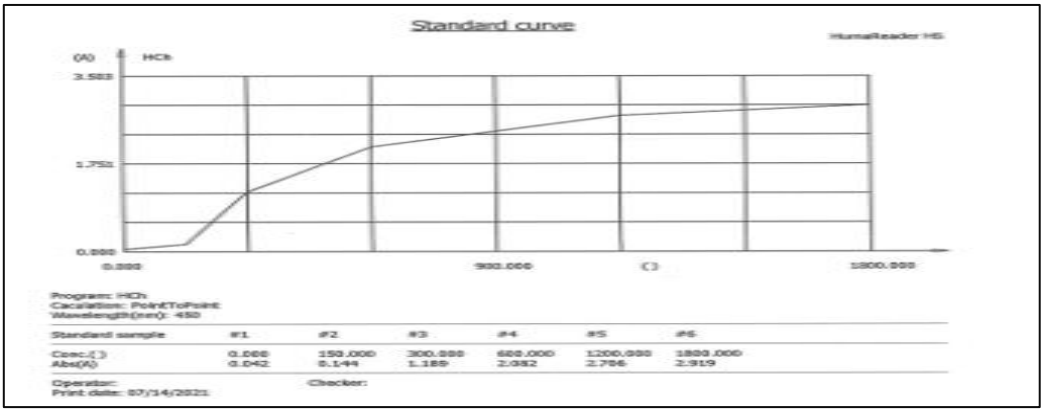

*Figure (1): Standard curve of Elisa*

| Table 8: Comparison between patients and control groups in Chemerin serum level | |
|---|---|
| Group | Mean ± SE |
| | Chemerin ELISA |
| Patients | 1285.56 ±188.54 |
| Control | 366.63 ±18.28 |
| T-test | 533.84 ** |
| P-value | 0.0010 |
| **: Highly Significant (P≤0.01). | |

## Results of Quantitative Real Time PCR

In the present experiment Real time PCR quantification applied utilized the SYBR green a fluorescent dye which distinguish any double stranded DNA including (cDNA)the amplification was taped as a Ct value (cycle threshold). The lower Ct value signified the inclusion of higher copies of the target and vise – versa, When it regards to gene expression, high Ct values denote low gene expression, whereas low Ct values imply strong gene expression. 11.

## Fold expression of *Chemerin* gene

In the present research work, quantitative RT-PCR test evaluated the mRNA expression of Chemerin and compared its expression between PCOS women to perfectly healthy control groups. The fold change in gene expression was calculated using relative quantification (Livak and Schmittgen, 2008). Folds of Chemerin gene expression depending on 2 -ΔΔCt method This depends on normalization of Ct values calculating the ΔCt which is the difference between the mean Ct values of replica of Chemerin cDNA amplification of each single case and that of the ß-actin and calculate the ΔΔCt then 2 -ΔΔCt .

In calculation of the relative expression of Chemerin gene in all study groups the 2-ΔΔCt results was applied. A calibrator was used and the expression of Chemerin as shown in table (9) the mean of 2-ΔΔCt values of women with PCOS was (38.30 ±6.20) and for apparently healthy control was (1.678 ±0.51). When the gene expression was calculated, it was found to be considerably greater in women with PCOS than in appeared to be healthy. Chemerin expression was higher in obese PCOS women than in normal-weight women, and it was significantly associated with BMI. 12. Figures (2,3) exhibit the amplification plots and dissociation curves for Chemerin.

| Table (9). Fold of Chemerin gene expression depending on 2 -ΔΔCt method in studied groups. (mean ± SD) | | | | | |
|---|---|---|---|---|---|
| groups | Means Ct of *B-actin* | Means Ct of Chemerin | ΔCt | ΔΔCt | Fold of gene expression 2^- ΔΔCt |
| Patient | 19.23632 | 25.39316 | 6.156842 | -4.78043 | 38.30 ±6.20 |
| Control | 18.65182 | 29.58909 | 10.93727 | 2.72727E-06 | 1.678 ±0.51 |
| T-test | --- | --- | --- | --- | 16.857 ** |
| P-value | --- | --- | --- | --- | 0.0001 |
| *: Significant (P≤0.05), **: Highly Significant (P≤0.01). | | | | | |

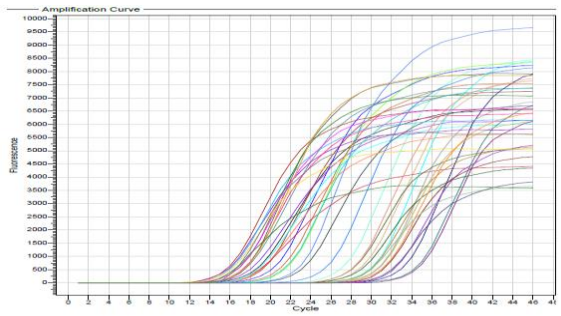


*Figure (2): Chemerin amplification plots by qPCR Samples included all study groups. The photograph was taken directly from (Rotor-Gene qPCR machine).*

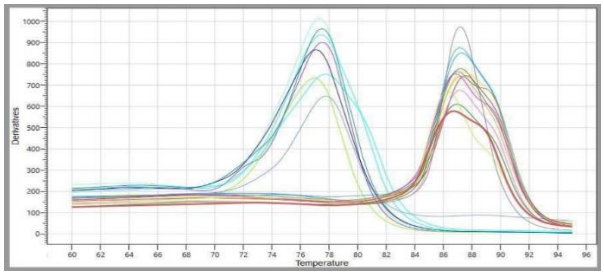


*Figure (3): Chemerin dissociation curves by qPCR Samples included all study groups. Melting temperature ranged from 55°C to 96°C. The photograph was taken directly from Rotor-Gene qPCR machine.*

## 4. Conclusions

According to the findings, this study PCOs can be characterized as a complicated, heterogeneous metabolic syndrome produced by the interaction of genetic and epigenetic causes. High expression values and serum level of Chemerin in women with PCOS Although it is not a feature of PCOS, it might be a valuable monitoring indicator for PCOS treatmen. The results shown that women with PCOS have altered Hormones and lipid profile, with normal cholesterol and vLDL levels , higher Triglyceride and LDL levels and lower HDL cholesterol compared with healthy women.

## Reference

Teede, Helena, Amanda Deeks, and Lisa Moran. "Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the

lifespan." BMC medicine 8, no. 1 (2010): 1-10.
Bongrani, Alice, Namya Mellouk, Christelle Rame, Marion Cornuau, Fabrice Guérif, Pascal Froment, and Joëlle Dupont. "Ovarian expression of adipokines in polycystic ovary syndrome: a role for chemerin, omentin, and apelin in follicular growth arrest and ovulatory dysfunction?." International journal of molecular sciences 20, no. 15 (2019): 3778.
Teede, Helena J., Marie L. Misso, Michael F. Costello, Anuja Dokras, Joop Laven, Lisa Moran, Terhi Piltonen, and Robert J. Norman. "Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome." Human reproduction 33, no. 9 (2018): 1602-1618.
Saleem, Fatima, and Syed W. Rizvi. "New therapeutic approaches in obesity and metabolic syndrome associated with polycystic ovary syndrome." Cureus 9, no. 11 (2017).
Dumesic, Daniel A., Sharon E. Oberfield, Elisabet Stener-Victorin, John C. Marshall, Joop S. Laven, and Richard S. Legro. "Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome." Endocrine reviews 36, no. 5 (2015): 487-525.
Ohashi, Koji, Rei Shibata, Toyoaki Murohara, and Noriyuki Ouchi. "Role of anti-inflammatory adipokines in obesity-related diseases." Trends in endocrinology & metabolism 25, no. 7 (2014): 348-355.
Liew, Fong Fong, Sulagna Dutta, Pallav Sengupta, and Bhupender S. Chhikara. "Chemerin and male reproduction:'a tangled rope'connecting metabolism and inflammation." Chemical Biology Letters 8, no. 4 (2021): 224-237.
Wang, Xueying, Xiaofei Liu, Zifan Song, Xin Shen, Siying Lu, Yan Ling, and Haibin Kuang. "Emerging roles of APLN and APELA in the physiology and pathology of the female reproductive system." PeerJ 8 (2020): e10245.
Estienne, Anthony, Namya Mellouk, Alice Bongrani, Ingrid Plotton, Ingrid Langer, Christelle Ramé, Claire Petit, Fabrice Guérif, Pascal Froment, and Joëlle Dupont. "Involvement of chemerin and CMKLR1 in the progesterone decrease by PCOS granulosa cells." Reproduction 162, no. 6 (2021): 427-436.
Roche, Jennifer, Christelle Ramé, Maxime Reverchon, Namya Mellouk, Marion Cornuau, Fabrice Guerif, Pascal Froment, and Joëlle Dupont. "Apelin (APLN) and apelin receptor (APLNR) in human ovary: expression, signaling, and regulation of steroidogenesis in primary human luteinized granulosa cells." Biology of Reproduction 95, no. 5 (2016): 104-1.
Schmittgen, Thomas D., and Kenneth J. Livak. "Analyzing real-time PCR data by the comparative CT method." Nature protocols 3, no. 6 (2008): 1101-1108.
Bongrani, Alice, Namya Mellouk, Christelle Rame, Marion Cornuau, Fabrice Guérif, Pascal Froment, and Joëlle Dupont. "Ovarian expression of adipokines in polycystic ovary syndrome: a role for chemerin, omentin, and apelin in follicular growth arrest and ovulatory dysfunction?." International journal of molecular sciences 20, no. 15 (2019): 3778.