The Relationship of Urocortin 2 with Insulin Resistance, Clinical, and Biochemical Hyperandrogenism in Women with Polycystic Ovary Syndrome

Alia Jasim Mohamad Ali, Najah Shaker Yassen***

ABSTRACT:

BACKGROUND:

Polycystic ovary syndrome is the most common endocrine and metabolic disorder in women. Urocotin-2 is thought to be involved in the pathophysiology of Polycystic ovary syndrome by paracrine and autocrine pathways.

OBJECTIVE:

To assess the Urocortin-2 level in women with Polycystic ovary syndrome and to assess the relation of urocotin-2 with insulin resistance, clinical and biochemical hyperanderogenism.

PATIENTS AND METHOD:

a case control study that involved; forty-five polycystic ovary patients according to Rotterdam criteria as a case group, and forty-five healthy women as a control group. Ferriman-Gallwey score was used to evaluate hirsutism, in addition to measurement of Body mass index and Waist/Hip ratio, levels of: Follicle stimulating hormone, luteinizing hormone, Prolactin, testosterone, insulin, fasting blood sugar, homeostatic model assessment of insulin resistance and urocotine-2 also assessed.

RESULT:

urocotin-2 was significantly higher in Polycystic ovary syndrome compared to control women using Receiver Operating Characteristic Curve analysis.

CONCLUSION:

Urocortin-2 may have a role in the pathogenesis of Polycystic ovary syndrome for its correlation with testosterone, homeostatic model assessment of insulin resistance, serum insulin, and Body Mass Index, since there was higher levels than that of normal women.

KEYWORDS: Polycystic ovary syndrome, urocortin-2, Insulin resistance.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is an exceptionally common ýdisorder of premenopausal women, its etiology remains unknown. [1] The diagnosis of polycystic ovary syndrome is usually made on the basis of a combination of clinical, ultrasonographic, and biochemical criteria [2]**.** Urocortin-2 (UCN-2) is a protein that in humans is encoded by the UCN gene, located in chromosome 2 (band: 2p23.3) [(3)]. Urocortin belongs to the corticotropin-releasing hormone (CRH) family of proteins which includes CRH, urocortin I, sauvagine, urocortin II and urocortin III peptides, are emerging as important central and peripheral regulators of ýenergy homeostasis and metabolic functions.

The CRH/UCN gene family members share similar genomic organization and sequence homology. like CRH , all the three UCN gene contain tow axon and single intron varying greatly in size from 200 to 8000bp in human UCN and their rodent orthologs' [(4)]

The insulin resistance can be seen in patients with PCOS and in these patients, the risk of type 2 diabetes is considerably higher than healthy woman. The levels of CRFR2 were found higher in insulin resistant muscle cells [(5)]. An increased level of testosterone and follicular atresia is generally associated with PCOS, UCN2 was shown to prevent estrogenic transformation of androgen by disrupting aromatization in mice ovaries resulting with a decrease in estradiol levels. In addition to that, it is reported that UCN2 damages ovulation by effecting ovarian folliculogenesis in mice. UCN2 may have an effect as a CRF antagonist [(5)].

*Al-Alawia Teaching Hospital/Baghdad, Iraq
**Baghdad Teaching Hospital /Baghdad, Iraq

AIM OF THE STUDY:

To assess Urocortin-2 level in women with PCOS and to assess the relation of urocotin-2 with insulin resistance, clinical and biochemical hyperandrogenism in PCOS patient.

PATIENTS AND METHODS:

A case control study had been carried out in Baghdad Teaching hospital. The data collection phase extends over a period of 8 months from March to November 2018**.** The study included 45 women with PCOS and 45 women without PCOS criteria served as normal control. They are either a companion of patients, medical and paramedical staff volunteers to enter the study.

A full history and clinical examination were done to both groups. Measurement of: weight (Kg), Height (cm) to calculate the BMI which universally expressed in units of kg/m2, resulting from mass in kilograms and height in meters. Waist circumference (cm), Hip circumference (cm) to measure Waist/Hip ratio. Modified Ferriman-Gallwey score was used to evaluate hirsutism. Pelvic Ultra sound was done for all woman with PCOS & control group by the same ultrasound doctor of the outpatient clinic the device used (HD11 philips with the resolution of probe 3.45 MH & of 220-230 voltage). Both groups exposed to the following lab investigations: 5cc of blood was taken, at the 2-3 day of menstrual cycle (induce withdrawal bleeding in case of amenorrhea) in fasting state (about 12 hours fasting) FSH, LH, total Testosterone, prolactin, estradiol, DEHEA, SHBG TSH, T4, T3FBS, Insulin and Urocortin-2. That sample had been centrifuged to collect the serum, through which part of it used for analysis of biochemical test listed above and part (2cc) were frizzed at -20 temperature centrifuge the sample again after thawing before assay (avoiding repeated freeze thaw cycles) to be used for Urocortin-2 assessment by using the Kit (normal value is 108-120 ng/dl), the laboratory kit provided from (Shanghai Yehua Biological Technology Co. Ltd.) with kit number (YHB3163Hu), the procedure uses ELISA assay in which the sera of patients bind to monoclonal antibodies for (UCN2).

- Insulin blood samples was stored after the serum was separated within no more than 5 h, and then stored in a refrigerator for up to 24 h.

Insulin blood samples was stored after the serum was separated within no more than 5 h, and then stored in a refrigerator for up to 24 h. Insulin was measured by immunoassay. The laboratory kit for insulin provided from (Demeditec/Germany.) with kit number (DE2935) enzyme immunoassay for the quantitative measurement of Insulin in serum and plasma. Chemical methods for blood glucose assay relied upon stages involving enzymes (e.g. glucose oxidase, glucose dehydrogenase) linked to chromogenic reactions or to reactions featuring changes in electron flow that can be measured by suitable electronic meters.

- HOMA-IR stands for Homeostatic Model Assessment of Insulin Resistance It is a method used to quantify insulin resistance HOMA-IR = (insulin * glucose) / 405 for glycemia in mg/dL the insulin is in mU/L

The ethical clearance was obtained from Baghdad teaching hospital and management directorate to perform the study. An informed verbal consent also was taken from all participants. The forma of research proposal had been proved by the higher ethical committee of research in the hospital directorate to do laboratory investigations in the laboratories of Baghdad Teaching hospital.

Statistical analysis

Discrete variables presented using there number and percentage, Two samples t test used to analyze the differences in means between two groups (if ýboth follow normal distribution with no significant outlier). Binary logistic regression analysis used to calculate the odd ratio (OR) and their 95% confidence intervals, when the outcome can be categorized into 2 binary levels, and if appropriate probability plot used to present the relationship. Linear regression analysis performed to assess the relationship between different variablesý, r (correlation coefficient or standardized beta is a representative of magnitude and direction of the relationship), 0.00-0.29 = little or no correlation; 0.30-0.49 = weak; 0.50-0.69 = moderate; 0.70-0.89 = strong; and 0.90-1.00 = very strong. Negative sign indicate inverse relationship, but positive sign represent direct relationship. Receiver operator curve used to see the validity of different

parameters in separating active cases from control (negative cases) and area under the curve i.e. AUC and its p value prescribe this validity (if AUC ≥ 0.9 mean excellent test, 0.8 – ٠.89 means good test, 0.7 – 0.79 fair test otherwise unacceptable). Trapezoidal method used for calculate the curve. Sensitivity: Probability that a test result will be positive when the disease is present (true positive rate).

RESULTS:

There was no statistically significant difference in age between both studied groups (P-value = 0.056). The mean BMI of the PCOS cases was statistically significant higher than that of controls. (P= 0.001). The mean W/H ratio of the PCOS cases was significantly higher than controls.(P = 0.001).as illustrated in table 1

Table 1: Demographic parameters of PCOS and control groups.

Group	PCOS	Control	p-value
Number	45	45	
Age (years), mean ± SD	26.22±5.60	28.73±6.64	0.056
BMI (kg/m^2), mean ± SD	30.77±5.92	24.48±1.25	<0.001 [S]
Waist: hip ratio, mean ± SD	0.88±0.06	0.79±0.02	<0.001 [S]
SD: standard deviation; PCOS: polycystic ovary syndrome; BMI: body mass index			

Fasting blood sugar, insulin and HOMA-IR were significantly higher in PCOS patients compared to control as illustrated in table 2. (P = 0.003). The mean Insulin of the PCOS cases was significantly higher than controls (P =< 0.001). The mean HOMA-IR of the PCOS cases was significantly higher than controls (P = 0.003) as illustrated in table 2.

Table 2: Biochemical assay of PCOS cases and control group.

Group	PCOS	Control	p-value
Number	45	45	-
FBS (mg/dL), mean ± SD	93.56±15.05	84.56±13.24	0.003 [S]
Insulin (IU/ml), mean ± SD	16.16±3.29	8.62±3.97	<0.001 [S]
HOMA-IR, mean ± SD	5.09±2.09	3.83±1.78	0.003 [S]
FBS: fasting blood sugar; HOMA-IR: homeostasis model assessment of insulin resistance; PCOS: polycystic ovary syndrome			

The mean level of Urcortin-2, was statistically higher in PCOS cases than control group (<P=0.001), as illustrated in table 3 and figure 1

Table 3: Level of Urocortin 2 in PCOS and control groups.

Group	PCOS	Control	p-value
Number	45	45	-
Urocortin 2 (pg/ml), mean ± SD	144.11±19.18	83.84±21.52	<0.001 [S]
PCOS: polycystic ovary syndrome			

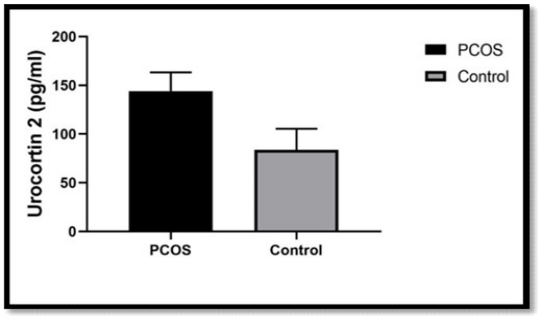

Figure 1: Histogram that demonstrates serum levels of urocortin 2 (ng/dl) in both PCOS patients & controls.

Urocortin-2 had excellent ability, with optimal cut point of equal 112 from Groups, in table 4, 5 and figure 2

Table 4: ROC analysis of Urocortin ý2ý as predictor of PCOS.

AUC (95%CI)	p-value	Cut-off	+LH	-LH
0.989 (0.940-1.0)	<0.001	≥112	11.25	0
AUC: area under the curve, LH: likelihood ratio, CI: confidence interval				

Table 5: Clinical validity of Urocortin 2 for predicting PCOS.

SN	SP	Accuracy	PPV	NPV
100%	91.1%	95.6%	91.8%	100%
SN: sensitivity, SP: specificity, AC: accuracy, PPV: positive ýpredictive value, NPV: negative predictive value				

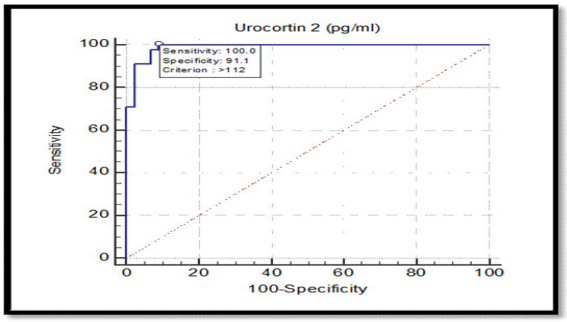

Figure 2: Receiver Operating Characteristic curve (ROC curve) was used to determine the cut-off value of urocortin 2

Significant correlation between Urocortin-2 and insulin level and HOMA-IR calculated index in PCOS group (P-values < 0.001, < 0.003) respectively table 6.

Table 6: Correlation between Urocortin 2 and insulin, HOMA-IR in PCOS group.

Variables	Urocortin 2	
	PCOS	
	(r)	p-value
FBS (mg/dL)	0.231	0.147
Insulin (IU/ml)	0.354	0.001 [S]
HOMA-IR	0.454	0.003 [S]
FBS: fasting blood sugar; HOMA-IR: homeostasis model assessment of insulin resistance; PCOS: polycystic ovary syndrome		

Significant correlation between Urocortin-2 and Testosterone in PCOS group (P- values <0.012) table 7.

Table 7: Correlation between Urocortin 2 with hormonal assay in PCOS.

Variables	Urocortin 2	
	PCOS	
	(r)	p-value
FSH	0.124	0.417
LH	-0.109	0.475
Prolactin	0.028	0.856
Estradiol	-0.103	0.501
Testosterone	0.342	0.012 [S]
PCOS: polycystic ovary syndrome; FSH: follicle stimulating hormone; LH: luteinizing hormone		

In multivariate analysis; UCN-2 was independently correlated with PCOS after excluding the effect of age, BMI and HOMA-IR. This model had overall coefficient of determination (which determine the overall ability of these variables: UCN-2, age, BMI, HOMA-IR, mFG score of hirsutism; to account for PCOS) had very high value = 0.014, indicating these variables explain 70% of the effect of UCN-2 on PCOS, as illustrated in table 8.

Table 8: Evaluation of the effect of age, BMI, HOMA-IR and mFG score on UNC-2 ýrelationship to predict PCOS.

Predictors	OR (95% CI)	p-value
Urocortin 2	1.293 (1.052-1.589)	0.014
Age	0.933 (0.744-1.170)	0.549
BMI	1.564 (0.803-3.047)	0.189
HOMA-IR	1.051 (0.583-1.892)	0.870
mFG	1.098 (0.971 – 1.345)	0.234
OR: odd ratio, CI: confidence interval. R^2 (Cox % Snell) = for multivariate analysis, Ferriman–Gallwey score.		

DISCUSSION:

In the current study 90 women equally distributed as PCOS and control groups, age match, there where statistically significant differences in BMI and waist to hip ratio, being higher for PCOS than control groups.

In the present study FBS, insulin and insulin resistance (measured using HOMA-IR a marker derived from both FBS and fasting insulin; [5.09±2.09 vs. 3.83±1.78]) was significantly higher in PCOS women, these findings were in agreement with multiple studies like Temur et al. [7], and in agreement with Ates et al with HOMA-IR p-value = 0.011) [8], and in agreement with Behboudi-Gandevani et al. [9].

These studies and the current findings indicate that PCOS is related to insulin resistance (IR) and an increased IR is present in PCOS. Ordinary obesity is the most common cause of insulin resistance. In the present study circulating urocortin 2 (UCN2) was significantly higher in PCOS patients compared to normal matched control p-value <0.001), this finding was in agreement with Temur et al: a Turkish study examined 38 PCOS patients and compared to control; in this study circulating UCN2 also found to be significantly higher in PCOS compared to control, p-value = 0.002) [5], In present study after examining UCN2 using ROC analysis, UCN2 shows excellent ability to discriminate PCOS from control (since AUC >0.9), additionally at cut-off ≥112 pg/ml UCN2 shows very high sensitivity, NPV, and accuracy (100, 91.8% and 100%, respectively) with high specificity and PPV (91.1, and 91.8%, respectively). Unfortunately, no study available currently examined UCN2 using ROC analysis, this make these findings the first reported in the literature concerning this maker.

The current study shows a direct significant correlation between UCN2 and insulin, HOMA-IR (*r = 0.354, 0.284*, respectively), which is in agreement with Temur et al (*r = 0.295, 264,* respectively) [5], , In present, study Testosterone (TT) was significantly higher in PCOS, which was in agreement with Li et al [6], and in agreement with Chen et al [10], and in agreement with Termur et al [5]. While in Ates et al there was no significant difference between PCOS and control [8].

An increased level of testosterone and follicular atresia is generally associated with PCOS like disorder is shown in experimental studies by androgen application [11]. In the study of Spyroglou et al. [12], UCN2 was shown to prevent estrogenic transformation of androgen by disrupting aromatization in mice ovaries resulting with a decrease in estradiol levels. Using multivariate analysis UNC-2 was associated with PCOS independently (independent of the effect of age, BMI. HOMA-IR, and hirsutism) with OR (95%CI) = ý1.293 (1.052-1.589) *p-value* = 0.014, indicating the effect of UCN2 on PCOS pathogenesis is unrelated to age, obesity and insulin resistance, which is in agreement with both Temur studies involving UNC-2 and UNC-3 [5,7].

CONCLUSION AND RECOMMENDATIONS

Urocortin 2 may have a role in the pathogenesis of PCOS for its correlation with testosterone, HOMA-IR, serum insulin, and BMI. The serum levels of Urocortin 2 in PCOS were higher than normal control women. Urocortin-2 can be used as an excellent predictor for PCOS with high level of sensitivity and specificity.Alarge sample size may be required with testing the other member of urocortin-2 and measuring its level in the follicular fluid to get more information about the pathogenesis whether they react centrally or periphery at ovarian level.

REFERENCES:

1. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Human reproduction (Oxford, England). 2004;19:41-47.
2. Kashanian M, Fazy Z, Pirak A. Evaluation of the relationship between gestational diabetes and a history of polycystic ovarian syndrome. Diabetes Res Clin Pract. 2008;80:289-92.
3. Maia LM, Rocha AL, Del Puerto HL, Petraglia F, Reis FM. Plasma urocortin-1 as a preoperative marker of endometriosis in symptomatic women. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2018;34:202-5.
4. Sossey-Alaoui K, Head K, Nowak N, Cowell JK. Genomic organization and expression profile of the human and mouse WAVE gene family. Mammalian genome : official journal of the International Mammalian Genome Society. 2003;14:314-22.

5. Temur M, Yilmaz O, Aksun S, Calan M, Ozun Ozbay P, Kumbasar S, et al. The relationship of urocortin-2 with insulin resistance patients having PCOS. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2017;33:124-27.
6. Li L, Zhang F, Cui J, Shi Y, Xiang J, Wang X, et al. Association of betatrophin with metabolic characteristics in overweight/obese and lean women with PCOS. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2017;33:238-43.
7. Temur M, Yilmaz O, Aksun S, Ozun Ozbay P, Calan M, Kume T, et al. Increased circulating urocortin-3 levels is associated with polycystic ovary syndrome. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2016;32:218-22.
8. Ates S, Aydin S, Ozcan P, Soyman Z, Gokmen Karasu AF, Sevket O. Clinical and metabolic characteristics of Turkish adolescents with polycystic ovary syndrome. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 2018;38:236-40.
9. Behboudi-Gandevani S, Ramezani Tehrani F, Cheraghi L, Azizi F. Could "a body shape index" and "waist to height ratio" predict insulin resistance and metabolic syndrome in polycystic ovary syndrome? European journal of obstetrics, gynecology, and reproductive biology. 2016;205:110-4.
10. Chen X, Ni R, Mo Y, Li L, Yang D. Appropriate BMI levels for PCOS patients in Southern China. Human reproduction (Oxford, England). 2010;25:1295-302
11. Ikeda K, Baba T, Noguchi H, Nagasawa K, Endo T, Kiya T, et al. Excessive androgen exposure in female-to-male transsexual persons of reproductive age induces hyperplasia of the ovarian cortex and stroma but not polycystic ovary morphology. Human reproduction (Oxford, England). 2013;28:453-61.
12. Spyroglou A, Riester A, Mueller-Peltzer K, Lu A, Rohde J, Hantel C, et al. Adrenal and Ovarian Phenotype of a Tissue-Specific Urocortin 2-Overexpressing Mouse Model. Endocrinology. 2015;156:2646-56.