

## Cross-Talk between Apelin, Insulin Resistance, Thyroid Hormones, and Cardio-metabolic Risk Factors in Polycystic Ovary Syndrome

Ragaa Abdelshaheed Matta\*<sup>1</sup>, Ahmed Abdelfadel Saedii<sup>2</sup>

Departments of <sup>1</sup>Internal Medicine and <sup>2</sup>Clinical Pathology, Faculty of Medicine, Minia University, Minia, Egypt

\*Corresponding author: Ragaa Abdelshaheed Matta, Telephone: 01008792833, Email: ragaamatta2017@gmail.com, Ragaa Matta ORCID iD /<https://orcid.org/0000-0002-3516-2566>

### ABSTRACT

**Background:** Apelin and its receptor are located in hypothalamo-pituitary axis and inhibit TSH secretion. Insulin resistance (IR) is linked to apelin, thyroid function, and polycystic ovary syndrome (PCOS). Apelin levels in obese and non-obese PCOS women display contradictory results.

**Objective:** To compare serum apelin in IR PCOS and non-IR PCOS groups, evaluate the cross-talk of apelin, IR, thyroid function, and cardiovascular risk factors in both groups, and identify the diagnostic value of apelin for IR in PCOS.

**Patients and Methods:** The study included 60 young non-obese euthyroid PCOS women: IR PCOS (n=28) and non-IR PCOS (n=32), and age-matched healthy fertile groups. Serum apelin-36, calculated homeostatic model assessment-IR (HOMA-IR), and thyroid hormones were evaluated.

**Results:** IRPCOS had significantly higher apelin compared to non-IR PCOS and healthy control groups with insignificant differences between the two latter groups. The bivariate analysis demonstrated a significant positive correlation between apelin and glucose, insulin, HOMA-IR, atherogenic lipid profile, and TSH and a significant negative correlation with free T4 in IR PCOS while inverse relations were observed in non-IR PCOS. After controlling for HOMA-IR, only HDL in IR PCOS and TSH and cholesterol in non-IR PCOS preserved the significant correlations. Cut-off value  $\geq 39$  pg/dl of apelin showed high specificity (88%) and sensitivity (86%) to identify IR in PCOS

**Conclusion:** Serum apelin is higher in IR than in non-IR PCOS patients. The relations between apelin and thyroid function, and cardio-metabolic risk factors are IR dependent. Apelin is a marker of IR in PCOS and had a direct talk with TSH, HDL-c, and cholesterol.

**Keywords:** Apelin, TSH, HOMA-IR, polycystic ovary syndrome. Cardiovascular risk factors.

### INTRODUCTION

Polycystic ovary syndrome (PCOS) as the most common endocrinopathy in women is the main etiology of anovulatory infertility. Insulin resistance (IR) is common in women (50-80%) with PCOS, even if they are not obese. IR is linked to reproductive and metabolic abnormalities, dysfunctional adipokine secretion, and high normal thyroid-stimulating level (TSH) in PCOS. IR and hyperinsulinemia are associated with low-grade chronic inflammation and increased risk of hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM). TSH level even in euthyroid PCOS women is linked to insulin resistance and traditional cardiovascular risk factors<sup>(1-3)</sup>.

Apelin is an endogenous peptide and acts through its G protein-coupled receptor (APJ). Apelin-APJ system is expressed in a range of body tissues including adipose tissue predominantly, hypothalamus-pituitary axis, ovary, heart, blood vessels, liver, skeletal muscle, and kidney. It is an adipokine and linked to obesity and IR. Similar to adiponectin, it stimulates glucose utilization, decreases insulin secretion, and negatively regulates catecholamine-mediated lipolysis. Apelin synthesis in adipocytes is stimulated by insulin, and plasma apelin levels are markedly increased under IR states such as obesity and T2DM, and metabolic syndrome<sup>(4)</sup>.

Local ovarian expression of apelin-APJ system is significantly increased with ovarian follicle size,

decreased at the late luteal phase, and declined sharply during corpus luteum (CL) regression. Apelin-APJ system is involved in CL formation, function, and regression during the menstrual cycle. Notably, progesterone stimulates the APJ expression in granulosa cells. Furthermore, apelin may participate in PCOS pathogenesis<sup>(5)</sup>. Reports regarding serum apelin levels in PCOS are controversial as elevated or decreased levels were reported in previous studies<sup>(6-9)</sup>. Up to date, most of these studies classified PCOS women into obese and non-obese groups and not upon IR and non-IR despite more accurately of later classification.

Apelin also plays an important role in the hypothalamic regulation of water and food intake and the endocrine axis. Apelin decreases TSH secretion by the pituitary gland<sup>(10)</sup>. In euthyroid and hypothyroid rats, apelin administration produces a mild and marked reduction in TSH levels respectively probably through the APJ receptor in the hypothalamo-pituitary axis. Apelin administration enhances thyroxin catabolism and subsequent reduction in its level in the hypothyroid rat. In addition, the apelin level is elevated in propylthiouracil-induced hypothyroid rats<sup>(10,11)</sup>. So, the relation between thyroid and apelin may be bidirectional. Expression of thyroid receptor and TSH receptor in adipose tissue are linked to IR state<sup>(12)</sup>. Also, high normal TSH is linked to IR even in PCOS<sup>(3)</sup>. Clinical human studies reported either

unchanged or decreased serum apelin levels in subclinical and severe hypothyroidism. Furthermore, after 12-weeks of levothyroxine therapy, the apelin level is increased significantly in subclinical hypothyroid (SCH) patients<sup>(13-14)</sup>. Cross-talk of apelin, IR and thyroid hormones in PCOS patients needs further exploration.

This study aimed to evaluate serum apelin level and its relationships with cardio-metabolic risk factors, IR, and thyroid function in euthyroid non-obese PCOS women who were stratified based on the presence of IR, to investigate whether apelin can serve as an indicator of IR, and to determine the optimal cut-off value of serum apelin concentration in detecting IR PCOS subjects, an aspect is not explored before.

## PATIENTS AND METHODS

The present observational case-control study was conducted from March 2015 to February 2017. PCOS women were recruited from Endocrinology out-patient clinic, Internal Medicine Department, El-Minia – University Hospital. The study involved three groups: IR PCOS group (n=28), non-IR PCOS groups (n=32), and healthy fertile group (n=30) as a control group. The healthy group was age-matched to other groups and BMI matched to the non-IR PCOS group. Diagnosis of PCOS was based on the revised Rotterdam diagnostic criteria after exclusion of other causes of hyperandrogenism and menstrual irregularity as well as thyroid disorders<sup>(15)</sup>.

Patients with homeostatic model assessment – insulin resistance (HOMA IR)  $\geq 2.5$  were IR-PCOS group while those with HOMA-IR  $< 2.5$  were non-IR PCOS group. These groups were euthyroid with normal TSH levels ranging from 0.3 up to 4.5uIU/ml. All the women in the control group had a regular menstrual cycle every 21-35 days. On days 22-23 of the menstrual cycle, serum progesterone was measured to assess normal ovulation in the healthy group

### Exclusion criteria:

Women who had any of the following disorders were not allowed to participate: Thyroid disorders, hyperprolactinemia, Cushing's syndrome, androgen-secreting tumors, non-classical congenital adrenal hyperplasia, and other concurrent medical illness. Women who were under the age of 18 and over the age of 35, smokers, pregnant or lactating, or had a BMI of more than 28 kg/m<sup>2</sup> were also excluded

**On a standardized questionnaire:** age, marital status, menstrual cycle, hirsutism, acne, family history, and medications were all asked about. A thorough physical examination was performed, with a focus on signs of hyper-androgenism. Anthropometric measurements were taken according to a set of guidelines. Body mass index (BMI) was calculated using the following formula: weight in kilograms/height in squared meters (kg/m<sup>2</sup>). Measurement of arterial blood pressure was

measured with a mercury sphygmomanometer following seating for fifteen minutes.

### Laboratory Investigations:

5 ml venous blood samples were withdrawn from each woman during the early follicular phase (day 3-5 of the menstrual cycle). The samples were left to be clotted then centrifuged at 3000 rpm for 15 minutes, the serum was used for the determination of blood glucose and complete lipogram using a fully automated chemistry analyzer (Konelab 20i, Thermo Electron Incorporation, Finland), hormonal profile (including plasma insulin was done using mini VIDAS analyzer (Biomerieux Italia S.P.A), the remaining serum was kept frozen at -20°C for determination of apelin by ELISA (Genasia BiotechCo., Ltd, Shanghai China). Homa-IR was calculated according to Matthew's formula (fasting plasma glucose (mg/dl)  $\times$  fasting plasma insulin ( $\mu$ IU/ml))  $\div$  405. The cut-off value for IR was HOMA-IR  $\geq 2.5$ <sup>(16)</sup>.

### Ethical consent:

**Our study was approved by the local ethical committee of our institute Faculty of Medicine, Minia University, Egypt. All procedures performed in our clinical study including human participants were following the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration. All individual participants gave informed consent to be included in the study.**

### Statistical analysis

In all the statistical analyses, the SPSS software version 22.0 was used. Data for parametric and non-parametric data were presented as mean standard deviation and median (interquartile range), respectively. Between groups, the comparisons of the parametric data were assessed using the ANOVA and a post hoc Tukey test, while the comparisons of the non-parametric data were analyzed with the Kruskal–Wallis test and the Mann–Whitney test as appropriate. The relationships between the parameters were evaluated by the Pearson and Spearman correlation coefficient analysis methods as appropriate. Multivariate analysis was performed with partial correlation analysis using HOMA-IR. A receiver operating characteristic (ROC) curve was used to determine the diagnostic performance of apelin for IR. Specificity, sensitivity, and cutoff values were detected via areas under the ROC curve (AUCs). P 0.05 was considered significant.

## RESULTS

The baseline characteristics of the three studied groups are presented in **Table 1**. The groups differed statistically regarding anthropometric measurements (BMI), systemic arterial blood pressure, lipogram profile, glycemic indices, and chosen

hormonal indices. As might be expected, the IR – PCOS group had the highest fasting plasma glucose concentration and the highest fasting plasma insulin levels. Serum apelin levels were significantly higher in the IR- PCOS group (51±11.7pg/dl) than in non- IR PCOS and healthy control groups 30.9±7.80; 30.4±5.1 pg/dl; p<0.001 for both). Both non -IR PCOS patients and healthy group had comparable apelin levels.

Correlation coefficient analyses showed serum apelin had a significant positive correlation with glycemic indices (fasting glucose, fasting insulin, HOMA-IR), atherogenic lipid profile, diastolic blood pressure, TSH, and a significant negative correlation free T4 levels in IR-PCOS patients (Table 2, Figures 1, 2). However, partial correlation controlling for HOMA- IR demonstrated that only HDL-c preserved the significant correlation with apelin (r= 0.42, p=0.041).

Correlation coefficient analyses among non- IR PCOS patients presented inverse associations. Serum apelin had a significant negative correlation with BMI, glycemic indices (fasting glucose, fasting insulin, HOMA-IR), cholesterol, LDL-c, and TSH levels (Table 2, Figures 3, 4). However, partial correlation controlling for HOMA- IR demonstrated that cholesterol and TSH preserved the significant negative correlations with apelin (r= -0. 56, p<0.001; r= -0. 44, p=0.01 respectively).

The ROC curve assessing the ability of apelin to distinguish between PCOS women with and without IR had an AUC of 0.95 (95% CI: 0.92–0.99) and a threshold value of 39 pg/dl or higher to identify insulin-resistant women in the PCOS group (Table 3, Figure 5). At this cut-off point, apelin assay showed high specificity (88%) and sensitivity (86%) to identify IR in women with PCOS.

**Table (1): Clinical and laboratory Parameters, and Serum Apelin of the Studied Groups**

	IR –PCOS (Group1) (n=28)	Non- IR PCOS (Group II) (n=32)	Healthy control (Group III) (n=30)	P-value			
				All groups	I vs II	I vs III	II vs III
Age	25.9±3.04	25.1 ± 3.5	24.6±3.05	0.22	0.11	0.23	0.36
BMI (Kg/m <sup>2</sup> )	26.1±1.2	25.3±2	24.6±2.2	0.01	0.09	0.003	0.21
SBP(mmHg)	122±8.1	112.9±8.6	113±8.4	<0.001	<0.001	<0.001	0.98
DBP(mmHg)	78.2±5.4	72.9±5.7	72.8±5.2	<0.001	0.001	<0.001	0.92
Cholesterol(mg/dl)	176.7±12.9	161.2±14.7	162.3±16	<0.001	<0.001	<0.001	0.77
Triglyceride(mg/dl)	132.9±28.9	100.4±23.9	93.7±21.2	<0.001	<0.001	<0.001	0.24
HDL-c(mg/dl)	50.9±4.88	53.1±4.69	56.± 3.97	<0.001	0.09	<0.001	0.01
LDL-c (mg/dl)	100.5±12.8	89 ±11	90.6±15.2	0.005	0.002	0.009	0.67
Fasting glucose (mg/dl)	95.5±9.7	78.2±6.2	80.5±7.8	<0.001	<0.001	<0.001	0.20
Fasting insulin (µU/L)	12.9±1.8	7.34±2.3	6.09±2.1	<0.001	<0.001	<0.001	0.03
HOMA-IR*	4.2[2.7-5.1]	1.2[0.91-1.4]	0.83[0.51-1.5]	<0.001	<0.001	<0.001	0.39
FSH (mIU/ml)	4.1±0.79	5.2 ± 1.3	4.3 ± 1.5	0.002	0.03	0.52	0.02
LH (mIU/ml)	7.9±1.5	10± 1.7	3.8 ± 0.6	<0.001	<0.001	<0.001	<0.001
Total testosterone (nmol/L)*	3.2[1.8-4.0]	2.7[1.8-4.2]	1.2[0.77-1.4]	<0.001	0.61	<0.001	<0.001
TSH(µIU/ml)	2.7±0.29	2.1±0.40	2.04±0.26	0.02	0.04	0.009	0.49
Free T4(ng/dl)	0.93±0.13	1.36±0.19	1.34±0.26	<0.001	<0.001	<0.001	0.71
Apelin (pg/dl)	51±11.7	30.9±5.80	30.4±5.1	<0.001	<0.001	<0.001	0.79

Parametric quantitative data are expressed as mean ± SD and compared using the One-way ANOVA test for comparison between the three groups followed by the post hoc Tukey analysis between two groups.\*= non-parametric quantitative data are expressed as median (25 -75% interquartile range) and compared using the Kruskal Wallis test between the three groups followed by Mann Whitney test between two groups. Significant level at p-value < 0.05.

IR= insulin resistance; PCOS=polycystic ovary syndrome; BMI= body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; HDL-c= high density lipoprotein cholesterol; LDL-c= low density lipoprotein cholesterol; HOMA-IR=homeostatic model assessment of insulin resistance; LH=luteinizing hormone; FSH= follicle stimulating hormone; TSH= thyroid stimulating hormone; Free T4= free thyroxin

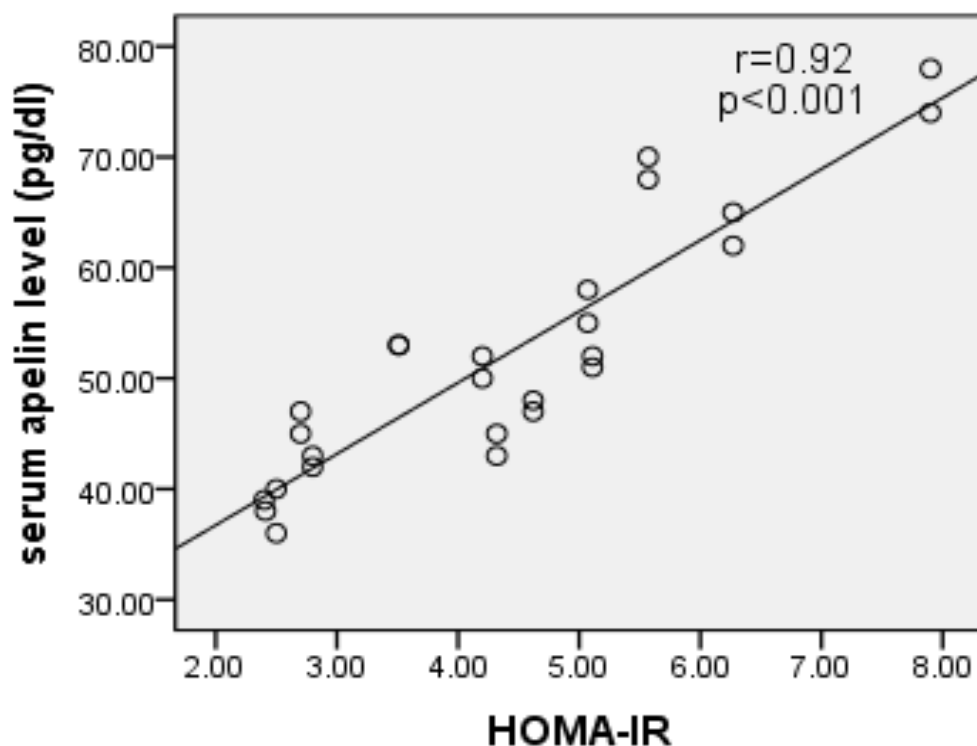
**Table (2): Correlation between serum Apelin levels and Clinical, Metabolic, and Hormonal Parameters among PCOS Women.**

Variables	IR PCOS		Non- IR PCOS	
	r	P	r	P
Age(years)	-0.013	0.94	-0.08	0.63
Body mass index (Kg/m <sup>2</sup> )	0.183	0.35	-0.39	0.02
Systolic blood pressure (mmHg)	0.05	0.80	-0.10	0.56
Diastolic blood pressure (mmHg)	0.39	0.04	-0.05	0.78
Total cholesterol(mg/dl)	0.38	0.05	-0.67	<0.001
Triglyceride(mg/dl)	0.51	0.007	-0.194	0.28
High density lipoprotein- cholesterol (mg/dl)	-0.41	0.03	0.24	0.17
Low density lipoprotein- cholesterol(mg/dl)	0.42	0.03	-0.49	0.004
Fasting glucose(mg/dl)	0.34	0.008	-0.48	0.005
Fasting insulin (μU/L)	0.59	<0.001	-0.55	0.001
Homeostatic model assessment of IR	0.92	<0.001	-0.60	<0.001
TSH(uIU/ml)	0.63	<0.001	-0.4	0.01
Free T4(ng/dl)	-0.67	<0.001	0.20	0.26

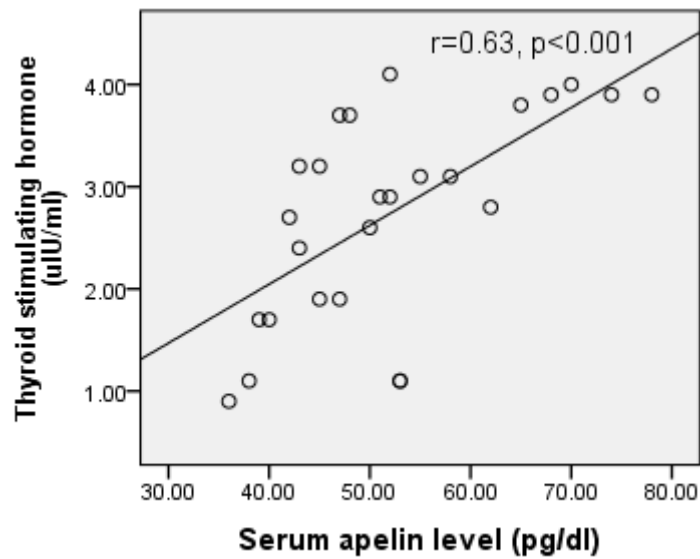
IR= insulin resistance; PCOS=polycystic ovary syndrome TSH= thyroid-stimulating hormone; Free T4= free thyroxin Pearson's and Spearman correlation as appropriate: Significant level at P-value < 0.05.

**Table (3): Diagnostic performance of serum apelin for insulin Resistance in polycystic ovary syndrome women**

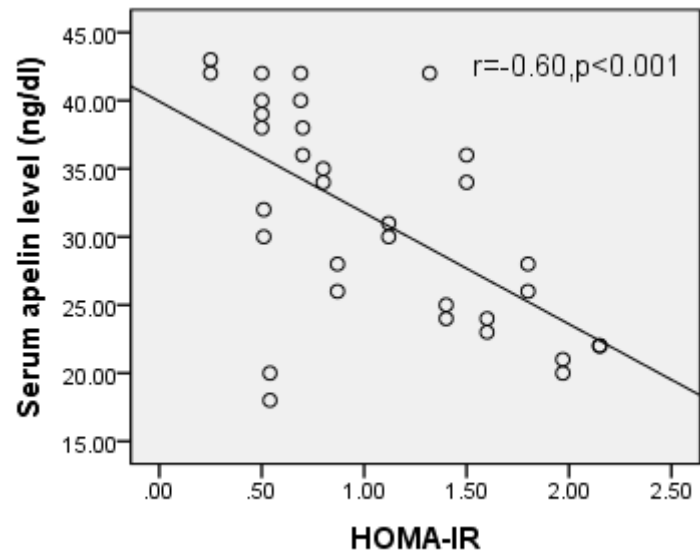
Cut-off value of apelin	Area Under the Curve	Asymptotic Sig. <sup>b</sup>	Asymptotic 95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
≥ 39(pg/dl)	0.95	0.001	0.92	0.99	86%	88%



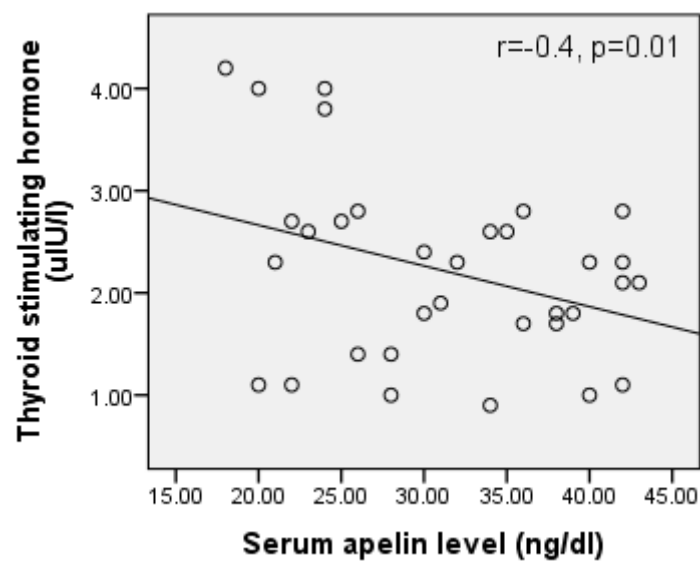
**Figure (1): Correlation between serum apelin and homeostatic model assessment of insulin resistance in insulin resistance- polycystic ovary syndrome group.**



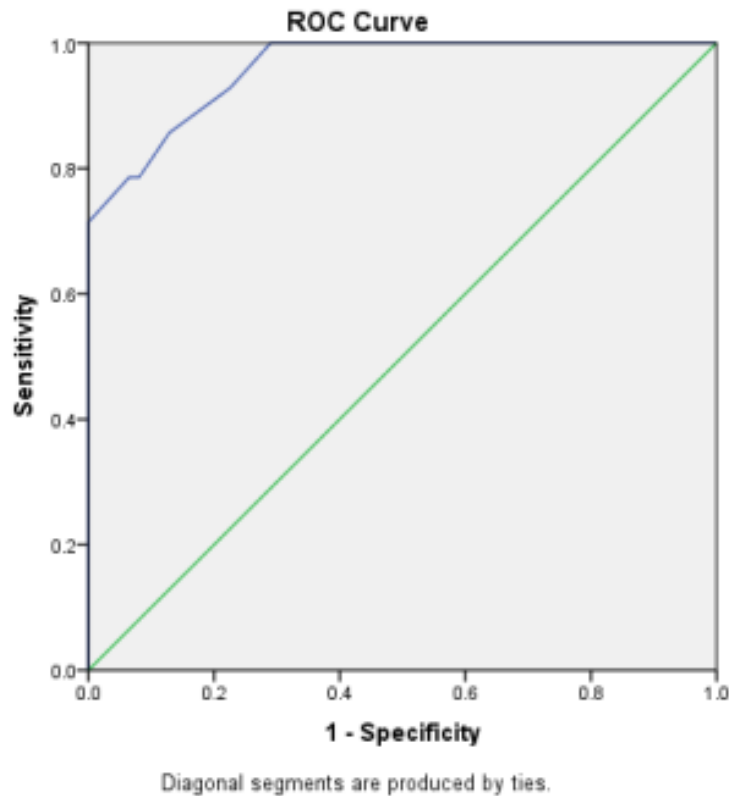
**Figure (2):** Correlation between serum apelin and thyroid-stimulating hormone in insulin resistance- polycystic ovary syndrome group



**Figure (3):** Correlation between serum apelin and homeostatic model assessment of insulin resistance in non-insulin resistance polycystic ovary syndrome group



**Figure (4):** Correlation between serum apelin and thyroid-stimulating hormone in non-insulin resistance polycystic ovary syndrome group



**Figure (5):** ROC curve for diagnostic performance of serum apelin for insulin resistance in polycystic ovary syndrome women using a cut-off value of  $\geq 39$  pg/dl for apelin, the AUC, sensitivity, and specificity were 0.92, 86%, 88% respectively with p-values  $\leq 0.001$ .

## DISCUSSION

Our study was the first to use the presence of IR to determine the level of apelin in PCOS. Previous studies looked at apelin levels in PCOS patients based on whether or not they were obese. Because not all obese women are IR, and many lean women with PCOS are also IR<sup>(17)</sup>. As a result, we chose non-obese PCOS patients who were divided into groups based on IR to see if IR had an effect independent of BMI. In our research, we were also the first to look at the relationship between thyroid hormone and apelin. We found that IR PCOS women had higher serum apelin levels than non-IR PCOS and healthy women, with no significant difference between the two groups.

In addition, the relations between apelin levels, atherosclerotic risk, IR indices, and TSH levels by the bivariate analysis were found to be contradictory in IR PCOS and non-IR PCOS women. In IR PCOS women, apelin was positively correlated with atherogenic lipid profile, DBP, fasting glucose, insulin, HOMA-IR, and TSH, and negatively correlated with free T4 levels, whereas in non-IR PCOS women, apelin was negatively correlated with many of the aforementioned parameters. Apelin maintained the significant correlation with HDL-c in IR PCOS and with TSH and cholesterol in non-IR PCOS in a multivariate analysis controlling for HOMA-IR. Apelin has a high specificity of 88 percent and a sensitivity of 86 percent in identifying insulin-resistant women in PCOS women at this cut-off point of 39 pg/dl or higher.

Our study involved non-obese PCOS women. Reports regarding serum apelin levels in PCOS are controversial some authors have found higher plasma apelin concentrations in patients with PCOS compared with healthy controls<sup>(6,7)</sup>, while others reported decreased apelin levels in PCOS women<sup>(8,9)</sup>.

IR may play a role in elevated apelin levels in PCOS patients. Dyslipidemia and IR cause CL dysfunction, resulting in a reduction in its capacity to produce and secrete progesterone. Because progesterone enhances apelin receptor expression, a low level of progesterone may result in a decline in apelin receptor expression. Apelin levels have risen as a compensatory mechanism to the story. Secondly: insulin growth factor-1 (IGF-1) promotes the production of estrogen by stimulating the apelin system. The increased insulin levels reduce the synthesis of insulin growth factor binding protein - 1(IGFBP-1), which in turn enhances the bioactivity of IGF-1. As a result of the constant low estradiol level in the early to mid-follicular range, with no mid-cycle increases in PCOS, IGF-1 and apelin may show a compensatory increase as an attempt to raise estradiol level in PCOS women<sup>(5)</sup>. A for mentioned data illustrated the role of IR in elevated apelin levels among IR PCOS in our study. Elevated apelin levels in other IR states such as obesity, impaired glucose tolerance (IGT), and T2DM, as well as in metabolic syndrome, with a decrease in apelin levels accompanying weight loss, were reported. In a similar way to insulin and leptin, these findings point to the

possibility of apelin resistance<sup>(4, 18)</sup>. Insulin stimulates apelin secretion, whereas apelin inhibits insulin secretion while increasing glucose utilization. As a result, the increased apelin seen in IR PCOS may be a compensatory mechanism aimed at reducing insulin resistance directly, because apelin has many metabolic actions to enhance insulin sensitivity in various tissues. When insulin resistance is reduced, apelin levels may be reduced as well<sup>(4)</sup>.

In our study apelin positively correlated to glycemic indices (fasting blood sugar, insulin level, and insulin resistance). Our results were similar to **Hasan et al.**<sup>(7)</sup>, as they subdivided PCOS women into lean and obese subgroups who all were IR according to HOMA- IR. They reported positive correlations between apelin and fasting insulin level, and HOMA-IR in both obese and lean subgroups. Other studies which display elevated apelin levels in PCOS did not demonstrate similar findings that may be related to different subgrouping of PCOS patients. Up to date, No study classified PCOS based on IR but almost always according to obesity. For example, the HOMA IR in PCOS women was  $2.58 \pm 1.65$  means nearly equal numbers of IR and non- IR PCOS were included in study of **Gören et al.**<sup>(6)</sup> Of note, the authors did not find any significant correlation between plasma apelin levels and cardiovascular risk factors nor IR parameters in PCOS women in this study. Similarly, serum apelin 36 levels were positively correlated with HOMA-IR in metabolic syndrome patients<sup>(18)</sup>.

Apelin inhibits insulin secretion and enhances glucose utilization by the tissues<sup>(4)</sup>. These data explain the presence of a negative association between apelin and glucose, insulin, and HOMA-IR in non- IR PCOS while hyperinsulinemia in IR PCOS stimulates apelin secretion as a compensatory mechanism to apelin resistance, the missing relationship between plasma apelin and BMI in IR-PCOS might be explained by a narrow range of BMI in the present study, different sources of apelin secretion or down regulation of apelin receptors. Other organs (e.g., the central nervous system, the heart, the lungs, the testis, the ovary, the mammary gland, and the gastrointestinal system) also secrete apelin, so it is not solely determined by adipose tissue<sup>(4)</sup>.

The arcuate and paraventricular nuclei of the hypothalamus, which control feeding, behavior, and energy expenditure, have also been found to express apelin and its receptor<sup>(19)</sup>. Central injection of apelin reduced food intake in normal rats but had no effect in high fat-fed rats owing to down regulation of the APJ receptor in high fat diet-fed rats<sup>(20)</sup>. Further information can be found in **Krist et al.**<sup>(21)</sup> they wanted to see if changes in circulating apelin during weight loss are primarily due to lower body fat mass or if they reflect enhanced insulin sensitivity. First, the elevated serum apelin concentration was reduced in all weight loss intervention studies (hypocaloric diet, bariatric surgery, or exercise program). Second, there

still are significant BMI-independent relations between lower apelin levels and improved insulin sensitivity after controlling for HOMA IR.

In IR PCOS, we found a significant positive correlation between diastolic blood pressure and apelin level, which was lost after controlling for HOMA IR. In this context, apelin is a vasodilator hormone because it promotes nitric oxide-dependent vasodilation and prevents angiotensin II-induced vasoconstriction<sup>(22,23)</sup>. As a result, increased apelin could be a compensatory mechanism for high blood pressure. A previous study found no link between apelin and systolic and diastolic blood pressure in obese and obese hypertensive patients<sup>(24)</sup>. Low apelin concentrations in the blood have been linked to an increased risk of hypertension, particularly in Caucasians<sup>(25)</sup>.

In our study, elevated apelin level is associated with atherogenic lipid profile in IR –PCOS but associated with favorable lipid profile and protective effect in non- IR PCOS. The inverse association of apelin with insulin resistance between both groups may explain the contradictory effects. Only HDL in IR PCOS and cholesterol in non-IR PCOS maintain a significant association with apelin after controlling for HOMA-IR. These results suggest an association of apelin with lipid profile is IR dependent. Similar to our result, elevated apelin level was associated with atherogenic lipid profile among morbid obese Egyptian women (about 75% of women were insulin resistant and among PCOS patients despite lower levels of apelin in PCOS women<sup>(26,27)</sup>. Another study showed lack of association between apelin level and lipogram in PCOS<sup>(28)</sup>, while another study reported a significant positive correlation between insulin, triglyceride, and IR but a negative correlation with HDL levels in PCOS patients who had lower apelin levels than healthy controls<sup>(9)</sup>.

Plasma levels of apelin were negatively correlated with serum levels of LDL-C in pregnancy<sup>(29)</sup>. Plasma apelin is decreased in non-obese, non-diabetic and normotensive patients with elevated LDL-cholesterol in addition to a positive correlation between HDL and apelin<sup>(30)</sup>. Apelin inhibits lipolysis and enhances fatty acid oxidation<sup>(31)</sup>. There is evidence that apelin-13 reduces TG levels by overexpressing AQP7 expression and decreasing lipid storage in hypertrophic adipocytes by initiating the PI3K signaling pathway<sup>(32)</sup>. This substance aids in glycerol efflux from adipose tissue and also encourages glycerol transport, leading to reduced serum TG levels<sup>(33)</sup>. Plasma TG and insulin levels were also observed to be lower in apelin-treated normal and obese mice<sup>(34)</sup>. The administration of apelin to hypothyroid rats led to a rise in HDL-C levels<sup>(11)</sup>. Further researches addressing the relationship between HDL and apelin are necessary.

The presence of APJ receptors in the hypothalamus and the pituitary gland has been

demonstrated previously <sup>(4)</sup>. However, to date, the relation of apelin with thyroid function in PCOS, particularly that of the apelin–APJ system and pituitary thyroid axis as neuroendocrine pathways has not been explored before. So in our study, we studied the correlation between thyroid hormones and apelin in euthyroid PCOS stratified based on HOMA-IR. Apelin had a significant positive correlation with TSH and a significant negative correlation with free  $T_4$  in IR – PCOS meanwhile apelin had a significant negative correlation with TSH in non IR –PCOS. After controlling for HOMA-IR, the preserved relation was a negative correlation between TSH and apelin in non IR PCOS.

Many experimental studies confirmed the inhibitory effect of apelin on TSH level in inclusion or exclusion of thyroid hormone. In euthyroid rats, an intra-cerebro-ventricular injection of pyroglutamate apelin-13 non-significant decrease in serum TSH levels. Apelin administration in hypothyroid rat decrease TSH and free thyroxin levels. Therefore appears that apelin can affect TSH levels through the APJ receptor and may enhance thyroxin catabolism <sup>(10, 11)</sup>. Apelin level in hypothyroidism or SCH displayed controversial results. Apelin level is elevated in propylthiouracil-induced hypothyroid rats <sup>(11)</sup>. A clinical study demonstrated insignificant increased apelin levels in subclinical and overt hypothyroidism while other demonstrated decreased levels of apelin in subclinical hypothyroidism that increased after thyroxin replacement therapy <sup>(13,14, 35)</sup>.

Also, the expression of TSH and thyroid receptor in adipocyte support the direct cross-talk of thyroid and apelin as the adipose tissue represent a major source of circulating apelin <sup>(4, 12)</sup>. Positive correlation of TSH with apelin is IR dependent in IR PCOS means the presence of apelin resistant and compensatory increased level as a mechanism to adjacent the TSH levels. Preserved negative relation between apelin and TSH in non –IR PCOS means direct effect between pituitary and apelin in euthyroid state and may suggest the combined use of apelin with thyroxin replacement therapy in hypothyroid. However effect of apelin on thyroid hormone levels should be further explored.

The differences in results between the studies can be attributed to ethnicity, study design, genetic characteristics of populations, and different PCOS phenotypes. The current study's cross-sectional design cannot provide cause-and-effect relationships, and our study's sample size is a limitation. We cannot be subgrouped women based on phenotype subtypes due to the small sample size.

## CONCLUSION

We concluded that IR is the underlying etiology for elevated apelin in PCOS. In presence of insulin resistance, the compensatory rise of apelin improves elevated blood pressure, atherogenic lipid profile,

elevated glucose, and insulin and decreases TSH and thyroid hormone levels in PCOS. Apelin is a specific and sensitive marker for IR in PCOS. Apelin is a bridge across adipose tissue, thyroid, and ovary. Inhibitory effect of apelin on TSH level is an independent on HOMA-IR.

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**Conflict of interest:** Nil.

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