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ORIGINAL ARTICLE

Serum Adropin and Vaspin Levels in Obese Rats with Polycystic Ovary Syndrome and after Metformin Treatment

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ABSTRACT

Background: Poly cystic ovary syndrome (PCOS) is the principal cause of anovulatory infertility.Adropin is a nutrient-regulated metabolic hormone shown to encourage glucose oxidation over fatty acid oxidation.Vaspin expression in visceral adipose tissue is associated with metabolic derangement in rat model of obesity and T2DM.Some studies addressed adropin and vaspin levels in PCOS, however, the results are controversial.

Aim of the study: This study was designed to evaluate both serum adropin and vaspin levels in obese rats with PCOS and to detect any possible association of these levels with metabolic and hormonal changes in this condition with and without metformin treatment.

Material and Methods: Twenty four female albino rats were divided into three equal groups**.** Group I:Control,Group II:obese rats with PCOS fed HFD for 9 weeks and given letrozole(1mg/kg BW)by gavage daily for the last 21 consecutive days.Group III:Metformin pretreated obese PCOS rats,at a dose of 200 mg/kgBWdaily along with letrozole for the last 21days.At the end of the experiment,Serum adropin,vaspin,sex hormones,insulin,glucose,lipid profile,TNF,plasma D-dimer and ovarian MDA levels were estimated.BMI, HOMA-IR were calculated.Ovarian histopathology was done.

Results: Obese PCOS group showed significantly lower serum adropin and higher vaspin levels when compared to control group.Metformin pretreated group showed significantly higher serum adropin and lower vaspin levels when compared to obese PCOS group**.**Adropin was negatively correlated with all parameters except sex hormones.Vaspin was positively correlated with all parameters except sex hormones in all groups.

Conclusion: Adropin and vaspin may represent a novel link between obesity and metabolic disturbance in obese PCOS rats.

Keyword: Adropin,vaspin,obesity,PCOS,metformin.

INTRODUCTION

PCOS is a common endocrine disorder affecting 6-18 % of women of reproductive age.It is usually associated with insulin resistance (IR), obesity, hyperlipidemia, with increased prevalence of type II diabetes (T2DM)**,** however, the exact mechanisms underlying PCOS are not clear [1]**.**

Adropin is a peptide hormone consists of 76 amino acids and expressed in liver and brain**.** It is concerned with glucose and lipid metabolism, adiposity and insulin resistance**.** Treatment with adropin can decrease blood glucose and improve insulin resistance in type 2 diabetic rats [2].

Vaspin has been known as a visceral adipose tissue-derived serine protease inhibitor. Its circulating level markedly increases in obese individuals**.** In overweight women with PCOS and insulin resistance, metformin decreases serum vaspin levels in parallel to improvement of insulin sensitivity [3].

In addition, vaspin has been reported to have anti-inflammatory actions, as administration of this adipokine suppresses circulating TNFα. demonstrating the inhibitory effects of vaspin on cytokine-induced expression of proinflammatory molecules in vascular endothelial cells [4].

Therefore, adropin and vaspin may be involved in the pathogenesis of obesity and PCOS.Some studies have found a link between adropin & vaspin levels and PCOS. However, these studies are limited and controversial [5].

Metformin is a synthetically derived biguanide used in treatment of metabolic disorders and insulin resistance in PCOS. It was found that metformin ameliorates reproductive abnormalities and restores cyclicity & ovulation in women with PCOS.Yet the effect of metformin on adropin and vaspin levels in PCOS patients is still unclear [6].

This study was designed to evaluate both serum adropin and vaspin levels in obese rats with PCOS and to detect any possible association of these levels with metabolic and hormonal changes occurring in this condition with and without metformin treatment.

MATERIAL AND METHODS

This study was carried out on 24 female of local strain albino rats 6 weeks old weighing 90-105gm.They were obtained from the animal house faculty of veterinary medicine Zagazig University.

The rats were kept in steel wire cages (6/cages) in the animal house of the faculty of medicine Zagazig University under hygienic conditions.The rats had free access to water and chow, were kept at room temperature on a 12 h light/ dark cycle.

The study design was approved by Institutional Review Board (IRB) NO.4289-4-2-2018, faculty of medicine, Zagazig University. All animal experiments were with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

The animals were randomly divided into 3 equal groups: Group I : Control group (n=8), fed on commercial rat standard chow consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat,Group II: Experimentally induced obese rats with PCOS (n = 8), those rats fed high fat diet (HFD) for 9 weeks [7] and given letrozole by gavage (1mg/kg BW dissolved in 1% carboxy methyl cellulose) daily for the last 21 consecutive days [8].Group III: Metformin pretreated experimentally induced obese rats with PCOS(n = 8),treated with metformin(200 mg / kg BW) dissolved in distilled water daily along with letrozole for the last 21 days [9].

Smears were obtained daily by vaginal washing with saline daily at 10 am and evaluated under the microscope. Cycles with duration of 4 to 5 days were considered regular estrus phases and were determined according to Marcondes et al.[10] and Goldman et al.[11] as follow:

The proestrus phase: the vaginal smear consists of apredominance of nucleated epithelial cells with smooth margins,the estrus phase: the vaginal smear shows large anucleated cornified (keratinized) cells with irregular margins,the met estrus phase: the vaginal smear shows many cornified cells plus infiltration of leukocytes and the diestrus phase: the vaginal smear shows absence of the cornified cells and presence of small leukocytes.

The observation of cornified cells in the smears during a minimum of 10 consecutive days was defined as persistent estrous, indicating anovulation and development of follicular cysts[8].

Twenty four hours after the end of the study (after the last dose of letrozole), and after overnight fasting, rats weighed and BMI and AC/TC ratio were calculated according to the equation: body weight (gm)/length^2 (cm^2) [12].

Rats were anaesthetized by ether inhalation, blood samples were collected from orbital sinus (sampling of controls taken in the estrus phase) and ovaries were dissected and immediately fixed in 10% buffered formalin. Each blood sample was divided as follow:

1mL of the blood was collected in a citrated tube,centrifugated at 3000 rpm for 15 min. The supernatant plasma was immediately used for determination of D-dimmer levels [13] and the remaining amount of the blood was allowed to clot. Serum was separated by centrifugation of blood at 3000 rpm for 15

minutes and stored at -20c until used for assay [14].

Serum was examined for adropin levels using rat adropin ELISA Kit (Catalog number:E-EL-R2566, MyBiosource, USA) according to the instruction of the manufacturer.

Serum vaspin levels was determined by using rat ELISA kits from Wkea Med Supplies Corp., (USA), according to Hida et al [15].

Glucose level was determined according to Tietz[16] using glucose enzymatic-liquizyme rat kits (Biotechnology, Egypt) and insulin level was estimated according to Temple et al.[17].

Lipid profile was estimated as follows: Total serum cholesterol level: according to Tietz [16] ,serum TG level: according to Fossati [18] , serum HDL levels according to Nauck et al. [19] and serum LDL levels was calculated according to Friedewald et al. [20] as follows: LDL = TC -HDL – TG/5 (Kits for estimation of serum insulin, cholesterol, TG and HDL levels were purchased from Biosource Europe S.A.Belgium).

LH, FSH, estradiol, progesterone and free testosterone levels were detected according to Tietz [16] using rat kits: BC-1031, BC-1029, BC-1111, BC-1113 and BC-1115, respectively, Bio Check Inc 323 Vintage Park Dr. Foster City.

Serum Tumor Necrosis Factor- α (TNF-α) was determined according to Fernando et al. [21] using KRC3011 rat kits, BioSource International Inc. 542 Flynn Road Camarillo, California 93012 USA.

Ovarian lipid peroxidation (MDA) was estimated according to Ohkawa et al.[22]. Plasma D-dimer levels was detected by ELISA kit, GenWay Biotech, Inc, ca 40-88-234402, USA according to Declerck et al. [23]

Homeostatic model assessment of insulin resistance index (HOMA-IR) was calculated according to the formula described by Sun et al. [24] as follows:

[HOMA-IR] = fasting serum glucose (mg/dl) × fasting serum insulin (μIU/ml)/405.

Histopathological examination: The abdominal cavities of the rats were opened. Ovaries were dissected and fixed in 10% buffered formalin for 6 hours at room temperature and washed in a phosphate buffer saline solution.For light microscopy,fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin.5 μm thick sections were mounted in slides previously treated with 3-aminopyropyl triethoxysilane and stained with hematoxylin-eoisin preliminary observation [25]**.** The pathologist was blinded to the treatment.

Statistical analysis:

Results were presented as mean ± standard deviation (SD). Statistical analysis of differences between groups was performed using one way analysis of variance (ANOVA). Pearson's correlation analysis was performed to screen potential relations between serum adropin and vaspin and all parameters. For all statistical tests done, P value < 0.05 was considered to be statistically significant. SPSS version 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

In group II (obese pco group), while the mean values of final BMI, final AC/TC ratio, serum vaspin, glucose, insulin, HOMA- IR, triglycerides, total cholesterol, LDL-cholesterol, TNF-α, MDA, LH, testosterone and plasma d-dimer levels were significantly high (P<0.001 for all), the mean values of serum adropin,estradiol,progesterone and HDL-cholesterol were significantly low (P<0.001) in comparison to those of group I (control group) (Table 1).

However, the mean values of serum FSH levels showed non-significant change when compared with those of group I (P > 0.05) (Table 1).

Moreover, in group III, metformin administration resulted in a significant decrease in vaspin, LH, testosterone, glucose, insulin, cholesterol, triglycerides, LDL levels, BMI and calculated HOMA-IR, TNF-α in addition to ovarian MDA and plasma D-dimer (p<0.001 for all), but significantly increased adropin, estradiol, progesterone and HDL (P value: < 0.01, < 0.001, < 0.001 < 0.01

respectively) when compared to group II (Table 1).

As regards the correlation with adropin levels, final BMI, final AC/TC ratio, serum glucose, insuln, HOMA-IR, total cholesterol, TG, LDL, TNF-α, plasma D-dimer, ovarian MDA were significantly negatively correlated with adropin in all studied groups. Where as, serum HDL levels were significantly positively correlated with adropin in all studied groups (Tables 2).

While final BMI, final AC/TC ratio, serum glucose, insuln, HOMA-IR, total cholesterol, TG, LDL, TNF-α, plasma D-dimer, ovarian MDA were significantly positively correlated with vaspin in all groups and serum HDL levels were significantly negatively correlated with vaspin in all groups (Tables 3).

Finally, there was a significant negative correlation between serum adropin and vaspin in all studied groups.

Histopathological examination: Ovaries from the control group had follicles in various stages of development including secondary follicles, graafian follicles, and recently formed corpus luteum surrounded by normal ovarian stroma (photo 1).Ovaries from PCO rats showed increased numbers of large cystic follicles, and the cystic wall was thickened, characterized by a thickened theca cell layer and a diminished granulosa cell layer with no antral follicles or corpus luteum indicating anovulation (photo 2). Ovarian tissue from group III shows decreased number of cystic follicles and increased thickness of granulosa cell lining in addition to the appearance of several follicles at different developmental stages (photo 3).

Table (1):The mean ± SD of all measured parameters in all studied groups

Groups / Parameters		Group I (control)	Group II (Obese PCOS)	Group III (Obese PCOS+metformin)
adropin (ng/mL)	$\overline{X}$ ± SD	29.14±4.5	14.79±1.4 a ***	20.25 ± 1.28 a *** b **
vaspin (ng/mL)	$\overline{X}$ ± SD	0.4 ± 0.07	3.8±0.46 a ***	1.04 ± 0.26 a ** b ***
final BMI (gm/cm2)	$\overline{X}$ ± SD	0.49 ± 0.008	0.93±0.04 a ***	0.75 ± 0.03 a *** b ***
final AC/TC ratio	$\overline{X}$ ± SD	1.13 ± 0.01	1.55 ± 0.02 a ***	1.33± 0.02 a *** b ***
glucose (mg/dl)	$\overline{X}$ ± SD	78.88±10	192.5±3.6 a ***	142.13 ±8.2 a *** b ***
insulin (µIU/ml)	$\overline{X}$ ± SD	7.67±0.58	28.7±1.08 a ***	12.4 ± 1.07 a *** b ***
HOMA-IR	$\overline{X}$ ± SD	1.52± 0.29	13.65±0.75 a ***	4.38± 0.62 a *** b ***
total cholesterol (mg/dl)	$\overline{X}$ ± SD	68.4 ±14.6	261.2 ±17.5 a ***	110.5 ± 16.2 a *** b ***
triglycerides(mg/dl)	$\overline{X}$ ± SD	73.75 ± 9.4	192.4±14.2 a ***	112.6 ± 12.16 a *** b ***
HDL (mg/dl)	$\overline{X}$ ± SD	51.25±6.5	29.5±3.3 a ***	36.6 ± 3.03 a *** b **
LDL (mg/dl)	$\overline{X}$ ± SD	78.5 ± 8.3	167.5 ±13.33 a ***	89.25 ± 6.6 a * b ***
FSH (IU/ml)	$\overline{X}$ ± SD	7.03 ± 0.2	6.8 ± 0.4	6.93 ± 0.23
LH (IU/ml)	$\overline{X}$ ± SD	2.1 ± 0.37	6.7± 0.59 a ***	4.6 ± 0.31 a ***b ***
testosterone (pg/ml)	$\overline{X}$ ± SD	77.1 ± 0.42	244.2 ± 0.55 a ***	139.6 ± 7.1 a *** b ***
estrogen (pg/ml)	$\overline{X}$ ± SD	36.33±2.29	14.71±.1.8 a ***	28 ± 2 a *** b ***
progesterone (ng/ml)	$\overline{X}$ ± SD	7.94 ± 0.33	4.76±0.49 a ***	5.8 ± 0.57 a *** b ***
TNF-α (pg/ml)	$\overline{X}$ ± SD	13.22±1.5	86.38 ±7.6 a ***	45.25 ± 6 a *** b ***
MDA (mmol/gm)	$\overline{X}$ ± SD	86.13±2.9	181.5±7.34 a ***	110 ± 7.8 a *** b ***
D-dimer (mg/dl)	$\overline{X}$ ± SD	58.4±2.3	207.2± 5.1 a ***	140 ± 7.15 a *** b ***

a = versus group I, b = versusgroup II, * = significant (P<0.05), ** = significant (P<0.01), *** = significant (P<0.001)

BMI:body mass index, **AC/TC ratio:**abdominal circumference/thoracic circumference ratio,
HOMA-IR: Homeostatic model assessment of insulin resistance index, **HDL**:high density lipoprotein, **LDL**: low density lipoprotein, **FSH:** follicular stimulating hormone, **LH**:Lutenizing hormone, **TNF-α:** Tumor Necrosis Factor- α, **MDA:** Ovarian lipid peroxidation

Table (2):Correlation between serum adropin levels and the levels of the other measured parameters

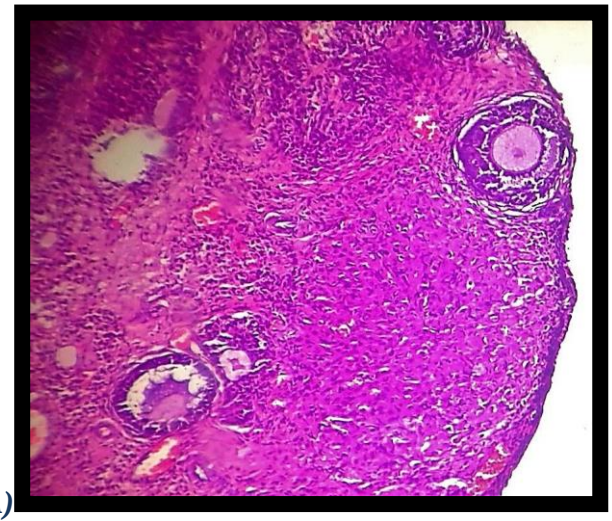
Parameters	Group I (Control)	Group II (Obese PCOS)	Group III (Obese PCOS+metformin)
	r	r	r
BMI (gm/cm2)	-0.931*	-0.947***	-0.91*
AC/TC ratio	-0.952 ***	-0.969 **	-0.944***
LH (IU/ml)	-0.275	-0.132	-0.169
FSH (IU/ml)	0.256	0.145	0.313
Estradiol (pg/ml)	0.059	0.21	-0.557
Progesterone (ng/ml)	0.236	-0.594	-0.261
Testosterone (pg/ml)	-0.089	-0.2	-0.199
Glucose (mg/ dl)	-0.955***	-0.937**	-0.959***
Insulin (µIU/ml)	- 0.899 **	-0.982***	-0.92***
HOMA-IR	- 0.989 ***	-0.984***	-0.957***
TC	- 0.957 ***	-0.988***	-0.957***
TG	- 0.903***	-0.953***	-0.946***
HDL	0.954 ***	0.95***	0.975***
LDL	-0.978 ***	-0.976***	-0.977 ***
TNF-α	- 0.952 ***	-0.977***	-0.958***
MDA	-0.956 ***	-0.976***	-0.926***
D-dimer	-0.985 ***	-0.97***	-0.966***
vaspin	-0.987***	-0.952***	-0.884***

r:Correlation coefficient * = significant (P<0.05); ** = significant (P<0.01); *** = significant (P<0.001)

Table (3): Correlation between serum vaspin levels and the levels of the other measured parameters.

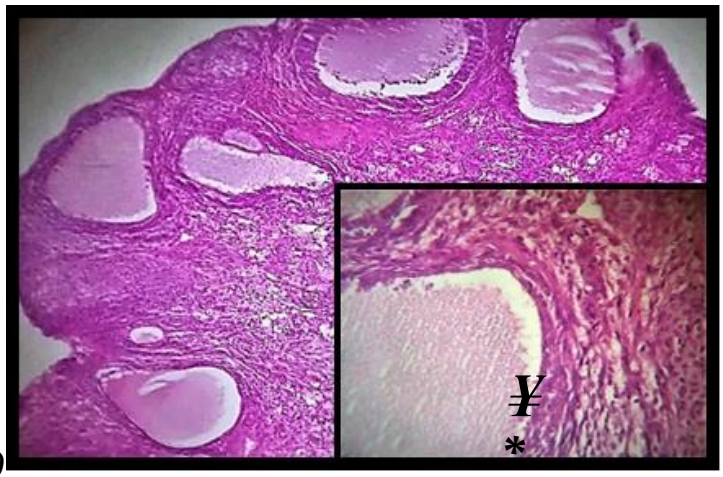
Parameters	Group I (Control)	Group II (Obese PCOS)	Group III (Obese PCOS+metformin)
	r	r	r
BMI (gm/cm2)	0.921*	0.972***	0.87**
AC/TC ratio	0.918 ***	0.949***	0.793*
LH (IU/ml)	0.354	0.16	0.76
FSH (IU/ml)	-0.309	-0.063	0.124
Estradiol (pg/ml)	0.062	0.276	0.437
Progesterone (ng/ml)	-0.158	-0.466	0.383
Testosterone (pg/ml)	0.27	0.15	0.505
Glucose (mg/ dl)	0.985 ***	0.962 ***	0.921**
Insulin (µIU/ml)	0.863 **	0.931 **	0.887**
HOMA-IR	0.992 ***	0.96 ***	0.908**
TC	0.974 **	0.95 ***	0.92**
TG	0.947 ***	0.944 ***	0.93**
HDL	- 0.982***	- 0.951***	- 0.843**
LDL	0.963***	0.984***	0.893**
TNF-α	0.984 ***	0.977***	0.904**
MDA	0.979 ***	0.95***	0.901**
D-dimer	0.986***	0.955***	0.909**
Adropin	-0.987 ***	-0.952***	-0.884***

r:Correlation coefficient * = significant (P<0.05); ** = significant (P<0.01); *** = significant (P<0.001)



(A)

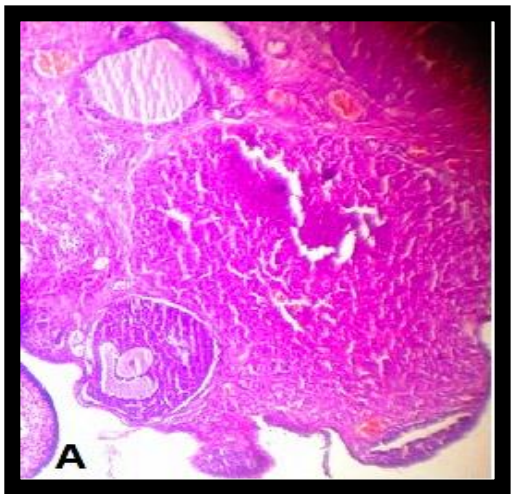
Photo (1): Ovarian tissue from group I (H&E × 200)



(B)

(A) (H & E × 200) ***(B) (H&E ×400)***

Photo (2): Ovarian tissue from group II
(*) very thin layer of granulosa cell
(¥)hyperthecosis of the stroma



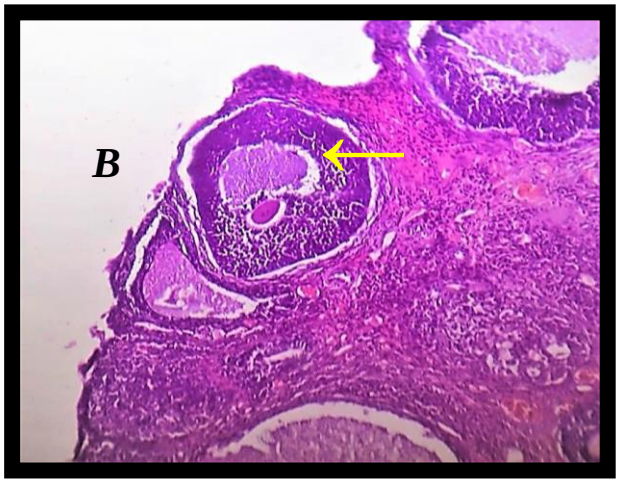


Photo (3): Ovarian tissue from group III (H & E × 200)
(←) increased thickness of granulosa cell lining (A & B).

DISCUSSION

The results of this work showed that HFD for 9 weeks started at age of 6 weeks (to produce early onset obesity) was found to induce a significant increase in BMI and AC/TC ratio denoting occurrence of obesity in addition to insulin resistance and dyslipidemia in rats of group II compared with control.

The signs of PCOS induced by letrozole in obese rats was proved by the significant hyperandrogenism accompanied by significant reduction in both estradiol and progesterone levels in comparison to control group, in addition to persistent estrus observed in vaginal smear and multiple ovarian cysts detected in histopathological examination, as letrozole blocked cytochrome P450 aromatase which is essential for aromatization of testosterone to estradiol [26].

Interstingly, metformin treatment significantly reduced BMI and AC/TC ratio in group III.Metformin's effects on body weight may be due to its anorexigenic effect via pathway involving neuropeptide Y in human & animal models and pancreatic polypeptide in human subjects in addition to its insulin sensitizing effect [1].

Regarding serum adropin, there was a significant decrease in its levels in obese PCOS rats compared with controls. However, treatment with metformin significantly increased these levels in group III. Furthermore, a significant negative correlation was found between adropin levels and BMI, and AC/TC ratio in all groups.

In line with these findings, Kume et al. [27] found that serum adropin levels were significantly decreased in lean and obese PCOS and were negatively correlated with BMI and waist circumference. The decreased adropin levels could be explained by results who reported that chronic exposure to HFD resultrd in reduced expression of adropin levels, perhaps revealing deregulation of liver *Enho* gene with obesity [2].

In the present study, obese PCOS rats showed significantly higher serum levels of glucose and insulin, TC, TG and LDL in addition to HOMA-IR index when compared with control rats, while, there was a significant decrease in HDL levels in the same group.Treatment with metformin produced a significant decrease in fasting serum levels of glucose, insulin, TC, TG, LDL and HOMA-IR associated with a significant increase in HDL levels in group III when compared with group II.

In addition, there was also a significant negative correlation between serum adropin level and glucose, insulin, TC, TG, LDL serum levels, and HOMA-IR in all groups, while a significant positive correlation between serum adropin and HDL levels was found.

These results are in line with other study which showed that serum adropin levels were positively correlated with HDL-C levels and negatively correlated with LDL-C levels in women with polycystic ovary syndrome [28].

In this study, serum level of TNF-α was significantly higher in obese PCOS group compared with control group. However, treatment with metformin produced a significant decrease in TNF-α. Furthermore, there was a negative correlation between adropin and TNF-α in all groups.This is in accordance with Ghowsi et al**.** [29] who reported that PCOS is a chronic low grade inflammatory disorder characterized by increased levels of pro inflammatory cytokines, IL-6 and TNF-α.

So, increased inflammatory condition could be due to reduced adropin level as adropin decreased the mRNA expression levels of TNF-α and IL-6 in the pancreas of diabetic rats and may play a protective role in diabetic nephropathy development through anti-inflammatory effects [30].

In this work, a significant increase in levels of ovarian tissue MDA was found in obese PCOS group compared with controls. While metformin treatment produced a significant decrease in these levels. In addition, a significant negative correlation between adropin and MDA was found in all groups.

This study supposed that increased oxidative stress in obese PCOS rats could be due to reduced adropin level .This could be supported by the finding that the reduced plasma adropin levels in aged rats were

associated with increased oxidative stress markers in the brain [31].

The present study found a significant higher levels of D- dimer in obese PCOS group compared with controls, while, metformin treatment produced a significant decrease in those levels. Furthermore, a significant negative correlation between adropin and D- dimer was found in all groups.

The relationship between adropin and D-dimer proved in our study can be supported indirectly by other studies showed decreased adropin levels in coronary artery disease While, chronic administration of adropin weakened the development of atherosclerotic lesions in the aorta, with reduction in the monocyte/macrophage infiltration and smooth muscle cell content inside the plaque [32].

Regarding vaspin, there was a significantly higher circulating level in obese PCOS rats compared with controls, while, metformin treatment produced a significant decrease in these levels. Furthermore, there was a significant positive correlation between vaspin and BMI & AC/TC ratio in all groups.

This increased levels can be explained by diet induced obesity as vaspin is mainly expressed in visceral adipose tissue [33].

The results of the present work also showed that circulating vaspin levels were positively correlated with circulating levels of glucose, insulin and HOMA-IR in all groups.

Our study suggested that the increased vaspin level may be a compensation for the disturbed glucose metabolism in obese PCOS group. This notion is supported by Hida et al.[15] who reported that administration of vaspin to obese mice fed with H.F.D. improved glucose tolerance and insulin sensitivity reflected by normalized serum glucose levels.

After metformin treatment, there was a significant decrease in serum vaspin levels. This result is supported by Tan et al.[34] who reported that Metformin therapy through improving insulin sensitivity and decreasing in circulating glucose levels, caused decrease in serum vaspin levels as glucose was found to cause a significant dose dependent increase in vaspin production and secretion from human omental adipose tissue, which indicates that vaspin is induced by hyperglycemia and explains the significant correlation between vaspin and glucose levels in the current study.

Regarding the relationship between circulating vaspin levels and lipid profile in this study, circulating vaspin levels were found to be positively correlated with TG, TC, LDL-C and negatively correlated with HDL in all groups. Our finding comes in agreement with other study that reported positive correlation between vaspin levels and triglycerides levels in controls and obese subjects [35]**.**

Our study suggested that the increased vaspin level may be a compensation for the disturbed lipid metabolism in obese PCOS group. This supported by Gao et al. [36] who reported that vaspin is known to suppress development of atherosclerosis via its antilipidemic properties and also suppress cholesterol efflux.

Additionally, the present study found a significant positive correlation between vaspin and TNF-α levels in all groups.The increased vaspin levels in group II might be a compensation for inflammation through its anti-inflammatory, anti-apoptotic, properties and as through its activity as a serine protease inhibitor. Consistent with this, it was reported that vaspin inhibited the expression of pro inflammatory cytokines; leptin, resistin and TNF- α in white adipose tissue [37].

Additionally, the present study found a significant positive correlation between vaspin and ovarian tissue MDA levels in all groups.This relation can be explained by the inhibitory effect of vaspin on ROS generation and NADPH oxidase activity [38].

Also, the present study found a significant positive correlation between vaspin and D-dimer levels in all groups. Experiments also indicated that vaspin could inhibit the release of many inflammatory markers in vascular smooth muscle cells and reduce FFA-induced apoptosis in endothelial cells [39].

In the present study, we did not find any significant correlations between serum levels of adropin & vaspin and testosterone, estrogen, progesterone, FSH or LH in all

groups. Our findings suggest that vaspin levels in PCOS might be affected by factors other than disturbance in the hormones of pituitary ovarian axis, and in turn this adipokine not affect those hormones directly

Finally, there was a significant negative correlation between adropin and vaspin levels in all groups. This finding can suggest that vaspin might decrease serum adropin levels as explained indirectly by results of who concluded that vaspin regulates the genetic expression of hormones involved in the pathogenesis of metabolic disorders, such as leptin, resistin and adiponectin [15].

In Conclusion, Adropin and vaspin may represent a novel link between obesity and metabolic disturbance in obese PCOS rats. Since these hormones have been associated with obesity, IR, inflammation, oxidative stress and hypercoagulability in those rats. Furthermore, adropin and vaspin may be involved in the beneficial effects of metformin in case of PCOS associated with obesiy.

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