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RESEARCH ARTICLE

ANTI OXIDANT ROLE OF ELEVATED APELIN LEVELS IN RATS WITH POLYCYSTIC OVARY

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ABSTRACT

Background: polycystic ovary syndrome (PCOS), one of the most common causes of infertility with increased incidence of inflammatory state and oxidative stress. Apelin is unique among adipose tissue hormones in being up regulated in the obese state and exert antioxidant activity.
Objective: This research aims to evaluate serum apelin Levels in Rat models of letrozole induced PCOS and asses its relation to oxidative stress markers, TNF-α, hormonal state, and insulin resistance.
Design: 48 Adolescent healthy female albino rats were used. It was divided into two equal groups; group I (normal diet) and group II (high fat diet) for 12 weeks, after the ninth week each group was subdivided into group A (control) and group B (PCOS induced). Fasting serum levels of apelin, insulin, glucose, estradiol, progesterone, LH, FSH and TNF-α were measured and ovaries oxidative stress markers were estimated.
Results: Serum apelin levels are increased in Letrozole induced PCOS in lean and HFD obese rats. While serum apelin levels positively correlated with TNF-α, insulin, HOMA-IR, BMI and ovarian tissues antioxidant markers, it correlated negatively with ovarian tissues oxidant markers. Hyperandrogenemia, Irregular cycles, incidence of subcapsular ovarian cysts with a diminished granulosa cell layer were detected in PCOS groups.
Conclusion: Inflammatory markers as TNF- α is one of the major mechanisms which increase apelin levels in lean and obese PCOS, beside the synergistic role of hypeinsulinemia. Increased serum apelin levels in PCOS can be considered as compensatory against risk factors of PCOS as it induced increase in ovarian antioxidant markers.

INTRODUCTION

Apelin is a recent adipokine isolated from the bovine stomach (Tatemoto *et al*., 1998). It is the endogenous ligand of the G protein-coupled receptor, (APJ) (Habata *et al*., 1999), apelin is unique among adipose tissue hormones in that is up regulated in the obese state and exert beneficial effects (Bełtowski, 2006). Schilffarth *et al*. (2009) and Shimizu *et al*. (2009) demonstrated up-regulation of mRNA of the apelin/APJ system during development of bovine follicles of normal ovaries, it may be involved in the proliferation of capillaries that accompanies the selection of the preovulatory follicle, resulting in an increased supply of nutrients and precursors, and therefore supporting growth of the dominant follicle, moreover, increased expression levels of apelin and APJ during corpus luteum (CL) formation and function, that led to the assumption that this system may be associated with vascular function in the ovary.

PCOS, is endocrine disease that affects 4% to 8% of women in their reproductive age, is characterized by hyperandrogenism, ovulatory dysfunction and infertility (Cardozo *et al*., 2011), with increased ovarian stroma and subcapsular follicular cysts (Chen *et al*., 2010). It is usually associated with insulin resistance (IR), obesity, hyperlipidemia, with increased prevalence of type II diabetes (T2DM) (Manco *et al*., 2014), however, the precise mechanisms underlying PCOS are not clear (Moran *et al*., 2010).

Reactive oxygen species (ROS) are important signal molecules in regulation of physiological functions in female reproduction, including folliculogenesis, oocyte maturation, steroidogenesis, CL function, and luteolysis (Agarwal *et al*., 2005), however, they also have a key role in pathological processes in female reproduction (Agarwal *et al*., 2003, Agarwal *et al*., 2008). Oxidative Stress (OS) is disequilibrium between body antioxidant capacity and toxic oxygen and/or nitrogen-derived products, so free radicals are insufficiently detoxified by cellular antioxidants causing tissue damage (Ruder *et al*., 2009; Dikmen *et al*., 2012).

OS play a role in the pathophysiology of PCOS and its related complication (Vakilian *et al*., 2009; Mohamadin *et al*., 2010; Kocer *et al*., 2014). Interestingly, apelin has antioxidant activity in the heart as it preserved cardiac function by inhibiting OS and stimulating catalase, glutathione peroxidase

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(GPx) superoxide dismutase (SOD) activity in cardiomyocytes (Zeng *et al*., 2009; Foussal *et al*. 2010; Pisarenko *et al*., 2014). In view of all previous reports, this study is designed to evaluate serum apelin Levels in letrozole-induced PCOS in rats, and asses its correlation with hormonal levels, insulin resistence, TNFα and OS markers to clarify the possible role of apelin in the pathogenesis of PCOS.

MATERIALS AND METHODS

Animals

This study was conducted on 48 young virgin healthy female albino wistar rats, 6 weeks old with body weight 80-90 gm, were obtained from the animal house of faculty of veterinary medicine- Zagazig University. Rats were kept in steel wire cages (6/cage) in the physiology animal house in faculty of medicine -Zagazig University under hygienic conditions. Animals had free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle. All rats received care in accordance with the national health guidelines and the study protocol was approved by the Institutional Review Board and ethics committee of faculty of medicine-zagazig University.

Methods

Rats were divided into two main groups (n= 24): Group I (lean rats, fed ordinary diet) Group II (high fat diet induced obese rats "HFD") rats fed high fat diet generally contain protein 20%, carbohydrates 35% and fat 45%, mainly in form of lard and soy bean for 9 weeks (Cha *et al*., 2000; He *et al*., 2012). Then each group remain on its diet and further subdivided into subgroup A (control) given 1ml water oral by gavage daily for 21 days (3 weeks), subgroup B (PCOS induced): oral administration of letrozole (non-steroidal aromatase inhibitor, ACDIMA international) (Daily 0.5 mg/kg dissolved in water by gavage) for 21 consecutive day (Kafali *et al*., 2004).

Determination of Sexual cycle

Smears were obtained daily by vaginal washing with saline and fresh unstained samples were evaluated microscopically during the treatment period, cycles with duration of 4 to 5 days were considered regular (Kafali *et al*., 2004*)*. Estrus phases were determined according to Marcondes *et al*. (2002) and Goldman *et al*. (2007) as follow:

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

At the end of the study all rats were weighed 24 h after the last dose of letrozole and BMI were calculated according to the equation: body weight (gm)/length^2 (cm^2) (nose to anus length) (Novelli *et al*., 2008).

At last day of experiment and after overnight fasting, rats were anaesthetised using ether (ADWIC Laboratory Chemicals, Egypt), blood samples were collected from orbital sinus (sampling of controls taken in the estrus phase) and ovaries were dissected(right ovaries were immediately frozen at _70 °C until used for determination of antioxidant markers whereas, the left ovaries of each group were immediately fixed in 4% paraformaldehyde for histopathology). Serum was separated by centrifugation of blood at 3000 rpm for 15 minutes and examined for:

Serum apelin-12 levels: Acoording to Porstmann, and Kiessig (1992) using Phoenix Pharmaceuticals (Rat kits), INC. 330 Beach Rd Burlingame, California USA.
Serum TNF-α: by According to Hosseini-Tabatabaei *et al*., (2009) using KRC3011 rat kits, BioSource International Inc. 542 Flynn Road Camarillo, California 93012 USA.
Serum glucose level: According to Tietz *et al*. (1995) using glucose enzymatic (GOD-PAP) - liquizyme rat Kits (Biotechnology, Egypt).
Serum insulin level: according to Temple *et al*. (1992) using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) rat Kits (BioSource Europe S.A., Belgium).
Serum LH, FSH, Estradiol, Progesterone and Testosterone levels: according to Tietz, (1995) using rat kits: BC-1031, BC-1029, BC-1111, BC-1113 and BC-1115, respectively, BioCheck Inc 323 Vintage Park Dr. Foster City, CA 94404.
Ovarian lipid peroxidation (MDA): according to Ohkawa *et al*. (1979).
Ovarian superoxide dismutase (SOD): according to Kakkar *et al*. (1984).
Ovarian glutathione peroxidase (GPX): according to Reddy *et al*. (1995)
HOMA-IR was assessed by homeostasis model assessment (where HOMA= fasting serum insulin (μIU/mL) x [fasting serum glucose (mmol/L)/22.5] (Matthews *et al*., 1985).

Histopathological examination

The 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-aminopyropyltriethoxysilane and stained with hematoxylin-eoisin preliminary observation (Baravalle *et al.,* 2007).

Statistical analysis

Results were presented as mean ± standard deviation. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 19.0 (SPSS Inc., Chicago, IL, United States). Repeated measures of analysis of variance (ANOVA) were applied followed by the Student-

Newman-Keuls post hoc test to compare means of each two different groups. Pearson's correlation analysis was performed to screen potential relations between serum apelin and all parameters. Multiple linear regression analysis was performed to study the relationship between ovarian antioxidant marker (SOD) and serum apelin levels. For all statistical tests done, P value < 0.05 was considered to be statistically significant.

RESULTS

Sexual cycle

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. At the beginning of the experiments, all rats had regular cycles. While all control rats had regular sexual cycles, all letrozole-treated rats were completely acyclic and exhibited constant estrus.

Statistical analysis (Table 1)

Lean PCOS group (IB) had significant high levels of serum apelin, testosterone, TNF-α, ovarian SOD, MDA when compared with that of lean control group (IA), (mean±SD) (0.58±0.042, 237.04±15.03, 46.42±3.27, 13.04±1.91, 126.99±16.90 versus 0.38±0.036, 78.16±9.88, 13.46±2.07, 7.92±1.14, 84.28±6.59 respectively) (P value: < 0.001, < 0.001, < 0.001, < 0.01, < 0.001 respectively).

While there were significant low levels of serum estradiol and progesterone compared with controls (mean±SD) (15.61±3.62, 5.11±1.03 versus 32.15±5.20, 7.81±0.98 respectively) (P < 0.001), however, no significant change in serum insulin, glucose, calculated HOMA, BMI, LH/FSH ratio and ovarian GPx, (mean±SD) (20.70±3.60, 82.42±9.93, 4.30±1.25, 0.49±0.048, 0.55±0.09, 18.59±2.90 versus 18.39±3.03, 78.80±17.00, 3.69±1.35, 0.49±0.026, 0.59±0.14, 16.14±2.04 respectively) (P > 0.05).

Obese PCOS group (IIB) showed significant high levels of serum apelin, testosterone, TNF-α, ovarian SOD, MDA compared with that of obese control Group (IIA), (mean ± SD) (0.73± 0.092, 235.16±11.66, 87.50±12.51, 30.65±5.14, 181.37±11.73 versus 0.62± 0.030, 75.08±9.17, 54.50±7.31, 21.55±4.69, 156.99±28.18 respectively) (P value: <0.001, <0.001, <0.001, < 0.001, < 0.01 respectively).While there were significant low levels of serum estradiol and progesterone compared with that of controls, (mean ± SD) (15.71±2.71, 5.07±1.05 versus 34.61±3. 77, 8.09±0.76 respectively) (P<0.001), however, no significant change in serum insulin, glucose, calculated HOMA-IR BMI, LH/FSH ratio and ovarian GPx (48.22±2.64, 194.16±6.01, 23.14±1.72, 0.94±0.14, 0.52±0.12, 0.51±0.12, 24.28±4.12 versus 46.52±3.22 , 192.74±8.54, 22.17±2.19, 0.97±0.088, 0.59±0.15, 21.82±2.60 respectively) (P >0.05). Obese PCOS group (IIB) showed significant high levels of serum apelin, TNF-α, insulin, glucose, ovarian SOD, GPx, MDA, calculated HOMA-IR and

Table 1. All parameters measured in the all studied groups

Parameter	Group IA	Group IB	Group IIA	Group IIB
Apelin (ng\ml)	0.38± 0.037	0.58± 0.042[a]	0.62± 0.030[a]	0.73 ± 0.092[b,c]
BMI (gm/cm$^{2)}$	0.49±0.026 r= +0.797**	0.49±0.048 r=+0.821**	0.97±0.088[a] r=+0.699*	0.95±0.14[c] r=+0.866***
TNF-α (pg/ml)	13.46±2.07 r =+ 0.826**	46.42±3.27[a] r =+ 0.786**	54.50± 7.31[a] r =+0.780 **	87.50±12.51[b,c] r =+0.889 ***
Testosterone (pg/ml)	78.16±9.88 r=0.422	237.04 ±15.03[a] r=0.333	75.08± 9.17 r=0.420	235.16 ±11.66[b] r=0.326
estradiol (pg/ml)	32.15±5.20 r= 0.257	15.61±3.62[a] r=0.225	34.61±3. 77[a] r=0.116	15.71±2.71[b] r=0.070
progesterone ng/ml	7.81±0.98 r= 0.072	5.11±1.03[a] r= 0.146	8.09±0.76 r= 0.090	5.07±1.05[b] r= 0.363
LH (IU/ml)	2.16±0.41 r= 0.193	1.95±0.23[a] r= 0.501	2.01±0.35[a] r= 0.126	1.76±0.28[b,c] r= 0.045
FSH (IU/ml)	3.58±0.41 r= 0.385	3.58±0.41 r= 0.288	3.46±0.43 r= 0.431	3.47±0.46 r= 0.094
LH/FSH ratio	0.59 ±0.14 r =0.046	0.55±0.09 r =0.162	0.59±0.15 r =0.252	0.52±0.12 r =0.053
Glucose (mg/ dl)	78.80 ±17.00 r= +0 .778**	82.42 ±9.93 r= +0.665*	192.74±8.54[a] r= + 0.633*	194.16±6.01[c] r= +0 .655*
Insulin (µIU/ml)	18.39±3.03 r= +0.852**	20.80±3.86 r= +0.795**	46.52±3.22 [a] r= +0.714**	48.22±2.64 [c] r= +0.649*
HOMA-IR	3.69±1.35 r =+ 0.808**	4.30±1.25 r =+0.763**	22.17±2.19[a] r =+ 0.777**	23.14±1.72[c] r =+0.754**
SOD (U/gm)	7.92±1.14 r = +0.867**	13.04±1.91[a] r = +0.893***	21.55±4.69[a] r = +0.622*	30.65±5.14[b,c] r = +0.705*
GPx (ng/gm)	16.14±2.04 r =+ 0.621*	18.59 ± 2.90 r =+ 0.586*	21.82±2.60[a] r =+ 0.577*	24.28±4.12[c] r =+0.874 ***
MDA (mmol/gm)	84.28±6.59 r =-0.619 *	126.99±16.90[a] r =-0.721**	156.99±28.18[a] r =-0.616*	181.37±11.73[b,c] r =-0.667 *

N = 12 rats data represented as mean and standard deviation ($\overline{X}$ ± SD**).**

([a])= significant when compared with group IA. ([b])= significant when compared with group IIA,

([c])= significant when compared with group IB. r= correlation with apelin levels, * = p<0.05** = p< 0.01 *** = p< 0.001

BMI compared with that of lean PCOS Group (IB) (P value: < 0.001, < 0.001, < 0.001 < 0.001 < 0.01, < 0.001 < 0.001 < 0.001 respectively) however no significant change in testosterone, estradiol, progesterone and LH/FSH ratio between the two groups (P >0.05). In addition, apelin showed significant positive correlation with TNF-α, insulin, glucose, calculated HOMA-IR, BMI, ovarian SOD, GPx levels (Group IA, p value: <0.01, <0.01, < 0.01, < 0.01, < 0.01, < 0.001, < 0.05 respectively). (GroupIB, p value: <0.01, <0.01, <0.05, <0.05, <0.01, <0.001, <0.05 respectively). (Group IIA) p (< 0.01, < 0.01, < 0.05,< 0.01,< 0.05,< 0.05,< 0.05 respectively). (Group IIB), p(< 0.001,<0.05,<0.05,<0.01,< 0.001,<0.05,< 0.001 respectively), while apelin showed significant negative correlation with ovarian MDA levels in all studied group, Group (IA, IB, IIA, IIB, p value: < 0.05, < 0.01, <0.05, < 0.05 respectively), however, no significant correlation between apelin and testosterone, estradiol, progesterone, LH/FSH ratio in all studied group (P > 0.05).

Multiple linear regression analysis was performed to study the relationship between ovarian antioxidant marker (SOD) and serum apelin levels, HOMA-IR and oxidant marker (MDA). SOD was the dependent variable, and the others used as independent variables. Serum apelin levels were found to be significantly associated with SOD marker in all studied groups {In group IA: Apelin (P=0.045), HOMA-IR (P= 0.935) and MDA (P=0. 983). In group IB: Apelin (P=0.004), HOMA-IR (P= 0.128) and MDA (P=0.11). In group IIA: Apelin (P=0.046), HOMA-IR (P= 0.138) and MDA (P=0.842). Finally group IIB: Apelin (P=0.007), HOMA-IR (P= 0.639) and MDA (P=0.014)}.

Histopathologic findings

Ovaries from the control groups (IA and IIA groups) histologically (under 100_ magnification) had follicles in various stages of development, including secondary follicles, graafian follicles, and recently formed CL (Figure 1 and 3). In ovaries from PCO rats (IB and IIB groups), follicular cysts were visible as fluid-filled sacs on the ovarian surface and histologically (under 100 magnification), it showed increased numbers of atretic and large cystic follicles and the cystic wall was thickened, characterized by a thickened theca cell layer and a diminished granulosa cell layer. (Figure 2 and 4).

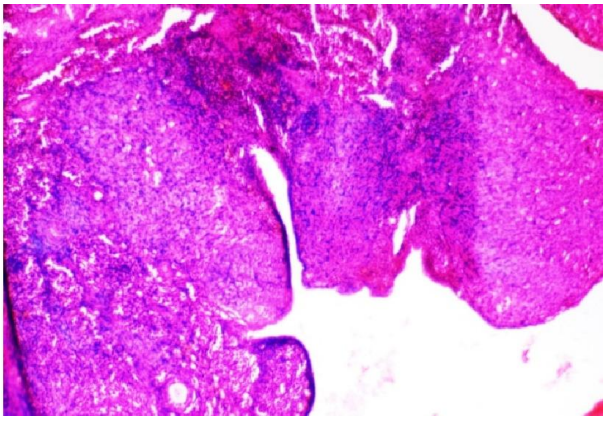

Figure 1. Histologic ovarian tissue sections dyed with H&E in group IA (lean control) under 100_ magnification

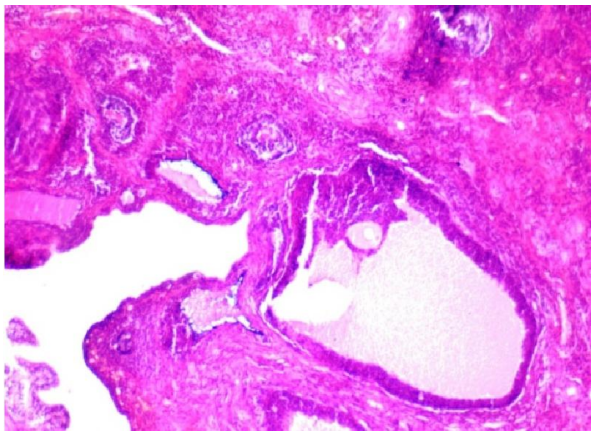

Figure 2. Histologic ovarian tissue sections dyed with H&E in group IB (lean PCOS) under 100_ magnification

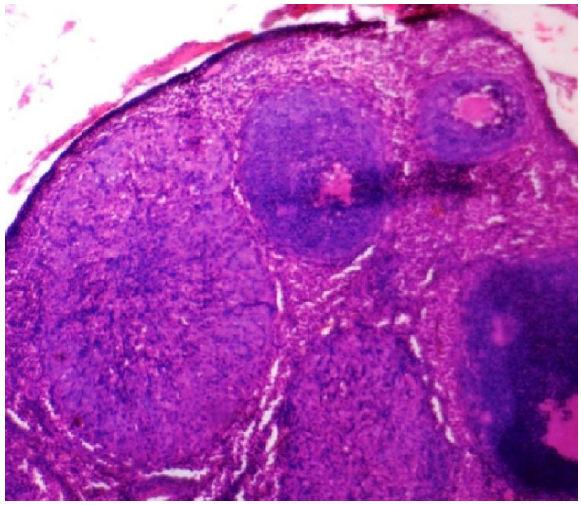

Figure 3. Histologic ovarian tissue sections dyed with H&E in group IIA (obese control) under 100_ magnification

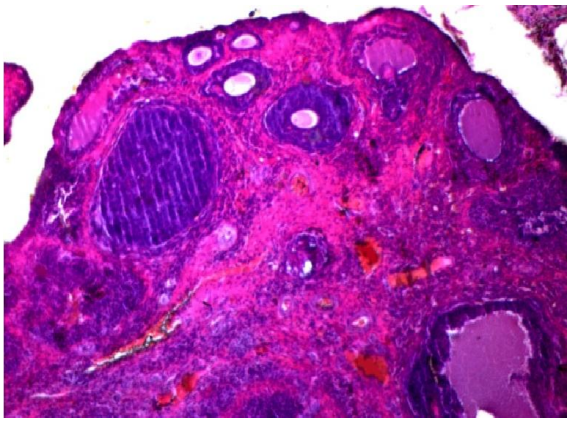

Figure 4. Histologic ovarian tissue sections dyed with H&E in group IIB (obese PCOS) under 100_ magnification

DISCUSSION

PCOS is the most frequent reproductive endocrinopathy in reproductive age women, associated with high prevalence of

obesity (Azziz *et al*., 2004). Patients with PCOS have long term risks such as, T2DM, dyslipidemia, endothelial dysfunction and Oxidative stress (OS) even in young and thin women (Wild *et al*., 2000; Chen *et al*., 2006 and Kocer *et al*., 2014). OS is considered to be one of the main causes of molecular damage to cellular and tissue structures, decreased levels of circulating antioxidants may favor endothelial dysfunction in PCOS patient (Pamuk *et al*., 2012). As apelin is a new link between PCOS, IR, obesity, and hyperlipidemia (Chen *et al*., 2006), we investigated the levels of apelin in letrozole-induced PCOS rat model and tried to explore its relation to hormonal state, IR, BMI, oxidative and inflammatory responses.

In the present study, the signs of PCOS induced by letrozole in lean and obese rats was proved by the significant hyperandrogenism (higher serum testosterone levels) accompanied by significant reduction in both estradiol and progesterone levels in comparison to control groups, in addition to persistent estrus and histopathological features of cystogenesis. These signs occurred because letrozole blocked cytochrome P450 aromatase which is responsible for aromatization of testosterone to estradiol (Van Voorhis *et al*., 1994). Anovulation was expected because there was a decrease in serum progesterone concentrations (Meenakumari *et al*., 2004), increase in the number of atretic and cystic follicles due to disturbed folliculogenesis, and persistent estrus (Rezvanfara *et al*., 2012).

Regarding apelin levels, we found significant higher levels in both lean and obese PCOS induced groups in comparison with control groups. In addition, obese PCOS group had significant higher levels in comparison to lean PCOS group. Our result is consistent with studies done on normal weights adolescents and women with PCOS that revealed significant higher apelin levels when compared with the controls, (Cekmez *et al*. 2011; Gören *et al*., 2012; Olszanecka-Glinianowicz *et al*., 2013). However, these studies did not investigate the mechanisms underlying this high apelin levels and did not declarate the possible role for apelin in this disease. In the contrary, chang *et al*. (2011) and Choi *et al*. (2012) revealed low serum apelin levels in lean women with PCOS, without declaration of the possible mechanism/s, also these studies were limited to lean patient although obesity is a high prevalent condition in PCOS. In the present study, serum apelin levels which was significantly higher in obese than lean rats, showed significant positive correlation with BMI and this is consistent with many studies on obese conditions (Boucher *et al*., 2005; Heinonen *et al*., 2005; Sorhede Winzell *et al*., 2005; Altinkaya *et al*., 2014).

Our result showed no significant correlation between Serum apelin levels and total testosterone or other pituitary ovarian axis hormones, however, Choi *et al*. (2012) reported that in PCOS women, serum apelin levels negatively correlated with total testosterone independent of insulin resistance. Our results suggest that factors other than disturbance in pituitary ovarian axis hormonal that affect the apelin levels in PCOS. Increased serum apelin levels in PCOS can be explained by more than one mechanism, as the increase in synthesis and release of inflammatory mediators, in this study, TNF-α levels were found to be significantly higher in both lean and obese PCOS induced groups in comparison to control group, moreover, TNF- α of obese PCOS group was significantly higher than that of lean PCOS group. Some studies coincide with our results evidenced that TNF-α levels were higher in patients with PCOS than healthy women (Toulis *et al*., 2011; Choi *et al*., 2012), moreover, Gonzalez *et al*. (2009) observed elevated serum TNF-α in patients with PCOS compared with age and BMI matched controls.

However, in controverse with us, Escobar-Morreale *et al*. (2011) showed no change in TNF-α levels in patient with PCOS. The levels of TNF- α were significantly positive correlated with that of apelin in all studied groups. Our hypothesis regarding the role of inflammatory marker (TNF-α) in elevating apelin levels is supported by Daviaud *et al*. (2006) a study which stated that TNF-α can promote directly both apelin expression and secretion in adipocytes, this expression is directly regulated by TNF-α via the MAPK, JNK and PI3-K in human and mouse adipose tissue. Moreover, Yamagata *et al*. (2012) demonstrated that TNF-α induced apelin gene expression in cultured human endothelial cell lines.These studies with our result confirm that inflammatory mediators as TNF-α are one of the mechanisms underlying increased apelin levels in PCOS.

Another explanation is hyperinsulinemia and IR, our result showed significant positive correlation between apelin levels and both of insulin levels and calculated HOMA-IR in all groups. Hyperinsulinemia has close correlation with apelin levels, furthermore, the expression of apelin increased by hyperinsulinemia in obese condition with IR in humans and rats (Boucher *et al*., 2005; Ronti *et al*., 2006; Cekmez *et al*., 2011; Altinkaya *et al*., 2014; Karbek *et al*., 2014) so we suggested that hyperinsulinemia plays a role in apelin increase in obese PCOS. However, in our present study, significant hyperinsulinemia and IR were proved only in HFD obesity induced groups, while there were significant high apelin levels in lean PCOS without significant hyperinsulinemia.

In the present study, MDA levels (a marker of lipid hydroperoxides “LPO” that assess the peroxidation process) in ovaries were found to be significantly higher in lean and obese PCOS induced rats which is consistent with studies revealing higher MDA circulating levels of lean and obese women with PCOS (Sabuncu *et al*., 2001; Gao *et al*., 2013; Bahmani *et al*., 2014; Kocer *et al*. 2014). Moreover, Blair *et al*. (2013) observed that oxidative status was increased in the PCOS subjects relative to their weight-matched controls. Significant increase in SOD levels was found in the present study in rats with PCOS, which was probably in compensation for the increase in oxidative status. In addition, there is increase in GPx activity in PCOS Induced rats compared with that of controls but it is insignificant. This result is consistent with Sabuncu *et al*. (2001) and Murri *et al*. (2013), who showed significant increase in SOD levels in PCOS with insufficient increase in GPx, they refered the insignificant increase in GPx to the substrate GSH amount of GPx, which was decreased.

In the contrary to us, Dikmen *et al*. (2012) and Dănească *et al*. (2014) showed decrease in antioxidant enzyme levels,

increased LPO activity but they used another rat model of PCOS using estradiol valerate, moreover, they also used sesame oil as a solvent for the estradiol in this study which may have caused antioxidant metabolic effects which have been suggested by Ahmad *et al*. (2006), this could explain the curious results in that regarding the decreased antioxidant enzyme levels, this study criticized itself and suggested the need for better designed experimental studies to elucidate the aetiopathogenesis of PCOS via novel techniques.

Apelin is significantly positive correlated with antioxidant SOD and GPx and significantly negative correlated with oxidant MDA levels in the ovary. Interestingly, there are many studies proved the antioxidant activity of apelin in preserving cardiac function by inhibiting OS and stimulating catalase, SOD activity and GPx in cardiac tissue (Zeng *et al*., 2009; Foussal *et al*., 2010; Pisarenko *et al*., 2014). In addition, it was found that potent antioxidant effects induced through increasing apelin expression in rat aorta (Jin *et al*., 2009). Recently It was evidenced that apelin suppresses production and release of reactive oxygen species (ROS) in adipocytes, this is further supported by the observations that apelin promotes the expression of anti-oxidant enzymes via mitogen-activated protein kinase (MAPK)/ extracellular regulated kinases (ERK) and AMP-activated protein kinase (AMPK) pathways, and suppresses the expression of pro-oxidant enzyme via AMPK pathway. Apelin is able to relieve OS-induced dysregulations of the expression of anti- and pro-oxidant enzymes, mitochondrial biogenesis and function, as well as release of pro- and anti-inflammatory adipocytokines (Than *et al*., 2014).

These data with our result suggest the antioxidant activity of apelin in the ovary. The results of our study show a clear increase in oxidant status and compensatory, increase in some antioxidants. Apelin was found to be the inducer of antioxidant activation as there was significant association between serum apelin levels and ovarian SOD marker activity in all studied groups.

In conclusion: serum apelin levels are increased in Letrozole induced PCOS in lean and HFD obese rats. While serum apelin levels positively correlated with TNF-α, insulin, HOMA-IR, BMI and ovarian tissues antioxidant markers, it correlates negatively with ovarian tissues oxidant markers and have no correlation with pituitary ovarian axis hormones. Now, we can hypothized that, inflammatory markers as TNF-α is considered one of the major mechanisms which increase apelin levels in lean and obese PCOS, beside role of hypeinsulinemia. Finally increased serum apelin levels in PCOS can be considered as compensatory against risk factor associated with PCOS as it increased antioxidant activity, but future role of apelin as therapy for PCOS need further investigation.

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