

SERUM LIPOCALIN-2 LEVELS IN EXPERIMENTALLY- INDUCED OBESE RAT WITH POLYCYSTIC OVARY: CORRELATION WITH METABOLIC AND HEMOSTATIC PARAMETERS

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine abnormalities in obese women of reproductive age that contributes to metabolic complications and increases the risk of hemostatic dysregulation. However, the effects of PCOS on coagulation and fibrinolysis have remained largely unexplored. Lipocalin-2 is an adipokine, elevates in obese and appears to play a role in the development of insulin resistance, which is one of cardinal characteristics of PCOS that predispose to hypercoagulable state.

Objective: Assessment of serum lipocalin-2 levels and correlate those levels with some metabolic and hemostatic features in experimentally-induced obese rats with PCOS.

Design: A total number of 20 young female albino local strain rats were divided into 2 main groups, group I (control group) and group II (obese polycystic group) that fed high fat diet (HFD) (45% fat) for 12 weeks and treated with oral letrozole (0.5 mg/kg BW) daily for the last 21 days. In both groups, serum levels of glucose, insulin (with calculation of HOMA-IR), lipocalin-2, total cholesterol, triglycerides, HDL, LDL, LH, FSH, estrogen, progesterone, testosterone, C-reactive protein, BT, WBCT, PT, aPTT, plasma fibrinogen, plasma D-dimers, platelet count, platelet aggregation and soluble vascular cell adhesion molecule-1 (sVCAM-1) were estimated.

Results: The results of this study showed a significant increase in serum lipocalin-2 levels in obese PCOS in comparison with that of control group, which was correlated positively and significantly with: BMI, serum glucose levels, insulin levels, HOMA-IR index, atherogenic lipid profile and markers of hyper-coagulability in the same group.

Conclusion: Elevated lipocalin-2 level in obese rats with PCOS may represent a novel link between metabolic signals, atherosclerosis, and hypercoagulability markers.

Keywords: Polycystic ovary, high fat diet, lipocalin, insulin, hemostasis, rat.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Women with PCOS commonly present with oligomenorrhea, hirsutism, subfertility and obesity

(Wild et al., 2000 and Fauser et al., 2012). Obesity may play a role in the etiology of PCOS and weight loss has been found to improve many clinical features of PCOS including menses regularity and fertility (Crosignani et al., 2003). PCOS is associated with obesity as

a cardinal sign in most cases, with increased cardiovascular (CV) risk markers including inflammation, and insulin resistance (IR) (Thomson et al., 2012).

Lipocalin-2 is a 25 kDa glycoprotein that consists of 178 amino acid residues and was first isolated in human neutrophils (Cowland and Borregaard, 1997). Lipocalin-2 mRNA has been isolated in the bone marrow as well as in tissues exposed to microorganisms. In addition, lipocalin-2 is expressed in several types of cells including adipocytes, endothelial cells, macrophages, vascular smooth muscle cells, hepatocytes, and bone marrow cells (Kratchmarova et al., 2002; Bu et al., 2006 and Sunil et al., 2007). Most investigators reported increased serum lipocalin-2 levels, as an adipokine, in obese patients (Choi et al., 2008 and Piouka et al., 2009). Moreover, lipocalin levels are elevated in patients with cardiovascular diseases and might represent an independent cardiovascular risk factor (Piouka et al., 2009).

Lipocalin-2 appears to play a role in the development of insulin resistance, as expression of this peptide is elevated by agents that promote insulin resistance and forced reduction of its expression in cultured adipocytes improves insulin action (Yan et al., 2007). Obesity and insulin resistance are cardinal characteristics of the polycystic ovary syndrome (PCOS). However, there are limited data on serum lipocalin-2 levels in patients with PCOS (Kahal et al., 2015).

Since a considerable proportion of patients with PCOS has obesity and insulin resistance, type 2 diabetes mellitus

(T2DM) and low-grade inflammation, i.e. disorders where lipocalin-2 secretion is affected (Wang et al., 2007 and Panidis et al., 2010). At the same time, there is a substantially increased risk of hemostatic disorders, including excessive activation of coagulation system, inhibition of fibrinolysis, decreasing endothelial thrombo-resistance and/or pro-inflammatory state in those conditions (Palomo et al., 2006 and Czestochowska, 2007). The present study was designed to assess serum lipocalin-2 levels and correlate those levels with some metabolic and hemostatic features in experimentally-induced obese rats with PCOS.

MATERIALS AND METHODS

Animals: This study was conducted on 20 young virgin healthy female albino local strain rats, 6 weeks old with body weight 80-90 g, were obtained from the animal house of faculty of Veterinary medicine-Zagazig University. Rats were kept in steel wire cages (5/cage, 50x60x60 cm) in the animal house in Faculty of Medicine - Zagazig University under hygienic conditions. Animals had free access to water and food (Diets were obtained from Faculty of Agriculture, Zagazig University), kept at room temperature and were maintained on a normal light/dark cycle. All procedures were approved by the Institutional Review Board and ethics committee of Faculty of Medicine, Zagazig University.

Methods: Rats were divided into two equal groups, Group I served as controls and received standard chow (25.8 % protein, 62.8 % carbohydrate and 11.4 % fat) (Ahren and Scheurink, 1998) and Group II were obese rats with PCOS fed high fat diet containing protein 20%,

carbohydrates 35% and fat 45%, mainly in form of butter and soy bean for 12 weeks to produce early onset obesity started at the age of 6 weeks (**Cha et al., 2000 and He et al., 2012**) and treated by oral letrozole (non-steroidal aromatase inhibitor, ACDIMA international, Egypt) in a dose of 0.5 mg/kg dissolved in water daily for the last 21 days (**Kafali et al., 2004**) for induction of PCOS in obese rats.

Initial and final body mass index (BMI) of the rats were calculated according to this equation: $BMI = \text{body weight (g)} / \text{length}^2 \text{ (cm}^2\text{)}$ (nose to anus length), to be used as an indicator of obesity where the cutoff value of obesity BMI is more than 0.68 g/cm² (**Novelli et al., 2007**).

Determination of Estrus cycle: After 70th day of age (the expected age of the female rats to be adult - **Marcondes et al., 2002**), vaginal smears of all females were taken daily at 1PM and analyzed under the microscope and the mean frequency of diestrus, metestrus, proestrus and estrus was determined and plotted in records of each labeled rat. 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)**.

Blood sampling: At the last day of the experiment and after overnight fasting, rats were anesthetized using ether (ADWIC Laboratory Chemicals, Egypt), blood samples (6-8 ml) were collected from orbital sinus (sampling of controls taken in the estrus phase) between 9-11 a.m. Blood was divided as follow: 2 ml of the blood was collected in a plastic centrifuge tube containing 3.2% sodium

citrate solution (0.1 ml/0.9 ml blood). plasma was separated by centrifugation of blood immediately at 1258 r.p.m for 10 min and divided into two parts: first part of plasma was used for determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and D-dimer levels and platelet count (platelet rich plasma-PRP), the other part of plasma was centrifuged again at 3000 r.m.p for 15 minutes to separate platelet-poor plasma (PPP), which was used with PRP to determine platelet aggregation. 3ml of the blood, each 1ml was placed in a separate test tube, for calculation of whole blood clotting time (WBCT). The remaining amount of blood was collected in clean plastic centrifuge tubes and allowed to clot. Serum was separated by centrifugation of blood at 3000 r.p.m. for 15 minutes (**Tietz et al., 1995**). The supernatant serum was pipetted off using fine tipped automatic pipettes and stored deep frozen at -20°C until assayed.

Hormonal and Biochemical Analysis:

- Serum lipocalin-2 levels: by using rat enzyme-linked immunoassay kits (Catalog Number: 201-11-5109, Shanghai Sunred biological technology, China) (Goetz et al., 2002).
- Serum glucose levels: by using glucose enzymatic (GOD-PAP)- liquizyme Kits (Biotechnology, Egypt) (Tietz et al., 1995).
- Serum insulin levels: by using rat insulin enzyme-linked immunosorbent assay kit, (Product Number: RAB0904, Sigma-Aldrich Chemie GmbH, U.S.A (Temple et al. (1992).
- Calculation of homeostasis model assessment of insulin resistance (HOMA-IR):

- The following equation was used; [insulin (?U/mL) x glucose (mmol/l) /22.5] (Matthews et al., 1985).
- Serum total cholesterol (TC) levels: using rat cholesterol enzyme-linked immunosorbent assay kit, (Catalog Number: 2011-11-0198, Shanghai Sunred biological technology, China) (Allain et al., 1974).
 - Serum triglycerides (TG) levels: using rat triglycerides enzyme-linked immunosorbent assay kit (Catalog Number: 2011-11-0250, Shanghai Sunred biological technology, China) (Naito, 1989).
 - Serum high density lipoproteins (HDL) levels: using rat HDL-cholesterol enzyme-linked immunosorbent assay kit, (Catalog Number: 2011-11-0255, Shanghai Sunred biological technology, China) (Warnick et al., 1983).
 - Serum low density lipoproteins (LDL) levels: LDL was calculated as follows: $LDL=TC-HDL-TG/5$ (Friedwald et al., 1972) .
 - Serum LH, FSH, Estradiol, Progesterone and Testosterone levels: using rat ELISA kits: (BC-1031, B C-1029, BC-1111, BC-1113 and BC-1115, respectively, Bio Check Inc 323 Vintage Park Dr. Foster City, Canada) (Tietz et al., 1995).
 - Serum C-reactive protein (CRP): by using rat Immuno-enzymometric assay kits, (Monobind Inc Lake Forest, Ca 92630, USA), (Kimberly et al., 2003).
 - Whole blood clotting time (WBCT) according to Quick, (1966).
 - Prothrombin time (PT) using using coagulometer according to Arkin, (1996).
 - Activated partial thromboplastin time (aPTT) using coagulometer according to Ansell, (1992).
 - Plasma fibrinogen levels using coagulometer (Cooper and Douglas, 1991).
 - Plasma D-dimer (a marker of hypercoagulability) levels by enzyme-linked immunosorbent assay kit, (GenWay Biotech, Inc, ca 40-88-234402, USA) (Declerck et al., 1987).
 - Platelet count by automated analyzer (Sysmex-KX 21N – Sysmex Corporation) (Brecher et al., 1953).
 - Platelet aggregation, using platelet aggregometer coultronics (Marcus, 1982) using Dia Med Kit. The aggregometric curves were recorded on the platelet aggregometer Apact II (Labitec, Ahrensburg, Germany) and the extent of platelet aggregation was defined as the slope of the aggregometric curve.
 - **Serum sVCAM:** Serum sVCAM-1 as indicator of endothelial dysfunction was measured using ELISA kits (CUSABIO BIOTECH, China) according to Lee et al. (2008).

The hemostatic parameters:

- Bleeding time (BT) according to Martin, (1981).

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-aminopyropyl-triethoxysilane and stained with hematoxylin-eosin dye (Baravalle et al., 2007).

Statistical analysis: Data were presented as mean ± SD. Statistical significance was determined by unpaired Student's "t" test, P values less than 0.05 were considered to be significant. The correlations between parameters were analyzed using Pearson's correlation. SPSS version 18.0 program for Windows (SPSS Inc. Chicago, IL, USA) was used.

RESULTS

Estrus cycle: Control rats (group I) showed regular estrus cycles (4-5 days) while letrozole-treated rats (group II) were completely acyclic and exhibited constant estrus for at least 10 days (indicating anovulation).

Histopathological findings: Histological examination of ovarian tissues from control rat (Group I) showed normal tunica albuginea and a cortical region with

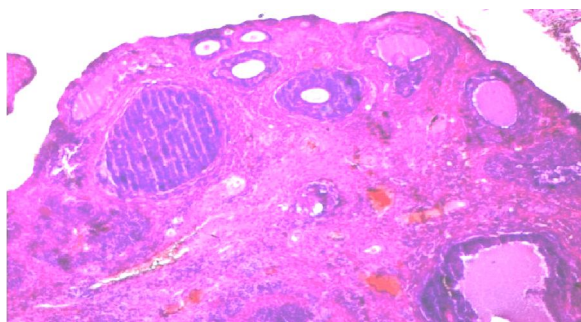


Figure (1): Ovarian tissue from group I. (H&E- ×50)

Biostatistical analysis (Table 1): Obese PCOS rats in group II showed a significant increase ($p < 0.001$) for all and ($P < 0.01$ for CRP) in BMI, serum levels of

numerous follicles at different stages, normal oocytes and several well-developed corpora lutea. There is also mild hyperemia and mild edema in the interstitial regions; (H&E ×50) (Fig. 1). While ovarian tissues from obese PCOS rats (Group II) showed:

- Multiple cystic follicles covered by a dense fibrous capsule, ovarian tissue was covered by dense fibrous connective tissue that is slightly vascularized.
- Luteinization of the theca interna (hyperthecosis), few corpora lutea or corpora albicantia denoting anovulation, atretic follicles simulate corpora albicantia.
- A prominent band of luteinized theca cells surrounds the cavity of an atretic follicle (follicular hyperthecosis); (H&E ×50) (Fig. 2).

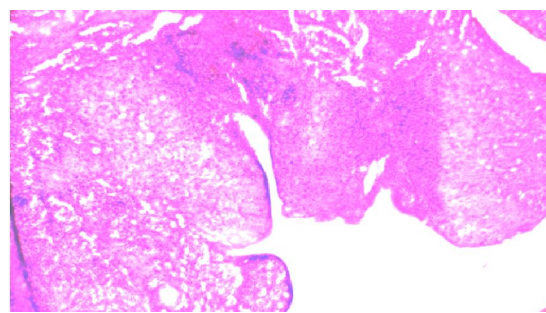


Figure (2): Ovarian tissue from group II. (H&E- ×50)

glucose, insulin, lipocalin-2, HOMA-IR, TC, TG, LDL and CRP when compared with the control group (group I). There was a significant decrease ($p < 0.001$) in

HDL levels in comparison with that of controls. In addition, a significant positive correlation was detected for lipocalin-2 serum levels with BMI ($r=0.83$; $p<0.001$), glucose levels ($r=0.77$; $P<0.01$), insulin levels ($r=0.91$; $P<0.001$), HOMA-IR ($r=0.95$; $P<0.001$), levels of TC ($r=0.71$; $p<0.05$), TG ($r=0.78$; $p<0.01$) and LDL ($r=0.75$; $p<0.01$). Moreover, in obese PCOS (group II) there was a

significant ($P<0.001$) increase in serum testosterone levels, while there was a significant decrease in levels of serum estradiol, progesterone and LH compared with controls ($P <0.001$). Regarding serum FSH levels, no significant change was detected. In addition, no significant correlation was detected between serum lipocalin-2 levels and any of the above mentioned parameters.

Table (1): BMI, hormonal and metabolic parameters of the studied groups (Mean \pm SD).

Parameters	Group I (control)	Group II (obese PCOS)
Initial BMI(g/cm ²)	0.36 \pm 0.03	0.35 \pm 0.05
Final BMI(g/cm ²)	0.48 \pm 0.04 $r=0.75^*$	0.89 \pm 0.14* $r=0.83^*$
Fasting serum glucose (mmol/l)	4.22 \pm 0.44 $r=0.65$	12.51 \pm 1.3* $r=0.77^*$
Fasting serum insulin (uIU)/ml	12.36 \pm 1.54 $r=0.03$	38.41 \pm 3.47* $r=0.91^*$
HOMA-IR	2.32 \pm 0.03 $r=0.03$	21.36 \pm 0.2* $r=0.95^*$
Lipocalin-2 (pg/ml)	48.99 \pm 3.44	96.55 \pm 5.89*
Serum TC levels (mg/dl)	110.75 \pm 14.65 $r=0.04$	208.6 \pm 15.66* $r=0.71^*$
Serum TG (mg/dl)	66.33 \pm 4.13 $r=0.25$	105.87 \pm 11.84* $r=0.78^*$
Serum HDL (mg/dl)	33.5 \pm 3.3 $r= -0.05$	21.19 \pm 4.4* $r= -0.15$
Serum LDL (mg/dl)	70.55 \pm 10.55 $r=0.01$	166.21 \pm 8.96* $r=0.75^*$
C-reactive protein(mg/l)	0.043 \pm 0.022 $r=0.221$	0.179 \pm 0.032* $r=0.91^*$
Serum testosterone (pg/ml)	81.16 \pm 10.11 $r=0.55$	215.13 \pm 12.66* $r=1.21$
Serum estradiol (pg/ml)	33.03 \pm 6.1 $r=0.76$	15.71 \pm 3.56* $r=0.065$
Serum progesterone ng/ml	7.55 \pm 1.1 $r=0.012$	4.93 \pm 0.88* 0.076
Serum LH (IU/ml)	2.13 \pm 0.55 $r=1.99$	1.36 \pm 0.13* $r=1.05$
Serum FSH (IU/ml)	3.43 \pm 0.38 $r=0.09$	3.46 \pm 0.41 0.08

r =correlation coefficient versus lipocalin-2 levels.

*=significant

In relation to the hemostatic changes in group II, BT, WBCT, PT and aPPT were found to be significantly ($p<0.001$) decreased, while, plasma fibrinogen and D-dimers levels were found to be significantly ($p<0.001$) increased. Concerning platelets, no significant difference in the platelets count was found between groups. There was a significant increase in the percentage of platelet aggregation in group II ($P<0.001$) when compared with that of group I. Furthermore, a significant ($p<0.001$) increase in sVCAM levels were reported

in the same group. Moreover, serum lipocalin-2 levels were found to be correlated negatively and significantly with BT ($r=-0.92$, $p<0.001$), WBCT ($r=-0.89$, $p<0.01$), PT (-0.83 , $p<0.05$), aPTT ($r=-0.93$, $p<0.001$) and positively with plasma fibrinogen ($r=0.92$, $p<0.001$), D-dimers ($r=0.74$, $p<0.05$), % of platelet aggregation ($r=0.95$, $p<0.001$) and sVCAM ($r=0.93$, $p<0.001$) levels in group II (Table 2).

Table (2): Hemostatic parameters of the studied groups (Mean \pm SD).

Parameters	Group I (control)	Group II (obese PCOS)
BT (sec)	210.5 \pm 21.2 $r= -0.88$	172.6 \pm 12.5* $r= -0.92^*$
WBCT (sec)	221.3 \pm 28.7 $r= -0.34$	163 \pm 14.2* $r= -0.89^*$
PT (sec)	12.2 \pm 2 $r= -0.71$	9.76 \pm 1.28* $r= -0.83^*$
aPTT (sec)	23.5 \pm 2.9 $r= -0.18$	12.8 \pm 3.5* $r= -0.93^*$
Fibrinogen (mg/dl)	285.9 \pm 59 $r=0.22$	458.9 \pm 71.1* $r=0.92^*$
D-Dimers (mg/dl)	149.88 \pm 19.96 $r= 0.67$	224.15 \pm 23* $r=0.74^*$
Platelet count (1000/mm ³)	218.5 \pm 19.9 $r=0.45$	219.1 \pm 15.8 $r=0.66$
% platelet aggregation	25 \pm 0.4 $r= 0.66$	59.7 \pm 2.5* $r=0.95^*$
Serum sVCAM (ng/ml)	3.21 \pm 0.66 $r=0.45$	8.01 \pm 0.91* $r=0.93^*$

r =correlation coefficient versus lipocalin-2 levels

*=significant

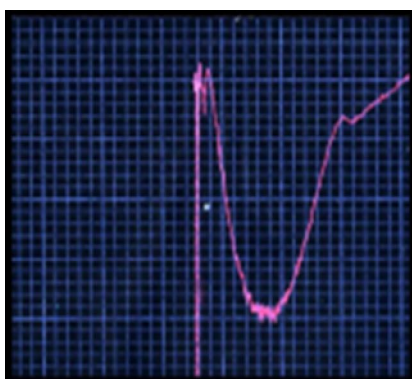


Figure (3): Platelet aggregation curve in group I
Platelet aggregation 23%.

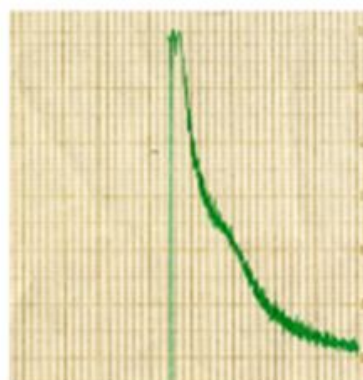


Figure (4): Platelet aggregation curve in
group II Platelet aggregation
59.9%

DISCUSSION

Polycystic ovary syndrome is one of the most common endocrine abnormalities in women of reproductive age, with a reported prevalence of 4% to 8% (Rotterdam, 2004). PCOS is characterized by hyperandrogenism, ovulatory dysfunction, insulin resistance (IR), and PCO morphology, also this syndrome is associated with high prevalence of obesity (Azziz et al., 2004). Women with PCOS exhibit many disorders, such as hyperinsulinemia, dyslipidemia, hypertension, endothelial dysfunction and inflammation, which are components of the metabolic syndrome (MetS) that increases the risk of cardiovascular events (Wild et al., 2000; Chen et al., 2006; Panidis et al., 2013 and Kocer et al., 2014).

The results of the present work showed that, HFD for 12 weeks (to produce early onset obesity started at the age of 6 weeks) was found to induce marked increase in body mass index (>0.68 , denoting obesity according to Novelli et al. (2007), insulin resistance and dyslipidemia in rats of group II. Moreover, the signs of PCOS induced by

letrozole in those early-onset obese rats was proved by the significant hyperandrogenism (higher serum testosterone levels) accompanied by a significant reduction in both estradiol and progesterone levels in comparison to control group, 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 in group II 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).

Current study revealed also a significant increase in serum lipocalin-2 level in obese PCOS rats, which was correlated positively and significantly with BMI, serum glucose levels, insulin levels and HOMA-IR index, serum TC, TG and LDL, while no significant correlation could be found between its levels and those measured parameters in

controls, except a significant positive correlation with BMI.

There are only few studies that assessed serum lipocalin-2 levels in patients with PCOS. However, they yielded conflicting results; in one study lipocalin-2 levels were lower in patients with PCOS than in controls (**Diamanti-Kandarakis et al., 2004**), while another studies showed that lipocalin-2 levels were elevated in patients with PCOS compared with controls and that lipocalin-2 may prove to be a useful marker of IR in those patients (**Diamanti-Kandarakis et al., 2008 and Cakal et al., 2011**). However, in a study by **Panidis et al. (2010)**, no significant differences in serum lipocalin-2 levels were observed between patients with PCOS and controls. The discrepancy between our results and those of others may be due to differences in: species, sample size, nutritional status, degree of metabolic disturbances or other currently undefined factors that may affect lipocalin-2 regulation.

Lipocalin-2 is a 25 kDa protein belonging to the lipocalin family and is produced by immune cells mainly neutrophils, and also by adipocytes being adipocytokine (**Kjeldsen et al., 1993**). Accordingly, many researchers reported elevated circulating levels of lipocalin-2 in adults with obesity and MetS (**Wang et al., 2007; Corripio et al., 2010; Auguet et al., 2011 and Liu et al., 2015**). Insulin resistance, via the resulting hyperinsulinemia, significantly contributes to the endocrine and metabolic disturbances observed in PCOS (**Brenner, 2004 and Manner's-Holm et al., 2011**). Insulin has been shown to stimulate theca cell androgen synthesis and suppress sex

hormone-binding globulin (SHBG) in the liver, further increasing the free portion of circulating androgens (**Coolman et al., 2006 and Kjerulff et al., 2011**). In addition, adiposity contributes to the conversion of androstendione to the most potent androgen, testosterone, because adipocytes have been shown to express significant amounts of the enzyme 17 β -hydroxy-steroid dehydrogenase-ketosteroid reductase (**Peltoketo et al., 1999; Glueck et al., 2003 and Yogev et al., 2010**).

Regarding insulin resistance, current results were supported by other investigators who concluded that lipocalin-2 was found to reduce insulin sensitivity in cultured adipocyte and hepatocyte cell lines via the increase in expression of both phosphoenolpyruvate carboxykinase and glucose-6-phosphatase mRNA, which led to increased glucose production (**Yan et al., 2007**). Moreover, it was shown that Lipocalin-2 was induced by factors that promote insulin resistance, such as tumor necrosis factor-alpha (TNF α) and dexamethasone (**Yan et al., 2007 and Kamble et al., 2016**), and that knocking down lipocalin-2 in cultured adipocytes enhanced insulin-stimulated glucose uptake (**Yan et al., 2007**).

It has been reported that lipocalin-2 levels are elevated in patients with CVD and might represent an independent cardiovascular risk factor (**Choi et al., 2008; Cakal et al., 2011; Katagiri et al., 2015; Soylyu et al., 2015 and Ito et al., 2016**). Lipocalin-2 is reported to be expressed in the atherosclerotic plaques and could be involved in creating local and systemic pro-inflammatory environment characteristic for atherosclerosis

(Hemdahl et al., 2006 and Eilenberg et al., 2016).

Choi et al. (2008) and Ni et al. (2013) reported that, lipocalin-2 serum levels have been positively correlated with serum TG and negatively correlated with serum HDL, indicating that, the related atherogenic mechanism of lipocalin-2 may involve disruption of lipid metabolism, which is in line with the results of this work.

Concerning sex hormones, no significant correlation was detected between serum lipocalin-2 levels and any of the following parameters in neither group I nor group II; serum FSH, LH, progesterone, estradiol and testosterone.

In relation to the hemostatic changes in group II, BT, WBCT, PT and aPTT were found to be significantly decreased, while, plasma fibrinogen, D-dimers levels and percentage of platelet aggregation were found to be significantly increased (indicating hypercoagulable state). However, no significant difference in platelets count was found. Furthermore, serum lipocalin-2 levels were found to be correlated negatively and significantly with BT, WBCT, PT and aPTT and positively with plasma fibrinogen, D-dimers levels and percentage of platelet aggregation in group II, but not in group I.

There is a relatively strong evidence suggesting that PCOS is associated with increased platelet aggregation and decreased plasma fibrinolytic activity. However, whether these hemostatic disorders are linked to the abnormal hormonal system in PCOS remains to be elucidated (Targher et al., 2014). Disturbances in the hemostatic system of women with PCOS were observed

including the prothrombotic state, as characterized by hypercoagulability, (as observed in the present study), and hypofibrinolysis (Carmassi et al., 2005 and Lindholm et al., 2010). In this regard, the increased body mass index of obese PCOS cases may influence the coagulation and fibrinolysis systems (Koioy et al., 2012).

Regarding hypercoagulable state of group II, observed in the current study, our results were supported by those of other investigators who reported that, the interaction between PCOS and obesity resulting in a significant increase in the activity levels of Von-Willebrand factor (vWF) & FVIII & FX and tissue plasminogen activator inhibitor-1 (PAI-1) concentration (Gerrits et al., 2010 and Shan et al., 2013). These findings may be attributable to the fact that those factors are released from the vascular endothelium, and hyperinsulinemia, IR, and dyslipidemia, which often accompany PCOS, predispose to endothelial injury. Additionally, free fatty acids, associated with visceral obesity, could increase the production of the endothelial clotting factors (Merten and Thiagarajan, 2000). Furthermore, dyslipidemia can lead also to an increase in tissue factor (TF) synthesis which enables cells to initiate the coagulation cascade (Basaran, 2009). In addition, hyperandrogenism and IR (the 2 major key features in PCOS women) were attributed to elevated PAI-1 concentration alongside with dyslipidemia (Sangle et al., 2008 and Basaran, 2009).

Regarding serum CRP as an inflammatory marker and sVAMC as endothelial dysfunction marker, there was a significant increase in those markers in

obese PCOS rats in comparison with that of controls, which was correlated positively and significantly with serum lipocalin-2 levels in those rats but not in controls. These findings were in line with the results of other investigators who found a significant correlation between serum lipocalin-2 and CRP in; patients with metabolic syndrome (**Wang et al., 2007**) and those with acute coronary disease (**Yan et al., 2007; Ni et al., 2013 and Singh et al., 2015**). Furthermore, **Liu et al. (2015)** indicated a significant associations of lipocalin-2 with the measures of inflammation: high-sensitivity C-reactive protein and white blood cell count, as well as with the markers of endothelial activation; intercellular adhesion molecule-1 (ICAM-1) and E-selectin, which mediates adhesion of activated platelets to neutrophils and monocytes and plays a central role in platelet aggregate size and stability, and may play an important role in the pathogenesis of inflammation and thrombosis (**Merten and Thiagarajan, 2000**).

Since lipocalin-2 has been associated with obesity, IR, inflammatory and endothelial activation markers in obese PCOS rats, which predispose to the observed hypercoagulable state, this peptide may act as a mediator that relates obesity and PCOS.

CONCLUSION

Lipocalin-2 level increased in obese rats with PCOS, and was positively correlated with BMI, HOMA-IR, and an atherogenic lipid profile, while it was correlated negatively with BT, WBCT, PT and aPTT. Furthermore, a significant positive correlation between lipocalin-2

level and levels of: fibrinogen, D-dimers, CRP and sVAMC had been detected, in addition to the significant positive correlation which was found between its level and percentage of platelet aggregation in the same group. These results suggested that lipocalin-2 may represent a novel link between metabolic signals and hypercoagulability in obese rats with PCOS.

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العلاقة بين مستويات الليبوكالين-2 وبعض دلائل الأيض وتخثر الدم في مصلى الجرذان التي تعاني من السمنة ومتلازمة المبيض المتعدد التكيسات المحدثه تجريبياً

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خلفية البحث: متلازمة المبيض المتعدد التكيسات هي واحدة من حالات الخلل الهرموني الأكثر شيوعاً في النساء البدنيات في سن الإنجاب. وتلك المتلازمة تتسبب في مضاعفات ناتجة عن الخلل الأيضي والتي تؤدي إلى الإخلال بآليات وقف نزف الدم. ومع ذلك، ظلت آثار متلازمة تكيس المبايض على التخثر إنحلال الفيبرين غير مستكشفة إلى حد كبير.

ليبوكالين-2 هو أحد هرمونات النسيج الدهني والذي يبدو انه يلعب دوراً في مقاومة الإنسولين، حيث أن مستوياته تزداد في مصلى المرضى الذين يعانون من السمنة ومقاومة الإنسولين، التي تعد من الخصائص الأساسية لمتلازمة تكيس المبايض التي تهيئ لحالة التخثر المفرط في الدم.

هدف البحث: تقييم مستويات الليبوكالين-2 في مصلى الدم وإستكشاف العلاقة بينها وبعض دلائل التمثيل الغذائي وتخثر الدم في الجرذان التي تعاني من السمنة مع متلازمة تكيس المبايض المحدثه تجريبياً.

مواد وطرق البحث: تم تقسيم عشرين من الجرذان الإناث إلى مجموعتين متساويتين: المجموعة الأولى: الضابطة والمجموعة الثانية: مجموعة تعاني من السمنة المفرطة الناتجة عن التغذية بنظام غذائي عالي الدهون لمدة اثنا عشر أسبوعاً، كما تم إعطاؤها عقار ليتروزول عن طريق الفم (0.5 مللى جرام / كيلوجرام من وزن الجسم) يومياً عن طريق الفم لمدة واحد وعشرين يوماً الأخيرة. وفي جميع الجرذان، تم قياس مستويات المصل من: الليبوكالين، و الجلوكوز، والإنسولين (مع حساب مؤشر مقاومة الإنسولين)، والكوليستيرول الكلي، والدهون الثلاثية، والكوليستيرول عالي، ومنخفض الكثافة، وكذلك هرمون الاستروجين، و البروجسترون، و التستوستيرون، و الهرمون المنشط لجريبات المبيض، و هرمون الليوتنيزين، والبروتين التفاعلي-ج، بالإضافة إلى بعض دلائل تخثر الدم مثل زمن النزف، وزمن تجلط الدم الكلي، وزمن البروثرومبين، وزمن البروثرومبين الجزئي النشط، والفيبرينوجين في البلازما، والبلازما D-dimers، وعدد الصفائح الدموية، وتجمع الصفائح الدموية، وجزء الإلتصاق بالخلايا الوعائية -1 الذائب في مصلى الدم.

نتائج البحث: أظهرت نتائج هذه الدراسة زيادة ذات دلالة إحصائية في مستويات الليبوكالين-2 في مصلى الجرذان الذين يعانون من السمنة مع متلازمة تكيس المبايض بالمقارنة مع المجموعة الضابطة كما وجد ارتباط إيجابي ذات دلالة إحصائية بين مستويات الليبوكالين-2، و كل من مؤشر كتلة الجسم، ومستويات الجلوكوز، ومستويات الإنسولين، ومؤشر مقاومة الإنسولين، ومستويات الدهون المسيبه لتصلب الشرايين ودلائل فرط تجلط الدم في نفس المجموعة.

الخلاصة: ارتفاع مستوى الليبوكالين-2 في الجرذان التي تعاني من السمنة مع متلازمة تكيس المبايض قد يمثل حلقة وصل بين مؤشرات التمثيل الغذائي، وتصلب الشرايين، وعلامات فرط تجلط الدم.