Original Research Article

Association of altered serum levels of Chemerin, Paraoxonase-1 (PON1), Asymmetric Dimethyl arginine (ADMA) and obesitin the development of Polycystic Ovarian Syndrome (PCOS) in Egyptian women

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ABSTRACT

Chemerinwas recently added to the adipokine family and was identified in human ovarian follicles and follicular fluid that suggests a direct correlation between chemerin and PCOS. Asymmetric dimethyl arginine (ADMA) is involved in endothelial dysfunctionthe atherogenic potential of ADMA has been investigated in young patients with PCOS. Oxidative stress is considered to be implicated in the pathophysiology of PCOS.Paraoxonase 1 (PON1) is an antioxidant enzyme and its concentration has been shown to be inversely associated with oxidative stress. Objectives: Evaluation of serum chemerin, ADMA, PON1in obese and non-obese polycystic ovarian patients to postulate their role in pathogenesis of PCOS. Methods: Ninetynuligravida women aged 20-35 (60 with PCOS and 30 controls) were recruited. Fasting blood was obtained on day 2 or 3 of the menstrual cycle. Clinical evaluation, hormonal profile, Chemerin, ADMA and PON1 were assessed. Results: There was a significant increase in serum chemerinlevels in PCOS obese group when compared with PCOS non obese patients and healthy controls non obese and obese respectively. Serum ADMA level was increased significantly in PCOS obese group as compared to the PCOS non obese group , control non obese and control obese. Paraoxonase was decreased stepwise significantly from the control non obese group and control obese group to PCOS non obese patients then PCOS obese patients to. Conclusions: it could be suggested that increased chemerin has a role in PCOS development andaltered ADMA and PON1 associated withobesity and oxidative stress may exacerbate the condition.

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder among women of reproductive age, affecting 5–10% of all women in their life span and it is in focus of research because of its increasing prevalence[1]. No single diagnostic criteria are sufficient for its clinical diagnosis. The revised diagnostic criteria of PCOS include any two of the following three features, oligoor anovulation,clinical and/or biochemical hyperandrogenemiaand lastly the finding of polycystic ovaries on ultrasound scan, with exclusion of other etiologies [2].

The etiology of this complex heterogeneous disorder is still uncertain. Environmental factors such as physical inactivity, malnutrition, obesity and insulin resistance

(IR) have crucial role in development of the disorder [3]. These factors might lead to the endothelial dysfunction which is observed before the onset of clinically manifested vascular diseases, mainly and cardiovascular risk in PCOS. The endogenous nitric oxide synthase inhibitor; asymmetric dimethyl arginine (ADMA) represents an independent marker for endothelial dysfunction and cardiovascular morbidity [4].

Dysfunction of adipose tissue has been implicated in the pathophysiology of PCOS. Increasing evidence shows that the dysregulated expression of adipokines, the secreted products of adipose tissue, plays an important role in the pathology of PCOS [5].

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These adipokines include adiponectin, leptin, omentin, resistin, retinol binding protein-4(RBP-4), tumor necrosis factor-α (TNF-α), interleukin-6 (IL6), vaspin, visfatin and chemerin[6].

Chemerin is a novel adipokine that regulates adipocyte development and metabolic function. In humans, plasma chemerin concentrations are correlated with body fat, glucose, lipidmetabolismand inflammation. Plasma chemerin concentrations are elevated in patients with obesity and / or DM [7]. Chemerin has been shown to be expressed in mouse ovary and placenta and in human placenta[8].Chemerinalso has been identified *in vivo* in human ovarian follicles and, more particularly, in granulosa and theca cells and follicular fluid. Chemerin inhibited IGF-1-induced progesterone and estradiol secretion as well as cell proliferation [9].

Oxidative stress has been identified to play a key role in the pathogenesis of subfertility in females. This imbalance between pro-oxidants and antioxidants can lead to a number of reproductive diseases such as endometriosis, PCOS, and unexplained infertility [10].

Human serum paraoxonase-1 (PON1) is a Ca2+dependent high-density lipoprotein (HDL) associated lactonase capable ofhydrolyzing a wide variety of lactones, thiolactones, arylesters, cyclic carbonates and organophosphate pesticides, nerve gases such as sarin and soman, glucuronide drugs and oestrogen esters [11].There is a paucity of data on the oxidative status of patients with PCOS. Reduced antioxidant status and increased oxidative stress in women with PCOS have previously beenreported[12, 13]. To the best of our knowledge the PON1 protein concentration has not been studied as yet in Egyptian PCOS patients. By knowing that PON1 activity is decreased in PCO patients, it was felt interesting to investigate quantitatively the enzyme protein in Egyptian PCOS patients.

The aim of the present study was to evaluate chemerin, ADMA and PON1 levels in obese and non-obese Egyptian women with PCOS and age-matchedhealthy controls and to correlate their relationship with each otherand with clinical, metabolic, and hormonal parameters.

Experimental

Subjects

In this prospective study, 60 patients with PCOS and 30age matched healthy controls were analyzed. The patients with PCOS were divided into two groups according to body mass index (BMI) as obese (BMI more than 30 kg/m^2, n=30) and non-obese (BMI less than 30 kg/m^2, n=30). They were attending the outpatient infertility clinic of Kasr Al Ainy Maternity Hospital, Cairo University, Cairo, Egypt. Control subjects were recruited from women visiting the clinic with infertility due to male factor.

The subjects included in our study with age range between 20 and 35 years,nuligravida, not takingany medications (oral contraceptives, glucocorticoids, antiandrogens, insulin sensitizers, ovulation induction agents, or antiobesity drugs) that could affectthe biochemical profile and metabolic variables. Subjects were excluded if not matching the age range (less than 20 or more than 35 years) and in cases of known medical problems as thyroid dysfunction, virilizing tumors, DM, hepatic dysfunction, renal dysfunction, and hypertension. Smokers were also excluded.

The diagnosis of PCOS was based on the revised criteria of the Rotterdam consensus conference by at least two of the following three features: (i) oligo- or anovulation; (ii) clinical and/ or biochemical signs of hyperandrogenism; and (iii) polycystic ovaries after excluding other etiologies including hyperprolactinemia, non-classical congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumor [14]. Oligomenorrhea and amenorrhea were defined as <8 spontaneous menstrual cycles per year and the absence of a menstrual period for three consecutive months. Clinical hyperandrogenismand hirsutism was evaluated using the modified Ferriman–Gallwey score (patients with score > 8 were considered as hirsute) [15].

Transvaginal ultrasonography examination was done to confirm diagnosis of PCOS, regularly menstruating women were scanned in the early follicular phase (cycle days 3–5), oligo-/amenorrhoeic women were scanned either at random or between days 3 and 5 after a progestin-induced withdrawal bleeding. The size of follicles <10 mm was expressed as the mean of the diameters measured on the two sections. The criteria for polycystic ovaries required visualization of 12 or more follicles in each ovary measuring 2–9 mm in a diameter, and/or increased ovarian volume (>10 cm3).[16].

Written informed consent was obtained from all subjects before entering the study and approved by the ethical comity.

Clinical characteristics and biochemical assays

All candidates were subjected to personal history (name, age, marital state, parity, address), menstrual history (duration, cycle, amount, regularity, last menstrual period) and medical history for acne and hirsutism. The BMI was calculated as weight (kg)/height squared (m^2).Blood pressure was measured in the sitting position after a rest period of at least5 min.

Initially, blood samples (10 mL) were taken between 8:00 and 10:00 a.m. Blood samples were obtained from the antecubital vein, after an overnight fasting on the second

or third day after a spontaneous or progesterone withdrawal menstrual cycle.

Blood samples were taken into plain vacutainer clotted tubes, where sera were obtained by centrifugation within 30 min at 4000 rpm for 10 min. Sera was separated, first for the measurement of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol determined using standard ELISA technique using a kit from ALPCO (USA). Other aliquoted sera were kept frozen at -80°C [17] until used for Chemerin (using standard ELISA technique using a kit provided by RayBio® (Norcross, GA, USA), ADMA (using standard ELISA technique using a kit provided by Sigma (Munich, Germany)and PON1 (using standard ELISA technique using a kit provided by Boster Immunoleader (Pleasanton, USA), determination. Intra-assay and interassay coefficients of variation were 4.7% and 7.3%, respectively for chemerin at the same time as they were 8.2% and 9.8%, respectively for ADMA.

Statistical Analysis

All analysis and graphics were performed using Graphpad prism (windows version 5; Graphpad software 2007). (GraphPad Software, San Diego, USA). Data are presented as mean ±standard deviation orpercentage.One-way analysis of variancewas performed to evaluate differences between the groups. The Tukey test was used as the post hoc test. All data wereanalyzed for normality of distribution. Spearman correlationcoefficient was used to test the strength of associationsbetween different variables.Statistical significance was considered at P≤0.05.

Results

Table (1)

Clinical, biochemical and hormonal characteristics of the PCOS and control obese and non obesegroups.			
Characteristics	**Control non obese**	**Control Obese**	**PCOS non obese**	**PCOS obese**
Number	18	12	30	30
Ageyears	26.3 ± 3.9	28.2 ± 4.1	24.7 ± 4.0	25.1 ± 4.5
BMIkg/m^2	23.8 ± 0.528	32.1 ± 0.562	25.8 ± 0.484	35.9 ± 0.763
LH mIU/mL	4.17 ± 0.235	3.44 ± 0.130	$7.032 \pm 0.191^{a,b}$	$6.892 \pm 0.217^{a,b}$
FSH mIU/mL	7.49 ± 0.261	7.43 ± 0.312	$5.96 \pm 0.170^{a,b}$	$5.52 \pm 0.180^{a,b}$
LH/FSH ratio	0.57 ± 0.15	0.48 ± 0.12	$1.2 \pm 0.32^{a,b}$	$1.3 \pm 0.36^{a,b}$
Estradiol pg/mL	50.2 ± 1.70	49.5 ± 1.38	$60.7 \pm 1.65^{a,b}$	$63.2 \pm 1.59^{a,b}$
Chemerin ng/mL	76.3 ± 3.57	119 ± 5.38	$114 \pm 4.52^{a,b}$	$171 \pm 14.7^{a,b,c}$
ADMAμmol/L	0.452 ± 0.027	0.422 ± 0.033	$0.585 \pm 0.018^{a,b}$	$0.679 \pm 0.027^{a,b,c}$
PON1pg/mL	214 ± 6.29	201 ± 9.39^{a}	178 ± 3.38^{a}	$166 \pm 3.96^{a,b}$

Note: Values are expressed as mean ± SD.

BMI: *body mass index*, LH: *luteinizing hormone*, FSH: *follicular stimulatinghormone*.
[a]significantly different from healthy control non obese group, at $P \leq 0.05$.
[b]significantly different from healthy control obese group, at $P \leq 0.05$.
[c]significantly different from PCOS non obese group, at $P \leq 0.05$.

Clinical characteristics and pituitary-gonadal hormone levels

In the present study, patients are distributed among groups as shown in table (1) and their clinical characteristics and biochemical parameters measured are listed in table (1).Data presented in table (1) revealed that LH levels in PCOS obese and non-obese patients were higher than healthy control non-obese subjects by (165% and 169%, respectively) (P≤0.05) and higher than healthy control obese subjects by (200% and 204%, respectively) (P≤0.05) Whereas, FSH levels decreased significantly in the PCOS groups to 5.96 ± 0.170 mIU/mL and 5.52 ±

0.180 mIU/mL for non-obese and obese womenrespectively (P≤0.05) from healthy control non obese level 7.49 ± 0.261 mIU/mL and healthy control obese level 7.43 ± 0.312 mIU/mL.

Also LH/FSH ratio were higher in PCOS obese and non-obese patients than healthy control non-obese subjects by (228% and 210%, respectively) (P<0.0001) and higher than healthy control obese subjects by (270% and 250%, respectively) (P<0.0001)

However estradiol levels in PCOS patients obese and non-obese were 63.2 ± 1.59 pg/mL and 60.7 ± 1.65 pg/mL, respectively, being higher than the healthy control

non obese subjects level (50.2 ± 1.70 pg/mL) (P≤0.05) and healthy control obese subjects level (49.5 ± 1.38 pg/mL) (P≤0.05).

### Chemerin, ADMA and PON1 levels

Our study revealed that PCOS obese patients showed a significant increase in serum chemerin levels (171 ± 14.7 ng/mL) when compared to PCOS non obese patients and healthy controls non obese and obese (114 ± 4.52, 76.3 ± 3.57, 119 ± 5.38 ng/mL) respectively at P ≤0.05. On the contrary, serum chemerin levels of healthy controls obese patients increased by 156 % and 104 % when compared to the healthy control non obese group and PCOS non obese respectively at P ≤0.05. To add more insight into chemerin results, there was significant

difference in serum chemerin levels between PCOS obese patients when compared to PCOS non obese patients by 150 % at P ≤0.05. These results are illustrated in figure (1). As obvious in figure (2) serum ADMA level was increased significantly in PCOS obese group (0.679 ± 0.027 μmol/L) as compared to the PCOS non obese group(0.585 ± 0.018 μmol/L) as well as the control non obese group (0.452 ± 0.027 μmol/L) and control obese group (0.422 ± 0.033 μmol/L) at P≤0.05. Paraoxonase as antioxidant marker was decreased stepwise significantly from the control non obese group level 214 ± 6.29 pg/mL and control obese group level 201 ± 9.39 pg/mL to PCOS non obese patients 178 ± 3.38 pg/mL then PCOS obese patients to 166 ± 3.96 pg/mL at P ≤0.05. These results are demonstrated in figure (3).
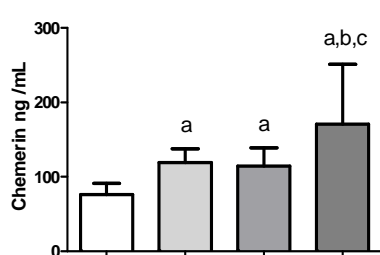
Figure (1): Chemerin levels of the studied groups
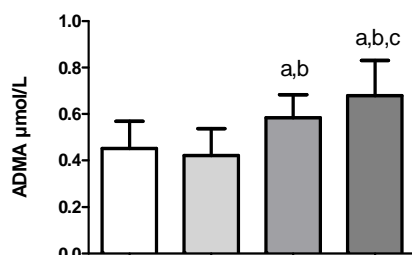
Figure (2): ADMA levels of the studied groups
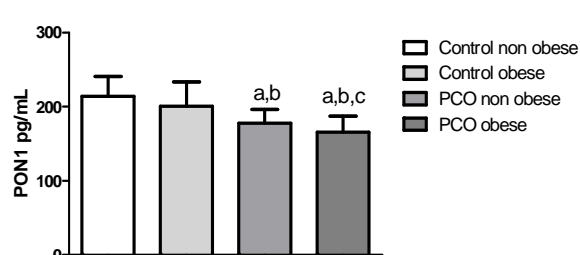
**Figure (3): Paraoxonase levels of the studied groups**

[a] Significantly different from healthy control non obese group, at $P \leq 0.05$.
[b] Significantly different from healthy control obese group, at $P \leq 0.05$.
[c] Significantly different from PCOS non obese group, at $P \leq 0.05$.

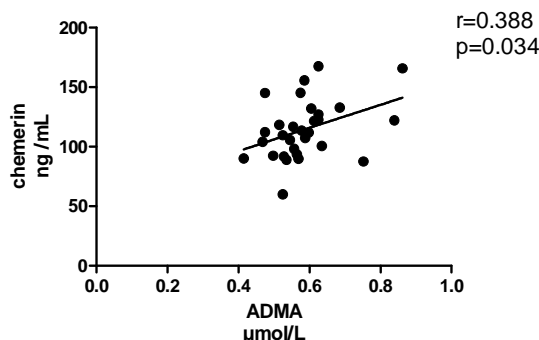### Correlation of serum Chemerin, ADMA andPON1levels

We correlated chemerin serum level with serum ADMA. In PCOS patients obese and non-obese, a significant positive correlation was observed between chemerin serum level and ADMA, whose r=0.388 at p=0.034 and r=0.393 at p=0.032, respectively, as illustrated in figures (4) and (5).Significant negative correlation was observed

between PON1serum level and ADMA, whose r=-0.499 at p=0.005 as demonstrated in figure (6).
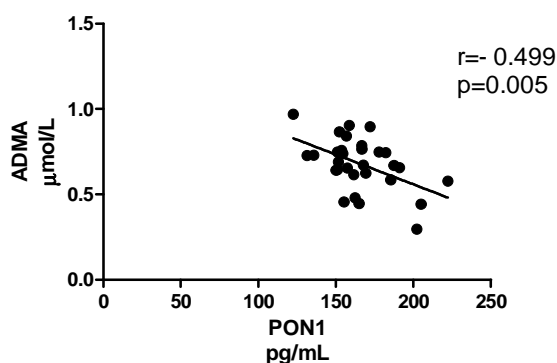
Moreover significant negative correlation was observed between PON1serum level and chemerin, whose r=0.402 at p=0.028 as demonstrated in figure (7).
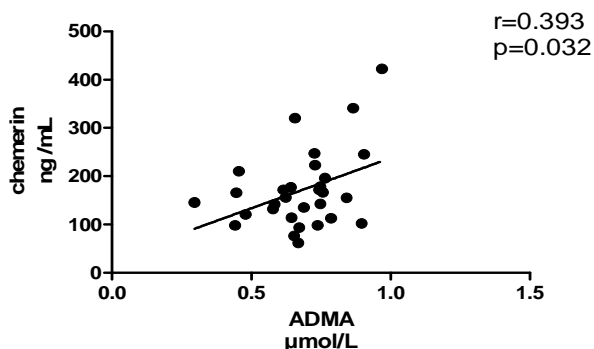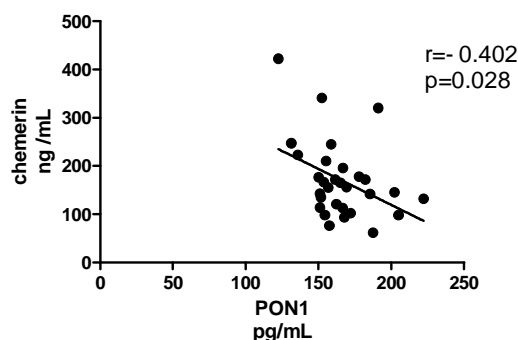

Figure (4): Correlation between serum chemerin and ADMA levels in obese women with PCOS

Figure (5): Correlation betweenserum chemerin and ADMA levels in non-obese women with PCOS

Figure (6): Correlation betweenserum PON1 and ADMA levels in obese women with PCOS

Figure (7): Correlation betweenserum chemerin and PON1 levels in obese women with PCOS

Discussion

Women with PCOS present a diverse combination of clinical complications including, psychological problems, reproductive alterations, and metabolic abnormalities including hyperinsulinemia, insulin resistance, dyslipidemia and obesity [18]. The relationships between PCOS and obesity, insulin resistance and endothelial dysfunction have complex features [19].

The present results revealed significant increase in serum LH and significant decrease in FSH in PCOS patients compared with control subjects.

These results agree withMohamadin *et al.*, (2010), Azziz *et al.*, (2009) and Lewandowski *et al.*, (2011) who postulated that this phenomenon might be potentially useful as an additional tool in the diagnosis of PCOS[20, 21 22]. In the present study it was found that LH is higher in patients with PCOS than in the control subjects, this was in accordance with a study done by Li and Lin, who found that LH was higher in obese women with PCOS when compared with non-obese PCOS patients and normal control subjects [23].Another study done by Samy et.al. who investigated hormonal profile in obese and non-obese PCOS patients and compared them to control normal subjects they found that increased levels of LH compared to healthy BMI matched controls [24].

There have been conflicts regarding the abnormalities of the hypothalamic-pituitary-ovarian (HPO) axis in women with PCOS. Anovulation is associated with disturbances in the feedback from the ovarian steroid hormones to the hypothalamus and pituitary, resulting in disturbances in the pulsatility of gonadotropin releasing hormone (GnRH). Gonadotropin-secretory changes, with a characteristic increase in LH relative to FSH release, have long been recognized in PCOS. It has also been suggested that the elevated concentrations of LH are due to an abnormal feedback by estrogen which may also

induce thecal hyperplasia as LH stimulates theca cell proliferation [25-28].

A significant increase in serum estradiol level was noticed among our PCOS group and this finding may support the postulation that adipose tissue aromatase is involved in this relationship. The excess of adipose tissue in obese patients creates the paradox of having both excess androgens (which are responsible for hirsutism and virilization) and estrone (which inhibits FSH via negative feedback) [29].

Our study revealed that PCOS obese patients showed a significant increase in serum chemerinlevels when compared to PCOS non obese patients and healthy controls non obese and obese subjects and there was significant difference in serum chemerin levels between PCOS obese patients when compared to PCOS non obese patients by 150 % .

Tan *et al* have demonstrated an increase of serum and subcutaneous and omental adipose tissue chemerin expression in women with PCOS [30]. Our finding was in harmony with results of Wang *et al* who have shown a higher level of chemein expression in the ovary of dihydrotestosterone induced PCOS rats [31]. Several experiments have demonstrated that chemerin may play a role in pathophysiology of PCOS in animal or human by direct action on ovary [32, 33]. It is well known that formation of polycystic ovaries is critically associated with abnormal steroidogenesis. It has been found that chemerin decreases estradiol secretion and suppressed FSH-induced progesterone and estradiol secretion in prenatal follicles and granulosa cells by inhibition of aromatase and p450scc expression [33]. It is also well recognized that development of polycystic ovaries is associated with new blood vessel formation although there is no data about the effect of chemerin on ovarian angiogenesis; however it is plausible that chemerin may

act as angiogenic factor [34]. By using an *in vitro* angiogenesis assay, it was shown that chemerin induced the formation of capillary like structures, a process which occur in obese patient and result in adipose tissue expansion [7]. These observations lead to the hypothesis that increasing the chemerin gene expression in ovary of PCOS rats may alter ovarian steroidogenesis or angiogenesis and may play a role in the development and progression of this reproductive disorder. Consistent with previous results, it could be hypothesized that chemerin has a role in PCOS development and manipulation of chemerin gene expression or its signaling may develop novel therapeutic approaches in the treatment of PCOS patients.

In our study, PCOS patients were found to have significantly higher plasma ADMA levels. These high levels of ADMA may be explained by endothelial dysfunction caused by insulin resistance. Paradisi *et al.* reported endothelial dysfunction and insulin resistance in women with PCOS [35]. Orio *et al.* evaluated young PCOS patients with no metabolic and cardiovascular disease and reported disturbances in endothelial functions in the early period of the disease [36].

Our results are in agreement with Heutling *et al.,* who demonstrated that plasma ADMA is increased in women with PCOS when compared to controls and decreased significantly after insulin sensitizer therapy (metformin) and suggested a close relationship between IR and plasma ADMA[37]. On the other hand, Demirel *et al.,* (2007) demonstrated that serum ADMA was not different in adolescent subjects with PCOS and controls[38] and Turkcuoglu *et al.,* (2011) who reported that plasma ADMA, nitric oxide levels and arginine/ADMA ratio were similar in PCOS and control groups[39]. In their study, plasma ADMA level did not correlate with the hormonal and metabolic parameters in patients with PCOS. Also, Pamuk *et al.,* (2010) noticed that there were no significant difference in plasma ADMA level in obese patients with PCOS and healthy controls[40].

The possible mechanism of elevation of serum ADMA in PCOS is not well understood. It may be due to reduced renal excretion [41]and decreased activity of hydrolase enzyme which metabolizes ADMA [42]. As consequence of IR, PCOS patients have an abnormal lipid profile so IR may, contribute in directly to endothelial dysfunction and cardiovascular risk [20].

Moreover, the Correlation between serum chemerin and ADMA levels in obese and non-obese women with PCOS shows that chemerin may be used as an early marker of endothelial dysfunction better than ADMA. This suggestion is based on the correlation results which clearly demonstrate a wider ascending chemerin range

that covers non-obese and obese PCOS patients than that of ADMA levels (figure 4 & 5).

The results of the current study indicate that PON1 levels was significantly decreased in patients with PCOS when compared with healthy controls. In previous studies, reduced serum PON1 activity has been reported to be associated with insulin resistance [13].These results agree with Mohamadin *et al.,* (2010)who found that PON1 activitywas significantly decreased in patients with PCOS whencompared with healthy controls[20].

Mohamadin *et al,* reported that serum PON1 activity, HDL-C and total antioxidant capacity were significantly lower in patients with PCOS than healthy controls [20]. Recently, PON1 became the focus of intense research after the identification of its antioxidant properties, particularly its capacity to protect LDL from oxidative damage [43].

Many studies that have addressed oxidative stress and PCOS to date have not given a definitive conclusion about their possible association [44]. Moreover, many different circulating markers used to estimate oxidative stress and the frequent finding of conflicting results across studies addressing the same markers may explain why the question of whether or not PCOS is associated with oxidative stress still remains open.

The resultant oxidative stress causes extensive cellular injury, demonstrated by protein oxidation, lipid peroxidation, and DNA damage. This oxidative stress may directly stimulate hyperandrogenism. Additionally, serum total antioxidant status, is diminished in women with polycystic ovary syndrome, decreasing the body's defense against an oxidative environment [45].

In the present study, there is a significant negative correlation between PON1 and chemerin in obese PCOS which indicate the association between obesity and decreased body antioxidant defense mechanisms.This concept is consistent with Aslan *et al.,* (2011) and Adriana, (2014)[46, 47].

In conclusion, our study showed that obese and non-obese women with PCOS have increased serum chemerin and ADMA with a strong positive correlation. In addition, decreased PON1 levels were observed in obese and non-obese PCOS with a strong negative correlation. These findings may indicate that women with PCOS are at high risk for oxidative stress, vascular endothelialdysfunction which is further aggravated by obesity.

However further studies are recommended to elucidate the role of chemerin gene expression in PCOS patients and its possible drug intervention.

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Conflict of interest: We declare that we have no conflict of interest.

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