Serum Levels of Vaspin and Osteoprotegerin in Premenopausal Women with the Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 5-10 % of reproductive-age women. It is characterized by menstrual dysfunction and hyperandrogenism and is associated with insulin resistance, impaired glucose tolerance, dyslipidemia and visceral obesity. Vaspin (visceral adipose tissue-derived serine protease inhibitor) levels increase with hyperinsulimenia and obesity. Osteoprotegerin (OPG) is a member of the tumour necrosis factor receptor superfamily. Recent data showed that obesity and insulin resistance result in decrease in serum OPG concentrations. The present study aimed to measure serum vaspin and osteoprotegerin levels in women with PCOS to show possible involvement in the pathogenesis of PCOS. Forty eight women with PCOS, 25 non-obese [body mass index (BMI) less than 30 kg/m^2] and 23 obese (BMI >30 kg/m^2) were enrolled for the study. Each group of them is compared to apparently healthy women as a control group matched for each in age and BMI. Clinical history, anthropometric measurements and biochemical and hormonal analysis were determined. The mean serum level of fasting blood sugar (FBS), insulin, homeostasis model assessment (HOMA-IR), triglyceride (TRIG) and high density lipoprotein-cholesterol (HDL-C) showed statistically significant difference between PCOS patients (non-obese and obese) when compared to control women (non-obese and obese) respectively. In both PCOS non-obese and obese patients groups as compared to the non-obese and obese control groups, the mean serum level of vaspin showed a statistically significant increase (P<0.001) in both PCOS groups, while the mean serum level of OPG showed a statistically significant decrease (P<0.001) in the same PCOS groups. Also, the levels of both previous two parameters (vaspin and OPG) showed significant differences between PCOS obese patients and PCOS non-obese ones. It is concluded that serum vaspin level increased in PCOS women particularly the obese, whereas serum OPG concentration reduced in the same patients group. These data suggest their involvement in the pathogenesis of PCOS.

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1. Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy of premenopausal women characterized by both reproductive and metabolic abnormalities. It affects 5-10 % of reproductive-age women (Economou *et al*., 2009). Women with PCOS are characterized by chronic anovulation and hyperandrogenism (Toulis *et al*., 2009). They commonly display a clustering of metabolic abnormalities, including impaired glucose tolerance, insulin resistance, dyslipidemia, increased prevalence of obesity, low-grade chronic inflammation and increased oxidative stress (Aroda *et al*., 2009). Obesity is present in approximately 44% of women with PCOS and it is characterized by central distribution of fat (Oris *et al*., 2003).

Insulin resistance (IR) is the most important pathophysiological factor in PCOS (Salley *et al*. 2007). It has been demonstrated in both obese and non-obese women with PCOS (Sepilian and Nagamani, 2005). The cellular and molecular mechanisms of insulin resistance in PCOS have not yet been elucidated, but they are considered to be distinct from those of other diseases associated with insulin resistance. Therefore, they have an increased prevalence of hypertension, diabetes and cardiovascular disease (Oh *et al*., 2009).

Osteoprotegerin (OPG) is a glycoprotein first described in 1997 by Simonet *et al., 1997*. It is produced in many different tissues, including bone, vasculature , heart, lung, kidney, and placenta , and also circulates in plasma, although the concentration

here is much lower than in bone and arterial tissue (Mand and Rasmussen, 2008).

Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily and is a secretory basic glycoprotein that exists in a 60 kDa monomeric form and a disulfide-linked homodimeric form of 120 kDa and, by binding and neutralizing the receptor activator of nuclear factor-κB ligand (RANKL), exerts an inhibitory effect on osteoclastic bone resorption (Escobar-Morreale *et al*., 2008). So, OPG-mediated pathways might have a role in osteoporosis because estrogen increases OPG gene expression. OPG maintains the structure of healthy bone and inhibits osteoclast activation and differentiation. In the vascular system, OPG inhibits pathological calcification in the media intima (Yi and Lin, 2007). OPG has been proposed for therapy of osteopenic disorders, such as postmenopausal osteoporosis, Paget disease, rheumatoid arthritis, hypercalcemia, and lytic bone metastases (Chen *et al*., 2001).

Besides, OPG has other biological functions, including anti-inflammatory actions, such as an anti-apoptotic effect resulting from the binding of TNF-related apoptosis-inducing ligand (TRAIL) with a consequent inhibition of the apoptosis process of susceptible cells (Escobar-Morreale *et al*., 2008). Interestingly, endothelial cells are one of the sites in which the anti-apoptotic effects of OPG have been demonstrated, suggesting a protective vascular role of the latter. Therefore, OPG has, apart from the aforementioned effects on osteoclastogenesis, other functions in vascular processes and immune responses that may be relevant to the pathogenesis of PCOS and its associated co-morbidities (Malyankar *et al*., 2000; Schoppet *et al.*, 2002).

Recently published data show that obesity and insulin resistance result in a decrease in serum OPG concentrations (Holecki *et al*., 2007).

The adipose tissue, predominantly visceral adipose tissue (VAT), produces and secretes a variety of bioactive adipocytokines. However, VAT accumulation induces adipocytes dysfunction, including oversecretion of interleukin-6, tumor necrosis factor-α, plasminogen activator inhibitor-1 and visfatin, and hyposecretion of adiponectin, which were supposed to be involved in the pathogenesis of insulin resistance and abnormal glucose metabolism (Yin *et al.,* 2009).

Recently Hida *et al.,* 2005 characterized vaspin as an interesting novel adipokine with insulin-sensitizing effects. Vaspin (visceral adipose tissue-derived serine protease inhibitor) belongs to the serine protease inhibitor (serpine) superfamily and is produced in the visceral adipose tissue depot of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of obesity with type 2 diabetes mellites (T2DM) (Hida *et al*., 2005; Tan *et al*., 2008). It is demonstrated convincingly in the initial report that administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity (Gulcelik *et al*., 2009). Furthermore, dysregulated expression of insulin sensitivity-modulating genes in adipose tissue including adiponectin and leptin was reversed after vaspin treatment (Seeger *et al.,* 2008; Glintborg *et al.,* 2006). Moreover, vaspin production was down-regulated with worsening of T2DM in OLETF rats (Hida *et al.,* 2000). In addition, it has recently shown that induction of vaspin mRNA expression in human adipose tissue is regulated in a fat depot-specific manner and could be associated with parameters of obesity, insulin resistance, and glucose metabolism (Kloting *et al.,* 2006). A few studies were reported about serum vaspin levels in humans and the correlation between vaspin serum levels and markers of insulin sensitivity, and glucose metabolism are unclear (Gulcelik *et al*., 2009).

The aim of the present study was to measure serum vaspin and osteoprotegerin levels in women with PCOS and asses possible correlations between each of them and clinical, biochemical and hormonal parameters of the syndrome as serum levels of vaspin and osteoprotegerin may show possible involvement in the pathogenesis of PCOS.

2. Subjects and Methods

Subjects:

Forty eight women with PCOS, 25 non-obese [body mass index (BMI) less than 30 kg/m2] and 23 obese (BMI >30 kg/m2) were enrolled for the study , all of whom were outpatients at Obstetrics and Gynecology Department, Faculty of Medicine, Minoufiya University and Internal Medicine, Faculty of Medicine, Al-Azhar University . The diagnosis of PCOS was made according to the National Institute of Child Health and Human Development criteria (Ferriman and Gallwey, 1961; Zawadski and Dunaif, 1992): 1) evidence of chronic anovulation or oligoamenorrhea (menstrual intervals > 35 days and or less than nine menstrual cycles per year); 2) clinical or biochemical evidence of hyperandrogenism [presence of acne, hirsutism or Ferriman-Gallwey (FG) score >8] and or elevated serum level of total testosterone more than 2.5 nmol/L and 3) exclusion of pregnancy, thyroid disease, prolactinoma, Cushing's syndrome and late onset nonclassic congenital hyperplasia.

Forty six healthy women (employees from Minoufiya University) matched for age, 24 non-obese (BMI <30 kg/m^2) and 22 obese (BMI > 30) participate to this study as controls. All controls were evaluated by a medical history and physical and

pelvic examination. Women with a menstrual cycle less than 26 days or more than 30 days were excluded. The normal ovulatory state was confirmed by TV-USG and plasma progesterone (P) assay during the luteal phase of the cycle. Exclusion criteria were pregnancy, hypothyroidism, hyperprolactiemia, Cushing's syndrome, current or previous (within the last 6 months) use of oral contraceptives, glucocorticoids, ovulation induction agents, antidiabetic and antiobesity drugs or other hormonal drugs. None of the patients or controls women were affected by neoplastic, metabolic or cardiovascular disorder. All the procedures used in this study were approved by the Research Ethics Committee of Faculty of Medicine, Minoufiya University and Al-Azhar University, Egypt. An informed consent was obtained from all subjects in this study.

All subjects were studied during the early follicular phase (second to fifth day) of the spontaneous or progesterone-induced menstrual cycle. 10 ml of venous blood were withdrawn after an overnight fasting from all subjects and allowed to clot in a plain sterile tube and then centrifuged. The separated serum was stored into aliquots at -80^{0}C for biochemical and hormonal determinations.

Anthropometric measurements:

Height and weight were measured with every subject. BMI was calculated as weight (kg)/ square height (m^2). Waist /hip ratio (WHR) was calculated by dividing the circumferences of the waist and hip.

Biochemical and hormonal analysis:

- Fasting blood sugar (FBS) and lipid profile including total cholesterol (T.Chol), triglyceride (TRIG) and high density lipoprotein-cholesterol (HDL-C) measurement using Beckman Coulter Automated Analyzer (Synchron CX9 ALX) clinical system, USA. Low density lipoprotein-cholesterol (LDL-C) was calculated by using Friedewald's formula as T.Chol (mg/dl) - HDL-C (mg/dl) – TRIG (mg/dl)/5 (Rifai *et al*., 1992).
- Luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), progesterone (PRG), Estradiol (E2) and total testosterone (TT) were measured by an automated quantitative enzyme immunoassay on the VIDAS instrument, BioMerieux, France using the Enzyme Linked Fluorescent Assay (ELFA).
- Fasting insulin, free testosterone (FT), 17-OH progesterone (17-OH PRG), androstendione, sex hormone-binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEA-S) were performed using a solid phase automated competitive chemluminescence's immunoassay (IMMULITE 2000, DPC, Germany).
- Serum OPG concentrations were measured using a commercial ELISA (BioVendor- GmbH-Laboratorni medicina, European Union) (Hofbauer, 1999). According to the manufacturer's directions , the amount of OPG present in the serum samples was determined from a calibration curve as pmol/ l.
- Serum level of vaspin (visceral adipose tissue-derived serine protease inhibitor) in sera were measured using a commercially available enzyme-linked immunosorbent assay (BioVender- GmbH-Laboratorni medicina, European Union). It is sandwich enzyme immunoassay for the qnuatitative measurement of human vaspin (Wada, 2008).
- Free androgen index (FAI) was defined as 100 times the molar ratio of total testosterone to SHBG [FAI= 100 × total testosterone (ng/ml) / SHBG (nmol/l)] (Fenkci *et al*., 2008).
- Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (uIU/ml) × glucose (mmol/l) / 22.5 (Matthews *et al*., 1985). Measurement of glucose level by mg/dl was multiplied by 0.555 to get result by mmol/l to calculate HOMA-IR.

Statistical Analysis:

Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis. The Pearson`s correlation coefficients were calculated for the normally distributed values. However, Spearman`s correlation coefficients were done for the not normally distributed values. Continuous normally distributed variables were tested for association by student's t-test. On the other hand, Mann–Whitney *U* test or z test is a non-parametric test for assessing whether two independent samples of observations have equally large values. It is one of the best-known non-parametric significance test used for the not normally distributed values. Data were presented as mean ± SD. p value <0.05 and <0.01 were considered statistically significant.

3. Results

Table 1 showed clinical and biochemical data in PCOS patients and control women according to BMI. As expected from appropriate matching, no differences were observed among PCOS patients and control women (non-obese or obese) in age, BMI and WHR. While, the mean serum levels of FBS, insulin, HOMA-IR, TRIG and HDL-C showed statistically significant difference between PCOS patients (non-obese and obese) when compared to control women (non-obese and obese) respectively. Regarding the mean serum level of T.CHOL and LDL-C, a statistically significant increase was observed only in

PCOS obese patients when compared to the obese controls.

There was statistically significant increase in the mean of serum levels of FT, TT, DHEA-S, prolactin, androstenedione and in FAI, FG score in patients with PCOS (non-obese and obese) when compared to the control women (non-obese and obese) who matched in BMI. While, there was no statistically significant difference in the mean serum level of LH, PRG and 17-OH-PRG in the same groups (Table 2).

In both PCOS non-obese and obese patients groups as compared to the non-obese and obese control groups, the mean serum level of vaspin showed a statistically (P<0.001) significant increase in both PCOS groups while the mean serum level of OPG showed a statistically (P<0.001) significant decrease in the same PCOS groups (Table 2).

Table 3 showed a statistically significant increase in the mean serum level of vaspin, BMI and HOMA-IR in the PCOS obese patients when compared to PCOS non-obese patients while, the mean serum level of OPG showed a significant decrease in the PCOS obese patients when compared to PCOS non-obese patients.

Serum vaspin level was negatively correlated with FT (r=-0.354, P=<0.05) but positively correlated with DHEA-S (r=0.812 & P=<0.001), FG-score (r=0.569 & p=<0.001), HOMA-IR (r=0.429 & p=<0.05), insulin (r=0.377 & p=<0.05) and BMI (r=0.736 &p=<0.001) as shown in table 4.

Considering the serum level of OPG as in table 5, it showed inverse correlation relationship with DHEA-S (r= -0.719 & P=<0.001), FG-score (r=-0.621 & P=<0.001), HOMA-IR (r=-0.399 & P=<0.05) and BMI (r=-0.721 & P=<0.001). While, it was positively correlated with FT (r=0.388 & P=<0.001) and insulin (r=0.346 & P=<0.05).

Table (1): Clinical and biochemical features of PCOS patients and controls according to BMI.

	PCOS (Non-obese) N=25 Mean±SD	**Control (Non-obese) N=24 Mean±SD**	**P-value**	**PCOS (Obese) N=23 Mean±SD**	**Control (Obese) N=22 Mean±SD**	**P-value**
Age (years)	29.5±0.9	29.3±2.0	NS	30.5±1.8	30.8±3.9	NS
BMI (Kg/m^2)	22.6±2.6	22.1±2.8	NS	43.0±4.0	42.1±5.9	NS
WHR	0.89±0.0	0.89±0.01	NS	0.89±0.03	0.89±0.01	NS
FBS (mg/dl)	93.8±8.3	89.4±7.3	<0.05*	99.8±13.6	92.7±5.9	<0.05*
Insulin (uIU/ml)	19.5±1.4	13.2±3.1	<0.001*	20.3±1.7	14.1±3.7	<0.001*
HOMA-IR	2.5±0.2	1.7±0.3	<0.001*	2.6±0.2	1.8±0.4	<0.001*
T.CHOL (mg/dl)	168.4±21.3	167.5±28.1	NS	197.0±41.8	177.0±19.6	<0.05*
TRIG (mg/dl)	208.8±29.5	108.0±12.7	<0.001*	247.9±43.0	143.2±9.4	<0.001*
HDL-C (mg/dl)	33.7±3.5	44.6±6.4	<0.001*	29.8±3.3	35.3±4.7	<0.001*
LDL-C (mg/dl)	96.1±21.5	93.9±34.5	NS	157.2±20.4	133.1±42.6	<0.05*

Values are given as mean ± SD. NS= non significant (P-value >0.05)

P-value <0.05 & P<0.001: statistically significant.

In this table, student's t-test was done for all the variables as they were normally distributed.

Table (2): Hormonal features of PCOS patients and controls according to BMI.

	PCOS (Non-obese) N=25 Mean±SD	Control (Non-obese) N=24 Mean±SD	P-value	PCOS (Obese) N=23 Mean±SD	Control (Obese) N=22 Mean±SD	P-value
FT (pg/ml)	7.1±1.4	0.6±1.1	<0.001*	6.2±1.2	0.2±0.1	<0.001*
TT (ng/ml)	4.7±0.7	2.4±0.6	<0.001*	4.8±0.9	2.6±0.4	<0.001*
SHBG (nmol/L)	28.2±2.3	89.9±27.1	<0.001*	24.6±4.2	85.1±21.4	<0.001*
FAI	16.8±2.2	2.8±0.7	<0.001*	19.5±2.2	3.4±1.3	<0.001*
FG score	10.9±0.6	3.9±0.1	<0.001*	12.5±0.5	4.9±0.3	<0.001*
LH (mIU/ml)	4.5±1.7	4.2±1.6	NS	4.1±1.4	3.9±1.7	NS
FSH (mIU/ml)	8.2±1.1	8.9±0.3	<0.05*	7.5±0.5	8.6±0.1	<0.001*
PRL (ng/ml)	15.2±0.8	11.5±0.5	<0.001*	13.5±0.5	12.6±0.5	<0.001*
E2 (pg/ml)	35.7±1.1	94.1±20.9	<0.001*	30.8±1.0	81.9±17.2	<0.001*
PRG (ng/ml)	0.99±0.2	0.90±0.3	NS	0.99±0.2	0.95±0.2	NS
17-OH-PRG (ng/ml)	1.8±0.7	1.4±0.5	NS	1.8±0.9	1.7±0.8	NS
Androstenedione (ng/ml)	4.8±0.7	2.3±0.8	<0.001*	5.2±0.7	1.8±0.6	<0.001*
DHEA-S (ug/dl)	522.2±62.4	209.6±94.9	<0.001*	548.1±41.2	156.2±82.7	<0.001*
OPG (pmol/l)	1.15±0.8	4.67±1.26	<0.001*	0.83±0.1	2.77±0.6	<0.001*
Vaspin (ng/ml)	1.78±0.1	1.11±0.1	<0.001*	1.92±0.0	1.30±0.08	<0.001*

Values are given as mean ± SD. NS= non significant (P-value >0.05)

P-value <0.05 & P<0.001: statistically significant.

In this table, student's t- test was done for all the variables except for DHEA-S (z-test was used because this variable isn't normally distributed).

Table (3): Clinical, biochemical and hormonal features of PCOS patients according to BMI.

	PCOS (Non-obese) N= 25 Mean±SD	PCOS (Obese) N= 23 Mean±SD	P-VALUE
Insulin (uIU/ml)	19.5 ± 1.4	20.3 ± 1.7	**NS**
HOMA-IR	2.5 ± 0.2	2.6 ± 0.2	**0.05* <**
BMI (kg/m^2)	22.6 ± 2.6	43.0 ± 4.0	**<0.001***
Vaspin (ng/ml)	1.7 ± 0.1	1.9 ± 0.1	**<0.001***
OPG (pmol/l)	1.1 ± 0.1	0.8 ± 0.1	**<0.001***

Values are given as mean ± SD. NS= non significant (P-value >0.05)

P-value <0.05 & P<0.001: statistically significant.

In this table, student's t-test was done for all the variables as they were normally distributed.

Table (4): Correlation analysis with serum vaspin level as the dependant variable.

	r	p value
DHEA-S (ug/dl)	0.812	<0.001*
FT (pg/ml)	-0.354	<0.05*
FG score	0.569	<0.001*
HOMA-IR	0.429	<0.05*
Insulin (uIU/ml)	0.377	<0.05*
BMI (kg/m^2)	0.736	<0.001*

P-value <0.05 & P<0.001: statistically significant.

In this table, Pearson's correlation coefficients was used for all the variables except for DHEA-S (Spearman's correlation was done as this variable isn't normally distributed).

Table (5): Correlation analysis with serum osteoprotegerin level as the dependant variable

	r	P value
DHEA-S (ug/dl)	-0.719	<0.001*
FT (pg/ml)	0.388	<0.001*
FG score	-0.621	<0.001*
HOMA-IR	-0.399	<0.05*
Insulin (uIU/ml)	0.346	<0.05*
BMI (kg/m^2)	-0.721	<0.001*

P-value <0.05 & P<0.001: statistically significant.

In this table, Pearson's correlation coefficients was used for all the variables except for DHEA-S (Spearman's correlation was done as this variable isn't normally distributed).

4. Discussion:

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy of premenopausal women that represents a major cause of infertility (Azziz *et al*., 2004). It is characterized by both reproductive and metabolic abnormalities including menstrual dysfunction, hyperandrogenism and is associated with insulin resistance, pancreatic β-cell dysfunction, impaired glucose tolerance, type 2 diabetes, dyslipidemia and visceral obesity (Azziz *et al*., 2004; Azziz, 2006; Tan *et al*., 2008).

The aim of the current study was to evaluate the levels of serum vaspin and osteoprotegerin in normal weight and obese PCOS women to find their possible involvement in the pathogenesis of this syndrome.

This study showed a statistically significant higher levels of FBS, insulin and HOMA-IR in (non obese) PCOS patients when compared to the (non obese) control subjects although BMI was in normal range in each group. The same statistically significant relationship in the previous parameters was observed when compared the PCOS obese patients with the obese control subjects. When compared PCOS non-obese women with PCOS obese women, a statistically significant decrease in HOMA-IR was found in non-obese group while, no significant difference was observed in the serum level of insulin in the same two groups when compared to each other. These results agreed with Economou and his colleagues, 2009 as they stated that insulin resistance was present in 41.6% of the overweight/obese PCOS women whereas it was present in 13.6% of the lean PCOS women. Also, Aigner *et al.,* 2009 stated that although the precise pathogenesis of PCOS remains unclear, a close link to insulin resistance (IR) and consecutive hyperinsulinemia, impaired glucose tolerance, type 2 diabetes mellitus, atherogenic dyslipidemia and visceral obesity has been well established. Dunaif, 1997 suggested that hyperinsulinemia occurs as a result of insulin resistance & accelerates ovarian androgen overproduction and it may also contribute to the development of diabetes and dyslipidemia in PCOS patients.

Regarding lipid profile, this study showed a statistically significant increase in the mean serum levels of T.CHOL, TRIG and LDL-C in PCOS-obese patients when compared to the obese controls and in the serum level of TRIG only in PCOS non-obese patients when compared to the non-obese controls. While, a statistically significant decrease was detected in the serum level of HDL-C in both groups of PCOS (non-obese & obese) when compared to the controls (non-obese & obese) respectively.

Fenkci *et al.,* 2008 agreed with the previous results as they reported that dyslipidemia is frequently accompanied by decreased HDL and increased T.chol, LDL-C, TRIG and can be observed in insulin resistance conditions as in PCOS.

In this study, like others (Aigner *et al.,* 2009; Alvarez-Blasco *et al.,* 2009; Ciaraldi *et al*., 2009), the mean levels of FT, TT, FAI, FG-score, androstenedione and DHEA-S were significantly higher in both obese and non obese PCOS women when compared to both obese and non obese control women respectively while, significant decrease in the levels of SHBG and E2 in the same groups were observed.

In the current study, the mean serum vaspin level showed a statistically significant increase in PCOS obese women when compared to PCOS non-obese women. In addition, significantly higher mean serum vaspin levels were detected in both the same previous groups [PCOS (obese & non-obese)] when compared to the control women (obese & non-obese) respectively. Furthermore, a statistically significant positive correlation was observed between serum vaspin level and each of DHEA-S, FG-score, HOMA-IR, insulin and BMI. Tan and his coworkers, 2008 agreed with these results as they found a higher serum and adipose tissue vaspin levels in women with PCOS and they reported that obese insulin-resistant subjects had higher serum vaspin levels and it showed a significant positive associations with BMI & insulin sensitivity. Also, Kloting *et al*., 2006 reported significant positive associations between

serum and omental adipose tissue vaspin levels with glucose and HOMA-IR and after metformin therapy given to the overweight PCOS women for 6 months, a significant decrease in circulating vaspin & glucose levels with a concomitant improvement in insulin sensitivity and a decrease in insulin resistance indexes. While, Escobar-Morreale and his colleagues, 2009 disagreed with this study as they found the serum vaspin concentration in premenopausal women are not affected by PCOS, obesity or glucose tolerance. Also, Ye *et al.*, 2009 stated that no significant correlations between vaspin and body fat indexes were detected.

From the previous data, this study hypothesize that the increased circulating vaspin levels may be a compensatory mechanism for insulin resistance and/or glucose metabolism in the PCOS subjects, with glucose playing a pivotal role as explained by Tan and his colleagues, 2008.

The present study showed that mean serum OPG concentration was statistically significant reduced in PCOS women (obese & non-obese) when compared to the control women (obese & non-obese) respectively and this finding is dependent from obesity as it showed a statistically significant decrease in obese PCOS women when compared to non-obese PCOS. Furthermore, OPG showed a statistically significant positive correlation with FT and insulin and a statistically inverse correlation with DHEA-S, FG-score, HOMA-IR and BMI.

Sandberg *et al*., 2006 and Kiech *et al*., 2007 explained the previous results as they stated that the reduction of serum OPG in PCOS and obesity might be related to the increased cardiovascular risk associated with these disorders, given that OPG may play a protective role in the vasculature by both inhibition of serum receptor activator of nuclear-κβ ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL), preventing plaque complication and apoptosis of endothelial cells. On one hand, by failing to neutralize RANKL, reduced OPG levels might favor cardiovascular disease, because RANKL overexpression is a prominent feature prone to rupture vulnerable atherosclerotic lesions, RANKL may also contribute to the transition from a stable to an unstable plaque and the serum level of RANKL is a highly significant predictor of cardiovascular disease (Kiech *et al*., 2007).

Also, Avignon *et al*., 2007 reported that increased circulating OPG levels have also found in association with the presence and severity of coronary artery disease, peripheral artery disease and stroke, suggesting that OPG may serve as a biomarker of established atherosclerosis in humans.

Saika and his coworkers, 2001 speculated that the lower levels of estradiol observed in PCOS women when compared with the controls in their study (as in this study) could have one of the pathophysiological factors involved because estradiol have been shown to upregulate OPG gene expression and production. Also, Chen *et al*., 2004 agreed with this study as they found that testosterone may indeed increase OPG expression and it correlates positively with serum OPG concentrations in men.

In conclusion, serum vaspin level increased in PCOS women particularly the obese and it showed a positive correlation with DHEA-S, FG-score, insulin, BMI and HOMA-IR. Whereas, serum OPG concentrations reduced in the same patients depending on obesity with an inverse correlation with DHEA-S, FG-score, BMI and HOMA-IR. These data suggest their involvement in the pathogenesis of PCOS. Further studies are needed to explain the pathophysiological roles of the increased serum vaspin and decreased serum OPG observed in PCOS.

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