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Serum Osteoprotegerin is Diminished in the Polycystic Ovary Syndrome and Associated with Insulin Resistance

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Abstract

Introduction: The polycystic ovary syndrome (PCOS) is a common endocrine disorder of reproductive age characterized by androgen excess and anovulation. PCOS is associated with insulin resistance (IR) and cardiovascular risk factors. Osteoprotegerin (OPG), an osteoclastogenesis inhibitor, has recently been related to metabolic and vascular disorders suggesting a possible link between OPG, PCOS and IR.

Objectives: To determine the association between OPG and IR in women suffering from PCOS and to review the existing literature.

Materials and Methods: Informed, written consent was obtained from 30 premenopausal women classified as PCOS (n = 13) or controls (n = 17) according to the Rotterdam criteria. Obese patients were excluded from the study. Biochemical analyses were performed including hormonal parameters, basal and after glucose overload determination of glucose and insulin. Serum OPG (pmol/L) was performed using a commercial enzyme immunoassay.

Results: PCOS patients showed higher fasting glucose and a tendency towards higher glucose concentration in the rest of the curve. Serum OPG concentration was significantly lower in PCOS patients than in controls (PCOS 1512.6 ± 95.7 vs Controls, 1952.5 ± 154.8 pg/mL, p = 0.023). Among PCOS women, those suffering from IR showed significantly diminished OPG levels (n = 9; 1365.3 ± 88 pg/mL; p = 0.023), compared to non-insulino-resistant PCOS (n = 4; 1844.0 ± 140.2 pg/mL; p = 0.03).

Conclusion: OPG concentration is diminished in PCOS independently of obesity. Further decrease is observed in those suffering from IR. All these findings suggest that OPG may not be implicated in the increased cardiovascular risk observed in PCOS.

Keywords: Insulin resistance; Osteoprotegerin; Insulin resistance; Polycystic ovary syndrome

Abbreviations: BMI: Body Mass Index; CIMT: Carotid Artery Intima Media Thickness; CV: Coefficient of Variation; DHEAS: Dehydroepiandrosterone Sulphate; DM: Diabetes Mellitus; ELISA: Enzyme Immunoassay; FSH: Follicule Stimulation Hormone; FMD: Brachial Artery Flow-Mediated Vasodilation; HDL: High Density Lipoprotein; HOMA: Homeostatic Model Assessment; IR: Insulin Resistance; LDL: Low Density Lipoprotein; LH: Lutenizing Hormone; OGTT: Oral Glucose Tolerance Test; OPG: Osteoprotegerin; PCOS: Polycystic Ovary Syndrome; PRL: Prolactin; QUICKI: Quantitative Insulin Sensitivity Check Index; RANK: Receptor Activator of κappa βeta; RANKL: Receptor Activator of κβ Ligand; RIA: Radio Immunoassay; SD: Standard Deviation; SHBP: Sex Hormone Binding Protein; SPSS: Statistical Package for the Social Science; TNF: Tumor Necrosis Factor

Introduction

The polycystic ovary syndrome (PCOS) is a complex and common endocrine disorder that typically develops in reproductive-age women. It is the most common cause of infertility and it is characterized by androgen excess and reproductive dysfunction [1].

The cause is multifactorial and characterized by interactions between susceptibility genes and environmental factors with known defects in pituitary secretion of luteinizing hormone (LH), disordered steroidogenesis with high production of androgens, impaired insulin signalling with resultant insulin resistance (IR) as well as ovulatory dysfunction. The interplay between hyperandrogenemia and IR is central to the pathophysiology underlying PCOS. Approximately 50–70% of women with PCOS have some degree of IR [2]. For the diagnosis of the syndrome, the Rotterdam criteria are widely used. These criteria require that patients have at least two of the following conditions: hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. The diagnosis of PCOS also requires exclusion of other potential etiologies of hyperandrogenism and ovulatory dysfunction [3].

The main features of the syndrome are anovulation, resulting in irregular menstruation, amenorrhea, ovulation-related infertility, and polycystic ovaries; excessive amounts or effects of androgenic hormones, resulting in acne and hirsutism; and IR [2]. Besides, women with PCOS exhibit an adverse cardiovascular risk profile characteristic of the cardio metabolic syndrome. These women, compared with age- and body mass index-matched women without PCOS, appear to present a higher risk of IR, glucose intolerance, and dyslipidaemia, and possibly a higher rate of type 2 diabetes mellitus (DM) and cardiovascular disease [4]. Many of these metabolic abnormalities that manifest in PCOS are worsened by the concurrent incidence of obesity. However, some of these metabolic perturbations including IR occur in women with PCOS regardless of weight [5,6].

IR is defined as a pathophysiological condition in which normal

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insulin concentration does not adequately produce a normal insulin response in target tissues because of the resistance to cellular actions of insulin. Under this condition, pancreatic β cells secrete more insulin to overcome the hyperglycaemia in these individuals leading to hyperinsulinemia. Over time, given the inability of the pancreatic β cells to produce sufficient insulin to correct the worsening of the tissue IR leads to hyperglycemia and overt type 2 DM [7]. Compensatory hyperinsulinaemia that develops in this context disrupts ovarian function, with enhanced androgen production and arrest of ovarian follicular development [8].

The mechanisms underlying the increased cardiovascular risk in this context may include not only metabolic aberrations, but also hormonal factors [9] and to date the mechanisms and factor implicated are no clearly understood.

Osteoprotegerin (OPG) is a soluble glycoprotein member of the tumour necrosis factor (TNF) receptor superfamily and exists in a 60 kDa monomeric form and a disulfide-linked homodimeric form. OPG was initially described as an anti-resorptive cytokine regulating the critical balance between bone formation and resorption through the OPG/receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK) pathway [10]. It is highly expressed in many different cell types such as osteoblasts, heart, kidney, liver, spleen, bone marrow [11]. In spite of being mainly involved in bone metabolism, several studies suggest that there is a potential role in mediating cardiovascular damage [12,13]. Moreover, OPG levels have been associated with different risk factors [14] such as DM [15], hypertension [16] and metabolic syndrome [17]. Furthermore it has also been associated with greater risk of cardiovascular events [18].

In this context, we wanted to evaluate OPG circulating levels in PCOS and the influence of IR on the OPG concentration in these patients, as there is scarce and contradictory information in the literature.

Objectives

In the present study, our aims were to assess the relationship between OPG concentrations in women with PCOS. Furthermore, another objective was to investigate whether there was an association between OPG levels in PCOS and IR. Moreover, a revision of the published literature was also performed.

Materials and Methods

Study population

Informed, written consent was obtained from 30 premenopausal women enrolled for the study. Of the 30 women, 13 fulfilled the Rotterdam criteria of PCOS according to the European Society for Human Reproduction (2003). The criteria were defined by at least presenting two of the following characteristics: oligomenorrhea and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries. Related disorders leading to secondary androgen excess were excluded before the diagnosis such as severe IR, androgen-secreting neoplasmas, Cushing's syndrome, hyperprolactinemia and thyroid abnormalities. Those who did not fulfil the criteria were considered controls (n = 17). Control patients had similar age than PCOS patients.

Body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/(height (m))^2. Obese patients according to their BMI were excluded from the study (Degree of obesity lean, BMI < 25 kg/m^2; overweight, BMI 25–29.9 kg/m^2; obese, BMI ≥ 30 kg/m^2 BMI SOP: 26 ± 4, BMI controls: 23 ± 5 Kg/m^2, p = 0.142). Besides, patients with impaired renal or liver disease as well as under medication (oral contraceptives, anti-androgens, infertility medications, metformin or drugs known to affect carbohydrate–lipid metabolism) were also excluded. The same exclusion criteria as in the PCOS group, were used for the control group. The study was conducted with the approval of the local ethics committee on clinical investigations.

Analysis

Clinical and anthropometrical variables, including hirsutism, clinical blood pressure, weight, height and body mass index (BMI) was determined in all subjects. Different biochemical analysis including routine analyses and hormones were performed. All routine biochemical analysis (Glucose, triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL)) and hormonal analysis (Insulin, testosterone, oestradiol, dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG), LH, follicle stimulating hormone (FSH), prolactin (PRL)) were determined by traditional laboratory methods. The ratio glucose/insulin and LH/FSH was also calculated.

17-hydroxyprogesterone was determined by radioimmunoassay (RIA) using a commercially available kit and according to the manufacturer instructions (Immunotech, Marseille, France. Beckman Coulter). Immediately after baseline blood sampling, an oral glucose tolerance test (OGTT) was performed; 75 g of glucose were administered orally and serum glucose levels were determined after 30, 60, 90, 120, 150 and 180 min. At the same time points and samples insulin was also determined.

The measurements of the IR included the homeostatic model assessment (HOMA), the quantitative insulin sensitivity check index (QUICKI) and the fasting glucose/insulin ratio. They were calculated using the following formulas:

HOMA-R = insulin (μU/mL) × glucosa (mmol/L)/22.5.

HOMA-B = 20 x insulin (μU/mL)/glucosa (mmol/L)-3.5.

QUICKI = 1 / (log (fasting insulin μU/mL) + log(fasting glucose mg/dL).

Serum OPG (pmol/L) was performed using a commercial enzyme immunoassay method (R&D Systems) according to the manufacturer instructions. The detection limit of the assay was 62 pg/mL and the mean intra- and inter-assay coefficients of variation (CV) were < 5%.

Samples were sent immediately to the laboratory, centrifuged and frozen at –20ºC until further analysis.

Literature search

A comprehensive literature search of PubMed was conducted from the inception to January, 2015 for human studies published in English. The following MeSH and key words were used: osteoprotegerin or OPG and polycystic ovary syndrome or PCOS.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the social sciences (SPSS) 15.0 package (SPSS, Chicago, IL). All parameters were given as mean and standard deviation (SD). Comparisons of continuous variables between two groups were done by independent sample Student's t test and Chi-square test when appropriate. Correlation was studied by bivariant Rho Spearman correlation. P < 0.05 was considered statistically significant.

Results

The clinical characteristics of the PCOS group and controls are shown in Table 1.

Compared with controls, PCOS patients presented with higher hirsutism score, polycystic ovaries, oligomenorrhea/amenorrhea, hyperandrogenism and IR. As expected, using two homogeneous groups no significant statistical differences were observed between both groups according to their age, body mass index (BMI) or overweight condition (Table 1).

PCOS patients presented increased fasting glucose levels glucose (4.78 ± 0.5 vs 4.07 ± 0.605 mmol/L, p = 0.003). Furthermore, PCOS showed a tendency towards higher glucose concentration in all the other samples during the glucose curve. Besides, basal insulin (PCOS, 8815 ± 1588 vs controls, 5201 ± 1077 µU/mL, p = 0.061) as well as HOMA-R (1.8 ± 1.1 vs 1.0 ± 0.9, p = 0.03) were more elevated in PCOS than in controls. The ratio glucose/insulin was lower in patients than in controls without statistical significance (6.5 ± 1.1 vs 8.6 ± 1.1, p = 0.257). Serum OPG concentrations were lower in PCOS patients compared with those of non-hyperandrogenic control women (PCOS, 1512.6 ± 95.7 vs controls, 1952.5 ± 154.8 pg/mL, p = 0.023). Among the PCOS group, patients with IR presented significant lower OPG concentrations (n = 9; 1365.3±88 pg/mL) compared to those non-insulin resistants (n = 4; 1844.0 ± 140.2 pg/mL; p = 0.03) (Figure 1).

Serum OPG concentrations showed statistically significant negative correlations with basal insulin (r = -0.577, p = 0.001), glucose (r = -0.444, p = 0.014), HOMA-R (r = -0.668, p < 0.0001), weight (r = -0.706, p < 0.0001) and body mass index (BMI) (r = -0.669, p < 0.0001). At different time points of the insulin curve there was a tendency towards significant correlation (30 minutes (-0.446, p = 0.056) and 90 minutes (-0.455, p = 0.050)) whereas no significant correlation was observed at any point of the glucose curve. Positive and significant correlation was observed between OPG and glucose/insulin ratio (r = 0.512, p = 0.004). All correlations are displayed in Table 2. No significant correlation was observed among OPG and the lipid profile as well as the hormonal profile including testosterone, oestradiol, DHEAS, SHBG, LH, FSH and prolactin.

Variable	PCOS	Control	P
Age, years	25 ± 1 (n = 13)	27 ± 1 (n = 15)	0.203 (n.s.)
Body mass index, Kg/m²	26.0 ± 1.1 (n = 13)	23.2 ± 1.4 (n = 12)	0.142 (n.s.)
Testosterone, ng/mL	1.28 ± 0.63 (n = 13)	0.55 ± 0.07 (n = 17)	0.267 (n.s.)
Oestradiol, pg/mL	37.1 ± 4.7 (n = 13)	61.9 ± 19.2 (n = 17)	0.277 (n.s.)
Dehydroepiandrosterone sulfate µg/mL	2.4 ± 0.32 (n = 13)	0.67 ± 0.16 (n = 17)	0.077 (n.s.)
17-hydroxyprogesterone ng/mL	1.04±0.13 (n = 13)	0.90 ± 0.20 (n = 17)	0.604 (n.s.)
Sex hormone binding globulin (SHBG), nmol/L	46.97 ± 5.77 (n = 13)	77.60 ± 12.90 (n = 17)	0.041
Overweight	70% (n = 13)	30% (n = 16)	Chi square 0.064 (n.s.)
Hirsutism	84.6% (n = 13)	15.4% (n = 17)	Chi square <0.0001
Polycystic ovaries	75% (n = 12)	25% (n = 15)	Chi square <0.0001
Oligomenorrhea/ Amenorrhea	66.7% (n = 13)	33.3% (n = 6)	Chi square 1 (n.s.)
Hyperandrogenism	76.9% (n = 13)	0% (n = 17)	Chi square <0.0001
Insulin resistance	81.8% (n = 13)	18.2% (n = 17)	Chi square 0.002
OPG, pg/mL	1512.6 ± 95.7	1952.5 ± 154.8	0.023

Table 1: Demographic and clinical characteristics of the study population.

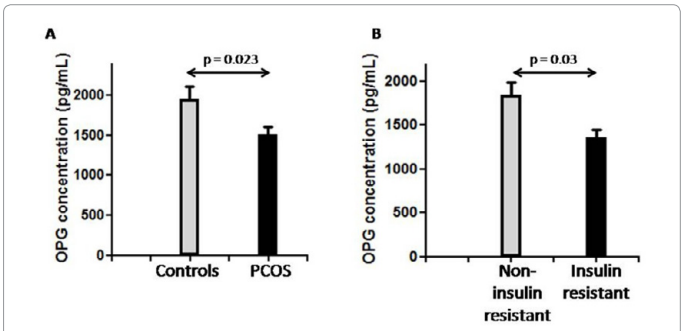

Figure 1: (A) OPG concentration (pg/mL) in controls and PCOS (Mean ± SD). (B) Differences in OPG concentration (pg/mL) according to the PCOS insulin resistant condition (non-insulin resistants versus insulin resistants (Mean ± SD).

Correlations (Serum OPG)		
Analysis	**r**	**p**
Insulin	-0.577	0.001
Glucose	-0.444	0.014
HOMA-R	-0.668	P < 0.0001
Insulin/Glucose ratio	0.512	0.004
Weight	-0.706	P < 0.0001
BMI	-0.669	P < 0.0001

Table 2: Significant correlations for OPG levels.

The review of the previously published literature was performed and few studies focused on the topic. Besides, the results obtained in the different published studies are not concluding and controversial.

The first report that assessed the relationship between serum OPG and PCOS was mentioned by Escobar-Morreale et al. [19] that found that circulating OPG was lower in patients with PCOS. After that, Pepene et al. [20], reinforced the relationship suggesting decreased OPG levels in PCOS patients. However, Glintborg et al. [21] reported no differences in both groups (PCOS and controls). Finally and contrary to the rest of studies, a recently published study by Abali [22] showed an increase in the OPG circulating concentration in PCOS patients. The different studies that have evaluated OPG levels in PCOS are summarized in Table 3.

Discussion

The present study shows that serum OPG concentrations are reduced in PCOS patients and that this finding is independent from obesity. Besides, OPG concentration is lower in insulinoresistant PCOS compared to non-insulinoresistants. IR is a characteristic of PCOS patients and it is unique and independent of obesity, although it is more pronounced in obese patients [5]. In this study, IR was evaluated without considering obesity as one of the exclusion criteria stated was BMI.

Different studies have previously evaluated OPG levels in PCOS, however, there is a great variability between studies. There is no agreement among studies whether PCOS increases OPG circulating concentration or diminishes it. In accordance with our study, both Pepene et al. [20] and Escobar-Morreale et al. [19], showed that OPG

Author	Year	Population of Study	OPG Values and Sample	Purpose	Findings
Abali et al. [22]	2015	37 PCOS 41 Controls (matched for BMI and age)	OPG was higher in PCOS (11.39 ± 2.29 vs. 10.22 ± 2.25 pmol/L, P = 0.026). SERUM	Relationships of OPG to endothelial dysfunction (BMD) and carotid intima media thickness (CIMT) in PCOS	No correlation between OPG and cardiovascular risk markers
Glintborg et al. [21]	2013	30 PCOS 14 Controls (matched for BMI and age)	OPG was comparable in PCOS and controls [12.0 (10.5-14.6) vs 12.9 (11.7-14.9) ng/mL]. PLASMA	To evaluate OPG levels during pioglitazone treatment in PCOS	OPG levels were comparable in PCOS and controls and unchanged during treatment with pioglitazone
Pepene et al. [20]	2011	64 PCOS 20 Controls (similar age)	OPG was significantly lower in PCOS SERUM	To investigate the relationships of OPG to insulin resistance, FMD, and CIMT in PCOS	In PCOS, OPG is related to endothelial dysfunction and insulin resistance
Escobar-Morreale et al. [19]	2008	40 PCOS 40 Controls (matched for BMI and age)	OPG was lower in PCOS (304 ± 120 vs 363 ± 105 pg/mL, P = 0.007) SERUM	To study serum OPG concentrations in PCOS	OPG concentrations are reduced in PCOS patients independently of obesity

Table 3: Review of the published studies regarding OPG concentration in PCOS.

levels were lower in PCOS compared to controls. However, other studies reported increased OPG concentration in PCOS [22] or no difference [21] between groups.

Inspite of not finding a lot of studies that focus on OPG circulating concentration in PCOS, the results are not concluding. In all these studies it is important to highlight that these wide differences present may be due to the lack of consensus regarding the units, sample type and probably different ELISA kits used. As stated in a previously published study performed by our group [23], it is vital to standardize the pre-analytical and analytical factors in OPG measurement. Besides, it is important to keep in mind that OPG exists both as a monomer and a dimer and the final concentration may differ depending on the kit used.

First of all, OPG results are expressed in different units: while Abali et al. uses pmol/L [22], others report the results in ng/mL [21] or pg/mL [19]. Another important factor is the lack of an international standard in OPG measurement, challenging the comparison among studies.

Furthermore, different samples are used in the different studies. Although in most studies the sample used is serum, Glintborg et al. [21] used plasma instead that may lead to differences in OPG concentration as previously stated [23]. In this regard, OPG determination should be standardized in order to be able to better compare the results from different studies.

Women with PCOS appear to have a higher risk of IR, hyperinsulinemia, glucose intolerance, dyslipidemia, and an increased prothrombotic state, possibly resulting in a higher rate of type 2 DM, fatty liver disease, subclinical atherosclerosis, vascular dysfunction, and finally cardiovascular disease and mortality [24]. Interestingly, PCOS patients who are known to have increased cardiovascular risk as well as a tendency towards IR have lower OPG concentrations. The actual underlying mechanism is still unknown. It may be due to the excessive amounts or effects of androgenic hormones or the impaired insulin signalling or a combination of both. Estradiol is known to upregulate OPG concentration [10,20,25] whereas OPG concentration negatively associated with free androgen index [26]. Hyperandrogenemia is the biochemical hallmark of PCOS and women with PCOS will have elevated levels of DHEAS [27] and testosterone levels [20,21]. Venujaru et al [10] concluded that there could be a regulatory role for sex steroids in the expression of OPG. This hormonal profile in PCOS patients could be one of the pathophysiological factors involved and may influence OPG concentration leading to decreased OPG levels. However, in our study no correlation was observed between OPG concentration and the hormonal profile. Instead, in this study, OPG correlated with parameters that measure IR. OPG negatively and significantly correlated with glucose, insulin and HOMA-R showing the relationship between OPG and insulin-resistance.

In this study, different factors which are known to influence OPG concentration were excluded. Obesity is known to alter OPG levels [17,28,29] that was the reason why this parameter was excluded based on BMI. However, a negative significant correlation was observed between OPG, weight and BMI. Another important factor that may be worth considering is the relationship between age and OPG that may affect the analysis. A variety of cross-sectional and epidemiological studies have shown a positive correlation of serum OPG levels with age explained as a compensatory response intended to counteract the bone loss or the dissolution from the skeleton with diminishing bone material in aging [30]. In our study, both groups (PCOS and controls) had similar ages which exclude the influence on OPG levels due to age.

IR is characterised by a diminished glucose response to the metabolic actions of insulin and consequently increased insulin levels are observed in these patients. In accordance with our study, Knudsen et al. [31] observed that acute hyperglycaemia did not seem to increase plasma levels of OPG in non-diabetic subjects, whereas hyperinsulinaemia may suppress plasma levels of OPG. Besides, Jorgensen et al. [32] in obese patients, concluded that acute hyperinsulinemia decreased plasma OPG. In different healthy subjects, OPG levels were negatively correlated with body weight, BMI, waist circumference, HOMA-IR and fasting plasma insulin [29] in accordance with our study. Ugur-Altun et al. [28] pointed out that IR in obesity was associated with decreased serum OPG levels. Moreover, a significant negative correlation was observed between OPG levels corrected for BMI and glucose, insulin and HOMA-IR. However, Gannage-Yared et al. [33] found a positively correlation between HOMA index and OPG.

Hyperandrogenism, the hallmark of PCOS, is associated with a higher risk of developing type 2 DM and appeared to correlate with cardiovascular risk [34,35]. So, PCOS subjects have an increased risk

of both impaired glucose tolerance and type 2 DM with a reported 3-7 fold greater risk overall and a 2-fold higher risk compared with age- and BMI-comparable women with normal cycles [24]. In our study the percentage of insulin resistant women was higher among the PCOS group.

IR and consequent hyperinsulinemia are related to many aspects of the syndrome such as hyperandrogenism, reproductive disorders, acne and hirsutism. In the long-term it may increase the risk of cardiovascular disease and negatively affect lipid profile and blood pressure [1]. OPG levels have been previously related to different cardiovascular risk factor as well as cardiovascular disease and mortality in different cohorts. Furthermore, human studies show a positive relationship between OPG and vascular damage. OPG concentration has been associated with higher cardiovascular risk, poorer outcomes and mortality [10,18,36-38]. Consequently, in PCOS patients who are more vulnerable to metabolic disorders and complications including IR, type 2 DM, dyslipidemia, metabolic syndrome, obesity and subclinical cardiovascular disease [39,40], it could be hypothesized that OPG may play a role in mediating this association. However, taking into consideration the previously discussed data, OPG may seem not to be a mediator in the increased cardiovascular risk observed in PCOS due to the diminished OPG values observed in these patients. Further studies in greater populations are needed to confirm the present results and to propose different and alternative mediators that contribute to the cardiovascular risk.

The main finding of our study is the observation of a negative correlation between OPG and IR in women with the PCOS. Besides, in PCOS patients the role of OPG as a mediator of the increased risk in these patients may be excluded. Precise clinical implications of this observation, so far, are not entirely clear and should be confirmed in larger cohorts.

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