Circulating osteoprotegerin is increased in the metabolic syndrome and associates with subclinical atherosclerosis and coronary arterial calcification

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Abstract

Context: The relationship between osteoprotegerin (OPG) a glycoprotein related to bone metabolism and the metabolic syndrome (MS) has not been established.

Objective: The aim of this study is to evaluate OPG concentration in patients with MS and its association with subclinical atherosclerosis and coronary arterial calcification (CAC).

Materials/methods: The study included 238 asymptomatic patients. MS was diagnosed according to the NCEP/ATPIII guidelines. OPG was measured by ELISA. All subjects underwent ultrasonography of the common carotid arteries to measure intima-media thickness (IMT) and evaluate the presence of atheroma plaques. In a subgroup (n = 39) CAC was quantified by ECG-triggered cardiac computed tomography. Adipose tissue was excised from 25 patients and OPG expression by RT-PCR and immunohistochemistry was studied.

Results: Patients with the MS (n = 60) had higher OPG than patients without (n = 178) (p < 0.05). OPG correlated with IMT (r = 0.2, p = 0.005) and patients with atheroma plaques had higher OPG (p = 0.008) and also those with coronary artery calcification (p < 0.05). OPG expression was confirmed in adipose tissue (n = 12) and the expression was significantly higher in patients with MS than in those without (p = 0.003).

Conclusions: This study shows that OPG may potentially be a biomarker for cardiovascular risk/damage in the MS and identifies adipose tissue as a potential source of OPG.

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Introduction

Osteoprotegerin (OPG) is a soluble glycoprotein member of the tumor necrosis factor (TNF) receptor superfamily, originally discovered as an inhibitor of osteoclastogenesis. Biochemically, OPG is a basic secretory glycoprotein composed of 380 aminoads and seven structural domains which exists as a more active monomeric form (∼60-kDa) and a homodimeric form [1].

OPG is part of the OPG/receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK) pathway. The RANKL/OPG/RANK axis has been shown to regulate bone remodeling. RANKL–RANK interaction leads to the transcription of specific genes required for osteoclast differentiation. OPG acts as a soluble decoy substrate to the receptor activator of RANKL and competes with RANK, inhibiting RANKL–RANK interactions and thus proliferation and differentiation of osteoclasts and consequently bone resorption [2].

In addition to being central to regulating RANK–RANKL interactions in bone metabolism, several studies suggest that there is a potential role of OPG in mediating cardiovascular damage [1,3]. In vitro studies indicate that OPG is expressed in cells involved in atheroma plaque development and progression, such as arterial smooth muscle cells [4], endothelial cells [5] and megakaryocytes [6]. Moreover, OPG expression is enhanced in explanted human carotid atherosclerotic plaques [7].

Human studies show a positive relationship between circulating OPG, vascular damage and cardiovascular disease. Indeed, elevated serum OPG levels have been found associated with atherosclerosis [8].
and carotid intima media thickness (IMT) in a general population [9] and with increased risk of cardiovascular disease and mortality [10,11].

There is scarce information of OPG circulating levels in the MS, a cluster of cardiovascular risk factors.

The aims of the present work were 1) to evaluate OPG circulating levels in patients with the MS and its association with the presence of subclinical atherosclerosis and coronary arterial calcification and 2) to explore whether adipose tissue is a source of OPG.

Methods

Study population

This case control study was performed in 238 apparently healthy subjects (51% males, 60 ± 1 years; 49% women, 59 ± 1 years) attending the Cardiovascular Risk Area of the Clinic Universidad de Navarra for a general check-up. The demographic and clinical characteristics of the study population are summarized in Table 1.

All participants underwent a complete medical examination and anthropometric measurements were taken. Subjects were free from clinically apparent atherosclerotic disease based on the absence of history of coronary disease, stroke or peripheral artery disease, and normal electrocardiogram. Exclusion criteria were osteoporosis, impaired renal or liver function, cancer, and inflammatory diseases.

In order to exclude osteoporotic subjects, females under treatment with antiresorptive therapy were excluded from this study. Furthermore, in all females over 50 years old without treatment, the FRAX risk score was calculated. FRAX is an algorithm that determines fracture probability in individuals by integrating the weight of important clinical risk factors for fracture and mortality risk, with or without information on bone mass density. Women with FRAX scores higher than 3% (hip) or 20% (major) 10-year fracture risk were excluded from the study.

Furthermore, women with confirmed osteoporosis by densitometry were excluded of the study.

Another group of patients (n = 25) who underwent elective surgery (for example abdominoplasty and laparoscopic gastric bypass) at the Surgery Department of the Clinic University of Navarra were recruited for the study in adipose tissue.

The MS was diagnosed according to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) guidelines. Individuals were classified as having the metabolic syndrome (MS) if they possessed three or more of the following criteria:

- High blood pressure: systolic and/or diastolic blood pressures ≥ 130/85 mmHg or patients receiving blood pressure lowering drugs
- Hyperglycaemia: fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dL) or patients receiving glucose lowering drugs
- Hypertriglyceridaemia: fasting plasma triglycerides ≥ 1.69 mmol/L (150 mg/dL)
- Low HDL-cholesterol: fasting HDL-cholesterol < 1.04 or 1.29 mmol (40 or 50 mg/dL) in males and females, respectively
- Central obesity: waist circumference > 88 or 102 cm in females and males, respectively.

Body mass index (BMI) was calculated using the following formula: weight (kg) / height² (m). Blood pressure was measured on the right arm, with the subjects in a seated position and after a 5 minute rest, with a mercury sphygmomanometer.

Waist circumference was measured at the superior border of the iliac crest.

Glycemic filtering rate was estimated from plasma concentrations of endogenous creatinine employing the abbreviated four variable Modification of Diet in Renal Disease (MDRD) study equation (MDRD-4).

All participants signed an informed consent document, and the study was approved by the Local Ethics Committee for Human Research. The percentage of patients without the MS taking statins was not significantly different compared with the number of patients with the MS on statins (23% vs 30%, non-significant).

Carotid ultrasonography

All subjects underwent ultrasonography of the common carotid arteries performed with color duplex equipment (ATL 1500 HDI) coupled to a high-resolution linear transducer at a frequency of 5–12 MHz. The measurement of intima-media thickness (IMT) was performed in the far wall of the common carotid artery (10 mm proximal to the bifurcation). From each individual, the IMT was determined as the average of near- and far-wall measurements of each carotid artery. In addition, the presence or absence of atheromatous plaques was determined.

Examinations were carried out by a sonographer who was trained and experienced in performing sonographic examination and IMT measurements, and who was blinded to the participants’ clinical information.

Computed tomography (CT) image acquisition protocol

A subgroup of 39 subjects underwent prospectively ECG-triggered cardiac computed tomography (CT) during a single breath hold using a DSCT system (Somatom Definition, Siemens Medical Solutions, Forchheim, Germany) at end inspiration. Acquisition started above the origin of the coronary arteries and ended at the dome of the diaphragm. The following protocol was used in all cases: tube voltage of 120 kV, current of 76 mA as for both tubes with online anthropomorphic tube current modulation (CareDose 4D, Siemens Healthcare), detector collimation of 6 × 3.0 mm, table displacement of 18 mm, and gantry rotation time of 0.33 ms (temporal resolution of 83 ms). ECG-gated cardiac CT studies were acquired at 70% or 40% of the cardiac cycle in individuals with heart rates < 80 or ≥ 80 beats per minute, respectively, to obtain motion-free images of the coronary arteries. After manual adjustment of the field of view (FOV) to the heart, all data were reconstructed with 3 mm slice thickness, 1.5 mm reconstruction increment, and dedicated soft-tissue convolution kernel (B35f) in a range extending from the main pulmonary artery to the dome of the diaphragm. No intravenous contrast media was administered.

Coronary artery calcium scoring

An experienced reader quantified the amount of coronary artery calcium (CAC) using dedicated software (CaScore, Siemens). Calcified lesions were identified as areas of at least 130 Hounsfield Units (HU) attenuation. The Agatston score was computed by multiplying the area of each lesion by a weighing factor that is dependent on the peak attenuation in the lesion. The scores of individual lesions were summed to obtain a global CAC Agatston score of all coronary vessels.

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Abdominal adipose tissue was obtained from 25 patients (13 healthy donors and 12 MS patients) undergoing elective surgery (for example abdominoplasty and laparoscopic gastric bypass) at the Surgery Department of the Clinic University of Navarra. Samples of adipose tissue were immediately transported to the laboratory. The tissue was washed with sodium chloride 0.9%, cut with scissors into small pieces (5–15 mg) and collected in trizol for RNA isolation.

**Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)**

Total RNA was extracted from adipose tissue using Trizol (Invitrogen) (1 mL/100 mg of tissue). Samples were centrifuged at 12,000 g for 10 min and the Trizol layer was transferred to fresh eppendorf tubes. 100 μL of chloroform was added and samples were incubated 2 to 3 min at room temperature. Samples were centrifuged at 12,000 g for 10 min at 4 °C. After centrifugation, the upper aqueous phase was transferred to a clean eppendorf and 250 μL of isopropl alcohol was added. After 10 minute incubation, samples were centrifuged at 12,000 g for 10 min at 4 °C. The supernatant was removed by inversion. The RNA pellet was washed with 1 mL 75% ethanol and then they were incubated in the thermomixer for 10 min at 60 °C. RNA concentration was quantified with a Nanodrop 2000 Spectrophotometer (Thermo Scientific). Subsequently reverse transcription was performed using Supercrop III Reverse Transcriptase (Life technologies) in a Veriti 96 well Thermal Cycler (Applied Biosystems) according to the manufacturer’s instructions.

Real time RT-PCR was performed with 50 ng of cDNA in a 7700 AbiReal-Time PCR (Abiprism) Sequence Detection System by using specific TaqMan fluorescent probes (Applied BioSystems, Foster City, California) suitable for relative genetic expression quantification. The commercially available and pre-validated TaqMan primer/probes sets used were as follows: endogenous control (β-actin and target OPG).

The reaction was performed in a final volume of 10 μL. The cycle program consisted of an initial denaturing of 10 min at 95 °C then 45 cycles of 15 s denaturizing phase at 95 °C and 1 min annealing and extension phase at 60 °C.

For each condition, the expression of the target gene and endogenous control was obtained in triplicate. The standard deviation (SD) among triplicates was always <0.2.

The threshold cycle (Ct value) was obtained for each amplification curve and a ΔCt value was first calculated by subtracting the Ct value for human β-actin (reference gene) from the Ct value for OPG in each sample. Fold changes compared with the endogenous control were then determined by calculating $2^{-\Delta\Delta Ct}$, so gene expression results are expressed as expression ratio relative to β-actin gene expression.

**Immunohistochemistry**

Biopsies were fixed (24 h, 4% paraformaldehyde (Panreac Quimica, Barcelona, Spain) and stored in 70% ethanol (Sigma-Aldrich) until paraffin embedding. Six-micron-thick sections were cut with a Leica microtome and mounted onto slides. After dewaxing and rehydration, slides were heated for 10 min in a solution containing 10 mM sodium citrate pH 6.0 at 95 °C to maximize antigen retrieval followed by permeabilization (triton 0.5%, 10 min) and blocking (milk 1%, 30 min). For OPG detection, slides were incubated overnight with the anti-OPG antibody (1:50, Abcam at 4 °C) washed three times, and subsequently incubated for 30 min with the biotinylated secondary antibody (Anti-rabbit IgG BA–10000 Vector IVD, at room temperature). The signal was revealed by using ABC complex (Vestatin ABC kit Elite PK–6100 Standard-Vector, 30 min at room temperature) and diaminobenzidine (DAB) chromogen substrate (Sigma Aldrich, 10 min at room temperature). Samples were counterstained using Carazzi’s Hematoxylin, dehydrated through graded alcohols to xylene and mounted using PDX mountant. Bright-field images were observed with a Nikon microscope and digitized using a coupled device camera. A positive control from kidney was run at the same time with the adipose tissue samples.

**Biochemical analyses**

Serum and plasma were collected in Vacutainer® tubes. Fasting serum glucose, cholesterol, triglycerides, HDL-C and LDL-C were measured by standard laboratory techniques.

Plasma OPG was measured by enzyme linked immuosorbent assay (R&D Systems) according to the manufacturer’s instructions. The inter and intra-assay variations were <5%. The detection limit was 60 pg/mL.

**Statistical analysis**

Analysis was performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of all variables was tested with the Shapiro–Wilks test. Not normally distributed variables were log transformed. Differences in OPG levels between patients with and without cardiovascular risk factors were assessed by unpaired Student’s t-test for continuous variables. Associations between OPG levels and other variables were examined by Pearson correlation test for continuous variables. Multiple linear regression analyses were used to examine associations between OPG levels and mean carotid IMT adjusting by age, sex, and traditional atherosclerotic risk factors (BMI, hypertension, diabetes, smoking status, and dyslipidemia). Mean carotid IMT was compared across OPG tertiles by ANOVA and the general linear model, followed by Bonferroni post hoc test. Data are expressed as mean ± standard error of mean (SEM).

The threshold cycle (Ct value) was obtained for each amplification curve and a ΔCt value was first calculated by subtracting the Ct value for human β-actin (reference gene) from the Ct value for OPG in each sample. Fold changes compared with the endogenous control were then determined by calculating $2^{-\Delta\Delta Ct}$.

OPG gene expression was not normally distributed and was expressed as median value and percentiles 25 and 75. Mann–Whitney test was performed to study difference between MS patients and controls in OPG expression in adipose tissue.

Statistical significance was established at $p < 0.05$.

**Results**

**Demographic and clinical characteristics of study population**

After complete clinical examination, subjects were divided in two groups: those with ($n = 60$) and those without ($n = 178$) MS. The demographic and clinical characteristics of the study population are displayed in Table 1. As expected, patients with MS exhibited significantly ($p < 0.001$) higher BMI, systolic arterial pressure, diastolic arterial pressure, waist circumference, glucose, and triglyceride levels and lower HDL-cholesterol than those without MS. Besides, total cholesterol was significantly higher in MS patients ($p < 0.01$) whereas no significant differences were observed in LDL-cholesterol between both groups.

**OPG circulating levels are increased in patients with MS**

Interestingly, mean serum OPG concentration was significantly higher in the group exposed to MS ($n = 60$) than in the control group ($n = 178$) (1255 ± 46 vs 1192 ± 47 pg/mL $p < 0.05$ (Fig. 1).

We further explored the association of OPG circulating levels with different parameters. In the whole population, OPG concentration correlated positively with age ($r = 0.50$, $p < 0.001$), glucose ($r = 0.15$,...
p < 0.05), creatinine ($r = 0.29, p < 0.001$), systolic arterial pressure ($r = 0.30, p < 0.001$) and waist circumference ($r = 0.16, p < 0.05$).

OPG significantly and negatively associated with total cholesterol ($r = −0.15, p = 0.02$) and MDRD-4 ($r = −0.35, p < 0.001$).

Patients were divided into two groups according to having or not each of the criteria of MS.

There were no statistically significant differences between patients with high and low triglycerides or low and high HDL-cholesterol. However, diabetic patients (16.5%) had higher OPG levels than those without diabetes (83.5%) ($1275 ± 64$ vs $1190 ± 42$ pg/mL, $p < 0.05$) and non-smokers (51.1%) compared with smokers (51.1%) ($1256 ± 56$ vs $1053 ± 40$, pg/mL, $p = 0.003$). These associations were not significant after adjusting for obesity and blood pressure. We also found that OPG levels were significantly increased in hypertensive patients (57.8%) than in normotensives (42.2%) ($1390 ± 64$ vs $1111 ± 44$ pg/mL, $p < 0.001$). Interestingly, OPG serum levels were higher in obese subjects (38.5%) compared with normal-weight subjects (61.5%) ($1318 ± 70$ vs $1137 ± 40$, pg/mL, $p = 0.023$) and the association was independent of diabetes and hypertension ($\beta = 0.14, p = 0.037$). Also, OPG was higher in non-smoker (51.1%) compared with smokers (51.1%) ($1256 ± 56$ vs $1053 ± 40$, pg/mL, $p = 0.003$).

A significant increase was found in OPG circulating levels with increasing number of risk factors for the MS (0 factors: $1080 ± 85$ pg/mL, 1–2 factors: $1225 ± 44$ pg/mL and 3–5 factors: $1261 ± 56$ pg/mL, $p$ for trend $<0.05$). Interestingly, OPG levels directly and significantly correlated with the number of factors of the MS ($r = 0.19, p < 0.05$). This association of OPG with the factors of the MS was independent of age and gender as well as independent of the medications.

**OPG associates with IMT and with the presence of atheroma plaques**

We next evaluated the possible associations of serum OPG with the presence of subclinical atherosclerosis. By univariate analysis, OPG levels directly and significantly correlated with mean carotid intima-media thickness in the overall population ($r = 0.20, p < 0.05$). By multiple regression analysis the association between OPG and IMT remained significant ($p = 0.047$) when controlling for age and gender.

Given the association between OPG and carotid IMT, mean carotid IMT was compared across tertiles of OPG. As shown in Fig. 2A, IMT was significantly greater in the highest tertile of OPG ($0.73 ± 0.01$ mm) than in the middle ($0.69 ± 0.01$ mm) and lowest tertiles ($0.65 ± 0.01$ mm, $p$ for trend $<0.05$).

Finally, OPG circulating levels were significantly higher in patients with carotid atheroma plaques compared with those without ($1357 ± 78$ vs $1124 ± 34$ pg/mL, $p < 0.01$) (Fig. 2B).

**OPG circulating levels and coronary artery calcification**

We next evaluated in a subsample ($n = 39$), the association of OPG concentrations with the prevalence of coronary artery calcification measured by computed tomography. Circulating OPG levels were significantly higher in patients with coronary artery calcification compared with those without ($1150 ± 86$ vs $970 ± 82$ pg/mL, $p < 0.05$) (Fig. 3).

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However, no significant correlation was found between the Agatston calcification score with OPG, nor the prevalence of coronary artery calcification significantly increased along OPG tertiles.

**OPG is expressed in adipose tissue**

We next explored potential sources of OPG. Total RNA was isolated from human abdominal adipose biopsies. Real time RT-PCR amplified cDNA for OPG from RNA obtained from the human biopsies. Proper reverse transcription was confirmed in all cases by amplification of beta-actin. OPG protein expression was also corroborated by immunohistochemistry (Fig. 4A).

We next aimed to compare OPG expression in patients with and without the MS. All patients with MS expressed mRNA OPG in their abdominal adipose tissue (n = 12). OPG mRNA expression was significantly higher in MS patients than in controls (5.0 (1.6, 9.3), n = 12 MS vs 0.8 (0.4, 1.4) n = 9 controls, p = 0.003) (Fig. 4B).

**Discussion**

The main findings of the current study are: 1) circulating OPG is increased in patients with the MS and associates with increasing number of cardiovascular factors, 2) carotid IMT is higher in patients with high serum OPG levels, 3) patients with carotid atheroma plaques or coronary artery calcification have higher OPG levels than those without and 4) OPG is expressed in adipose tissue samples and its expression is increased in MS patients. 

**Increased circulating OPG increased by risk factors and MS**

In the present study we demonstrated that OPG is increased in MS patients and that elevated OPG concentrations are related to different risk factors such as diabetes, hypertension, obesity or smoking.

In the literature there is scarce and contradictory information about the relationship between OPG and MS. Akinci et al. [12] showed that women with previous gestational diabetes mellitus developing MS had higher OPG than those without MS and healthy controls. However, Nabipour et al. [13] did not find significant differences between the mean serum OPG levels of postmenopausal women with and without the MS. As these two cohorts included exclusively women, results cannot be compared with results from this study. Our study confirms that OPG is elevated in MS patients and that is associated with different risk factors. In accordance with other studies, OPG concentration was increased in patients with diabetes [14,15]. In hypertension, we confirmed increased OPG concentration as previously reported [16,17]. In hypertensive subjects, markers of inflammation are elevated and the pressure of arterial blood may stimulate the endothelium promoting the inflammatory cascade and increasing OPG concentration.

Few studies have investigated the relationship between smoking and the RANK/OPG system. Both animals and human studies suggest that OPG levels are downregulated by smoke. Rats exposed to cigarette smoke inhalation had an upregulation in RANKL/OPG ratio compared to the rats without exposure [18]. In addition, two studies comparing smokers to non-smokers showed that smokers had lower OPG levels without differences in RANKL levels [19].

The controversial issue in this study is that in obese patients OPG concentration was increased while other studies have reported to be

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Fig. 4. OPG expression in human adipose tissue. Total RNA was isolated from human abdominal adipose biopsies. A) Representative microscopic views of OPG staining in human adipocytes using an antibody for human OPG identified in brown (magnification × 40). B) Comparative expression in adipose tissue from patients with and without the MS.

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diminished [20]. Obesity is related to low-grade inflammation in adipose tissue; thus, excessive release from adipose tissue could lead to increased OPG concentrations in these patients.

The source of increased circulating OPG remains unknown. OPG is produced by endothelial cells [5] and smooth muscle cells [4], regulated by multiple stimuli including pro-inflammatory cytokines [21]. Obesity, diabetes, and the MS are characterized by a proinflammatory state. But more research is required to elucidate the cells and mechanisms responsible of increased OPG release.

Circulating OPG associates with mean carotid IMT and the presence of carotid atheroma plaques

Although studies in animals show that OPG is an inhibitor of atherosclerosis [22], our study and other studies in humans show that OPG is strongly associated with numerous cardiovascular risk factors and it is related to carotid atherosclerosis and an independent risk factor for cardiovascular disease [1,23].

Two extensive studies have been performed evaluating the value of OPG in the prediction of plaque growth, cardiovascular disease and mortality [10,11]. Our finding that OPG levels directly and significantly correlated with mean carotid intima-media thickness (IMT) in the overall population confirms in Spanish population the results of previous studies [9,10] and suggests that OPG might play a role in the pathogenesis of atherosclerotic disease.

In human atherosclerotic plaque, the expression of inflammatory molecules such as OPG is increased and upregulated in a hypoxic status [7]. Moreover, OPG secreted by explants from endarterectomy samples from stable and unstable carotid atherosclerosis was higher in explants from symptomatic patients. And the upregulation of OPG in carotid plaque may play a role in plaque stability [24]. Our study expands these in vitro findings to circulating OPG, which was significantly higher in patients with carotid atheroma plaques compared with those without.

Circulating OPG associates with coronary artery calcification

Several biomarkers, including OPG, have emerged as surrogate markers of coronary calcification because of their role on vascular biology [25]. In this pilot study, circulating OPG levels were significantly higher in patients with coronary artery calcification (CAC) compared with those without. This finding is in accordance with previous studies in which OPG is independently associated with coronary artery calcification but in other cardiovascular risk groups [11,26]. In contrast with these studies we did not find association between OPG and calcification scores, which could be explained by the relatively lower number of patients studied.

In animals, OPG appears to be protective against vascular calcification. OPG knockout mice exhibited early onset osteoporosis, but unexpectedly, also exhibited calcification of the aorta and renal arteries, suggesting that regulation of OPG may play a role in the association between osteoporosis and vascular calcification [27]. Besides, the inactivation of OPG resulted in augmented vascular calcification and increased size of atherosclerotic lesions in OPG and apolipoprotein E knockout mice [28]. Besides, OPG expression was demonstrated in calcified plaques more frequently observed in carotid than in femoral plaques and the expression correlated with the macrophage infiltration [25].

The calcification process stimulates the secretion of pro-inflammatory cytokines capable of increasing OPG production and enhancing osteogenic differentiation, which could explain the increased circulating OPG levels observed in patients with coronary artery calcification and with atheroma plaques.

Adipose tissue as a potential source of OPG

Adipose tissue has been considered a passive energy storage; however, recent studies demonstrated that it is metabolically active that secreted adipokines with paracrine actions in other tissues [29].

In 2007, An JJ et al. [30] described an increase in OPG expression during the differentiation process of 3T3L1, while there were no differences in RANKL expression. Besides, a higher OPG/RANKL ratio was observed after stimulation with tumor necrosis factor alpha (TNF-α) and a decrease in that ratio when cells were stimulated with insulin and rosiglitazone. Afterwards, Harsof et al. [31] confirmed the expression of OPG in the adipose tissue. They observed an increased OPG expression with pro-inflammatory cytokines such as IL1-β and TNF-α and reduced OPG expression by cortisol or troglitazone. Moreover, Fain et al. [32] in explants from human adipose tissue from obese women observed that OPG was secreted by both fat and non-fat cells. To our knowledge, this study is the first to show by different techniques that human adipose tissue expresses OPG and that this expression is higher in adipose tissue from patients with the MS than in controls. Adipose tissue in patients with the MS secretes adipokines that contribute to the pro-inflammatory and prothrombotic state observed in these patients. Future studies will be needed to elucidate the mechanisms by which OPG is increased in the adipose tissue of these patients.

Limitations

Our study has some limitations that must be taken into consideration. First, serum concentration of RANKL was not measured. However, Lieb et al. [11] showed that OPG was associated with increased cardiovascular disease (CVD) and mortality whereas RANKL concentrations displayed inverse associations with cardiovascular risk factors and were not related to coronary artery calcification or incident CVD or mortality. Moreover, several population studies did not find relation between serum RANKL levels and CVD [33]. Another limitation is the influence of different treatments that were not studied separately.

Also, this is a cross sectional study and conclusions about causally related to atherosclerotic disease progression and acute coronary events cannot be drawn. Moreover, as recent studies have pointed out, the association of IMT and coronary calcium score and subclinical atherosclerosis is questionable [34]. Future studies will have to elucidate the intermediate steps in the pathways from elevated OPG to clinical events and to clarify whether OPG serves not only as a marker of atherosclerotic disease but also as a mediator of cardiovascular disease.

In summary, the present study revealed that serum OPG levels tended to be increased in patients with the MS and associated with subclinical atherosclerosis and coronary calcification. Furthermore, this article identifies for the first time, adipose tissue as a potential source of OPG and higher OPG expression in MS patients. These data suggest that more research is needed to validate OPG as a cardiovascular damage/risk biomarker.

Author contributions

Carmen Pérez de Ciriza, María Moreno and Patricia Restituto made substantial contributions to data acquisition, analysis and interpretation of data as well as drafting the article and revising it.

Gorka Bastarrika and Isabel Simón performed the intima–media thickness and coronary artery calcium measurements and revised the manuscript. Inmaculada Colina recruited the patients and revised the manuscript.

Nerea Varo made substantial contributions to conception and design, revised the article and gave final approval of the version to be submitted and any revised version.

Disclosure statement

The authors have nothing to disclose.
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References


