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The relation between osteoprotegerin, inflammatory processes, and atherosclerosis in patients with metabolic syndrome

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Abstract. – OBJECTIVE: To evaluate osteoprotegerin serum concentration (and compare with healthy controls), to estimate the relationship between serum osteoprotegerin and lipid parameters, insulin resistance, and selected inflammatory factors, and to assess the relationship between osteoprotegerin and intima media thickness in patients with metabolic syndrome.

PATIENTS AND METHODS: A total of 70 individuals aged 18-65 years with metabolic syndrome were enrolled. Anthropometric parameters, including body weight, body mass index, waist circumference, and waist-hip ratio, were assessed. The relative and absolute fat tissue contents were evaluated. Serum glucose, insulin, osteoprotegerin, C-reactive protein, and lipid profile were determined. Insulin resistance was calculated using Homeostasis Model Assessment. Intima media complex thickness was evaluated in each participant.

RESULTS: No significant differences were observed between patients and the controls with respect to lipid and carbohydrate profiles. Osteoprotegerin was significantly elevated in metabolic syndrome patients compared to the controls. Both C-reactive protein serum concentration and insulin resistance increased in the metabolic syndrome patients. Significant positive correlations between osteoprotegerin serum concentration and body mass index, waist-hip ratio, C-reactive protein serum concentration, and insulin resistance, were documented in patients with metabolic syndrome.

CONCLUSIONS: Patients with metabolic syndrome have increased osteoprotegerin serum levels than healthy individuals. Osteoprotegerin plays an important role in the development of arteriosclerosis, and the effect of osteoprotegerin on intima media thickness strongly depends on the extent of the arteriosclerotic changes that occur in metabolic syndrome.

Key Words:
Osteoprotegerin, Inflammatory processes, Atherosclerosis, Metabolic syndrome.

Abbreviations

% FAT = relative fat tissue content; BMI = body mass index; CRP = high-sensitivity C-reactive protein; CVD = cardiovascular diseases; DBP = diastolic blood pressure; ECs = endothelial cells; HDL = high-density lipoprotein; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; hsCRP = high-sensitivity C-reactive protein; IL-1B = interleukin-1B; IL-6 = inerleukin-6; IMT = intima media thickness; INS = insulin; kg FAT = absolute fat tissue content; LDL = low-density lipoprotein; MS = metabolic syndrome; NS = not significant; OPG = osteoprotegerin; PAI-1 = plasminogen activator inhibitor-1; PETIA = Particle-enhanced turbidimetric immunoassay; SBP = systolic blood pressure; SD = standard deviation; SNP = single nucleotide polymorphism; TCH = total cholesterol; TG = triglycerides; TNF-α = tumor necrosis factor-α; TRAIL = TNF-related apoptosis inducting ligand; VSMCs = vascular smooth muscle cells; WHR = waist-hip ratio; WOSCOPS = West of Scotland Coronary Prevention Study.

Introduction

Metabolic syndrome (MS) combines the most important cardiovascular risk factors, including visceral obesity, pre-diabetes and diabetes, elevated blood pressure, and imbalanced lipid homeostasis[1]. Almost 25% of the global adult population fulfills the conditions for MS. Early recognition of MS is necessary to launch an effective offensive against cardiovascular epidemic outcomes. Despite numerous studies, the leading cause of MS has not yet been settled. Both obesity and insulin resistance have been found to serve as important MS diagnosis criteria. Insulin resistance is integrally linked to MS, while visceral obesity has been regarded as its clinical manifestation[2,3]. Recent evidence suggests a relationship between obesity and inflammatory processes. Macrophages are the main reservoir of proinflammatory mediators within the fat tissue. Increased amoun-

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ts of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1B (IL-1B), are secreted by such cells. However, hsCRP (high-sensitivity C-reactive protein) still remains the most apparent indicator of inflammation. Fat tissue is also a source of a wide range of other cytokines, including resistin, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1). It is hypothesized that TNF-α may influence atherosclerosis development through its proinflammatory action, and in consequence lead to an increase in the frequency of cardiovascular outcomes. Novel candidates for cardiovascular risk factors have been considered, such as osteoprotegerin (OPG), discovered by Tsuda et al[4] in 1997. Other studies[4-6] have reported the discovery of similar proteins. One of OPG's functions is to inhibit osteoclastogenesis in human fibroblasts[4-6]. OPG is mostly secreted by osteoblasts, but its expression has also been found in kidneys, liver, spleen, and bone marrow[7]. OPG is present in the heart, veins, arteries, endothelial cells (ECs), and vascular smooth muscle cells (VSMCs)[8]. OPG favors the survival of endothelial cells, but the exact mechanism remains unknown. It affects metabolic processes in blood vessel walls by inhibiting the smooth-muscle cell apoptosis induced by TRAIL (TNF-related apoptosis inducting ligand). *In vitro* studies have revealed that proinflammatory cytokines are responsible for OPG increase both in ECSs and VSMCs. Those findings may suggest OPG involvement in cardiovascular diseases development. However, the doubt remains about whether OPG protects or favors the progression of atherosclerosis. Animal studies have shown that OPG inhibits blood vessel calcification, and this may indicate its protective role in ECS and VSMCs. It has been recently demonstrated that there is a connection between OPG, ischemic heart disease, and atherosclerosis[9]. Bennett et al[9] put forward the hypothesis that a lack of osteoprotegerin may lead to sclerosis of major blood vessels and their more rapid calcification[9]. It should be thus considered that OPG in circulatory system may act as a calcification inhibitor and a marker of atherosclerosis. Data on this in humans is scarce. Reports of aortic and blood vessel calcification have led to intense research on the potential relation between OPG and cardiovascular diseases. Epidemiological studies have demonstrated the association between processes of osteoporosis and atherosclerosis. Soufi et al[10] showed a correlation between increased cardiovascular outcomes and OPG gene polymorphism. A particular single nucleotide polymorphism (SNP) of the OPG gene seems to have a direct relationship with common carotid artery intima media thickness (IMT), and could be used as an early indicator of sclerosis[11]. It can thus be assumed that OPG has a protective role in blood vessels, and that this SNP negatively affects its function.

In the light of available data, the precise impact of OPG on cardiovascular risk development is unknown. It has been shown that OPG concentrations are significantly elevated in patients with both cardiac and peripheral blood vessels sclerosis[12], as well as in ischemic stroke. In this work, we investigate the relationship between OPG concentration, inflammation, and arteriosclerosis in patients with metabolic syndrome, a condition that involves multiple cardiovascular risk factors.

The aims of the study were:

1. To evaluate the OPG concentration in patients with MS, as compared to healthy controls.
2. To estimate the relationship between OPG and lipid parameters, insulin resistance, and selected inflammatory factors in patients with MS.
3. To assess the relation between OPG and IMT in MS."

Patients and Methods

Patients

A total of 70 individuals aged 18-65 years with metabolic syndrome were enrolled in the study. Consent from the Bioethical Committee of Poznań University of Medical Sciences was obtained for the research (number 19/10; 2010). Written agreement was achieved from all study participants. MS was diagnosed according to the guidelines of the International Diabetes Federation[13]. Patients with chronic or acute inflammatory processes, neoplasms, ischemic heart disease, type-2 diabetes, osteoporosis, or the secondary causes of obesity, were excluded from the study. The average body mass index (BMI) value in the group with MS was 35.7 ± 6.4 kg/m². The control group included 20 healthy subjects (ten women and ten men) aged 48.3 ± 10.6 years with average BMI 23.9 ± 1.9 kg/m².

Methods

Anthropometric parameters – including body weight, BMI, waist-hip ratio (WHR), and waist circumference, were assessed in all patients, who were wearing light clothes, without shoes, in the

morning. Relative (% FAT) and absolute (kg FAT) fat tissue contents were evaluated by bioelectrical impedance analysis using a Bodystat 1500 (Bodystat Ltd, Douglas, Isle of Man, UK)[14].

Peripheral blood (5 ml) was obtained from each individual to evaluate selected biochemical parameters. Glucose and insulin concentration were estimated using the radioimmunological method. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was determined (HOMA values > 2.9 indicate insulin resistance)[15]. The concentration of hsCRP was estimated using particle-enhanced turbidimetric immunoassay (PETIA) (Pointe Scientific Inc., Canton, MI, USA). Osteoprotegerin concentration was assessed using the ELISA immunoenzymatic method (Biomedica Medizinprodukte, Wien, Austria). Serum lipid profile was estimated using commercial kits (Abcam, Cambridge, MA, USA). Total serum cholesterol concentration was estimated using a reaction with cholesterol esterase, cholesterol oxidase, and peroxidase. HDL (high-density lipoprotein) serum concentration was estimated using the direct enzyme method. Triglycerides (TG) serum concentration was estimated using the enzymatic method with lipase, glycerol kinase, phospho-glycerol-3-oxidase, and peroxidase. The concentration of LDL (low-density lipoprotein) in the serum was calculated using the Friedewald formula: LDL (mmol/L) = TCH (mmol/L) – HDL (mmol/L) – TG (mmol/L) / 2.2[16].

Blood pressure was measured in the morning, after one hour of rest, in a sitting position, using a digital electronic tensiometer 705IT TM (Omron, Kyoto, Japan). During the measurement, the lower limbs were uncrossed, and the back and arms were supported.

Intima media complex thickness was evaluated in each participant using a GE Voluson 730 pro USG apparatus, head 12 MHz (General Electric, Boston, MA, USA). IMT measurement was performed at many different points on the external carotid artery, more or less 1 cm proximately from its sinus. Evaluations were carried out by independent specialists. The results are presented as an average of two measurements[17].

Statistical Analysis

The data are given as means ± SDs (standard deviations). Comparisons between groups were carried out using the Mann-Whitney U-test or the unpaired t-test if the data were normally distributed. The Shapiro-Wilk test was used to check for normal distribution. The analysis of correlation was carried out using the Spearman rank-correlation test, or the Pearson correlation test if the data had normal distribution. A p-value of less than 0.05 was regarded as significant. All calculations and statistics were performed using Statistica 6.0 PL software for Windows (StatSoft, Kraków, Poland).

Results

No statistically significant difference was observed between patients and controls with respect to lipid and carbohydrate profiles. A trend towards increased blood pressure values was seen in the MS group. Detailed data on the studied individuals is presented in Table I.

Compared to the control group, the patients with MS showed a statistically significant increase in the values of all anthropometric parameters, as shown in Table II.

OPG was found to be significantly elevated in MS patients compared to controls. There were

Table I. Characteristics of studied population.

Parameter	Metabolic syndrome (MS) N = 70	Control N = 20	p
Age (years)	52.7 ± 11.6	48.3 ± 10.6	NS
INS (μUI/ML)	29.2 ± 10.2	10.4 ± 3.4	NS
Glucose (mmol/L)	5.9 ± 1.0	5.4 ± 0.4	NS
TCH (mmol/L)	5.6 ± 1.2	5.1 ± 0.6	NS
LDL (mmol/L)	3.5 ± 1.0	3.0 ± 0.8	NS
HDL (mmol/L)	1.2 ± 0.3	1.5 ± 0.6	NS
TG (mmol/L)	2.2 ± 1.3	1.6 ± 0.5	NS
SBP (mmHg)	144.0 ± 12.8	129.9 ± 10.2	NS
DBP (mmHg)	89.6 ± 7.8	81.3 ± 6.7	NS

INS: insulin; TCH: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides; SBP: systolic blood pressure; DBP: diastolic blood pressure; NS: not significant.

Table II. Comparison of anthropometric parameters in the study groups.

Parameter	Metabolic syndrome (MS) N = 70	Control N = 20	p
BMI (kg/m)	35.7 ± 6.4	23.0 ± 1.9	$p<0.001$
WHR	1.03 ± 0.1	0.95 ± 0.07	$p<0.01$
Kg FAT (kg)	35.8 ± 7.9	21.0 ± 5.7	$p<0.001$
% FAT (%)	36.9 ± 7.4	24.2 ± 5.6	$p<0.001$

BMI: body mass index; WHR: waist-hip ratio; kg FAT: absolute fat tissue content; % FAT: relative fat tissue content.

Table III. The comparison of biochemical parameters in studied groups.

Parameter	Metabolic syndrome (MS) N = 70	Control N = 20	p
OPG (pmol/l)	5.7 ± 2.7	2.5 ± 0.5	$p<0.001$
hsCRP (mg/l)	4.1 ± 2.7	2.1 ± 1.2	$p<0.001$
HOMA-IR	7.7 ± 3.1	2.6 ± 0.9	$p<0.001$

OPG: osteoprotegerin; hsCRP: high-sensitivity C-reactive protein; HOMA: Homeostasis Model Assessment.

Table IV. Comparison of IMT values in the study groups.

Parameter	Metabolic syndrome (MS) N = 70	Control N = 20	p
IMT (mm)	1.0 ± 0.3	0.7 ± 0.2	$p<0.01$

IMT: intima media thickness.

also statistically significant differences in the concentrations of hsCRP and HOMA-IR between studied groups. Both parameters were increased in MS patients. Detailed results are present in Table III.

IMT values were significantly higher in patients with MS than in the controls (Table IV).

Statistically significant positive relations between OPG concentration and BMI, WHR (Table V), hsCRP (Figure 1, Table V) and HOMA-IR (Figure 2, Table V), were documented in patients with MS. No correlations were present between OPG and the examined parameters in the control group.

Discussion

Novel risk factors for cardiovascular diseases have continually been investigated. There are scarce data on the estimation of OPG concentrations in MS and discrepancies exist among the results. Our investigation shows that OPG concentration was higher in individuals with MS than in the control group. Our results are in line with those obtained by Bernardi et al[18]. We showed that OPG concentration is independent of age, systolic blood pressure, and glucose imbalance. We found a positive correlation between OPG and body mass index. Our results allow us to hypothesize that OPG might be involved in metabolic processes associated with atherosclerosis development. Studies showing that osteoprotegerin levels negatively correlate with abdominal obesity thus become interesting. Gannage-Yared et al[19] compared a group of 106

Table V. Relationship between OPG concentration and the evaluated parameters in patients with MS.

Correlation	R (Spearman)	p
OPG and BMI	0.29	$p<0.01$
OPG and WHR	0.46	$p<0.01$
OPG and hsCRP	0.35	$p<0.001$
OPG and HOMA-IR	0.54	$p<0.001$

OPG: osteoprotegerin; WHR: waist-hip ratio; hsCRP: high-sensitivity C-reactive protein; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

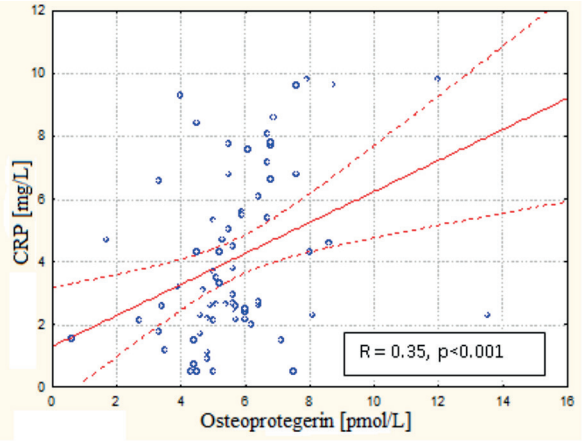

Figure 1. Correlation between osteoprotegerin and hsCRP concentrations in metabolic syndrome; hsCRP: high-sensitivity C-reactive protein.

obese people and 64 subjects with normal body weight and found no differences between them in terms of OPG concentration[19]. Conversely, Ugur-Altun et al20 presented results showing that OPG concentration in a group of young obese adults was significantly lower than in a control population[20]. On the other hand, Nabipour et al[21] did not find any association between obesity and OPG concentration in a group of 382 postmenopausal women[21], a finding not supported by the other previously mentioned authors. In our study, there was a positive relation between OPG concentration and BMI in patients with MS. In the research of Golledge et al[22], obesity was followed by increased OPG concentration in a group of patients with atherosclerosis in the lower extremities[22]. The discrepancy in results may thus be a consequence of the study populations being characterized by different parameters, such as age, gender, hormonal changes, level of atherosclerosis in the lower extremities, or history of bariatric surgeries.

CRP is one of the main markers of inflammatory state. In our study, its values correlated with serum blood OPG concentration, and the results were not associated with the age of the subjects. Positive correlations between OPG, CRP, and other proinflammatory markers, have previously been described by other authors. These observations were confirmed by Yaturu et al[23], which showed a positive correlation between OPG concentration and the main proinflammatory cytokine, TNF-α[23]. Our study also established a positive relation between OPG and the inflammatory state marked by CRP concentration, both found to be higher in MS patients than in the controls. The West of Scotland Coronary Prevention Study (WOSCOPS) revealed a higher risk of cardiovascular events in a subpopulation with MS with CRP above 3 mg/L[24].

Our results indicate the involvement of OPG in mild inflammatory processes in patients with MS. It has been described that proinflammatory cytokines induce the expression of the OPG gene in the smooth muscle cells and endothelium, as a result of the regulation of inflammatory process[25]. Since proinflammatory factors have been regarded as a key issue in the development of atherosclerosis, the OPG associated with their concentration might also play some roles in the development of atherosclerosis.

Numerous epidemiological studies show that insulin resistance increases the number of cardiovascular diseases outcomes[24]. HOMA-IR in our patients was almost three times greater than in the control group. It is thus possible to draw the conclusion that patients with MS might have a parallel higher risk of cardiovascular outcomes. Considering elevated HOMA-IR and CRP values as important CVD risk factors, OPG, which correlates with these parameters, should also be recognized as a one of components in the atherosclerotic process[26].

In our patients, OPG concentration also correlated with visceral obesity indicator (WHR) and BMI. Recent studies have paid much attention to OPG, and its role in the development of atherogenesis is still widely deliberated. Reports on the calcification of aortic endothelial and renal

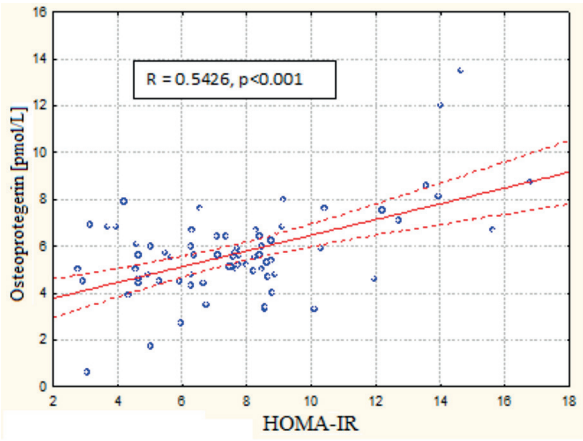

Figure 2. Correlation between osteoprotegerin concentration and HOMA-IR index in metabolic syndrome; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

arteries in mice lacking the OPG gene suggest that OPG may play a role in the development of atherosclerosis in humans. OPG is regarded as a prognostic marker of CVD outcomes and CVD-related mortality.

Kiechl et al[27], in the Bruneck study, performed ultrasonographic carotid artery measurements and assessed OPG concentrations; the same procedure was repeated ten years later. OPG levels positively correlated with the presence of sclerotic changes in arteries; however, the difference was not statistically significant[27]. The trial covered 6516 participants ranging from 25 to 85 years old. Analysis revealed the correlation between IMT and OPG concentration. The study also confirmed the relation between IMT and traditional CVD risk factors, including age, sex, total cholesterol, HDL, CRP, BMI, diabetes mellitus, and systolic blood pressure. Furthermore, a stronger correlation between IMT and OPG in patients 55 years of age and older was documented[27]. Conversely, no significant correlations between OPG and IMT in patients with MS have been found in our work. Nevertheless, both parameters were elevated in MS over the control group. Potentially, a relationship may exist between OPG and subclinical atherosclerosis. Bennett et al[9] showed that mice deficient in the OPG gene develop atherosclerosis much earlier than normal mice[9]; they suggested that there is a positive impact of OPG on the intima media. Some investigators have claimed that OPG is a marker, rather than a mediator, of atherosclerosis[28].

A serious discrepancy exists between the influence of OPG on the cardiac system: some authors have reported a positive impact, while others have claimed there to be no significant correlation between OPG and CVD. It is well known that the development and further growth of arteriosclerosis is associated with inflammatory cytokines. On the other hand, decreased serum OPG concentration exerts a positive feedback on IMT and inhibits atherosclerosis progression. OPG blocks TRAIL, which induces apoptosis. This may initiate atherosclerosis development. OPG is thus a factor that potentially inhibits apoptosis and takes part in atherogenesis[29].

The question arises of whether OPG will take a place in the global hierarchy of CVD risk factors. In the light of current research, we may suspect that OPG will be regarded as a future CVD prognostic marker. However, the exact role of OPG in the development of atherosclerosis and cardiovascular outcomes deserves more attention and needs further studies based on large population of patients with MS, similar to high-quality investigations on connection between OPG and osteoporosis[30].

Conclusions

Patients with MS have twice as high OPG serum levels than healthy controls. The relation between OPG and markers involved in the state of inflammation or lipid and carbohydrate balance suggests the significant role of OPG in the development of arteriosclerosis. The effect of OPG on IMT may strongly depend on the level of arteriosclerotic changes present in MS.

Conflict of interest

The authors declare no conflicts of interest.

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