ASSOCIATION BETWEEN DRUG RESISTANT HYPERTENSION AND INCREASED OSTEOPROTEGERIN LEVELS IN HYPERTENSIVE MALE PATIENTS WITH NON OBSTRUCTIVE SLEEP APNEA

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Introduction

Suboptimal doses of three antihypertensive medications including diuretics, anti-inflammatory therapy, renal artery stenosis, hyperaldosteronism and obstructive sleep apnea (OSA) may occur in patients with drug resistant hypertension(1-4). Non-dipping drug resistant hypertension is particularly accepted for a high-risk hypertensive stage and related to progressive atherosclerosis(5).

Osteoprotegerin (OPG) is a soluble member of the tumor necrosis factor (TNF) receptor superfAMILY and takes part in the suppression of osteoclast formation and immune functions such as leukocyte adhesion. The presence of an elevated OPG concentration in patients with atherosclerosis and its relation with cardiovascular mortality has been previously reported(6,7). In the last decade, although the role of OPG has been investigated in atherosclerosis and cardiovascular mortality(8,9), its role in OSA and hypertension has not been well documented. The association of OSA and hypertension is well known(10,11) and OSA is stressed as one of the frequent causes of drug resistant hypertension(12,13) and the prevalence of OSA has been found 17% of 50-70 year-old males by Peppard et al.(14).

Carotid intima-media thickness (IMT) is a useful diagnostic tool for determining atherosclerosis(15,16) and increased OSA related carotid IMT and stiffness has been reported previously(17). Therefore, 

ABSTRACT

Objectives: Increased carotid intima-media thickness (IMT) reflects subclinical vascular damage and is related to obstructive sleep apnea (OSA) syndrome and hypertension. Osteoprotegerin OPG is a member of the tumor necrosis factor superfamily and increases leukocyte adhesion. The relationship between increased OPG and atherosclerosis is well known, but there is still no evidence whether there is an association between OPG and resistant hypertension. This study investigated the association between OPG and drug resistant hypertension in patients with OSA and non-OSA.

Patients and methods: We investigated the association between serum OPG levels and carotid IMT in patients with drug resistant hypertensive OSA (ODR) patients (n= 39) and drug resistant hypertensive non-OSA(NODR) patients (n=34) and drug-responsive hypertensive non-OSA controls (n=36).

Results: OPG levels and carotid IMT were found to be higher in the ODR [12.4(5.9-19.5) and 0.89 ±0.5] and in the NODR group [11.9(5.7-17.6) and 0.88±0.6] compared with the controls [8.4(4.8-16.5) pmol/L and 0.73 ± 0.8 mm], (p= 0.01and p=0.01 for OPG and p<0.01 and p<0.01 for carotid IMT). The carotid IMT was positively correlated with OPG levels in the ODR group (r=0.412, p=0.016) and in the NODR group(r=0.321, p=0.024) and with apnea hypopnea index (r=0.462, p<0.001) in the ODR. There was no correlation between OPG and apnea-hypopnea index (r=0.07, p=0.564 ) in the ODR group.

Conclusions: Increased serum OPG levels were associated with subclinical carotid atherosclerosis in patients with ODR and NODR , but not with drug-response non-OSA controls.

Key words: Carotid intima-media thickness, Obstructive sleep apnea, Osteoprotegerin, drug resistant hypertension.

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in the present study we investigate the level of OPG to establish whether there are relationships between the apnea-hypopnea index (AHI) and carotid IMT and OPG in 50-70 year-old male drug resistant hypertensive patients with OSA and non-OSA.

Materials and methods

Subjects, Polysomnography, and Study design

We enrolled 109 hypertensive patients between 50-70 years-old undergoing polysomnography (PSG) for OSA suspicion between September 2012 and October 2013. After a routine cardiologic examination including blood pressure measurements and electrocardiograms, ambulatory blood pressure monitoring (ABPM) and echocardiographic evaluations were performed by one cardiologist to identify non-dipper hypertension and to exclude patients with congestive heart failure and any moderate to severe valvular disease. Then, all patients were hospitalized in the sleep laboratory to identify the presence of sleep disorders.

Our local ethical committee approved the study and a waiver of consent was obtained from all patients. All study patients had neither any systemic disease nor a history of previous myocardial infarction. Hypertension was defined as blood pressure (BP) value >140/90 mmHg according to two different sphygmomanometer measurements. Drug resistant non-dipper hypertension criteria were accepted as the following: under medical treatment with three or more antihypertensive agents, including diuretics such as angiotensin converting enzyme inhibitors, calcium antagonists, or beta blockers, those with 140> mmHg for systolic and/or >90 mmHg for diastolic blood pressure and those with the difference of <10% between day and night mean blood pressure values according to ABPM results. Patients were classified as non-dipper hypertensive if the mean nocturnal BP fell by less than 10 % compared with the mean daytime value on ABPM.

A computerized system (Embla N7000; Somnologica, Broomfield, Colorado) was used to obtain PSG findings, which were then visually re-examined to ensure accuracy of the data. Apnea was defined as cessation of airflow of ≥10 seconds and hypopnea was defined as oxygen desaturation of ≥3% or a reduction in thoracic excursion of ≥50%. The number of apnea and hypopnea events per hour of sleep was deemed as the AHI. The drug-resistant OSA group and the drug-resistant non-OSA group comprised 39 patients and 34 patients who have drug-resistant hypertension, respectively. The control group consisted of 36 non-OSA patients who had hypertension with drug-resistance and dipper, and they were matched to the OSA drug-resistant (ODR) group and the non-OSA drug-resistant (NODR) group for age and cardiovascular risk factors. The control group’s patients were under medical treatment with antihypertensive agents and their BP values were lower than 140/90 mmHg.

We included to the study those with an AHI score of ≥5 in the ODR group and those with an AHI score of < 5 in the NODR group and the control group. Patients with heart failure, renal artery stenosis, hyperaldosteronism, nasal continuous positive airway pressure therapy, medication non-compliance, anti-inflammatory drug users, chronic liver disease, kidney failure, heart valve disease and known coronary artery disease, or previous myocardial infarction were excluded from the study. None of the diabetic patients were taking insulin in either group. Age, body mass index, systolic and diastolic BPs, smoking, diabetes, and hyperlipidaemia were recorded for both groups. We used pharmacy prescription refill data and performed renal ultrasound. Urinary protein, serum sodium, creatinine, calcium, potassium, phosphorus and thyroid-stimulating hormone levels were analysed to exclude secondary causes of resistant hypertension from the study. All drug-resistant patients were on medication with angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), calcium antagonists, and diuretics. In addition these 3 medications, 26 patients and 29 patients were taking carvedilol and 7 patients and 8 patients were taking doxazosin in the ODR group and NODR group, respectively. Subjects with dyslipidaemia and diabetes in all groups were taking statin medication and oral antidiabetics. Control group patients were on ACEI or ARB. If a patient was on sub-optimal/sub-maximal doses of 3 medications, we did not include as resistant.

Biochemical Analyses

After overnight fasting for> 10 hours, antecubital venous fasting blood samples were taken for the measurement of fasting glucose, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), serum sodium, creatinine, calcium, potassium,
phosphorus and thyroid-stimulating hormone levels and OPG.

For OPG measurements, after blood was collected in separate tubes and centrifuged; plasma were separated and stored at -80 °C. Fasting glucose, TC, HDL-C, LDL-C, and TG levels were measured using a standard enzymatic method [AU680 auto-analyser (Beckman Coulter, Brea, CA). The CRP levels were measured using a standard nephelometry method (Cobas 311, Roche Diagnostics, Mannheim, Germany) with a sensitivity of 0.1 mg/L.

**OPG Assay**

OPG levels in the serum were measured using an enzyme-linked immunosorbent assay. The assay detects both monomer and dimeric forms of OPG, including OPG bound to its ligand. Briefly, Mouse anti-Human OPG was used to capture antibodies and a biotinylated polyclonal anti-Human OPG antibody was used for detection. Performance data were provided from the manufacturer. The minimum detectable concentration for OPG was 0.03 pmol/l. Its calibration range was 1.5 - 60 pmol/l. The measured intra and inter-assay variability coefficients were 3.5 and 5.8%, respectively. The manufacturer does not yet define a reference range for healthy subjects.

**Statistics**

Analysis of data was also performed on the Predictive Analysis Software (SPSS Inc., Chicago, Illinois). The variable distributions were checked using the Shapiro-Wilk normality test. One-way ANOVA, Tukey test and Kruskall-Wallist test were used to compare the non-normally and normally distributed variables, respectively, in the three groups. Categorical data such as smoking, diabetes, and hypertension were compared using chi-square test and expressed as numbers and percentages. P values <0.05 were considered significant. Pearson and Spearman correlation tests were used to evaluate the presence of correlation between parametric and nonparametric variables.

**Results**

**Anthropometric, Clinical Features, and Biochemical Variables**

The ODR group included 39 patients aged 62(50-70) years with a body mass index (BMI) of 29(26-45) kg/m2, the NODR group included 60.5(50-70) years with a body mass index (BMI) of 28(25-43) kg/m2 and the control group included 36 patients aged 61(50-70) with a BMI of 27.5 (21 - 37) kg/m2.

There were 14 subjects with dyslipidaemia, 8 diabetic individuals, and 15 subjects were smokers in the ODR group. There were 13 subjects with dyslipidaemia, 7 diabetic individuals, and 14 subjects were smokers in the NODR group. There were 12 subjects with dyslipidaemia; 7 diabetic individuals, and 13 subjects were smokers in the control group.

Age, BMI, fasting glucose level, TC, TG, HDL-C, LDL-C, and CRP levels did not differ between the three groups (p >0.05). Smoking, dyslipidaemia, and diabetes rates of the three groups were also similar. Systolic and diastolic BP values were higher in the ODR group and the NODR group than the controls. Systolic and diastolic BP values in the ODR group and the NODR group were similar (p >0.05) (Table 1). The percentages of mean nocturnal dipping in the control group were higher than that of NODR group and the ODR group (Table 2).

**Polysomnographic Values, OPG, and Carotid IMT**

Compared to the controls and the NODR group, the ODR group had a higher AHI score [27 (8-71) vs. 2.5 (1-4) and 2.8 (1-4), p<0.01 and p<0.01] a higher percentage of recording time spent (PRTS) of oxygen saturation (SaO2)<90% (28±12 vs. 1.3±0.7 and 1.5±0.8, p<0.01 and p<0.01) and the lowest SaO2 percentage values (71±11 vs. 94±3 and 93±3, p<0.01) (Table 2). AHI score and PRTS in the ODR group and the NODR group were similar (p >0.05) (Table 2).

The ODR group and the NODR group had higher OPG levels and carotid IMT values compared with the control group [12.4(4.7-19.5) and 11.9(5.7-17.6) vs. 8.4(4.8-16.5), p=0.01 and p=0.01] and (0.89 ±0.5 and 0.88±0.6 vs. 0.73 ± 0.8, p<0.01 and p<0.01) (Tables 1,2). OPG levels and carotid IMT values in the ODR group and in the NODR group were similar (p >0.05) (Table 2).

Both AHI and SaO2 < 90%-PRTS were positively correlated with BMI, CRP, and carotid IMT, but not with OPG in the ODR group (Table 3). OPG was correlated with age but did not correlate with CRP in three groups. In addition, a correlation was found between OPG and cIMT in the ODR and the NODR group (Figure 1 and 2), but not with in the control group (Table 4).
Control group, DBP- Diastolic Blood Pressure, MND- Mean Nocturnal Dipping. SBP- Systolic Blood Pressure, control group, Tukey test, of recording time spent at SaO2.<

Obstructive sleep apnea and drug-resistant, PRTS- Percentage of recording time spent (PRTS) of oxygen saturation (SaO2)<90%, *Spearman rank correlation test, SaO2 < 90%-PRTS-percentage of recording time spent (PRTS) of oxygen saturation (SaO2)<90%.

Table 1: Anthropometric, clinical features and biochemical parameters of the drug-resistant OSA and control groups.

<table>
<thead>
<tr>
<th></th>
<th>ODR group (n = 39)</th>
<th>NODR group (n = 34)</th>
<th>CONTROLS (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62(50-70)</td>
<td>60.5(50-70)</td>
<td>61(50-70)</td>
<td>0.678</td>
</tr>
<tr>
<td>Body mass index, kg/m2</td>
<td>29(26-45)</td>
<td>28(25-38)</td>
<td>27.5(21-37)</td>
<td>0.083</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>160(145-180)</td>
<td>165(140-190)</td>
<td>130(110-140)</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>100(80-115)</td>
<td>105(80-120)</td>
<td>75(60-90)</td>
<td>0.468</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>15 (38.4%)</td>
<td>14(41.1%)</td>
<td>12 (38.8 %)</td>
<td>0.974*</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>14(35.9 %)</td>
<td>13(38.2 %)</td>
<td>13(33.3 %)</td>
<td>0.793*</td>
</tr>
<tr>
<td>Diabetes Mellitus, n (%)</td>
<td>8(20.5%)</td>
<td>7(20.5%)</td>
<td>7(19.5%)</td>
<td>0.754</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>102 (78-159)</td>
<td>101(75-165)</td>
<td>98 (65 - 216)</td>
<td>0.643</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>199(93-36)</td>
<td>195(91-41)</td>
<td>188 ± 53</td>
<td>0.341</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>180(59-442)</td>
<td>181(40-415)</td>
<td>157(54-450)</td>
<td>0.156</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>40(18-72)</td>
<td>42(21-67)</td>
<td>41(25-69)</td>
<td>0.129</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>110(37-232)</td>
<td>118(41-243)</td>
<td>117(50-243)</td>
<td>0.679</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>3 (2-6)</td>
<td>4 (1-6)</td>
<td>3(1-5)</td>
<td>0.074</td>
</tr>
<tr>
<td>Osteoprotegerin, pmol/L</td>
<td>12.4(3.9-19.5)</td>
<td>11.9(5.7-17.6)</td>
<td>8.4(4.8-16.5)</td>
<td>0.01**</td>
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</tbody>
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Table 2: Comparison of Polysomnographic Findings and carotid IMT values of three groups.

<table>
<thead>
<tr>
<th></th>
<th>ODR group (n = 39)</th>
<th>NODR group (n = 34)</th>
<th>CONTROLS (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI score</td>
<td>27 (8-71)</td>
<td>28(1-94)</td>
<td>2.5 (1-4)</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Lowest SaO2, %</td>
<td>71±11</td>
<td>93±3</td>
<td>94±3</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>SaO2&lt;90%, PRTS</td>
<td>28±12</td>
<td>15±0.8</td>
<td>13±0.7</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Carotid IMT, mm</td>
<td>0.89±0.5</td>
<td>0.88±0.6</td>
<td>0.73 ± 0.8</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>MND- Mean Nocturnal Dipping</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>SBP-Systolic Blood Pressure</td>
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<tr>
<td>DBP-Diastolic Blood Pressure</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CRP</td>
<td>3.2(1-4)</td>
<td>4.1(1-6)</td>
<td>3(1-5)</td>
<td>0.074</td>
</tr>
<tr>
<td>Osteoprotegerin, pmol/L</td>
<td>12.4(3.9-19.5)</td>
<td>11.9(5.7-17.6)</td>
<td>8.4(4.8-16.5)</td>
<td>0.01**</td>
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Table 3: Correlations between Polysomnographic Findings, Osteoprotegerin, BMI, CRP and Carotid IMT in the obstructive sleep apnea and drug-resistant group.

<table>
<thead>
<tr>
<th></th>
<th>ODR</th>
<th>NODR</th>
<th>CONTROLS</th>
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<tbody>
<tr>
<td>Osteoprotegerin</td>
<td></td>
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<tr>
<td>BMI</td>
<td></td>
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<td></td>
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<tr>
<td>CRP</td>
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<tr>
<td>Carotid IMT</td>
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Table 4: Correlations between Osteoprotegerin, age, CRP and Carotid IMT in three groups.

<table>
<thead>
<tr>
<th></th>
<th>ODR</th>
<th>NODR</th>
<th>CONTROLS</th>
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<tbody>
<tr>
<td>CRP</td>
<td></td>
<td></td>
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<tr>
<td>Carotid IMT</td>
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Fig. 1: A positive correlation was found between osteoprotegerin and carotid intima-media thickness in patients with drug resistant hypertensive and with obstructive sleep apnea. 
(r=0.412; p=0.016)

Fig. 2: A positive correlation was found between osteoprotegerin and carotid intima-media thickness in patients with drug resistant hypertensive and with non-obstructive sleep apnea. 
(r=0.321; p=0.024)
Discussion

We studied for the first time the relationship between OPG and carotid IMT in 50-70 year-old male patients with OSA, who have drug resistant and non-dipper hypertension. We specifically compared patients with OSA who were hypertensive and drug resistant in a quest to determine how the association between OSA and cIMT was affected by serum OPG levels. We found elevated OPG levels in drug resistant hypertensive OSA and non-OSA patients and also a relationship between OPG and increased carotid IMT in the drug-resistant groups, but not with drug-response non-OSA. Although OPG correlated with carotid IMT in the drug resistant hypertensive OSA group, there was no relationship between AHI and OPG levels in the drug resistant OSA group. In agreement with previous studies(6,7), OPG levels correlated with age. There was also a positive correlation between AHI and carotid IMT in accordance with some previous studies(23).

OPG is a novel member of the tumor necrosis factor receptor superfamily and a soluble decoy receptor of the receptor activator of nuclear factor-κB ligand. Some studies has implicated that OPG is an independent risk factor for the progression of atherosclerosis and onset of cardiovascular disease(6,7). Our study does not suggest that OPG is a direct cause of hypertension. Abnormalities in extracellular fluid volume regulation in drug resistant hypertension are essential mechanism. OPG, as a calcification inhibitor, may influence aortic stiffening in atherosclerosis. Our findings potentially suggest that elevated OPG levels are related to increased mean carotid IMT.

It is more likely that uncontrolled hypertension results in increased inflammation, which in turn results in increase OPG levels and greater atherosclerosis. It has been shown as a possible link between OPG and vascular diseases that angiotensin II blockade down-regulates OPG in vitro(18) and elevated OPG levels are significantly associated with endothelial dysfunction(19).

Increased carotid IMT is a known marker of future cardiovascular diseases(20) and both the severity of OSA and age is associated with carotid IMT(16,17). Smoking, dyslipidaemia, diabetes, and hypertension are important risk factors for atherosclerosis and carotid IMT(21,22). In our study, age, the percentage of smoking, diabetes and dyslipidaemia, and the ratio of patients taking lipid-lowering agents and oral antidiabetics were similar in both groups.

Kiechl S et al.(6) found in their 10-year follow-up study that an increased OPG level was an independent risk factor for incident cardiovascular disease. OPG takes a crucial dual part in the process of activation of specific proinflammatory and pro-apoptotic signalling pathways, and increases leukocyte adhesion(23). The significance of increased OPG levels for vascular disease has been documented especially in advanced coronary artery disease(24,25). OSA results in intermittent hypoxia and continuous increased sympathetic tonus leading to endothelial dysfunction, and is associated with arterial stiffness and atherosclerosis, and triggers hypertensive mechanisms(11,12,26). In our study, there was no relationship between OPG and the severity of OSA, but we found that carotid IMT correlated with the severity of OSA and OPG. Whether the relationship between OPG levels in patients with OSA has not been investigated prior to this study, which showed higher levels in the ODR and NODR patients than in the control patients, possibly because of increased carotid IMT, and determined no relationship between the severity of OSA and OPG.

The first limitation of the study is the small sample size. Second, we used only carotid IMT and omitted carotid artery distensibility (change of diameter) values, because carotid IMT is more effective in determining between low- and high-risk patients than distensibility(20). Third, it would be better to study serum fibrinogen levels and inflammatory markers and mediators such as some interleukins (IL-1 and IL-6), interferon gamma, and growth factors, serum C3, C4, and high sensitivity CRP.

In conclusion, increased OPG levels did not correlate with OSA severity and are related to higher carotid IMT. OPG levels are no affected by OSA but are more closely associated with uncontrolled hypertension. In addition, higher AHI is associated with elevated carotid IMT in OSA. Further studies are needed to reach conclusions concerning OPG concentrations related to drug resistant hypertension.

References


2) Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism among black and


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