OSTEOPROTEGERIN PLASMA CONCENTRATIONS ARE CORRELATED WITH FORCED EXPIRATORY VOLUME AT 1 SECOND (FEV1) IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Introduction*: Several inflammatory biomarkers such as IL-1β, IL-6 and TNF-α have been evaluated during stable state and exacerbations of chronic obstructive pulmonary disease (COPD). Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor family that inhibits receptor activator of nuclear factor-kappa B ligand (RANKL). OPG mRNA is expressed in the lung and macrophages. The objective of the study was to evaluate the serum level of OPG and its correlation with forced expiratory volume at 1 second (FEV1) in patients with COPD versus healthy volunteers.*

Materials and methods*: The study was conducted between June 2011 and October 2012 in the pulmonology clinic of the medical school at Namık Kemal University. Previously diagnosed stable 47 COPD patients and non-smoking gender- and age-matched 23 healthy volunteers were included to the study. Osteoprotegerin levels in the serum were quantified by Biovendor enzyme linked immunosorbent assays.*

Results*: The level of FEV1 on average was 59.3±16.9. Plasma OPG concentrations of COPD patients (8.61±4.4.78 ng/ml. n=47) were higher than those of healthy control group (6.29±1.36 ng/ml. n=23). The difference was statistically significant (p<0.05). On the other hand, serum OPG levels had a significantly negative correlation with FEV1 (r= - 0.33 p<0.05).*

Conclusion*: OPG levels are increased in COPD patients and correlated with FEV1. After larger and longitudinal studies OPG may be used as a biomarker of the lung function in COPD patients.*

Key words*: COPD, Osteoprotegerin, FEV1, biomarker.*

Received *June 18, 2014;* ***Accepted*** *October 02, 2014*

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the airways and lung parenchyma that is estimated to affect 9-10% of adults over 40 years of age[(1)]. COPD causes both significant mortality[(2)] and morbidity[(3)]. It is estimated that it will be the third-leading cause of mortality by 2020[(4)]. Although in previous studies the prevalence and mortality of COPD were greater among man than women, recent data from developed countries show that the prevalence of the disease is now almost equal in men and women, probably reflecting the changing patterns of tobacco smoking[(5)].

COPD is defined as a preventable disease, and it is characterized by persistent airflow limitation that is usually progressive and related to an increased chronic inflammatory response in the airways and the lung to noxious particles or gases[(5)]. Major symptoms of COPD are chronic progressive dyspnea, cough and sputum production[(5)]. Chronic cough and sputum production may precede the development of airflow limitation by many years[(5)].

Although COPD is characterized by chronic inflammation, the relationship between COPD and the inflammatory pathway has not been well defined. Levels of inflammatory cytokines such as IL-1β, IL 6, and TNF-α are higher in the systemic circulation of COPD patients; systemic inflammation is implicated, or at the least it may worsen comorbid diseases[6]. Systemic inflammation leads to a decline in lung function, and triggers further exacerbations[7].

Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily (TNFRS); it prevents the biological effects of the receptor activator of NF-ϰB (RANK)/RANK ligand (RANKL). It has also been shown that this system interacts with IL-1β, IL-6, and TNF-α[8]. OPG is reportedly involved in lipopolysaccharide-induced acute inflammation in animal models[8]. Serum OPG level has been shown to be increased in coronary artery disease, rheumatoid arthritis, and some other diseases, and it has been used as a biomarker[9]. OPG levels also increase with ageing[10].

Forced expiratory volume in 1 s (FEV1) is the only validated clinical marker of COPD, but it poorly correlates with the clinical features of the disease[11]. There is a need for novel biomarkers by which to diagnose stable disease, predict its stage, and monitor progression. The objective of this study is to evaluate the serum level of OPG and whether there is a correlation between OPG and FEV1, among both individuals with COPD and healthy nonsmokers.

Materials and methods

Subjects

This observational, prospective, and cohort-based study was conducted between June 2011 and October 2012. All patients were selected from the outpatient clinic of Namık Kemal University Hospital. The inclusion criteria for COPD patients were age ≥ 40 years, history of smoking ≥10 packs/year, no exacerbations for ≥8 weeks, and the presence of no other clinically significant disease. Patients were excluded if they had ever had heart failure, or any overt/active hematological, renal, hepatobiliary, infectious, inflammatory or thyroid disorder. A total of 70 subjects (47 COPD patients aged 61.9 ± 9.5 years and 23 healthy nonsmokers aged 58.4 ± 8.7 years) were recruited into the study. All COPD patients were ex-smokers, and their smoking loads were recorded as pack-years.

Based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) severity criteria[5], the patients had GOLD stage I (n= 5), stage II (n = 25), stage III (n = 13), or stage IV (n=4) disease. No patients were receiving systemic corticosteroid therapy at the time of the study.

This study and its protocols were reviewed and approved by the local ethics committee. After all the subjects provided written informed consent, medical histories were gathered on the day of the screening visit, and both spirometry (including a reversibility test, when needed) and blood sampling were performed.

Pulmonary function tests

Pulmonary function tests including FEV1, forced vital capacity (FVC), and FEV1/FVC were measured with a Jaeger Master Scope spirometer (VIASYS Healthcare, Hochberg, Germany). The tests were repeated at least three times in the sitting position by the same technician and the highest value was included in the analyses as described by the protocol of the American Thoracic Society guidelines[12]. COPD diagnosis and severity were evaluated according to GOLD criteria[5].

Blood-sampling protocol

Peripheral venous blood was drawn into pyrogen-free blood collection tubes containing ethylenediaminetetraacetic acid, and centrifuged for 30 min at 2.150 × g (15 min at 4°C). All samples were stored at-80°C.

Osteoprotegerin measurement

Osteoprotegerin levels in the serum were quantified by BioVendor enzyme-linked immunosorbent assays (ELISA; BioVendor Laboratory Medicine, Modrice, Czech Republic). This assay detects both monomer and dimeric forms of OPG, including OPG bound to its ligand. Briefly, mouse anti-human OPG was used as a capture antibody, and a biotinylated polyclonal anti-human OPG antibody was used for detection. The detection limit of this assay was 0.03 pmol/L. Intra and inter-assay variability was less than 4.1% and 4.6%, respectively.

Statistical analysis

Data were analyzed using Predictive Analysis Software (PASW) Statistics, version 18 (SPSS Inc., Chicago, IL, USA). A Kolmogorov-Smirnov test was used to check the distribution of continuous variables. Descriptive statistics were expressed as a

mean ± standard deviation for the parametric data, or as a median (interquartile range) for the nonparametric data. To facilitate comparisons of the two groups, an independent t-test was used for normally distributed variables; a Mann-Whitney U test, meanwhile, was used for nonnormally distributed variables, and a chi-square test for categorical variables. Spearman rank correlation analysis was employed to estimate the relationship between the test parameters, such as FEV1%, FVC, and OPG. A value of $p<0.05$ was considered statistically significant.

Results

Clinical characteristics of subjects

The COPD group included 47 patients aged 61.9 ± 9.5 years with a body mass index (BMI) of 26.0 ± 5.1 kg/m2. The control group included 23 healthy subjects aged 58.4 ± 8.7 years with a BMI of 25.7 ± 2.2 kg/m2. As shown in Table 1, there were no significant differences between the COPD and the control group in terms of age, gender and BMI ($p>0.05$). The mean FEV1 of the COPD patients was 59.3% ± 16.9% of predicted, and the FEV1/FVC ratio was 55.7 ± 11.0; meanwhile, those of the control group were within normal ranges (100.4 ± 12.0 and 85.9 ± 6.5, respectively).

	COPD (n=47)	Controls (n=23)	p-value
Age.years	61.9±9.5	58.4±8.7	>0.05
Gender. Male.n (%)	38 (81)	17 (74)	>0.05
BMI(kg/m2)	26.0±5.1	25.7±2.2	>0.05
Duration of smoking.pack-yr	41.8±11.4	0	
FEV1 (%)	59.3±16.9	100.4±12.0	<0.01
FVC (%)	84.4±19.0	96.5±9.8	<0.01
FEV1/FVC	55.7±11.0	85.9±6.5	<0.01
CRP mg/L	4,72 ± 2,58	3,27 ± 1,24	<0.05

Table 1: Comparison of demographic parameters, pulmonary function results smoking load and CRP levels between two COPD and control groups.
Data are mean (SD) unless otherwise stated.
COPD: chronic obstructive pulmonary disease, BMI:body mass index. FEV1: forced expiratory volume in one second. FVC: forced vital capacity. CRP: C-reactive protein

Plasma osteoprotegerin concentrations

As shown in Figure 1, the mean concentrations of OPG values among the COPD patients (8.61±4.78 ng/ml, n=47, 95% CI = 7.71-9.36, p=0.002) were higher than those of individuals in the control group (6.29±1.36 ng/ml, n = 23, 95% CI = 5.75-6.96).

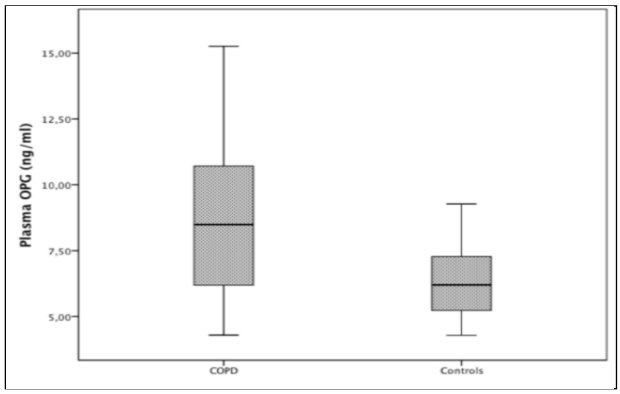

Figure 1: Plasma OPG levels in patients with COPD and control group. (Mann-Whitney U, p=0,002). OPG: Osteoprotegerin.

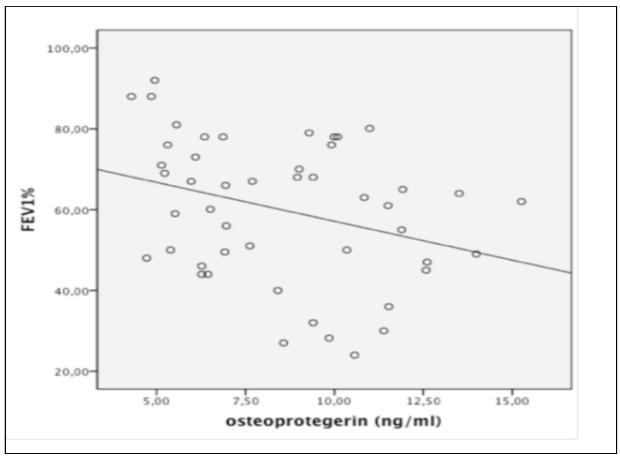

Figure 2: A negative linear correlation between serum OPG levels and FEV1 is shown on scatter diagram (Spearman rank correlation coefficient r= - 0.33, p <.05).

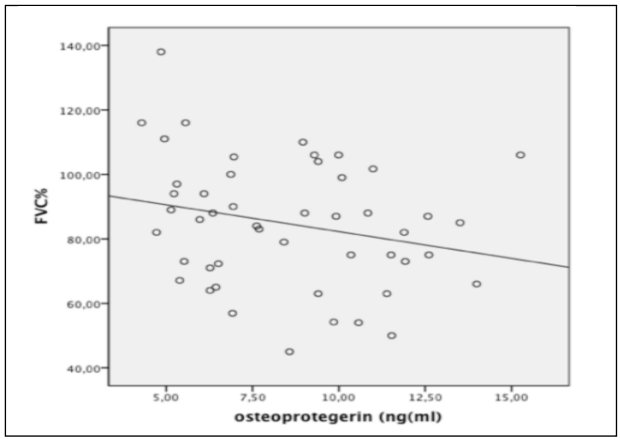

Figure 3: No correlation between serum OPG levels and FVC is shown on scatter diagram (Spearman rank correlation coefficient r= - 0.257. p >.05).

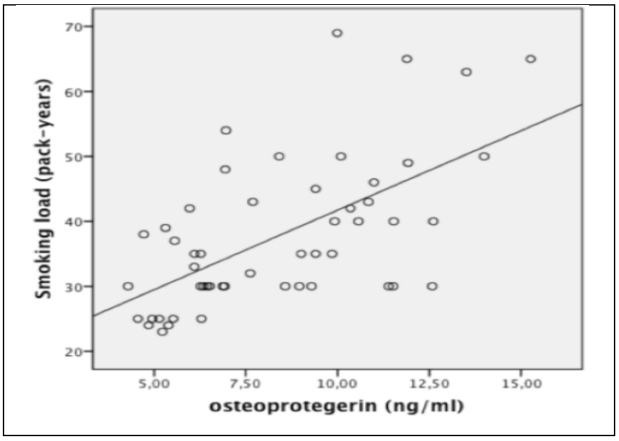

Figure 4: A positive linear correlation between serum OPG levels and smoking load is shown on scatter diagram (Spearman rank correlation coefficient r= 0.611, p <.05).

Associations among plasma osteoprotegerin, spirometer values, C reactive protein levels, and smoking load

Serum OPG concentrations in COPD patients negatively correlated with the FEV1% of predicted (r=-0.33, p< 0.05) (Figure 2). On the other hand, no significant correlation was detected between FVC and OPG (r=-0.257, p > 0.05) (Figure 3). No significant correlation was detected between serum OPG levels and C-reactive protein levels in COPD patients (r=0.056,p > 0.05). As shown in Figure 4, serum OPG concentrations in COPD patients positively correlated with smoking load (r= 0.611, p< 0.05).

Discussion

The present study observes the relationship between FEV1 and OPG. Our data demonstrate that serum OPG levels were higher in COPD patients than in healthy nonsmokers, and that they negatively correlated with FEV1% of predicted. We have also found that smoking load positively correlated with serum OPG levels.

After Celli et al.[13] found that both the BODE index (BMI, obstruction, dyspnea, and exercise] severity and prognosis of COPD patients were determined largely by FEV1, systemic manifestations and comorbidities considered to be mediated by systemic inflammation have been recognized, and multidimensional scales have been developed and proposed to describe the disease. Many studies have demonstrated that there is an increase in inflammatory cytokines not only in the lung, but also systemically. Many of the extra pulmonary effects of COPD seem to be mediated by systemic inflammation[14].

More recently, OPG was shown to be expressed in the atheromatous carotid plaques, and is associated with the affluence of macrophages in atheromatous lesions[15]. It was also shown that diabetic patients, especially those with poor glycemic control, introduced an increase of OPG levels, which is associated with the stimulation of atherosclerosis and vascular disease[16]. Clinical studies support the assertion that OPG is an active cytokine in vascular calcification, osteoporosis, arthritis, cancer, and bone-related disease, and can be considered a biological marker for these pathologies[17]. On the other hand, the best-recognized systemic manifestations of COPD, including cardiovascular comorbidities, systemic inflammation, osteoporosis, anemia, cachexia, and muscle dysfunction[18], may be related to OPG.

Contradictory results to the findings of the study, Eagan and et al(19) were found that plasma OPG levels were lower in COPD than in those of subjects without COPD. However, they report that among COPD patients, lower FEV1 was associated with higher OPG levels, and also that elevated serum OPG levels were associated with frequent exacerbations[19].

Another study[20] found a correlation in COPD patients between sputum OPG concentrations and FEV1, suggesting a link with severity of the disease or progression. In the same study, it was also found that patients with diffuse emphysema, as demonstrated by Computed Tomography (CT) scan, had higher sputum OPG levels than patients lacking radiographic emphysema[20]; additionally, a link was found between increased OPG concentrations and increased matrix metalloproteinase-9 production in alveolar macrophages, resulting in emphysema[20].

In the study of Duckers et al.[21], the OPG serum level was higher in COPD patients with osteopenia and osteoporosis than those without low bone-mineral density. Pobea et al.[22] suggest that osteoporosis of the hip is associated with increased circulatory levels of OPG in COPD patients.

An increase in CRP plasma concentration has been observed in COPD patients, suggesting that the inflammatory process persists even when exposure to a risk factor has ceased[23]. It was also shown that levels of inflammatory cytokines such as IL-1β, IL-6, and TNF-α are higher in the systemic circulation of COPD patients[6]. In the current study, we found that serum OPG levels correlated with smoking

load, and that patients who had quit smoking had higher serum CRP and OPG levels than healthy nonsmokers, thus suggesting that the COPD patients had persistent systemic inflammation.

Conclusion

COPD is a syndrome characterized by significant disease heterogeneity[24]. A limited number of studies indicate a relationship between OPG and COPD, but their results are conflicting. However, common findings of previous studies show that serum and sputum levels of OPG were directly associated with comorbidity, severity, and emphysematous phenotype of COPD. From this perspective, the first limitation of the current study is that it does not identify specific phenotypes or comorbidities of COPD patients. A second limitation is that all the COPD patients were ex-smokers: we did not compare serum OPG levels between current smokers and ex-smokers who were also COPD patients. However, in a previous study that compared sputum OPG concentrations between ex-smokers and current smokers among COPD patients, no significant difference was detected[20].

A final limitation of the current study is its relatively small sample size, which lowers the statistical power of the findings herein.

In conclusion, although we found that elevated OPG concentrations among COPD patients correlated with FEV1, larger and longitudinal studies are required to establish OPG as a useful biomarker for specific COPD phenotypes.

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