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Proinflammatory Cytokines and Bone Turn Over Markers in COPD Patients with Femur and Lumbar Osteoporosis

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Abstract : Osteoporosis is one of the systemic effects associated with chronic obstructive pulmonary disease (COPD). The etiology of osteoporosis in COPD is probably complex and various factors may contribute to its pathogenesis. Some of these are the consequences of chronic inflammatory lung disease. This study aims to investigate the cytokine levels and bone turnover markers in COPD patients complicated by osteoporosis. This study was conducted on 70 patients with severe COPD, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. Blood pro-inflammatory cytokines (TNF-α, IL-6, IL-17, IL-1B and CTX, RANKL, and OPG) were analyzed by ELISA. The Brinkman Index, duration of COPD, FEV1/FVC ratio, and FEV1 values were not significantly different between osteoporosis and non-osteoporosis patients in COPD groups ($P > 0.05$). The level of femur and lumbal BMD were significantly decreased in the COPD + OP group compared to the COPD group ($P < 0.05$). The levels of TNF-α, IL-6, and IL-17 were significantly greater in COPD + OP patients than in COPD patients. CTX levels were significantly increased in the COPD + OP group compared to the COPD group ($P < 0.05$). The levels of RANKL and OPG were not significantly different between groups ($P > 0.05$). In conclusion, our study suggested that the increased pro-inflammatory cytokines induce bone resorption in osteoporosis due to COPD.
Keywords: bone, cytokine, bone turnover markers, bone loss; pulmonary.

Introduction

Chronic obstructive pulmonary disease (COPD) is a disorder of the airways characterized by airway progression limitations due to pulmonary inflammatory response to various gases and particulate materials[1]. This disease affects approximately 10% of the population and its prevalence is likely to increase to 50%[2]. In addition to effects on the lungs and respiratory tract, these disorders also trigger systemic effects. One of the systemic effects on the musculoskeletal system is osteoporosis. The pathomechanism of osteoporosis in COPD is still being debated[3].

Osteoporosis is a skeletal disorder characterized by decreased bone mineral density and/or deterioration of the bone microarchitecture. This condition will trigger bone fragility and fracture sensitivity[4]. Systemic inflammation is the underlying mechanism of decreased bone mineral density in patients with COPD. In addition to inflammation, reduced physical capacity due to shortness of breath, decreased bone formation and increased bone resorption, and proteolysis also contributes to the development of osteoporosis in COPD patients[5,6]. Various studies have shown an increase in inflammatory cells in the circulation of patients with COPD, including neutrophils and lymphocytes[7,8]. These cells will produce cytokines (IL-1B, PGE2, bFGF, IL-

6, IL-17, and TNF-α which activates bone resorption by inhibiting the production of OPG. This effect would hinder the work of OPG as cytokines produce pre-osteoblasts and osteoblasts to trap RANKL as an early signal of osteoclast differentiation and bone resorption[9,10]. As far as we know, there have been no studies concerning the pro-inflammatory cytokines and bone turnover markers in osteoporosis-associated COPD patients. Therefore, the current study aimed to investigate the cytokine levels and bone turnover markers in COPD patients complicated by osteoporosis.

Materials and Methods

Subjects:

The research design was a cross-sectional study. This study was conducted on 70 patients with severe COPD, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria[1]. They were selected from Pulmonology Outpatient in Mohammad Hoesin Public Hospital Palembang from March 2014 to May 2015. Inclusion criteria were male patients, age above than 39 years old, with clinically stable COPD and provided written informed consent. Exclusion criteria were lung cancer, known psychiatric illness, maintenance treatment with systemic corticosteroids, active tuberculosis or insulin-dependent diabetes mellitus.

Anthropometry and pulmonary function test:

Height and weight were determined with the patient bare foot and in light-weight indoor clothing (Seca, Hamburg, Germany). From this, the body mass index (BMI) was calculated (kg/m^2). Spirometry was performed according to the ATS/ERS standardization guideline using a MasterScope spirometer (Viasys Healthcare GmbH, Germany) and measured forced respiratory volume in the first second (FEV1) and forced vital capacity (FVC)[11].

Bone mineral density:

BMD was measured by dual-energy X-ray absorptiometry (DEXA) with fan-beam technology using a total body scanner (Lunar Prodigy, GE Healthcare, United Kingdom). Individual measurements of the left hip (total femur), anteror-posterior lumbar spine (L1 to L4) were expressed in absolute values in grams of the mineral per unit area scanned (g/cm) and relative T-scores.

TNF α, IL-6, IL-17, and IL-1β assays:

Blood levels of cytokines under study were measured through an ELISA-based capture assay by using the commercial kits Human IL-6, Human IL-17, Human IL-1β (Quantikine1 ELISA, R&D Systems, Minneapolis, MN, USA), as well as Human TNF-α (Legend MaxTM, BioLegend Inc., San Diego, CA, USA), following the manufacturer's instructions.

RANK, OPG, and CTX assays:

Blood levels of bone markers under study were measured through an ELISA-based capture assay by using the commercial kits Human RANKL, Human OPG and Human CTX (Quantikine1 ELISA, R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions.

Ethics:

Human experimental procedures were approved by the Institutional Ethics Committee of Moehammad Hoesin Public Hospital/Medical Faculty of Sriwijaya University, Palembang, South Sumatera, Indonesia.

Statistical analysis:

The differences between groups were analyzed using bivariate and multivariate analysis with SPSS 17.0 statistical package. $P < 0.05$ was considered statistically significant.

Results

The age, body mass index, Brinkman Index, duration of COPD, FEV1/FVC ratio were not significantly different between groups (P > 0.05) (Table 1). The level of femur and lumbal BMD were significantly decreased in the COPD with osteoporosis group compared to the COPD group (P < 0.05). Levels of hemoglobin, leukocyte, LED, blood glucose, urea, creatinine and calcium were not significantly different between groups (p> 0.05).

Table 1: Baseline of patient characteristics

Characteristics	COPD with osteoporosis (N=35) (mean ± SD)	COPD without osteoporosis (N= 35) (mean ± SD)	P*
BMI ($kg/m^{2)}$	20.53±2.23	20.19±3.64	0.62
Age (years)	66.66±9.09	67.00±8.09	0.87
Brinkman Index (packs/years)	603.94±425.80	500.40±256.80	0.22
Duration of COPD (years)	3.63±2.95	3.86±4.29	0.79
BMD lumbal (gr/m^2)	0.72±0.10	0.93±0.09	0.00
BMD femur (gr/m^2)	0.66±0.09	0.86±0.09	0.00
FEV1/FVC	0.45±0.13	0.50±0.12	0.09
Hb (mg/dl)	13.18±1.31	13.64±1.13	0.12
Leukosit	8020±1627	8120±1665	0.8
LED (mm/hour)	30.63±28.11	22.43±22.11	0.18
Blood glucose (mg/dl)	109.80±19.11	105.06±18.91	0.3
Ureum (mg/dl)	28.83±10.47	27.51±8.54	0.57
Creatinin (mg/dl)	0.984±0.188	1.029±0.152	0.28
Calcium (mg/dl)	9.437±0.609	9.523±0.512	0.53

**Independent t test*

The levels of OPG were not significantly different between COPD with osteoporosis and COPD without osteoporosis. The levels of TNF-α, IL-1B, IL-6, IL-17, RANKL and CTx were significantly different between COPD patients with osteoporosis and those without. The levels of TNF-α, IL-1B, IL-6, IL-17, RANKL and CTx were significantly increased in COPD patients with osteoporosis compared to those without (Table 2).

Table 2: Marker of inflammation and bone turn over markers

	COPD with osteoporosis,(n)	COPD without osteoporosis,(n)	Total	Adjusted OR (95% CI)	adjusted p value
TNF α≥ 2,47 pg/ml	29	10	39	2,82-8,78	0,01
TNFα< 2,47 pg/ml	6	25	31		
IL-1B ≥ 0,76 pg/ml	27	25	52	0,11-6,07	0,11
IL-1B < 0,76 pg/ml	8	10	18		
IL-6 ≥ 0,74 pg/ml	29	8	37	2,11-8,57	0,00
Il-6 < 0,74 pg/ml	6	27	33		
IL-17 ≥ 1,8 pg/ml	25	8	33	2,17-9,58	0,02
IL-17 < 1,8 pg/ml	10	27	37		
RANKL ≥ 111,2 ng/ml	27	23	50	0,18-3,43	0,12
RANKL < 111,2 ng/ml	8	12	20		
OPG ≥ 4,0 ng/ml	34	30	64	0,02-1,58	0,88
OPG < 4,0 ng/ml	1	5	6		
CTx ≥ 0,4 ng/ml	30	5	35	2,62-13,66	0,00
CTx < 0,4 ng/ml	5	30	35		

**Logistic regression*

Discussion

Inflammatory cells that accumulate in the lungs of patients with COPD will secrete a variety of cytokines. This will trigger the release of cytokines and the remodeling of airway obstruction. In addition to local environment, the various mediators are also systemic in nature, although it remains unclear whether systemic cytokines may have an effect on the bone and a similar activity in the regulation of bone homeostasis physiology.

Interleukin-6 is an inflammatory interleukin involved in the acute phase response. In bone, IL-6 will trigger the differentiation and activation of osteoclasts[12-15]. We found that the levels of IL-6 were significantly higher in COPD with osteoporosis compared to COPD without osteoporosis ($P < 0.05$), but the IL-1 levels were not significantly different between groups ($P > 0.05$). In this study, circulatory IL-6 may induce osteoclast differentiation and activation, therefore playing a prominent role in lumbar and femur bone loss in COPD patients.

IL-17 is also an inflammatory cytokine, with an action in epithelial, endothelial and mesenchymal cells to induce pro-inflammatory chemokines and other factors. This action will trigger chemotaxis in neutrophils and aggravate the inflammatory status.[16] Our data showed that the IL-17 levels were significantly greater in COPD patients with osteoporosis than in those without ($P < 0.05$). The presence of IL-17 promotes the pro-inflammatory state in the circulation of COPD patients.

Tumor necrosis factor alpha (TNF-α) is classified as a pro-inflammatory cytokine that can activate the signal nuclear factor kappa-B (NF-κB) to aggravate the status of inflammation and apoptosis of bone cells[17,18]. We found that the levels of TNF-α were significantly increased in the COPD with osteoporosis group compared to the COPD without osteoporosis group ($P < 0.05$). This finding indicates that TNF-α will maintain a highly pro-inflammatory state in the circulation of COPD patients.

Osteoblasts are bone-forming cells which will secrete OPG as a trap against RANKL. If this homeostasis is adequate, osteoporosis does not develop. In other words, any factor, including pro-inflammatory cytokines that trigger a decrease in the number and function of osteoblasts will inhibit the formation of extracellular matrix disorders and trigger osteoporosis[19,20]. Our bone marker data showed that the levels of RANK and OPG were not significantly different between groups ($P > 0.05$). This finding indicates that systemic inflammation in COPD with or without osteoporosis does not affect the osteoblasts. We hypothesize that this finding is a hallmark of osteoporosis-related COPD. Our OPG results contradict and extend the previous finding that osteoporosis of the hip is associated with increased circulatory levels of OPG in patients with COPD[21].

Bone collagen can also undergo a series of non-enzymatic transformations including the isomerization of aspartic acid residues within the C-telopeptides[22-26]. In this study, the level of CTX was significantly increased in the COPD with osteoporosis group compared to the COPD without osteoporosis group. This finding indicated that the transformation of collagen through cross-linking was increased in COPD patients who developed osteoporosis[27]. This finding suggests that osteoporosis in COPD was determined by increased bone resportion, not decreased of bone formation. In conclusion, our study suggested that the increasing of pro-inflammatory cytokines induces bone resorption in osteoporosis due to COPD.

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