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Assessment of Osteoprotegerin and Receptor Activator of Nf-kb Ligand in Malaysian Male Patients with Chronic Obstructive Pulmonary Disease: A Cross-Sectional Study

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ABSTRACT

Background: Limited information exists regarding the pathophysiological interactions between osteoporosis and chronic obstructive pulmonary disease (COPD). **Objective:** To study the association of Osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL) in male COPD patients. **Methods:** An observational clinical study was conducted at Penang General Hospital in Malaysia. Participants were divided into three groups: COPD patients with osteoporosis, COPD patients without osteoporosis, and healthy participants of the same age groups. Serum OPG (sOPG) and RANKL (sRANKL) levels were investigated. **Results:** The mean age of COPD patients was 64.10 ± 10.04 years. COPD patients had lower body mass index (23.22 ± 6.43) than healthy participants (27.32 ± 6.80). The T-score was significantly lower among COPD patients than healthy participants ($p = 0.018$). The sOPG concentration among healthy participants was significantly higher (361.90 ± 29.10 pg/mL, $p < 0.001$) than in the other groups, while the sRANKL concentration was not significantly different. The serum OPG/RANKL concentration was markedly higher in the control group than in the COPD patient group ($p < 0.05$). The COPD patients with osteoporosis had significantly lower pulmonary parameters (forced expiratory volume in the first [FEV]1% and FEV_1/[forced vital capacity] (FVC), $p < 0.01$) and more dyspnea (modified medical research council = 2.60 ± 0.78 versus 1.90 ± 0.70, $p < 0.01$) than did the patients without osteoporosis. Furthermore, patients with severe COPD had a 3 times greater risk of developing osteoporosis (OR = 2.997 [95% CI = 2.181, 4.118], $p < 0.001$), while spirometric parameters had a significant inverse relationship with osteoporosis (FEV_1% OR = 0.970, [95% CI = 0.954, 0.986], $p = 0.001$; FEV_1/FVC OR = 0.984, (95% CI = 0.970, 0.999], $p = 0.035$). **Conclusion:** The study concluded that COPD patients had lower sOPG levels, leading to decreased OPG/RANKL ratio and faster bone resorption. Low bone mineral density was associated with more severe COPD. (REV INVEST CLIN. 2024;76(6):262-73)

Keywords: Chronic obstructive pulmonary disease. Osteoporosis. Osteoprotegerin. Receptor activator of nuclear factor kappa-B ligand. Molecular bone turnover markers. Osteoclastogenesis.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory life-threatening lung disease. It is usually identified by a progressive airflow limitation that can be irreversible and episodic[1]. COPD is caused by cigarette smoking and can be associated with asthma, chronic bronchitis, and emphysema[2]. Based on the latest reports, the World Health Organization (WHO) has ranked COPD as the third leading cause of death, with 3.23 million deaths and more than 300 million patients worldwide[3,4]. Osteoporosis is a bone disease that can cause bone weakness, increasing susceptibility to fracture. It is characterized by low bone mineral density (BMD) and is considered one of the comorbidities of COPD. However, recent research has shown that osteoporosis is more prevalent among COPD patients than anticipated and might be systemically correlated with COPD[5].

Human bones are constantly undergoing a restless process of remodeling[6]. Molecular bone turnover markers, such as receptor activator of nuclear factor kappa-B ligand (RANKL), also known as Tumor Necrosis Factor (TNF)-related activation-induced cytokine, reflect osteoclast activity during the bone resorption process. In contrast, TNF receptor superfamily member 11B, also known as Osteoprotegerin (OPG), is a cytokine that acts as a decoy receptor for RANKL. While OPG plays a crucial role in regulating bone remodeling by inhibiting osteoclast activity, it does not directly reflect osteoblastic activity or bone formation[7]. OPG, also known as osteoclastogenesis inhibitory factor, plays a significant role in suppressing the maturation and differentiation of osteoclast cells, known as bone-resorbing cells[8]. Mesenchymal cells assist in the differentiation of osteoclasts through intracellular contact[9,10]. When RANKL attaches to its receptor on the surface of pre-osteoclast cells, it triggers the differentiation and formation of polynuclear osteoclast cells responsible for bone resorption[11]. Furthermore, it plays a crucial role in prolonging the life span and maintaining osteoclast activity[12]. Thus, increased expression of RANKL can lead to faster bone resorption than to faster bone formation.

By competing with RANKL for the same receptor, OPG suppresses osteoclast cell differentiation and formation[13]. In addition to hormonal cytokines, OPG plays a major role in bone density regulation[14]. When the balance of the bone-regulating system (OPG/RANK/RANKL) is shifted toward faster resorption, osteoblast cells are overwhelmed, and the bone loses more material because of the increased number and activity of osteoclast cells, which leads to osteoporosis over time[15,16].

The link between COPD and osteoporosis is yet unclear. In this study, we aimed to elucidate the relationship between COPD and osteoporosis by evaluating the levels of osteoclastogenesis mediators in the blood and investigating the potential effect of an imbalanced ratio of the OPG/RANK/RANKL pathway on bone remodeling. Moreover, we sought to establish a correlation between the severity of COPD and the presence of osteoporosis.

METHODS

Study design and setting

This investigative observational clinical study was conducted at Penang General Hospital in Malaysia. Medical records from the chest clinic and chest ward were screened to identify eligible participants with COPD the study. The medical staff invited the selected patients to participate in a pre-recruitment interview, during which their medical history was confirmed, and they were then informed of the study details. Then, participants who agreed to be included were clinically examined and interviewed by a competent pulmonologist and a clinical investigator. The recruited COPD participants were divided into Group A: patients with COPD and osteoporosis; Group B: COPD patients with normal BMD; and Group C: healthy participants. We followed the STROBE recommendations for reporting observational studies (Please refer to Supplementary Material: STROBE Checklist). This study was performed in accordance with the principles of the Declaration of Helsinki. The study was registered with the National Medical Research Register under the following number: NMRR-19-239-46017 and ethical approval was obtained from the Medical Research and Ethics Committee, Ministry of Health Malaysia, under the following number: KKM/NIHEC/P19-528/11. This project was funded by the Bridging/ Bridging-Incentive Grant by Universiti Sains Malaysia (grant number: 304.PFARMASI.6316508).

Participants

Informed consent was obtained from all individual participants included in the study. Eighty clinically stable COPD patients and 40 healthy volunteers of the same age groups were recruited for the study. The patients were coded, their codes shuffled, and then selected randomly to participate in the study to avoid selection bias. The interview and clinical evaluation for each participant during the study typically lasted approximately 30 min.

Inclusion and exclusion criteria

Clinically stable male participants with COPD aged 40 years and older who visited the hospital or the clinics were enrolled (COPD was diagnosed based on the Global Initiative for Chronic Obstructive Lung Disease [GOLD]). Due to the effect of post-menopausal osteoporosis, female participants were excluded. In addition, patients with cancerous diseases, hepatic malfunction, kidney disease, Paget's disease, rheumatoid arthritis, mastocytosis, Addison's disease, Cushing's syndrome, Graves' disease, or osteogenesis imperfecta were excluded due to the impact of these diseases on bone health.

Similarly, TB patients were excluded if they were successfully treated at least 5 years ago. In addition, patients who were already diagnosed with osteoporosis or other bone diseases, who had a history of oral steroidal treatment (> 2 months), or who received bone-targeted supplements or medications (such as calcium, vitamin D, or bisphosphonates) were excluded from the study, as these supplements can alter bone density and other markers of bone health.

For the control group, healthy volunteers with no history of lung disease or bone disease and above 45 years of age and from the same ethnic groups were recruited. Furthermore, participants who were taking bone-targeted supplements, oral steroids, or bone treatment were excluded from the study.

Sample size

The sample size was calculated using the Kelsey and Fleiss formula for clinical studies for an odds ratio (OR) of 30.3 and a 95% confidence interval[17]. After continuity corrections, the minimum sample size needed to achieve a power of 80 (1-β, % chance of detection) was 13 per arm. We recruited and tested 40 participants in each group of the study.

Tests and data collection

The collected data included demographic and socioeconomic data, medical history, COPD exacerbation history, and comorbidities. Clinical evaluation was performed using the modified Medical Research Council (mMRC) dyspnea score, COPD assessment test (CAT) score, spirometry results, the BODE index for COPD Survival, and GOLD's COPD severity categories. Furthermore, participants' lifestyles and habits, including their level of daily activities and drug use, were recorded, if any. The participants were requested not to take any bronchodilators before spirometry (8 h for short-acting bronchodilator use and 24 h for long-acting bronchodilator use); for those who needed to take a bronchodilator, only the post-bronchodilator use was determined through spirometry.

Bone mineral density measurement

At each visit, the participants' BMD was checked immediately after the interview. The BMD was measured through quantitative ultrasonography (QUS) of the calcaneus area (SONOST 3000; OsteoSys Co., Ltd., Guro-Dong 152-848, Seoul, South Korea). The T Score was recorded, and the patients were categorized according to the WHO classification for osteoporosis[18].

Biological sample collection

Whole-blood samples were collected from each patient (10 mL) using serum separator tubes. Samples were collected from fasted patients in both groups according to the standard sampling protocol and then centrifuged within < 30 min at 2150× g for 15 min at 4°C. Serum was extracted into Eppendorf tubes, stored in a proper biological sample transport container, and shipped from the healthcare facility to the university to be stored at −80°C. The samples were thawed only once before conducting the enzyme-linked immunosorbent assay (ELISA) tests[19].

Assessment of molecular bone turnover markers

Two molecular bone turnover markers related to osteoporosis were investigated using 10 mL serum

samples collected from all participants. The tests were run using colorimetric double sandwiched Human sOPG ELISA Kits following the attached protocol (Cat. No: E-EL-H1341, 96T, Elabscience Biotechnology Co., Houston, Texas 77079, USA) with a detection range of 0.16-10 ng/mL coefficient of variation of < 10%, and colorimetric sandwiched ELISA kits for Human sRANKL (Soluble RANKL) according to the attached protocol (Cat. No: E-EL-H5558, 96T, Elabscience Biotechnology Co., Houston, Texas 77079, USA.) with a detection range of 15.63-1000 pg/mL and a coefficient of variation of < 10%. The optical density (OD) of the produced microplates was scanned using a Promega GloMax®-Multi+ (Promega BioSystems Sunnyvale, Inc., Sunnyvale, California 94085, USA). The same serum samples were used for both molecular bone turnover markers tests (OPG and RANKL); each test was performed in triplicate, and the averaged results were taken to ensure accuracy.

Statistical analysis

The statistical analyses were conducted using the latest version of the Statistical Package for the Social Sciences (SPSS) (version 27.0; IBM Corp.). Descriptive statistics were performed to determine the nature of the study population. The Chi-square test was performed for categorical variables (e.g., smoking), and a t-test was used to compare the means of continuous variables (e.g., age) to detect any significant difference between the two points and establish an association. Linear regression was conducted to examine the relationship between the parameters. Logistic regression was performed to calculate the risk of osteoporosis among COPD participants. All the data and variables were double-checked to ensure there were no missing data before the tests were conducted, and the adopted cutoff point was $p < 0.05$. Microsoft Excel and Word were used to store and arrange the data and to generate figures and tables.

RESULTS

The initial screening through the hospital records resulted in 500 patients, who were subsequently coded and shuffled. A total of 250 participants were then invited by hospital staff to participate. Among them, 166 were reluctant to contact, and 17 were lost to follow-up. After an additional 13 participants were recruited, the final number of enrolled participants was 80 (Fig. 1). The majority of participants were 44 Chinese (55%), 22 (27.5%) were Malay, and 14 (17.5%) were Indians. Similarly, 40 healthy participants of the same age group were enrolled in the healthy control group (Group C). Of these, 22 (55%) were Chinese, 12 (30%) were Malay, and 6 (15%) were Indians.

Sociodemographic characteristics

The overall mean age (± SD) of the COPD participants was 64.10 ± 10.04 years, with no significant difference between them and the healthy controls. The body mass index (BMI) of the osteoporosis group was significantly lower than that of the other group (21.94 ± 6.41 and 24.56 ± 6.71, respectively), while the BMI of the healthy participants was significantly higher than that of the COPD patients (27.32 ± 6.80) (Table 1). There was a significant association between participant income (more than USD 400.0) and both osteoporosis incidence and regular alcohol consumption (more than two units a day). In addition, osteoporosis was significantly associated with low physical activity (< 30 min per day). According to the QUS T scores, the BMD of the normal control participants was significantly higher than the BMD of the COPD patients ($p = 0.018$).

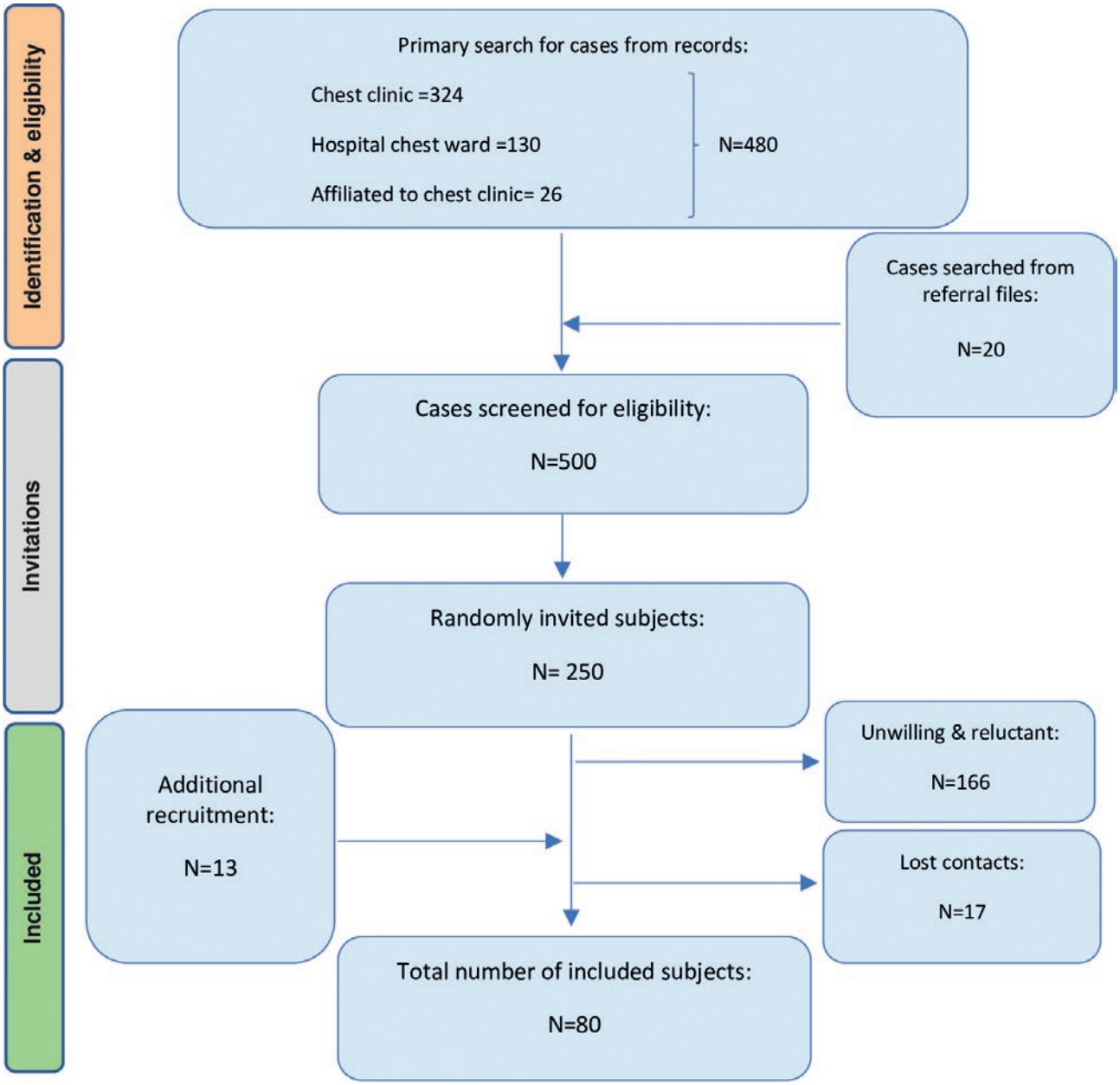
Molecular bone turnover markers

The mean SD for the serum OPG concentration was significantly higher in the healthy control group than in the other groups (361.9 ± 29.10 pg/mL, $p < 0.001$). Furthermore, COPD patients with osteoporosis had significantly lower OPG levels than the patients without osteoporosis did (180 ± 16.2 pg/mL versus 282.30 ± 21.40 pg/mL, $p < 0.001$). In contrast, the serum concentration of RANKL did not significantly differ between the groups. The ratio of OPG/RANKL was markedly higher among in the control group than in the COPD patient groups ($p < 0.05$) (Fig. 2).

COPD severity analysis

Among COPD patients, those with osteoporosis suffer from more severe symptoms. The spirometric parameters were significantly lower among COPD patients with osteoporosis (FEV_1/FVC: 62.30 ± 14.10 and FEV_1% predicted: 55.70 ± 15.80, $p < 0.01$ in both) than among the non-osteoporosis group (FEV_1/FVC:

Figure 1. Flow chart of the search, screening, and recruitment process for chronic obstructive pulmonary disease patients.



67.78 ± 10.30 and FEV_1% predicted: 65.30 ± 12.90) (Table 2). Furthermore, the mMRC dyspnea scale showed that COPD patients with osteoporosis had significantly greater scores (2.60 ± 0.78 versus 1.90 ± 0.70, $p < 0.01$), while the CAT score was not significantly different. A significantly greater number of osteoporotic patients had a history of acute respiratory failure, while the number of exacerbations per year was not significantly different.

The predictors of osteoporosis among COPD patients

Multiple variables from predictor analysis showed a significant result. Patients with lower serum OPG levels were also at higher risk of developing osteoporosis (OR = 0.981, [95% CI = 0.964-1.009], p = 0.021), while the serum level of RANKL did not show any significantly different (OR = 0.876, [95% CI = 0.684-1.085],

Table 1. Sociodemographic characteristics and BMD of the COPD patients and healthy participants

Parameters	Patients				Normal control, (n = 40)	p
	Non-OST, (n = 40)	(n = 40)	Overall, (n = 80)	p		
Age (Mean ± SD, years)	63.90 ± 10.70	64.28 ± 10.30	64.10 ± 10.04	0.731	61.50 ± 9.70	0.162
BMI (Mean ± SD, kg/m^2)	24.56 ± 6.71	21.94 ± 5.41	23.22 ± 6.43	< 0.01*	27.32 ± 6.80	< 0.01#
QUS (T-Score)	−1.30 ± 0.86	−3.20 ± 0.70	−2.21 ± 0.75	< 0.001*	−1.46 ± 0.63	0.018#
Marital status, n (%)						
Married	28 (70)	24 (60)	52 (65)		27 (65.7)	
Single	5 (12.5)	7 (17.5)	12 (15)	0.378	6 (15)	0.663
Divorced/widowed	7 (17.5)	9 (22.5)	16 (20)		7 (17.5)	
Residential area, n (%)						
Rural	5 (12.5)	4 (10)	9 (11.3)		8 (20)	
Urban	35 (87.5)	36 (90)	71 (88.7)	0.791	32 (80)	0.841
Education, n (%)						
Non-university	13 (32.5)	15 (37.5)	28 (35)		13 (32.5)	
University	27 (67.5)	25 (62.5)	52 (65)	0.129	27 (67.5)	0.782
Alcohol consumption, n (%)						
Yes	1 (2.5)	4 (10)	5 (6.2)	0.017*	2 (5)	0.048#
No	39 (97.5)	36 (90)	75 (93.7)		38 (95)	
Average income, n (%)						
≤ 400USD	18 (45)	10 (25)	18 (22.5)	0.006*	15 (37.5)	0.030#
> 400USD	22 (55)	30 (75)	52 (77.5)		25 (62.5)	
Smoking history, n (%)						
Yes	29 (72.5)	27 (67.5)	56 (70)		29 (72.5)	
No	11 (26.5)	13 (32.5)	24 (30)	0.771	11 (27.5)	0.583
Physical activity, n (%)						
≤ 30 min/day	4 (10)	7 (17.5)	11 (13.7)	0.050	2 (5)	0.016#
> 30 min/day	36 (90)	33 (82.5)	69 (86.3)		38 (95)	

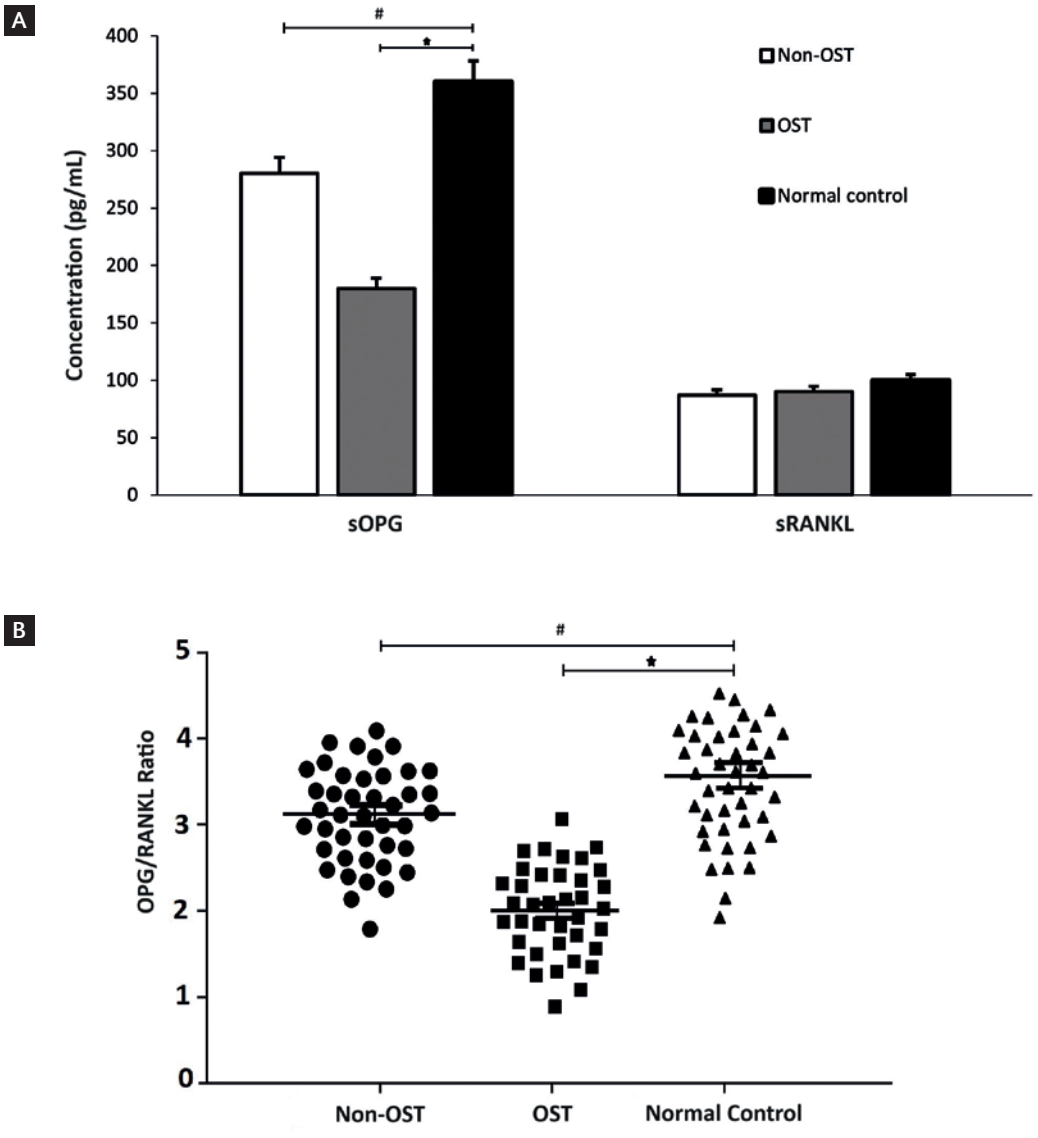
Data are expressed as mean ± SD and frequency (%). *Categorical variables were compared using the Pearson χ^2 test (for variables with more than two categories) and Fisher's exact test (for binary variables). Continuous variables were analyzed using the Mann–Whitney test. *,#P < 0.05 indicated statistical significance compared among COPD patients (non-OST and OST) and normal control, respectively. OST: COPD patients with osteoporosis; Non-OST: COPD patients without osteoporosis; QUS-T: quantitative ultrasonography; COPD: chronic obstructive pulmonary disease; BMI: body mass index.

p = 0.389). COPD patients in severe GOLD categories (C and D) had a three-fold greater risk of developing osteoporosis (OR = 2.997, [95% CI = 2.181-4.118], p < 0.001]; table 3. The FEV_1% predicted and the FEV_1/FVC ratio showed a statistically significant inverse relationship with osteoporosis; the lower these spirometric values were the greater the risk of developing osteoporosis (OR = 0.970, [95% CI = 0.954, 0.986], p = 0.001 and OR = 0.984, [95% CI = 0.970, 0.999], p = 0.035, respectively). Patients who had a higher mMRC scale (second grade and above) had twice the risk of developing osteoporosis (OR = 1.872, [95% CI = 0.947, 3.699], p = 0.002).

DISCUSSION

Osteoporosis is highly prevalent among COPD patients and significantly affects their quality of life. Unfortunately, osteoporosis in COPD patients remains

Figure 2. Comparison of osteoprotegerin and receptor activator of nuclear factor kappa-B ligand serum levels among the three groups. *,#A p < 0.05 indicated statistical significance compared among chronic obstructive pulmonary disease patients (non-OST and OST) and normal control, respectively. Non-OST: COPD patients without osteoporosis; OST: COPD patients with osteoporosis; sOPG: serum osteoprotegerin; sRANKL: serum receptor activator of nuclear factor kappa-B ligand; COPD: chronic obstructive pulmonary disease.



underdiagnosed and undertreated until fractures occur, contributing to worsened lung function and higher mortality. Although the exact mechanisms are unclear, both general and COPD-specific factors, such as reduced physical activity and chronic inflammation, play roles in the increased risk of osteoporosis, making early detection crucial[20]. In this study, neither the COPD patients nor the healthy control participants differed in terms of most of the sociodemographic characteristics. However, COPD patients had a significantly lower BMI than normal controls. Furthermore, COPD patients with osteoporosis had even lower BMIs than patients without osteoporosis. However, after multivariate analysis, the BMI had borderline statistical significance (p = 0.069), revealing the BMI paradox among COPD patients[21]. Unlike patients with other COPD comorbidities, such as cardiovascular diseases, COPD patients with osteoporosis tend to have a lower BMI[22]. This can be attributed to the breathing limitations among COPD patients, which

Table 2. COPD severity profile of the enrolled participants

Parameters	Non-OST, (n = 40)	OST, (n = 40)	Overall, (n = 80)	p
FEV_1/FVC ratio (Mean ± SD)	67.78 ± 10.30	62.30 ± 14.10	65.00 ± 12.20	< 0.010*
FEV_1% pred (Mean ± SD)	65.30 ± 12.90	55.70 ± 15.80	60.50 ± 14.30	< 0.010*
SpO_2% (Mean ± SD)	96.10 ± 0.810	96.20 ± 1.00	96.00 ± 0.90	0.317
mMRC Dyspnea Scale (Mean ± SD)	1.90 ± 0.70	2.60 ± 0.78	2.20 ± 0.740	< 0.010*
CAT score (Mean ± SD)	14.20 ± 9.51	13.30 ± 8.90	13.70 ± 9.20	0.421
Exacerbations (Mean ± SD, years)	0.91 ± 0.63	0.90 ± 0.66	0.90 ± 0.64	0.631
P. Rehab., n (%)	4 (10)	3 (7.5)	7 (8.7)	0.679
BODE index (Mean ± SD)	3.40 ± 1.10	4.20 ± 1.20	3.80 ± 1.10	0.360
LTOT, n (%)	6 (15)	5 (12.5)	11 (13.7)	0.711
GOLD, n (%)				
A	5 (12.5)	4 (10)	9 (11.2)	
B	12 (30)	8 (20)	20 (25)	
C	15 (37.5)	21 (52.5)	36 (45)	0.002*
D	8 (20)	7 (17.5)	15 (18.8)	
ARF, n (%)	1 (2.5)	4 (10)	5 (6.2)	< 0.010*

Data are expressed as mean ± SD and frequency (%). Categorical variables were compared using the Pearson χ^2 test (for variables with more than two categories) and Fisher's exact test (for binary variables). Continuous variables were analyzed using the Mann-Whitney test. *A $p < 0.05$ indicated statistical significance compared among COPD patients (Non-OST and OST). Non-OST: COPD patients without osteoporosis; OST: COPD patients with osteoporosis; mMRC dyspnoea scale: modified Medical Research Council dyspnoea scale; CAT Score: COPD Assessment Test score; P. Rehab: pulmonary rehabilitation patients; ARF: acute respiratory failure; LTOT: long-term oxygen treatment; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity of the lungs; FEV_1% pred: predicted forced expiratory volume in the first second; SpO_2%: oxygen saturation. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GOLD: Global Initiative for Chronic Obstructive Lung Disease.

can negatively impact their appetite and eating capacity[23]. In Turkey, a study showed that COPD patients with a lower BMI had a 28% greater risk of developing osteoporosis (OR = 1.28, [95% CI = 1.14-1.45], $p < 0.001$)[24]. In addition, Zhao et al.[25] noted that participants with a BMI above 24 kg/m^2 had a better BMD than those with a BMI below 24 kg/m^2, while Lee et al.[26] reported that the optimal BMI for determining BMD is between 23 kg/m^2 and 24.9 kg/m^2.

In our work, osteoporosis was associated with more severe COPD. Kameyama et al.[27] reported that the rapid decline in COPD patients correlates with a greater likelihood of vertebral fracture, and BMD was associated with a low level of physical activity in severe patients, severe airflow limitation, or obstruction[28]. Furthermore, lower spirometric values and more severe dyspnea increase the risk of developing osteoporosis. Similarly, Inoue et al. reported a significantly lower FEV_1 in COPD patients with osteoporosis than in healthy controls[29], and Raherison et al.[30] reported a significant association between the severity of dyspnea and osteoporosis among COPD patients. In addition, patients with more severe dyspnea were at higher risk of osteoporosis, which is logical because higher mMRC scores indicate more difficulties in breathing and will limit the patient's physical capacity, which increases osteoporosis incidence[31,32].

Furthermore, the limited capacity to move and perform physical activities can drastically reduce sunlight exposure among COPD patients, increasing the risk of osteoporosis[33]. Cheng et al.[34] reported that the CAT score could be a good representative of COPD severity in outdoor patients, while the mMRC was more accurate in patients who needed hospital admission. One study noted that osteoporosis was more prevalent among patients with severe COPD with frequent exacerbations than among the less severe and stable patients[29].

A decreased level of OPG in COPD patients causes a decreased OPG/RANKL ratio, leading to a greater

Table 3. The predictors of osteoporosis among COPD patients

Variable	Crude OR (95% CI)	p
Race		
Malay	1	
Chinese	0.701 (0.354-1.385)	0.671
Indian	0.895 (0.537-1491)	0.301
Age	0.982 (0.962-1.002	0.078
BMI	0.962 (0.923-1.003)	0.069
GOLD category	2.997 (2.181-4.118)	0.001*
FEV_1% pred	0.970 (0.954-0.986)	0.001*
FEV_1/FVC_1	0.984 (0.970-0.999)	0.035
mMRC	1.872 (0.947-3.699)	0.002*
SPO_2	0.853 (0.678-1.075)	0.117
CAT-Score	0.966 (0.928-1.005)	0.090
sOPG	0.981 (0.964-1.009)	0.021
sRANKL	0.876 (0.684-1.085)	0.389
OPG/RANKL	0.873 (0.691-1.068)	0.003*

Logistic regression was performed to calculate the risk of osteoporosis among COPD participants. Categorical variables were compared using the Pearson χ^2 test (for variables with more than two categories) and Fisher's exact test (for binary variables). Continuous variables were analyzed using the Mann-Whitney test. *A $p < 0.01$ indicated statistical significance compared among COPD patients (Non-OST and OST). Non-OST: COPD patients without osteoporosis; OST: COPD patients with osteoporosis; mMRC dyspnoea scale: modified Medical Research Council dyspnea scale; CAT Score: COPD Assessment Test score; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity of the lungs; FEV_1% pred: predicted forced expiratory volume in the first second; SpO_2%: oxygen saturation; OR: odds ratio; CI: confidence interval; BMI: Body mass index; GOLD: Global Initiative for Chronic Obstructive Lung Disease; sOPG: serum osteoprotegerin; sRANKL: serum receptor activator of nuclear factor kappa-B ligand.

number of mature and active osteoclasts and faster bones resorption[35]. While several factors that decrease OPG levels may also lead to an increase in RANKL, it is important to note that decreased OPG does not directly increase RANKL levels. Instead, factors, such as increased ROS and pro-inflammatory cytokines, such as TNF-α, interleukin (IL-1), and IL-6, can stimulate RANKL and may contribute to both outcomes simultaneously[36]. Therefore, the complexity of the relationship between OPG and RANKL clarifies that the decrease in OPG does not necessarily cause an increase in RANKL.

Moreover, Wang and his group reported that the increased expression of RANKL in patients with osteoporosis was due to increased DNA methylation in the OPG/RANK/RANKL pathway[37]. According to Zheng et al.[38] a balanced OPG/RANK/RANKL level is essential for healthy bone remodeling; thus, the bone becomes more prone to resorption when the OPG/RANKL ratio decreases. When RANKL binds to its receptor on the osteoclast surface, it stimulates the cell, leading to increased activity and delayed apoptosis, while OPG blocks this pathway by binding to RANKL and preventing it from binding to the receptor RANK[39]. Thus, a higher OPG/RANKL ratio was associated with a greater BMD[40], which is similar to our findings. On the other hand, Zheng et al.[38] reported that a reduced OPG/RANKL ratio increased RANK ligand expression, induced osteoclast differentiation, and facilitated maturation, which caused faster bone resorption. Bone resorption by osteoclasts and formation by osteoblasts are ongoing processes in bone tissue. OPG inhibits osteoclastogenesis by blocking the RANKL-RANK interaction, thus preventing bone resorption41. Zhou et al.[42] reported that cytokines such as RANKL and macrophage colony-stimulating factor modulate osteoclastogenesis in inflammation-induced bone resorption. These cytokines play crucial roles in the differentiation and activation of osteoclasts, which drive bone resorption.

Other proinflammatory cytokines on osteoclasts, such as TNF-α and IL-1, also contribute to this process. Conversely, the immune system produces regulatory cytokines, such as IL-4, IL-10, and interferon-β which help limit excessive osteoclastogenesis activation and prevent excessive bone loss during inflammation. A recent qualitative study concludes that matrix metalloproteinase-9, TNF-α, and the OPG/RANK/RANKL system are interrelated and likely contribute interactively to the pathogenesis of osteoporosis in male COPD patients[43].

Notably, in our study, osteoporosis was more common among COPD patients in advanced stages than among those in mild or moderate stages. This seems reasonable because patients with severe COPD usually have movement limitations due to chronic lung conditions, which increase the risk of osteoporosis due to a lack of proper exercise and exposure to the sun. After all, patients with severe COPD are usually bound to stay at home[44]. Ugay et al.[45] highlighted a direct correlation between OPG levels and lung function parameters such as FEV_1% while also showing an inverse relationship between OPG and inflammatory

markers, such as TNF-α. The current study reinforced these findings, noting that COPD patients with lower pulmonary function had a higher risk of osteoporosis, and lower OPG levels were linked to decreased OPG/RANKL ratio and faster bone loss. Both studies conclude that reduced OPG and elevated RANKL contribute to bone deterioration in COPD patients.

The majority of the patients included in this study were categorized as GOLD C and D. Based on GOLD guidelines, the standard treatment for patients classified as GOLD C is primarily LAMA, and LABA can be added for poorly controlled COPD. For patients in GOLD D, it is LAMA + LABA, and for patients with frequent exacerbation, inhaled corticosteroids (ICS) could be added intermittently or for a short period[46]. Although one study indicated that prolonged ICS use was associated with a slightly lower hip BMD, the association was limited to a small population of older women with asthma or COPD, and no association on BMD was seen from low to moderate ICS exposure[47]. In addition, The European Respiratory Society Study on COPD (EUROSCOP) involving a large group of patients with COPD found that long-term treatment with budesonide 800 μg·day-1 through Turbuhaler® had no clinically significant effects on BMD or fracture rates[48]. Moreover, based on a recent Japanese study, ICS has no impact on patients' BMD since the concentration is very low, and its effect is limited to the lungs, with a negligible amount reaching the systemic circulation[49].

The study had some limitations. This work identified the association between COPD and osteoporosis and investigated several of its characteristics. Unfortunately, this approach does not precisely show the direction of the effect or which disease occurred first. However, one could argue that COPD promotes the onset of osteoporosis due to the reportedly high levels of inflammatory markers in COPD patients[50]. The lower OPG level in COPD also decreased the OPG/RANKL ratio, which induces faster bone resorption. However, further molecular research is needed to establish the causal mechanism involved. Long-term studies are essential for monitoring the impact of COPD on bone health, and randomized controlled trials are needed to strictly control variables and confounders that might influence the results. A recent scoping review by Penedones et al.[51] highlights established links between COPD and osteoporosis, particularly regarding shared risk factors, but underscores the need for further research on COPD severity, corticosteroid use, and quality of life impacts to better guide clinical management. Another limitation of this study is the use of QUS for bone density measurement. Even though QUS is a convenient, radiation-free, and cost-effective option, it has limitations in accuracy and precision compared to Dual-energy X-ray Absorptiometry (DEXA), which is considered the gold standard for assessing BMD. DEXA could provide more detailed information on bone density and is better suited for identifying changes in bone health, which could have strengthened the reliability of our findings. COPD patients in advanced stages usually have limited capacity to do activities due to the breathing difficulties that they usually encounter[52], so considering the activities in a small population or sample might not be significant. Additional studies should be conducted to investigate the effect of physical activity among COPD patients.

In this study, COPD patients had significantly lower percentages of sOPG, leading to decreased OPG/RANKL ratio and faster bone resorption. In addition, an imbalanced OPG/RANKL ratio was observed among patients with severe COPD, and a low BMD was associated with more severe COPD. This study adds to the growing body of scientific research investigating the relationship between COPD and osteoporosis; however, additional research is needed to clarify the link between these two conditions.

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SUPPLEMENTARY MATERIAL

The raw data were obtained, recorded, and saved in English. The datasets obtained in Bahasa Malaysia, Mandarin, Tamil, or any other languages were transcribed and translated into English. The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Supplementary data are available at 10.24875/RIC.24000192. These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

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