Original Article

Serum Matrix Metalloproteinase 9 Level, Blood Absolute Neutrophil Count in Correlation with Diffusion Capacity and Exercise Capacity of Stable Chronic Obstructive Pulmonary Disease Patients at Universitas Sebelas Maret Hospital

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is the leading cause of morbidity and mortality worldwide. Exposure to cigarette smoke activates alveolar macrophages producing neutrophils and proteases such as MMP-9 and NE that damage the extracellular matrix triggering emphysema as well as spill over into the systemic circulation. This study aimed to determine the correlation of serum MMP-9 level and blood absolute neutrophil count with diffusion capacity and exercise capacity of stable COPD patients.

Methods: A cross-sectional observational study was conducted in stable COPD patients visiting the pulmonary outpatient clinic of Universitas Sebelas Maret (UNS) Hospital in October 2022. Consecutive sampling was applied for sample collection. We assessed diffusion capacity and exercise capacity with the DLCO method and 6MWT, respectively. We examined serum MMP-9 level and blood absolute neutrophil count. All data were statistically analyzed with SPSS 22.0 and $P<0.05$ was considered significant.

Results: Thirty stable COPD patients were included in the study comprising 19 males (63.3%), and 11 females (36.7%). With a mean age of 61.90±9.99. Serum MMP-9 level did not correlate with either diffusion capacity and blood absolute neutrophil count ($P=0.898$ and $P=0.589$, respectively). However, serum MMP-9 level had a significant correlation with exercise capacity ($P=0.014$). There was no correlation between blood absolute neutrophil count with diffusion capacity and exercise capacity ($P=0.0281$ and $P=0.592$, respectively). Diffusion capacity related to exercise capacity ($P=0.001$).

Conclusion: Serum MMP-9 level is not related to diffusion capacity, but it is related to exercise capacity. Blood absolute neutrophil count is not related to diffusion capacity or exercise capacity. Serum MMP-9 level is not associated with blood absolute neutrophil count. Diffusion capacity is related to exercise capacity.

Keywords: 6MWT, DLCO, COPD, MMP-9, Neutrophil

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disease that is associated with an increased chronic inflammatory response in the airways and lungs to particles or gases and is characterized by persistent airflow limitation that is usually progressive.[1] Deaths caused by COPD were estimated at 3.23 million cases globally in 2019 according to WHO.[2,3]

Damage to the lung tissue in COPD is a complex interaction between oxidative stress, the imbalance of protease-antiprotease, inflammation, and apoptosis.[1,4] Inflammatory cells activate macrophages and epithelial cells releasing proteases such as matrix metalloproteinase 9 (MMP-9) and neutrophil elastase (NE) causing elastin degradation, emphysema, and mucus hypersecretion.[5,6] Exposure to cigarette smoke activates alveolar macrophages which induce the production of matrix metalloproteinases (MMPs).[1] The increased number of neutrophils in the airways is related to the severity of COPD.[4] Neutrophils produce a series of inflammatory cytokines that play a role in elastin tissue damage which causes emphysema.[7,8]

Pathological changes in COPD result in impaired gas exchange function in the lungs due to reduced area of diffusion and permanent abnormal

dilation of the airspaces distal to the terminal bronchioles.[1,9] The movement of gas from the alveoli to the capillaries can be measured by the diffusing capacity of the lung for carbon monoxide (DLCO).[10] Chronic obstructive pulmonary disease patients experience a significant decrease in DLCO which is directly related to the loss of alveolar membrane-capillary surface area due to parenchymal damage.[5,9,10]

Muscle weakness often occurs in COPD patients due to shortness of breath and fatigue which can reduce muscle exercise activity.[1,4,6] Peripheral lung inflammation causes a spillover effect so that cytokines such as interleukin 6 (IL-6) enter the circulation and eventually cause systemic inflammation which can lead to skeletal muscle atrophy.[5,7]

In this study, the authors planned to conduct a study which such topic that has never been studied before, namely the absolute blood neutrophil levels and MMP-9 protease levels with diffusion and exercise capacity, which, if proven to have a correlation, is expected to be a breakthrough in contributing to therapeutic interventions with antiproteases and other strategies that can be done such as promoting smoking cessation in health programs.[11–13]

METHOD

This research is an analytic observational study with a cross-sectional approach. The research was conducted at the pulmonary clinic of Universitas Sebelas Maret (UNS) Hospital in October 2022 until the number of study samples was reached. The targeted population is stable COPD patients undergoing outpatient care at the UNS pulmonary clinic in October 2022.

Stable COPD patients are defined as COPD patients who are not experiencing an acute exacerbation (defined by worsening respiratory symptoms characterized by increased shortness of breath, sputum production, and change in sputum color).

The selection of research samples used a consecutive sampling method, namely patients who meet the inclusion criteria are included in the study until the number of samples are sufficient. The sample size was using total sampling.

Inclusion criteria for stable COPD were outpatients at UNS Hospital who had post-bronchodilator spirometry test results and were willing to participate in the study. The exclusion criteria included patients with acute pulmonary infection, sepsis, lung malignancy, former pulmonary tuberculosis, interstitial lung disease, hearing loss, heart disease, and patients who were uncooperative and unable to perform the required examination. The criteria for discontinuation are COPD patients who experience acute exacerbations, resigned or patients who died.

Patients diagnosed with stable COPD who were undergoing outpatient control at the pulmonary clinic of UNS Hospital and met the inclusion criteria were included in the observation group. The research subjects were confirmed not to be in an exacerbation state. Measurement was conducted for vital signs, height, and weight. The patient's diffusion capacity was then assessed by the DLCO single-breath method. Also assessed was the exercise capacity by the six-minute walking test (6MWT) procedure and blood was taken from the median cubital vein for examination of serum MMP-9 levels and blood absolute neutrophil count. Enzyme-linked immunosorbent assay (ELISA) kit from Elabscience (E-EL-H6075) was used to measure MMP-9 serum.

The data were analyzed with SPSS 22.0 for Windows. The independent variables in this study were serum metalloproteinase 9 levels and blood absolute neutrophils count. The dependent variable in this study is diffusion capacity and exercise capacity. Bivariate analysis was conducted to see the correlation between the independent with dependent variables. The correlation between numeric and numeric variables uses Pearson's correlation test. Results are said to be significant if the value of $P<0.05$. The value of "r" represents the strength of the correlation.

RESULT

This study was conducted on stable COPD patients who came to the Pulmonary clinic of UNS Hospital. Our study was conducted from 11 October to 27 October 2022 with a sample size of 30 patients. The description of the characteristics of the research subjects includes gender, age, MMP-9, diffusion capacity, and exercise capacity with the results shown in Tables 1 and 2.

Based on Table 1, it is known that the majority of the samples are male with 19 subjects (63.3%), and female samples consist of 11 subjects (36.7%). The average age of the research subjects was 61.90±9.99 years with a range between 41 years to 77 years. Most of the COPD patients were included in group D (46.7%), then the rest were within group C (33.3%) and B (20.0%).

Table 1. Characteristics of sex and COPD group of the research subject

Variables	N	%
Sex		
Male	19	63.3
Female	11	36.7
COPD Group		
B	6	20.0
C	10	33.3
D	14	46.7

The results of the MMP-9 examination obtained an average of 1118.22±340.62 ng/mL with a range between 316.10 ng/mL to 1791.90 ng/mL. The results of the absolute neutrophil examination obtained an average of 4.77±1.53 µL with a range between 2.52 µL to 8.69 µL. The results of the DLCO examination obtained an average of 73.47±34.9% with a range between 11-136.00%. The results of the 6MWT examination got an average of 349.33±84.44 m with a range between 200m to 510m (Table 2).

Table 2. Characteristics of age, MMP-9, absolute neutrophil, DLCO and 6MWT the research subject

Variables	Mean±SD	(Min-Max)
Age	61.90±9.99	(41.00–77.00)
MMP-9 (ng/mL)	1118.22±340.62	(316.10–1791.90)
Absolute neutrophil (µL)	4.77±1.53	(2.52–8.69)
DLCO (% prediction)	73.47±34.92	(11.00–136.00)
6MWT (m)	349.33±84.44	(200.00–510.00)

Based on Table 3, it is known that the correlation between MMP-9 with diffusion capacity has a value of r=0.024. This means that there is a positive and very weak correlation (r=0.000–0.199) between MMP-9 with diffusion capacity. The value of *P*=0.898 means that the correlation is not statistically significant. So the hypothesis which states that "there is a correlation between serum MMP-9 level with diffusion capacity" is rejected.

Based on Table 3, it is known that the correlation between absolute neutrophils with diffusion capacity (DLCO) has a value of r=0.204, which means that there is a positive and weak correlation (r=0.200–0.399) between absolute neutrophils with diffusion capacity, and value of *P*=0.281 means that the correlation is not statistically significant. The hypothesis that states "there is a correlation between blood absolute neutrophil count level with diffusion capacity" is rejected.

Table 3. Correlation between serum MMP-9 level with diffusion capacity (DLCO).

Variable	Diffusion Capacity (DLCO)	
	r	*P*
[a]MMP-9	0,024	0.898*
[b]Absolute Neutrophil	0,204	0.281*

Note: [a]Pearson product-moment correlation test: *significant if *P*≤0.05; [b]Spearman rank correlation test: *significant if *P*≤0.05

Based on Table 4, it is known that the correlation between MMP-9 with exercise capacity has a value of r = -0.442 which means that there is a negative and moderate correlation (r=0.400–0.599) between MMP-9 with exercise capacity (6MWT), and the value of *P*=0.014 means that the correlation is statistically significant. The hypothesis which states that "there is a correlation between serum MMP-9 level with exercise capacity" is proven.

Based on Table 4, it is known that the correlation between blood absolute neutrophil count levels with exercise capacity (6MWT) has a value of r=0.102, which means that there is a positive and very weak correlation (r=0.000–0.199) between absolute neutrophils with exercise capacity. The value of *P*=0.592 means that the relationship is not statistically significant. The hypothesis that states that "there is a correlation between blood absolute neutrophil count with exercise capacity" is rejected.

Based on Table 4, it is known that the correlation between diffusion capacity with exercise

capacity has a value of r=0.650 which means that there is a positive correlation and a strong category of correlation (r=0.600–0.799) between diffusion capacity (DLCO) and exercise capacity (6MWT), and value of *P*=0.001 means that the correlation is statistically significant. The hypothesis that states "there is a correlation between diffusion capacity with exercise capacity" is accepted.

Table 4. Correlation between serum MMP-9 level, neutrophil absolute and diffusion capacity with the exercise capacity (6MWT).

Variable	Exercise capacity (6MWT)	
	r	*P*
MMP-9[a]	-0.442	0,014*
Neutrophil absolute[b]	0.102	0.592*
Diffusion capacity[a]	0.650	0.001*

Note: [a]Pearson product-moment correlation test: *significant if *P*≤0.05; [b]Spearman rank correlation test: *significant if *P*≤0.05

Based on Table 5, it is known that the correlation between MMP-9 and absolute neutrophils has a value of r=0.103, which means that there is a positive and very weak correlation (r=0.000–0.199) between MMP-9 with absolute neutrophils, and a value of *P*=0.589 means that the correlation is not statistically significant. The hypothesis which states that "there is a correlation between serum MMP-9 level with blood absolute neutrophils count" is rejected.

Table 5. Correlation between serum MMP-9 level with blood absolute neutrophils count.

Variable	Absolute Neutrophil	
	r	*P*
MMP-9	0,103	0.589*

Note: Spearman rank correlation test: *significant if *P*≤0.05

DISCUSSION

Chronic obstructive pulmonary disease is a respiratory disease associated with inflammation, due to exposure to harmful particles such as cigarettes and other pollutants.[1,3] Exposure to cigarette smoke activates alveolar macrophages which induce the production of matrix metalloproteinases (MMPs).[1,14] An increase in the number of neutrophils in the airways is related to the degree of COPD. Neutrophils produce NE, protein serine cathepsin G, proteinase-3, MMP-8, and MMP-9 which contribute to elastin tissue damage that causes emphysema.[15] The worsening airway inflammatory process is correlated with the degree of airflow limitation, inflammatory reactions, lung hyperinflation, impaired carbon monoxide (CO) diffusion, and emphysema on radiological features.[1,5,9]

Measurement of lung diffusion capacity uses a diffusion-influenced CO displacement.[5] Carbon monoxide is a gas that can illustrate the concept of diffusion capacity. The process of diffusion must pass through the walls of the alveoli, interstitial tissue, capillary endothelium, plasma, and erythrocyte walls.[1,9] Chronic obstructive pulmonary disease patients experience a significant decrease in DLCO directly related to the loss of alveolar-capillary membrane surface area due to parenchymal damage.[9]

Exposure to cigarette smoke, genetic predisposition to systemic inflammation, and spillover of airway inflammation to the systemic circulation produce systemic inflammation, that causes skeletal muscle atrophy.[1,9] Muscle weakness often occurs in COPD patients due to shortness of breath and fatigue so it can reduce muscle exercise activity.[1,6] This study had difficulty finding references in five years because the research on the topic is still rare.

Several risk factors for the prevalence of COPD in Indonesia include male gender, age >40 years, and smokers.[15,16] The results of this study showed that the majority of sex is male as much as 63.3% than female 36.7%. This is in accordance with the research of Firdausi et al in Indonesia. The epidemiological data on the prevalence of COPD in Indonesia was 4.2% higher in males than 3.3% in women according to the 2013 Basic Health Research (RISKESDAS).[16] Chronic obstructive pulmonary disease is more prevalent in men compared to women because of the smoking habit in men, and the exposure to outdoor and indoor air pollution that increases the risk of COPD.[11,16]

The age result obtained a mean of 61.90±9.99 with a range between 41 to 77 years, this is under the research of Firdausi et al in Indonesia which stated that the age group >40 years had a 1.20 times higher risk of suffering from COPD.[16] Another factor that

plays a role is the accumulation of exposure to harmful gases and particles during a previous life, which causing damage to the lungs and makes it easier to develop COPD.[16,17] This is associated with a decrease in lung function followed by the increasing age of the patient.[17]

Stable COPD according to GOLD in 2022 is divided into groups A, B, C, and D.[1] This study showed that the number of group B was 20.0%, group C was 33.3%, and group D was 46.7%. The average control patients in the pulmonary clinic were group B, C, and D patients. In this study, the majority of the patients are included in group D.[1]

Based on the 2022 GOLD, states that there is an increase in MMP-9 in COPD. Matrix metalloproteinase-9 and NE produced by macrophages mutually degrade their inhibitors, so that the capacity to damage the extracellular matrix becomes stronger and further triggers emphysema.[1] In this study, the mean results were 1118.22±340.62 ng/mL, which was by previous studies. Linder et al, 2015 research in Sweden stated that MMP-9 which was measured using the DuoSet kit ELISA Development System plays an important role in lung remodeling and is a potential biomarker in COPD.[18]

Neutrophil is one of the many types of PMN cells in the airways and systemic circulation.[19,20] Blood neutrophil measurements are a procedure that can be used as a predictor of the progression and mortality of COPD patients. The results of the current study obtained a neutrophil value of 4.77±1.53 µL. Research by Aini et al in Riau stated that there was an increase in blood neutrophils in COPD patients.[19]

The diffusion capacity of the lung for carbon monoxide is a pulmonary function test that is useful for measuring the ability of the lungs to exchange gas that is transported into red blood cells through the pulmonary capillaries.[21] The normal value of DLCO is 20–30 milliliters per minute of millimeters of mercury (ml/min mmHg). The current research results obtained 73.47±34.92% prediction. Sin et al, a study in Turkey reported that 50% of COPD patients experienced a significant decrease in DLCO.[22]

Exercise capacity was assessed using 6MWT to measure the distance that the subject could cover while walking on a flat, hard surface with a 30-meter track length in six minutes. Six-minute walking test (6MWT) is used to measure lung function in COPD patients.[1,5] The results of this study obtained a mean of 349.33±84.44 meters. Research by Nusdwinuringtyas et al in Jakarta explains that 6MWT is a recommended test that is reliable and valid to measure the functional capacity of adults with COPD.[23]

Matrix metalloproteinase-9 is the main proteolytic enzyme that can damage the extracellular matrix and elastic fibers.[1] Matrix metalloproteinase-9 is produced by macrophages and neutrophils, as well as by epithelial cells, mast cells, fibroblasts, and myocytes. Matrix metalloproteinase-9 regulates extracellular matrix turnover which influences the severity of COPD and then triggers mucus hypersecretion and causes emphysema. The cause of COPD is excessive secretion of MMP-9 from cigarette smoke exposure stimuli that can cause lung tissue degradation.[1]

The degree of airflow limitation, inflammatory reactions, lung hyperinflation, impaired carbon monoxide (CO) diffusion, and emphysema on radiological features can worsen the airway inflammatory process.[1] Pathological changes in COPD result in impaired gas exchange function in the lungs due to reduced diffusion area and permanent abnormal widening of the air spaces distal to the terminal bronchioles.[1,9] The decrease in DLCO value was caused by a decrease in the gas exchange surface area of the alveolar-capillary membrane, blood volume, membrane thickness, and hemodynamics. An increase in MMP-9 can cause a decrease in the value of DLCO.[1,9,10]

Research by Zhang et al in China explained that MMP-9 was associated with the degree of COPD, marked by a decrease in FEV1% and DLCO values due to the pathological changes in the alveolar septa. The study of D'Armiento et al explained that serum MMP-9 level was not associated with the severity and progression of emphysema.[37]

The results in this study obtained a value of *P*=0.898 meaning that the correlation is not statistically significant. This is not under the research

of Zhang et al in which several factors could be considered, due to the small number of samples, we did not input smoking history data which is one of the factors that can increase MMP-9 expression so that it might be a confounding factor in this study. It is not only MMP-9 that can affect DLCO, but also the control level of COPD that we do not explore further will be a confounding factor in this study.[11]

Matrix metalloproteinase-9 regulates the gelatinolytic, elastolytic and collagenolytic activities, and is the main key in extracellular matrix turnover that affects the severity of COPD. MMP-9 also increases neutrophil chemotactic activity by tenfold. Plasma MMP-9 levels are associated with α-1-antitrypsin deficiency associated with emphysema in COPD.[1]

Muscle weakness often occurs in COPD patients due to shortness of breath and fatigue, so it can reduce muscle exercise activity. Muscle weakness is also caused by smooth muscle apoptosis, oxidative stress, hormonal changes, corticosteroid use, and lactic acidosis. Systemic inflammation causes skeletal muscle atrophy.[1]

Chronic obstructive pulmonary disease is characterized by irreversible airway obstruction and shortness of breath. Patients with severe COPD commonly experience repeated exacerbations, peripheral muscle dysfunction, and significant activity restriction. COPD shows an increase in functional parameters including shortness of breath and exercise capacity which can be measured by walking distance. COPD patients with low levels of exercise capacity have a poor prognosis.[1,5]

Linder et al in Sweden stated that MMP-9 plays an important role in lung remodeling and is a potential biomarker in COPD that can affect the quality of life in COPD patients.[18] The study of Boschetto et al in Italy explained that there was a correlation between a decrease in 6MWT and an increase in serum MMP-9 in emphysema patients.[24] The results of this current study showed that there was a correlation between MMP-9 and exercise capacity, with a value of r= -0.442, which means that there was a negative and moderate correlation (r=0.400-0.599) between serum MMP-9 level and exercise capacity (6MWT), and the value of *P*=0.014 means that the relationship is statistically significant. These findings are under previous research.

The degree of COPD is related to an increasing number of neutrophils in the airways. Neutrophils produce NE, cathepsin G protein serine, proteinase-3, MMP-8, and MMP-9 which contribute to elastin tissue damage that causes emphysema.[1] Neutrophil elastase will increase oxidative stress resulting in epithelial cell metaplasia and mucus gland hyperplasia that results in mucus hypersecretion. Stimulation of releasing the neutrophils and macrophages is mediated by proteolytic enzymes.[1]

Neutrophil cells are thought to play an important role in the pathogenesis of chronic bronchitis and pulmonary emphysema in COPD. Neutrophils affect goblet cells and submucosal glands.[1,14] Chronic obstructive pulmonary disease patients experience a significant decrease in DLCO which is directly related to the loss of surface area of the alveolar-capillary membrane due to pathological changes in parenchymal damage.[7,14]

Research by Aini et al in Riau stated that there was an increase in blood neutrophils in COPD patients.[19] A 2020 study by Lonergan et al in England, stated that an increase in the neutrophil count is a good indicator of exacerbation risk and mortality in COPD.[25] Research by Oudijk et al in the Netherlands stated that COPD is associated with neutrophil activation in the systemic compartment.[20] Research by Saydain et al in England reported that the DLCO value decreased in COPD patients.[21]

Research on absolute neutrophils with stable COPD diffusion capacity (DLCO) has never been carried out either abroad or in Indonesia. The results of this study obtained a value of r=0.204, which means that there is a positive and weak correlation (r=0.200–0.399) between absolute neutrophils and diffusion capacity, and a value of *P*=0.281 means that the correlation is not statistically significant.

This could be caused due to the small number of samples, and circulating neutrophils in the blood which play a role in inflammation thus affecting the results of this study which could be a confounding

factor. We did not input data on medical and non-medical therapy in this study. Not only that absolute neutrophils can affect DLCO, but also the degree of controlled disease and physical activity of the patients which we did not explore deeper will be a confounding factor in this study.[13,26–28]

Neutrophils act as the first defense response of the natural immune system that releases neutrophil chemotactic factors so that neutrophil cells in the blood can migrate into the damaged airways. Pathological conditions can cause an increase in neutrophils including acute infection, inflammation, tissue damage, and metabolic disorders.[17,18,29]

Patients with severe COPD commonly experience repeated exacerbations, peripheral muscle dysfunction, and significant activity restriction. COPD shows an increase in functional parameters including shortness of breath and exercise capacity which can be measured by walking distance. The six-minute walking test has been widely used for clinical evaluation in COPD patients. The six-minute walking test is a simple test to measure lung function and can be used as a prognosis in COPD patients.[1,7,30,31]

Research on absolute neutrophils with exercise capacity (6MWT) in stable COPD has never been conducted either abroad or in Indonesia. The results of this study obtained a value of r=0.102 which means that there is a positive and very weak correlation (r=0.000–0.199) between absolute neutrophils and exercise capacity, and the value of *P*=0.592 means that the correlation was not statistically significant. This may happen because of the small sample factor, circulating neutrophils in the blood that play a role in inflammation that affects the results of this study which can be a confounding factor. This study did not input medical and non-medical therapy data in this study. The degree of controlled disease and patient physical activity that we do not explore will also be a confounding factor.[13,26,27,30]

The increase in the number of neutrophils in the airways is related to the degree of COPD. Neutrophils produce NE and the stimulation release of neutrophils and macrophages is mediated by proteolytic enzymes. Neutrophil elastase activates MMPs causing a decrease in α-1AT1 activation.[1,4,9,32]

Matrix metalloproteinase-9 also increases neutrophil chemotactic activity by tenfold. Plasma MMP-9 levels are associated with α-1-antitrypsin deficiency and may be the underlying cause of emphysema in COPD. Matrix metalloproteinase-9 is secreted by macrophages and neutrophils, then triggers mucus hypersecretion and causes emphysema.[16–20,22,33]

Research by Koo et al in South Korea explained that there was a significant positive correlation between MMP-9 and blood neutrophils with *P*<0.001.[34] However, in this study, the results of the correlation between MMP-9 serum and absolute neutrophils obtained a value of r=0.103, which means that there is a positive and very weak correlation (r=0.000–0.199) between MMP-9 and absolute neutrophils, and a value of *P*=0.589 means that the correlation is not statistically significant.[25]

Due to the insufficient number of study samples, we did not input the smoking history data which is one of the factors that can increase MMP-9 expression so this might be a confounding factor in this study. Circulating neutrophils in the blood which play a role in inflammation can also influence this result. We also did not input data about therapy and how long the patient have had COPD so that we could have assessed the role of lung function, and maybe there are many other data about the effect of other proteases in this study that have not been obtained so that they can also be a confounding factor.[25]

Pathological changes in COPD results in the impaired gas exchange function in the lungs due to reduced diffusion area. Pathological changes in the lung parenchyma contribute to airflow limitation by reducing elastic recoil of the lung. The worsening airway inflammatory process correlates with the degree of airflow limitation, inflammatory reactions, lung hyperinflation, impaired carbon monoxide (CO) diffusion, and emphysema on radiological features. Cigarette smoke exposure, genetic predisposition to systemic inflammation, and spill over of airway inflammation into the systemic circulation makes

COPD patients vulnerable to comorbidities that are often found to affect outcome and quality of life. Muscle weakness often occurs in COPD patients due to shortness of breath and fatigue which can reduce muscle exercise activity. Muscular dysfunction is expressed by fatigue and weakness.[1,4,9]

Research Farkhooy et al in Sweden explained that DLCO is a good predictor for assessing exercise capacity in COPD, in that study a significant correlation was found.[31] Research by Ijiri et al explained that there is a significant correlation between 6MWT and DLCO.[35]

The results of this study found a relationship between diffusion capacity and exercise capacity with a value of r=0.650, which means that there is a positive correlation and a strong correlation category (r=0.600–0.799) between diffusion capacity (DLCO) and exercise capacity (6MWT), and the value of *P*=0.001 means that the correlation is statistically significant. So it is in accordance with previous studies.[34,35]

LIMITATIONS

The study has the following limitations: (1) no smoking data is being included in the research; (2) there are no data of exposure to cigarette smoke and dangerous pollutants in this research; (3) we did not take research data on therapy and duration of suffering from COPD; (4) we did not analyze the use of knowing the degree of good control and controlled patient activity; (5) we did not use the influence of sampling MMP-9 levels, especially from the respiratory tract, namely BAL in this study; (6) the degree of emphysema type in this study was not analyzed; (7) not all comorbidities in research subjects have been analyzed. Further studies are needed using BAL MMP-9 to improve significance and deficiency in this research.

CONCLUSION

Serum MMP-9 level have a correlation with exercise capacity, but not with diffusion capacity in stable COPD patients. Blood absolute neutrophil count level is not related to diffusion capacity or exercise capacity in stable COPD patients. From additional analysis, there is a correlation between the diffusion capacity with exercise capacity in stable COPD patients.

REFERENCES

1. Agustí A, Beasley R, Celli BR, Chen R, Criner G, Halpin D, et al. Global initiative for chronic obstructive lung disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Vol. 61, European Respiratory Journal. 2022. p. 1–165.
2. World Health Organization. Chronic obstructive pulmonary disease (COPD) [Internet]. 2021 [cited 2022 Jan 20]. Available from: https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd)
3. Akinbami LJ, Liu X, American Lung Association. Trends in COPD (Chronic bronchitis and emphysema): Morbidity and mortality. American Lung Association Epidemiology and Statistics Unit Research and Health Education Division. 2013;1.
4. Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. Respir Res. 2006;7:53.
5. Perhimpunan Dokter Paru Indonesia. Penyakit paru obstruktif kronik diagnosis dan penatalaksanaan. 1st ed. Antariksa B, Susanthy D, Wiyono WH, Sutoyo DK, Damayanti T, Syafiuddin T, et al., editors. Jakarta: UI Press; 2016. 1–88 p.
6. Singh D. Small airway disease in patients with chronic obstructive pulmonary disease. Tuberc Respir Dis (Seoul). 2017;80(4):317–24.
7. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(1):16–27.
8. Abboud RT. Pathogenic mechanisms in emphysema: From protease anti-protease imbalance to apoptosis. In: Dr. Mahadeva R, editor. Emphysema. InTech; 2012.

9. Balasubramanian A, MacIntyre NR, Henderson RJ, Jensen RL, Kinney G, Stringer WW, et al. Diffusing capacity of carbon monoxide in assessment of COPD. Chest. 2019;156(6):1111–9.
10. Rosyid AN, Marhana IA. Faal paru difusi. Jurnal Respirasi. 2018;4(2):61–70.
11. Zhang Y, Li Y, Ye Z, Ma H. Expression of matrix metalloproteinase-2, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and changes in alveolar septa in patients with chronic obstructive pulmonary disease. Med Sci Monit. 2020;26:e925278-1-e925278-10.
12. Ria DO, Suradi S, Aphridasari J, Reviono R, Indarto D. Uji DLCO sebagai prediktor penting prognosis penderita penyakit paru obstruktif kronik. Jurnal Respirologi Indonesia. 2018;38(2):83–92.
13. Deesomchok A, Webb KA, Forkert L, Lam YM, Ofir D, Jensen D, et al. Lung hyperinflation and its reversibility in patients with airway obstruction of varying severity. COPD. 2010;7(6):428–37.
14. Hikichi M, Mizumura K, Maruoka S, Gon Y. Pathogenesis of chronic obstructive pulmonary disease (COPD) induced by cigarette smoke. J Thorac Dis. 2019;11(Suppl 17):S2129–40.
15. Perez-Padilla R, Menezes AMB. Chronic obstructive pulmonary disease in Latin America. Ann Glob Health. 2019;85(1):7.
16. Firdausi NL, Artanti KD, Li CY. Analysis of risk factors affecting the occurrence of chronic obstructive pulmonary disease in Indonesia. Jurnal Berkala Epidemiologi. 2021;9(1):18–25.
17. Fukuchi Y. The aging lung and chronic obstructive pulmonary disease: Similarity and difference. In: Proceedings of the American Thoracic Society. 2009. p. 570–2.
18. Linder R, Rönmark E, Pourazar J, Behndig A, Blomberg A, Lindberg A. Serum metalloproteinase-9 is related to COPD severity and symptoms - cross-sectional data from a population based cohort-study. Respir Res. 2015;16(1):28.
19. Hurul Aini QS, Adrianison A, Fridayenti F. Gambaran jumlah neutrofil darah tepi pasien penyakit paru obstruktif kronik (PPOK) di ruang rawat inap RSUD Arifin Achmad provinsi Riau tahun 2017. Jurnal Ilmu Kedokteran. 2019;13(2):134–40.
20. Oudijk EJD, Nijhuis EHJ, Zwank MD, Van De Graaf EA, Mager HJ, Coffer PJ, et al. Systemic inflammation in COPD visualised by gene profiling in peripheral blood neutrophils. Thorax. 2005;60(7):538–44.
21. Saydain G, Beck KC, Decker PA, Cowl CT, Scanlon PD. Clinical significance of elevated diffusing capacity. Chest. 2004;125(2):446–52.
22. Sin BA, Akkoca O, Saryal S, Oner F, Misirligil Z. Differences between asthma and COPD in the elderly. J Investig Allergol Clin Immunol. 2006;16(1):44–50.
23. Nusdwinuringtyas N, Widjajalaksmi, Yunus F, Alwi I. Reference equation for prediction of a total distance during six-minute walk test using Indonesian anthropometric. Acta Medica Indonesiana - The Indonesian Journal of Internal Medicine. 2014;46(2):90–6.
24. Boschetto P, Quintavalle S, Zeni E, Leprotti S, Potena A, Ballerin L, et al. Association between markers of emphysema and more severe chronic obstructive pulmonary disease. Thorax. 2006;61(12):1037–42.
25. Lonergan M, Dicker AJ, Crichton ML, Keir HR, Van Dyke MK, Mullerova H, et al. Blood neutrophil counts are associated with exacerbation frequency and mortality in COPD. Respir Res. 2020;21(1):166.
26. Polosukhin V V., Gutor SS, Du RH, Richmond BW, Massion PP, Wu P, et al. Small airway determinants of airflow limitation in chronic obstructive pulmonary disease. Thorax. 2021;76(11):1079–88.
27. Higham A, Quinn AM, Cançado JED, Singh D. The pathology of small airways disease in COPD: Historical aspects and future directions. Respir Res. 2019;20(1):1–11.
28. Efriadi, Yunus F, Damayanti T. Hubungan antara hasil pemeriksaan kapasitas difusi paru terhadap karbonmonoksida (DLCO) metode napas tunggal dengan derajat obstruksi pasien PPOK di RSUP

Persahabatan Jakarta [Thesis]. Universitas Indonesia; 2015.

29. D'Armiento JM, Goldklang MP, Hardigan AA, Geraghty P, Roth MD, Connett JE, et al. Increased matrix metalloproteinase (MMPs) levels do not predict disease severity or progression in emphysema. PLoS One. 2013;8(2):e56352.
30. Salcedo-Posadas A, Villa Asensi JR, De Mir Messa I, Sardón Prado O, Larramona H. Measurement of CO diffusion capacity (II): Standardization and quality criteria. An Pediatr (Engl Ed). 2015;83(2):137.e1-7.
31. Farkhooy A. Lung function in relation to exercise capacity in health and disease [Disertation]. Uppsala University; 2017.
32. Kato A, Hanaoka M. Pathogenesis of COPD (Persistence of airway inflammation): Why does airway inflammation persist after cessation of smoking? In 2017. p. 57–72.
33. Simpson JL, McDonald VM, Baines KJ, Oreo KM, Wang F, Hansbro PM, et al. Influence of age, past smoking, and disease severity on tlr2, neutrophilic inflammation, and MMP-9 Levels in COPD. Mediators Inflamm. 2013;2013:462934.
34. Koo HK, Hong Y, Lim MN, Yim JJ, Kim WJ. Relationship between plasma matrix metalloproteinase levels, pulmonary function, bronchodilator response, and emphysema severity. International Journal of COPD. 2016;11(1):1129–37.
35. Ijiri N, Kanazawa H, Yoshikawa T, Hirata K. Application of a new parameter in the 6-minute walk test for manifold analysis of exercise capacity in patients with COPD. International Journal of COPD. 2014;9:1235–40.