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Association of Fibrinogen and Neutrophil Levels with Incidence and Severity of Chronic Obstructive Pulmonary Disease

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Abstract

The major characteristic of COPD is systemic inflammation. This inflammatory process stimulates hematopoietic system, especially bone marrow, to release leukocytes and stimulates the liver to produce acute-phase proteins such as fibrinogen. Fibrinogen levels tend to increase with severe airflow obstruction. High neutrophil levels are associated with a decrease in FEV_1 even beyond exacerbations. In this study, we evaluated the relationship between fibrinogen and neutrophil levels with severity of COPD. The objective of this study was to analyze the correlation between fibrinogen and neutrophil levels with the incidence and severity of COPD. Design of this study was observational with a cross-sectional approach to population of COPD patients from May to July 2017 and healthy people as control. Consecutive sampling did a sampling of COPD patients. We evaluated clinical, CAT score, history of exacerbations, number of cigarettes consumed, fibrinogen, and neutrophil levels. From 35 COPD subjects and 21 healthy controls, we found that fibrinogen and neutrophils levels increased in COPD subjects compared to control ($p < 0.001$), there was no significant correlation of fibrinogen and neutrophil levels with smoking status on COPD subjects ($p > 0.05$), Fibrinogen levels was significantly higher in exacerbation COPD than stable ($p < 0.001$) but not with neutrophil, there was no significant correlation between smoking status and amount of cigarettes with COPD severity($p > 0,05$), there was significant correlation between fibrinogen levels with COPD severity ($p < 0.001$) but not with neutrophils. Conclusion of this study is there was a correlation between fibrinogen with COPD incidence and severity. However, neutrophils levels associated with COPD incidence.

Keywords: COPD; neutrophil; fibrinogen; COPD severity.

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1. Introduction

Currently, chronic obstructive pulmonary disease (COPD) is a global health problem. *Global initiative for chronic Obstructive Lung Disease* (GOLD) defineCOPDis a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases [1]. Pulmonary component is characterized by airflow obstruction that is not entirely reversible. Airflow obstruction is usually progressive and associated with pulmonary inflammatory responses to noxious particles or gases [2,3]. The chronic inflammatory response in COPD may induce parenchymal tissue destruction (caused emphysema), and disrupt normal repair and defense mechanisms (caused small airway fibrosis) [1]. This inflammatory process stimulates hematopoietic system especially bone marrow to release leukocytes and platelets and stimulates the liver to produce acute-phase proteins such as CRP and fibrinogen. Fibrinogen is a glycoprotein found in plasma, with a half-life about 100 hours and released in large quantities into circulation in response to stimulation of interleukin 6 (IL-6) [4,5]. While neutrophils are a type of polymorphonuclear leukocytes, which have a role during the acute inflammatory phase, with a half-life in the circulation about 8 hours in humans [6]. One of fibrinogen degradation products is fibrinopeptide A and B which have a special proinflammatory effect as a neutrophil, monocytes, and macrophages chemoattractants [5]. In general, the inflammatory process increases with the severity of disease and persists despite quit smoking [7,8]. Fibrinogen levels tend to increase with severe airflow obstruction [9]. High neutrophil levels associated with decrease in FEV_1 even beyond exacerbations [10,11] Neutrophil and fibrinogen levels are predictive markers for future exacerbations (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints, ECLIPSE). Currently, fibrinogen considered as a prognostic biomarker in COPD patients [16,17]. In this study, we intend to explore the correlation between fibrinogen and neutrophil levels with incidence and COPD severity. Our hypothesis that fibrinogen and neutrophils levels associated with COPD severity.

2. Methods

Study Design

This cross-sectional study was performed from May to July 2017. The flowchart showing the enrollment, screening, and outcome of this study was depicted in Figure 1. We enrolled outpatients and in patients diagnosed with COPD in Wahidin Sudirohusodo Hospital, and it's network.

Subject

We enrolled patients who were at least 40 years old diagnosed with COPD, which is stable and exacerbation stage with severity from mild to very severe. The diagnostic criteria were defined as an FEV_1/FVC ratio <70%, determined when stable in accordance with the GOLD criteria. Excluded from the study were participants with a history of hypertension, stroke, Diabetes Mellitus, heart disease, autoimmune disease, asthma bronchial, pulmonary tuberculosis, chronic kidney disease, cancer, hematology disease, liver disease, and pregnancy. The subjects in the control group were non COPD healthy people who were older than 40 years with or without

smoking history.All participants were informed about the nature and purpose of the study and gave their written consent. The study was approved by the Ethics Committee of Medical Faculty, Hasanuddin University, Makassar.

Assesment

Baseline demographic information, smoking history, medication history, and patient-reported history of exacerbations were collected. Exacerbations were defined according to the criteria of Anthonisen and his colleagues Patients who had deterioration in the symptoms of the respiratory tract that caused a change in medical treatment beyond normal daily variations were classified as “exacerbation COPD”. Patients who had not had any significant changes in their symptoms in the last 3 months and the ones who did not need additional inhaler treatment dosages or any other additional treatments were defined as “stable COPD”. At all visits, spirometry and symptom assessment using COPD Assesment Test, CAT and modified Medical Research Council,mMRC, were undertaken.

The RBTand fibrinogen in blood was measured for both of group. After the collection of all databases, the differences among the parameters such as fibrinogen and neutrophil between the COPD group and those of the healthy group were compared. All the COPD patients received standard medication in accordance with the GOLD guidelines. Spirometry examination was performed on stable condition by using Spirovit Sp-1, Swiss-made Schiller AG which has been calibrated first. Blood samples for fibrinogen examination inserted into a citrate tube (blue cap) containing the Na citrate buff, the tube inserted into the STA Compact tool by previously entering the patient data. Blood samples for neutrophil examination were inserted into a tube with EDTA potassium.

Levels of hemoglobin, hematocrit, platelets, as well as white blood cells and types (neutrophils, lymphocytes, eosinophil, and monocytes), were identified with automatic blood counters (Siemens Advia 2120, Diagnostic Solutions, Milan, Italy) with electrical impedance methods. All measurements will auto-exit from the tool.

Statistical analysis

The data obtained were analyzed with a computer using Statistical Package for Social Science (SPSS) version 22. Statistical analysis was descriptive statistical analysis and Chi-Square, Fisher Exact, Mann-Whitney, Kruskal-Wallis and Kendall's tau-b statistic tests. Statistical test results are significant if the p-value of test <0.05.

3. Result

Our study recruited 56 subjects with age range 40 to 85 years, from 56 subjects we have 35 COPD subjects (62.5%) and 21 healthy subjects (37.5%) as controls with characteristics can be seen in following tables:

Table 1: Characteristics of Sex Distribution by COPD and Controls

Sex		Groups		
		COPD	**Control**	**Total**
male	n	32	12	44
	%	91,4%	57,1%	78,6%
female	n	3	9	12
	%	8,6%	42,9%	21,4%
Total	n	35	21	56
	%	100,0%	100,0%	100,0%

Fisher Exact test (p=0,005)

Comparison of COPD group subjects between male and female was 91.4% and 8.6%, respectively, as well as with the control group, male greater than female in 57.1% and 42.9%. Distribution of sex differed significantly by group ($p < 0.01$). The percentage of a male was higher in COPD than in controls (91.4% with 57.1%), whereas the percentage of females was higher in control than COPD (42.9% with 8.6%).

Table 2: Characteristics of Age Distribution by COPD and Control Group

Age		Groups		Total
		COPD	**Control**	
<50 years	N	2	14	16
	%	5,7%	66,7%	28,6%
50-59 years	N	5	5	10
	%	14,3%	23,8%	17,9%
60-69 years	N	18	2	20
	%	51,4%	9,5%	35,7%
>=70 years	N	10	0	10
	%	28,6%	0,0%	17,9%
Total	N	35	21	56
	%	100,0%	100,0%	100,0%

Chi-Square test (p=0,000)

In COPD group, subjects with age $\geq$ 60 years were much more than age < 60 years (80% vs. 20%) while in the control group, subjects with age $\geq$ 60 years were only two persons (9.5%). Age distribution was significantly different by group ($p < 0.001$). Percentage of subjects with age < 60 years was higher in control than COPD, whereas the percentage of subjects with age $\geq$ 60 years was higher in COPD than in controls.

Table 3: Characteristic of Body Mass Index Distribution by COPD and Control Group

BMI			Groups		Total
			COPD	Control	
	Low BMI	n	10	0	10
		%	28,6%	0,0%	17,9%
	Normal BMI	n	17	21	38
		%	48,6%	100,0%	67,9%
	High BMI	n	8	0	8
		%	22,9%	0,0%	14,3%
Total		n	35	21	56
		%	100,0%	100,0%	100,0%

Chi-Square test (p=0,000)

In the COPD group, the number of subjects with less BMI was ten people (28.6%), and high BMI were eight people (22.9%). While in the control group, all subjects had normal BMI (100%). The distribution of BMI differed significantly by group (p <0.001). Percentage of subjects with less nutrition and obese were higher in COPD than controls.

In table 4, all subjects of COPD and control groups were divided into smokers and nonsmokers. Seven subjects were nonsmokers, and 28 subjects were smokers in the COPD group while in the control group, 14 subjects were nonsmokers and seven subjects were smokers. The percentage of subjects who smoked was significantly higher in COPD than in controls (80.0% and 33.3%) (p <0.001).

Table 4: Characteristic of Smoking Status Distribution by COPD and Control Group

Smoking Status			Groups		Total
			COPD	Control	
	Non-smokers	n	7	14	21
		%	20,0%	66,7%	37,5%
	Smokers	n	28	7	35
		%	80,0%	33,3%	62,5%
Total		n	35	21	56
		%	100,0%	100,0%	100,0%

Chi Square test (p=0,000)

Comparison of Fibrinogen and Neutrophil Levels between COPD Group and Control Group

In Table 5 shows that fibrinogen levels were also significantly higher in COPD than in controls group (p <0.001), whereas fibrinogen levels in the COPD group compared with the control group (461.8 vs. 341.9) (p<0.001). The detailed results are displayed in the graph (Figure 2). While neutrophils levels were significantly higher in COPD than the control group (p <0.001), where neutrophil levels in the COPD group compared with the control group (66.5 vs. 51.0). The detailed results are displayed in the graph (Figure 3).

Table 5: Fibrinogen and Neutrophil Levels in COPD and Control Group

Variable	Groups	N	Median	Mean	SD	p
Fibrinogen	COPD	35	415,0	461,8	126,4	0,000
	Control	21	336,0	341,9	74,3	
Neutrophil	COPD	35	67,9	66,5	13,1	0,000
	Control	21	50,8	51,0	7,9	

Mann-Whitney test

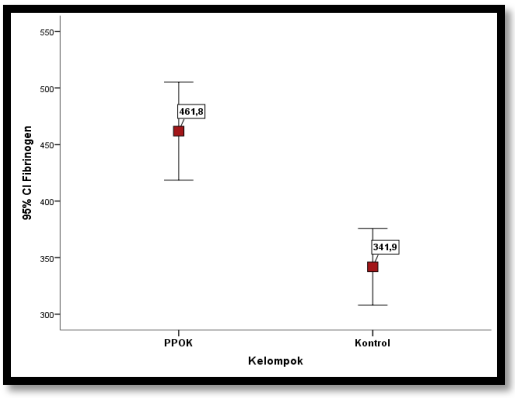

Figure 2: Fibrinogen levels in COPD and Control Groups

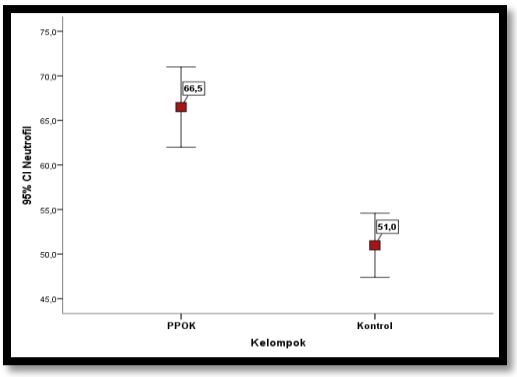

Figure 3: Neutrophils levels in COPD and Control Groups

Correlation of Fibrinogen and Neutrophil Levels with Smoking Status in COPD Group

Table 6: Correlation of Fibrinogen and Neutrophil Levels with Smoking Status in COPD Group

Variable	Smoking Status	N	Median	Mean	SD	p
Fibrinogen	Non-Smoker	7	386,0	393,0	105,4	0,087
	Smoker	28	454,0	479,0	126,9	
Neutrophils	Non-Smoker	7	65,5	62,8	14,1	0,606
	Smoker	28	68,4	67,4	13,0	

Mann-Whitney test

Table 6 shows that fibrinogen and neutrophil levels did not differ significantly according to smoking status (p> 0.05).

Correlation of fibrinogen and neutrophil levels with exacerbations in the COPD group

Table 7 shows that Fibrinogen levels were significantly higher (median and mean) in COPD exacerbations than in stable COPD (p <0.001).

Table 7: Correlation of Fibrinogen Levels with Exacerbation in COPD Group

COPD	N	Median	Mean	SD	p
Stable	23	392,0	385,1	56,7	0,000
Exacerbation	12	597,0	608,9	83,8	

Mann-Whitney test

While in table 8 showed that neutrophil levels did not differ significantly with exacerbations in COPD (p> 0.05).

Table 8: Correlation of Neutrophil Levels with Exacerbations in COPD Group

COPD	n	Median	Mean	SD	p
Stable	23	67,600	65,026	13,8729	0,362
Exacerbation	12	72,900	69,308	11,5829	

Mann-Whitney test

Correlation of Smoking Status with COPD Severity

In Table 9. shows that no significant difference in the distribution of smoking status with COPD severity (p> 0.05).

Table 9: Correlation of Smoking Status with COPD Severity

Smoking Status			COPD Severity		Total
			Grade A/B	Grade C/D	
	Non Smokers	n	2	5	7
		%	50,0%	16,1%	20,0%
	Smokers	n	2	26	28
		%	50,0%	83,9%	80,0%
Total		n	4	31	35
		%	100,0%	100,0%	100,0%

Fisher Exact test (p=0,171)

Table 10: Correlation of Cigarettes Distribution (Brinkman Index) with COPD Severity in COPD Smokers Group

Cigarettes (IB)			COPD Severity		Total
			Grade A/B	Grade C/D	
	Light Smokers	n	0	1	1
		%	0,0%	3,8%	3,6%
	Mild Smokers	n	1	3	4
		%	50,0%	11,5%	14,3%
	Heavy Smokers	n	1	22	23
		%	50,0%	84,6%	82,1%
Total		n	2	26	28
		%	100,0%	100,0%	100,0%

Chi-Square test (p=0,321)

Whereas in table 10. shows that no significant difference in cigarettes distribution (Brinkman Index) with COPD severity in smokers COPD (p> 0.05).

Correlation of Fibrinogen and Neutrophil Levels with COPD Severity

In Table 11. shows that fibrinogen levels highest significantly on grade D and lowest on grade A (p <0.001). This suggests a significant correlation between fibrinogen levels and COPD severity.

Table 11: Correlation of Fibrinogen and Neutrophil Levels with COPD Severity

Variable	COPD Severity	n	Median	Mean	SD	p
Fibrinogen	Grade A	2	285,0	285,0	32,5	0,000
	Grade B	2	297,0	297,0	12,7	
	Grade C	19	396,0	407,8	43,1	
	Grade D	12	597,0	604,2	91,9	
Neutrophil	Grade A	2	55,3	55,3	26,9	0,484

Kruskal-Wallis test

Figure 4 also shows that fibrinogen levels were going higher with increasing severity of COPD.

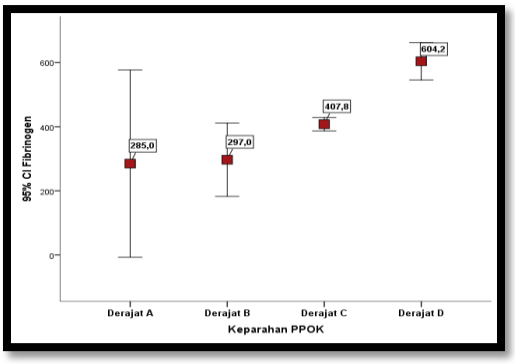

Figure 4: Correlation of Fibrinogen Levels with COPD Severity

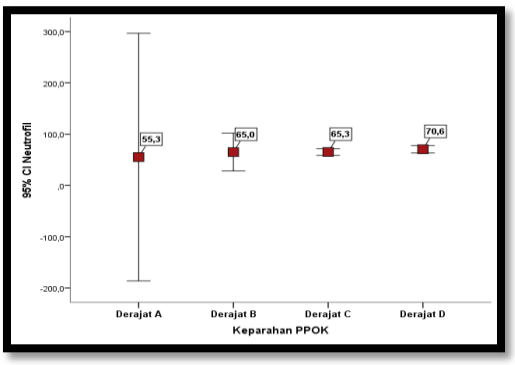

Figure 5: Correlation of Neutrophil Levels with COPD Severity

While neutrophil levels did not differ significantly with COPD severity ($p > 0.05$). This shows that no significant correlation between neutrophils with COPD severity. The detailed results are displayed in the graph (Figure 5).

4. Discussion

In this study, our COPD group divided into smokers and nonsmokers. A number of smokers were 28 people, and non-smokers were seven people. Similarly, the control group divided into smokers and non-smokers, with a number of smokers were seven people and nonsmokers were 14 people. Percentage of subjects who smoked was significantly higher in COPD than in controls, 80.0% and 33.3% ($p < 0.001$). Percentage of men was higher in COPD than in controls groups (91.4% with 57.1%) with percentage of subjects with age > 60 years higher in COPD than in control groups (80% with 9.5%). Based on BMI, percentage of subjects with less and high BMI was higher in COPD than in controls groups, 28.6%, and 22.9%, respectively. Our findings are in agreement with recent studies by Garciario and his colleagues which found that compared with control group, COPD group were more men and smokers, with older age and a higher body mass index [26]. Similarly, in the ECLIPSE study, found that COPD patients are older than control and have more intense smoking than smokers with normal lung function [60].

Comparison of Fibrinogen and Neutrophil Levels between COPD and Control Groups

Fibrinogen is a major plasma protein coagulation factor associated with adverse health effects when the levels are low or high. While COPD is a predictor of increased levels of fibrinogen [8]. In this study, fibrinogen levels were significantly higher in COPD than in controls groups ($p < 0.001$), whereas fibrinogen levels in the COPD group compared with the control group (461.8 vs. 341.9). This finding is consistent with a shift in hemostatic balance that will activate coagulation in COPD. Various studies have demonstrated the prothrombotic conditions that occur in COPD [8,41]. Mona Fattouh and his colleagues also found a statistically significant increase of fibrinogen levels in COPD patients compared with the control group [4]. Neutrophils play an essential role in the pathogenesis of COPD and considered to be the primary effector cells involved in inflammatory process. In COPD, peripheral blood neutrophils activated by different inflammatory mediators then extravasation into lung tissue. Neutrophilia caused by neutrophil demarginations, slow neutrophil apoptosis, and bone marrow stimulation by growth factors [61,62]. In this study, neutrophil levels were significantly higher in COPD than in controls groups (66.5 vs. 51.0). Our findings are in agreement with recent studies by Erdal in and his colleagues which found that statistically, neutrophil levels were significantly higher in COPD than in control group. Several studies have shown that neutrophils are a key mediator of decreased lung function in COPD patients. When activated, neutrophils release several proteolytic enzymes, elastase and metalloproteinase matrices, which contribute to the formation of emphysema. Milara and his colleagues reported that peripheral blood neutrophils are constantly increasing in patients with COPD despite smoking cessation for many years. Increase the level of neutrophils associated with development of COPD. Increased levels of neutrophils in COPD patients are in line with previous studies and support the concept of systemic inflammation [36,55,62].

Correlation of Fibrinogen and Neutrophil Levels with Smoking Status in COPD Group

Long-term smokers can inhale more than 5,000 different compounds over several decades while slowly developing the pulmonary disease. In this study, we found that fibrinogen levels in smokers were higher than non-smokers in the COPD group (479,0 vs 393.0) but not statistically significant ($p > 0.05$). While neutrophil levels in smokers were higher than nonsmoker subject in COPD group (67,4 vs 62,8) but not statistically significant ($p > 0,05$). This is consistent with a study conducted by Agusti A. and his colleagues found that fibrinogen didn't affected by smoking activation, which in later studies concluded that IL-8 and TNF-α were strongly influenced by smoking while hs-CRP, IL-6 and fibrinogen were inflammatory biomarkers associated with COPD whereas WBC affected by both smoking and COPD [9]. YunitaArliny and his colleagues also found that there was no significant correlation between fibrinogen levels with smoking history and Brinkman Index [7]. Our results show elevated levels of fibrinogen and neutrophils in smokers COPD versus non-smokers although not statistically significant. Based on this study, we concluded that an increase of neutrophil level in COPD didn't only influence by smoking alone. Similarly, insignificant elevated fibrinogen levels in these patients are likely due to our COPD subjects still have comorbid factors such as hypertension and diabetes mellitus which unable to exclude It may also affect inflammatory biomarkers in COPD patients outside of smoking. Miller and his colleagues suggests that comorbid diseases such as heart disease, hypertension, and diabetes associated with increased systemic inflammation [63].

Correlation of Fibrinogen and Neutrophil Levels with Exacerbations in COPD Group

Alteration in various systemic inflammatory indicators observed during COPD exacerbation, which accompanied by significant pulmonary function decline. The basic mechanism of COPD exacerbation episodes is "pulmonary inflammatory flareup," regardless of the "trigger" that causes it (infection, air pollution, etc.). Increased levels of airway inflammation during exacerbations are also accompanied by greater systemic inflammation, which has a role in higher cardiovascular morbidity. In our study, fibrinogen levels were significantly higher (median and mean) in exacerbations than in stable ($p < 0.001$). Our findings are in agreement with recent studies by Kersul and his colleagues and Fattouh and his colleagues who found that during COPD exacerbations, plasma fibrinogen levels were elevated compared with stable phases or smokers control subjects [4, 57]. Several studies have shown that peripheral blood neutrophils are increasing during exacerbations [64]. Peripheral blood neutrophils increased during exacerbations, with levels correlated with exacerbations severity. The percentage of blood neutrophil apoptosis in COPD exacerbations decreases. In our study, we found that levels of neutrophils in stable COPD (65.026) increases during exacerbations (69.308), but this result was not statistically significant ($p > 0.05$). An acute exacerbation can be caused by various causes including infection (bacteria or virus), bronchospasm, air pollution, or sedative class drugs [22]. While an increase in neutrophils itself only dominated by more severe exacerbations caused by bacterial infections [65]. In addition, in some exacerbated subjects, we did not immediately take blood samples on the same day our subject admitted to the hospital. At the time we took blood samples, the subjects had already received antibiotic therapy. This may affect levels of neutrophils in COPD subjects. Neutrophils are not acute-phase reactions, short-lived cells with a half-life in circulation about 8 hours in humans, compared with fibrinogen that has a half-life about 100 hours [5,6]. The nature of neutrophil, which is faster vanish in circulation, affect the neutrophil levels in our study.

Correlation of Smoking Status with COPD Severity

In our study, we found that no significant difference in smoking status distribution with COPD severity ($p > 0.05$) and no significant difference in cigarettes distribution (Brinkman Index) with COPD severity in smokers COPD ($p > 0, 05$). Our findings are in agreement with recent studies by Agusti A. and his colleagues in ECLIPSE, found that the number of cigarettes which consumed almost the same at different stages of GOLD. Smoking is a major risk factor for COPD, but it has been suggested that not all smokers develop into this disease because of the presence the vulnerable and non-vulnerable smokers. Various 'levels of vulnerability' have been proposed by Fletcher and Peto in 1977, and have recently been confirmed in the Framingham Offspring Cohort, which there is a potential difference or genetic interaction with other risk factors, such as nutrition or infection [60].

Correlation of Fibrinogen and Neutrophil Levels with COPD Severity

Our study showed that the highest fibrinogen levels were in grade D and the lowest levels were in grade A ($p < 0.001$). This suggests a significant relationship between fibrinogen levels and COPD severity. Our findings are in agreement with recent studies by Cockayne and his colleagues found that fibrinogen increases with COPD severity, consistent with its role as a chronic inflammatory marker [66]. Several mechanisms proposed as the origin of increased systemic inflammation in COPD. First, inflammatory mediator 'spillover' from the lung compartment; Second, inflammatory reaction due to tissue hypoxia, and third, the reaction caused by the

proinflammatory product of bacterial lipopolysaccharide [65]. In our study, neutrophil levels were higher in line with COPD severity, where the lowest score (55.3) was found in grade A while the highest score (70.6) was found in grade D but not statistically significant ($p > 0.05$). It shows no significant correlation between blood neutrophils and COPD severity. Our findings are in agreement with recent studies by Z He and his colleagues found that in peripheral blood analysis showed no significant difference between groups of COPD subjects based on GOLD criteria with the proportion of white blood cells or neutrophils [67]. Xiong and his colleagues and Lee and his colleagues found that neutrophils did not correlate with severity of respiratory tract obstruction and death in COPD [55, 65]. Based on these studies, it concluded that Neutrophil to Lymphocytes Ratio reflects severity and activity of COPD better than neutrophilia or lymphopenia alone where this ratio integrates neutrophilia as an indicator of inflammation and lymphopenia as an indicator of overall immune deficiency found in COPD [65]. Insignificant increase neutrophil levels with COPD severity in our study is likely due to blood sampling was done by random, not specified at any one time. Circadian variation of neutrophil in circulation has been reported, with neutrophil levels being highest in the blood during the day [68]. Also, in this study, we do not exclude any subject who use antibiotics that can affect neutrophils levels.

Limitations

Several potential limitations of this study are worth discussing. First, our COPD subject still accompanied by a comorbid disease which included exclusion criteria, this may lead to confusion in the results of the study. Several studies have suggested that this disorder is more common in COPD patients and, therefore, may contribute to its proinflammatory state. Secondly, COPD subjects in our study, 28% used inhaled and systemic corticosteroids. This can lead to underestimation of systemic biomarker levels. However, the effect of inhaled corticosteroids on inflammatory biomarkers remains controversial. Thirdly, the number of samples in our study is small so that it could not generalize some findings.

5. Conclusion

There is a significant correlation between increased levels of fibrinogen with the incidence and severity of COPD. In addition, there is a significant correlation between elevated levels of neutrophils with COPD incidence

Acknowledgment

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6. Competing Interest

The authors declare that they have no competing interests.

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