ASSOCIATION BETWEEN BACTERIAL COLONIZATION AND SERUM LEVEL OF INTERLEUKIN-8 IN STABLE CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN MAKASSAR

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ABSTRACT

Background: Chronic Obstructive Pulmonary Disease is a progressive systemic and airway chronic inflammatory disease. Stable chronic obstructive pulmonary disease is a condition of chronic obstructive pulmonary disease that is not being exacerbated. Chronic inflammation of the airways and lung damage facilitates the colonization of bacterial pathogen in the airways. The bacteria in the lower airway activate the immune system by stimulating inflammatory cells, cytokines, proinflammation and chemokine (Interleukin-8). Bacterial colonization can aggravate inflammation and increase inflammation in the airways and systemic including Interleukin-8. Association between bacterial colonization and interleukin-8 has not been widely reported. **Objective:** Determined the association between bacterial colonization and serum level of interleukin-8 in stable chronic obstructive pulmonary disease at Wahidin Sudirohusodo Hospital Makassar. **Methods:** The study design was a cross sectional design. Subjects were 61 subjects who were examined for sputum culture and serum levels of interleukin-8. **Results:** The mean of serum interleukin-8 level was significantly higher, respectively in gram-negative, gram-positive, and no bacterial growth. **Conclusion:** There is an association between bacterial colonization and serum levels of interleukin-8 in stable chronic obstructive pulmonary disease.

KEYWORDS Stable chronic obstructive pulmonary disease, Sputum culture, Interleukin-8

Introduction

Chronic Obstructive Pulmonary Disease is a progressive systemic and airway chronic inflammatory disease. Chronic inflammation and lung damage facilitates the bacterial colonization in the airways. This colonization can induce further inflammation which contributes to the progression of the obstruction degree and exacerbates lung damage.[3] The bacteria in the lower airway activate the immune system by stimulating inflammatory cells, cytokines, proinflammation and chemokine (Interleukin-8).[2,3] Bacterial colonization can aggravate inflammation and increase inflammation in the airways and systemic including Interleukin-8. Stable chronic obstructive pulmonary disease is a COPD condition that is not currently exacerbating. [1] Chronic obstructive pulmonary disease is the 4th largest cause of morbidity and death in the world. Based on data from the 2013 Basic Health Research (Riskesdas) the highest prevalence of COPD was in East Nusa Tenggara (10.0%), followed by Central Sulawesi (8.0%), West Sulawesi and South Sulawesi respectively

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Table 1 Distribution of Research Variable Categories (n=61).

Variable		n	%
Gender	Male	56	91,8
	Female	5	8,2
Age	≥60 years old	40	65,6
	<60 years old	21	34,4
Nutritional Status	Underweight	16	26,2
	Normal	45	73,8
Smoking Status	Light	5	8,2
	Moderate	18	29,5
	Heavy	38	62,3
Severity of COPD	Grade A	14	23,0
	Grade B	47	77,0
Degree of Obstruction	Mild	10	16,4
	Moderate	11	18,0
	Severe	21	34,4
	Very severe	19	31,1
Sputum Culture	Positive-Gram	8	13,1
	Negative-Gram	40	65,5
	No Bacterial Growth	13	21,3

6.7%.[4] Zhang M et al., found the bacterial colonization in stable COPD patients was significantly associated with elevated IL-8 levels.[5] Fujimoto K et al., reported an IL-8 levels in stable COPD patients are significantly higher compared to healthy smokers, and higher levels in exacerbated COPD.[6] Currently research on bacterial colonization in sputum of COPD patients and serum level of IL-8 in Indonesia is limited.

Materials

This study was cross sectional design conducted at Wahidin Sudirohusodo Hospital in Makassar from November 2018 until February 2019. It has been approved by the ethical committee of Hasanuddin University Medicine Faculty with reference number: 1001/H4.8.4.5.31/PP36-KOMETIK/2018.

Methods

Examination methods are sputum and blood serum for interleukin-8. The plate used by Elisa Max Standard Set Human IL-8 by Biolegend. The number of samples is 61 subjects.

A. Population

The population of this study were all inpatients and outpatients with the stable chronic obstructive pulmonary disease at Wahidin Sudirohusodo Hospital in Makassar and its networks. The inclusion criteria were patients diagnosed with stable COPD, didn't have a pulmonary infection and other obstructive diseases, did not have heart failure and inflammatory diseases (Rheumatoid Arthritis), willing to take part in this study and sign a research approval letter.

Table 2 Serum levels of Interleukin-8 to Bacterial Colonization.

Sputum Culture	n	Mean	SD	p
Positive-Gram	8	103,4	22,3	
Negative-Gram	40	145,3	45,2	0,011
NBG	13	117,0	34,6	
*NBG=No Bacterial Growth				

B. Methods and Data Collection

Sampling was done based on non-random sampling, all patient with respiratory complaints that did chest x-ray and spirometry. Subjects that met the inclusion criteria (stable COPD) were examined for sputum culture and serum level of IL-8.

C. Statistical Analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) program version 22. The statistical analysis performed was the calculation of descriptive statistics and frequency distribution as well as the Paired-t, Independent-t and Chi-Square test. The test results are significant if the value is $p < 0.05$.

Results

Subject characteristics based on variable categories are shown in table 1. Most of the subjects studied were male (91.8%), aged ≥60 years (65.6%), normal nutritional status (73.8%) and heavy smokers (62.3%). Clinical and laboratory examination results showed that most COPD grade B (77.0%), gram-negative bacterial colonization (65.6%), and no bacterial growth in 13 subjects (21.3%).

The subjects studied were aged between 27-83 years with an average of 63.6±11.8 years and the mean of IL-8 level was 133.7. (shown in table 2)

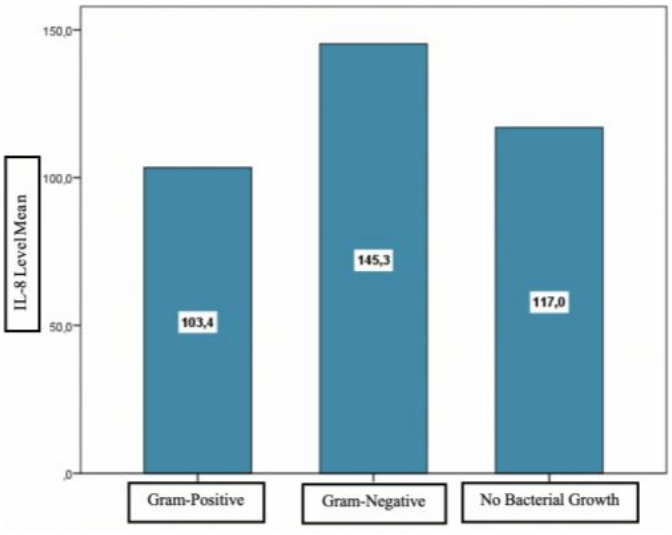

Figure 1: Bacterial colonization with mean IL-8 levels.

Mean serum level of IL-8 was found significantly higher in negative-gram compared to positive-gram and no bacterial growth ($p < 0.05$). The mean serum level of IL-8 was found to be

Table 3 Serum levels of Interleukin-8 to Bacterial Colonization.

Sputum culture	N	Mean	SD	p
Gram-positive	8	103,4	22,3	
Gram-negative	40	145,3	45,2	0,011
NBG	13	117,0	34,6	
*NBG=No Bacterial Growth				

lowest in positive-gram, i.e. 103.4. These results indicate a significant correlation between negative-gram bacterial colonization with high IL-8 levels.

Discussion

The subjects in this study were dominated by male 56 patients. According to the GOLD 2018 guidelines, it was stated that female gender increased the risk of having COPD compared to men[1], but it was found to be different because the subjects in this study used COPD smoking subjects which affected the sex distribution in this study. Based on WHO, the main risk factor for COPD is cigarette smoke, both active smokers and passive smokers, so in this study we used male subjects who smoked. From a study conducted by Terzikhan et al., said that the prevalence of COPD was found to be higher in men and smokers.[7] Zhang et al., also found that COPD patients with smokers had a more severe clinical appearance and heavier degrees of obstruction than nonsmokers.[8] WHO Report on the 2017 Global Tobacco Epidemic in Indonesia found that the percentage of smokers in men is greater than women (64.9% vs 2.1%).[9] The same distribution is also shown in Burden of Obstructive Lung Disease (BOLD) Study involving multinational subjects (Philippines, China, Turkey, Austria, South Africa, Iceland, Germany, Poland, Norway, Canada, America, and Australia) there were more COPD subjects in men than women.[10]

Based on age group, the distribution of stable COPD subjects in this study was obtained more at age $\geq$60 years compared to age <60 years (65.6% and 34.4%) with a mean 63.6 ± 11.8 years. This study used a 60-year-old benchmark according to the age limit of the elderly although various literature shows different values for the age of risk of suffering from COPD.[11] This study correspond with the results of a study conducted by Kim et al., that age >60 years is a significant risk factor for COPD (OR 4.05; 95% CI).[12] Other studies also found almost the same results such as Patel et al 65.9 ± 7.84 years[13] , Miravitlles et al 68,1 ± 9 years[14] , and Intania et al 67.98 ± 7.18 years.[15] Along with increasing age, the ratio of FEV1/FVC in nonsmoker individuals has decreased physiologically at the age of >50 years. Forced expiratory volume for a second decreases 25-30 ml/year from the age of 35-40 years and decreases twice as much to 60 ml/year after 70 years of age. Older age is also related to the possibility of longer duration of cigarette exposure compared to younger COPD subjects.[16] Although no analysis of these factors is done, osteoporosis, depression, cardiovascular disorders, and obesity in the elderly can affect exercise capacity even though it is not directly affect the COPD itself.[17] Thus this can affect the age distribution in COPD patients.

The COPD subjects in this study were all smokers, where heavy smokers dominated by 62.3%, moderate smokers 29.5% and light smokers by 8.2%. This correspond with theory that pollutants from cigarettes cause chronic inflammation of the airways. This chronic inflammation destroys the pulmonary parenchyma which causes emphysema and damages the lung defensive mechanism due to small airway fibrosis. The amount and duration of cigarette exposure will determine the severity of inflammation that occurs.[1] This theory was demonstrated in epidemiological studies in Japan which showed that smoking was a strong risk factor for COPD and showed a dose-response relationship between COPD and smoking in the male population in Japan. [18]

Bacterial colonization in the distal airway is often found in stable COPD patients. Decreased phagocytic activity of alveolar macrophages increases bacterial colonization. Bacteria such as Hemophilus Influenza, Streptococcus pneumonia, and Moraxella catarrhalis are found in 25% of stable COPD patients and more than 6 50% in exacerbation COPD.[19] This shows that microbiomes in humans vary. The composition of the lung microbiome is at least determined by three factors. The first factor is microbial immigration, including microaspiration, inhalation of bacteria, and direct mucosal dispersion. The second is microbial elimination, such as cough, mucociliary clearance, and innate – adaptive host defences. The third is regional growth conditions (nutrient availability, oxygen tension, temperature, pH, concentration of inflammatory cells, activation of inflammatory cells, local microbial competition, and host epithelial cell interactions). New evidence shows that the composition of microbiota in the airways differs according to the degree of disease, inhaled corticosteroids used, inhaled bronchodilators, and previous antibiotic. Chronic obstructive pulmonary disease is characterized by persisting inflammatory. Inflammatory is associated with reduced microbiome complexity.[20] Desai et al., found higher serum IL-8 levels in COPD patients with bacterial colonization. Bacterial colonization will increase the inflammatory response of the host which increases serum IL-8 levels.[21] Tumkaya et al., also found an association between the number of bacterial colonization and markers of inflammation in the airways in stable COPD.[22] Another study by Liu et al., found IL-8 levels higher in COPD patients compared to Bronchial Asthma, but the COPD sample in this study was exacerbated COPD.[23] Zhang M et al., examined bacterial colonization in lower airway from sputum induced with 3% NaCl in stable COPD patients, concluding that bacterial colonization in the lower airway is significantly associated with elevated IL-8 levels. [5] Fujimoto K et al., found IL-8 levels in stable COPD patients is significantly increased compared to healthy smokers, and higher levels in exacerbated COPD patients.[6] These results are in line with this study where the mean IL-8 levels were found to be the highest at negative-gram bacteria compared to positive-gram colonization and no bacterial growth, which is 145.3 ($p < 0.05$).

Conclusion

There is an association between bacterial colonization and serum levels of interleukin-8 in stable chronic obstructive pulmonary disease.

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Competing Interests

The authors declare that there is no conflict of interest in this study.

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Ethics Committee

The ethical committee has approved it of Hasanuddin University Faculty of Medicine with reference number: 1001/H4.8.4.5.31/PP36-KOMETIK/2018.

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