Analysis on the Relationship Between Chronic Asthma Based on Spirometry, and Interleukin 4 and Interleukin 13

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Abstract. Asthma is one of the global health issues that occurs in both developed and developing countries. There are a few ways to classify asthma, based on clinical presentation and spirometry, based on immunologic reaction using Interleukin 4 (IL-4), Interleukin 13 (IL-13) and Eosinophil Cationic Protein as chronic respiratory tract inflammation markers. Observational study was held using a cross-sectional design among 46 patients diagnosed with chronic asthma at Dr. M. Djamil General Hospital. Data analysed was using t-test to found relationship between chronic asthma with IL-3 and IL-4. Among 46 patients, 23 samples were mild chronic asthma, 72% of the sample were women (p=0,190), age of the patients with mild chronic asthma was 24,96 years old ± 7,64 year SD, while severe chronic asthma were 29,52 years old±8,88 year (p= 0,232). Interleukin 4 (IL-4) was found less in mild chronic asthma than the severe one, 3,44 (±0,73) pg/ml and 3,64 (±1,11) pg/ml respectively.IL-13 in mild chronic asthma was in the higher level than in the severe ones. The level of IL-4 was 295,23 (±2,38) pg/ml in mild chronic asthma, while the level of IL-13 in severe chronic asthma was less than the mild ones, 294,21 (±3,24) pg/ml. There is no significant relationship between the severity of chronic asthma based on spirometry and level of Interleukin 4. Interleukin 13 was significantly related to chronic asthma.

Keywords: Asthma, Interleukins, Respiratory Function Test.

1 Introduction

Asthma is one of the global issues in the world, both in developed and developing countries. According to GINA (Global Initiative for Asthma) in 2012, approximately there are three hundred million people with asthma around the world, with an increasing mortality rate up to 180.000 cases per year [1]. WHO (World Health Association) share the same result, with an increased asthma prevalence within thirty years. GINA estimated 300 millions of people. Diagnosed with asthma and will be around 400 millions or more by the year 2025, due to underdiagnosis. Poor air-quality and lifestyle changes might be the cause of increasing asthma prevalences. Asthma prevalences in the world are around 1-18% [1].

Asthma is a chronic respiratory tract inflammation, with a lot of contributing elements. The chronic inflammation causes hyperresponsiveness, bronchoconstriction, edema, and hypersecretion of the gland, resulting in airway limitation with periodic clinical manifestations of wheezing, shortness of breath, chest tightness, and, coughing especially at night/dawn.

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Spirometry is one of the many ways to measure the function of the lungs, by calculating the expiratory volume after maximum inspiration. This test determines whether it is an obstructive disease (such as asthma or COPD) or restrictive (size of the lungs shrinks like in fibrotic lungs). Spirometry is the most effective way to show the severity of asthma [2]. A study conducted by Efren (2011) argued that IL-13 was one of the main targets in identifying asthma. This study was by Grunig (2012) suggested that IL-13 affects asthma prognosis. Based on the previous study, the author would like to conduct a study to investigate the relationship between chronic asthma severity based on spirometry with interleukin 4 and interleukin 13 [3].

2 Materials and Methods

This was a cross-sectional observational study. Asthma patients were categorized into 2 groups, mild and severe chronic asthma who came to Dr. M. Djamil Padang Central Hospital from January 2017 to June 2017. Data was collected using consecutive sampling method, and numeric univariate data was presented in mean tendential, standard deviation, minimum and maximum value. The t-test was done to analyze single data with normal distribution. Meanwhile, Mann Whitney test was done to analyze data with abnormal distribution. A logistic regression model was done to analyze multivariate data.

3 Results

Based on spirometry result, total respondents with mild and severe chronic asthma is presented in table 1

Table 1. Proportion of Respondent

Asthma severity	FEV1 value	Total (n)
Mild chronic asthma	≥ 80	23
Severe chronic asthma	<80	23

Table 4.1 shows mild (FEV 1 ≥ 80) and severe (FEV 1 < 80) chronic asthma has a similar proportion, 23 patients for each group.

The respondent's characteristics include as age, sex, and occupation are presented in table 2

Table 2. Characteristic of respondent

No	Characteristic	Asthma severity		P
		Mild	Severe	
1.	Sex {n (%)}			
	Male	4 (17,4)	9 (39,1)	0,190
	Female	19 (82,6)	14 (60,9)	
2.	Occupation {n (%)}			
	House wife	1 (4,3%)	2 (8,7%)	
	Civil servant/retired	5 (21,7%)	5 (21,7%)	0,152
	Private sector	1 (4,3%)	6 (26,1%)	

	Students	16 (69,6%)	10 (43,5%)	
3.	Age			
	{Mean (SD)}	24,96 (±7,64)	29,52 (±8,88)	0,232

Table 2 above shows that there are more female compared with male, as many as 33 out of 46 (72%). Male and female who had mild chronic asthma were 19 and 4, respectively, meanwhile in severe chronic asthma were 14 and 9, respectively. Comparative test between two groups showed p-value of 0,190 (p > 0,05). Therefore there was no significant sex difference between mild and severe chronic asthma.

Table 4.2 also showed mean of age in mild and severe chronic asthma were 24,96±7,64 and 29,52±8,88, respectively. Comparative test showed p-value of 0,232 (p > 0,05). This result showed there was no significant age difference between both groups.

All 46 respondents underwent an examination of Interleukin 4 (IL-4) using *Human IL-4 Elisa Kit,* and the result is shown in table 3:

Table 3. Mean of cytokine IL-4 in mild and severe chronic asthma.

Dependent variable	Mean (SD) (pg/mL)	Min-Max
Mild chronic asthma	3,44 (±0,73)	2,39 – 5,66
Severe chronic ashtma	3,64 (±1,11)	2,39 – 7,62

Based on the previous table, we can conclude that mean of IL-4 in mild chronic asthma was lower compared with severe asthma, 3,44 (±0,73) pg/ml and 3,64 (±1,11) pg/ml, respectively.

Table 4. Mean of cytokine IL-13 in mild and severe chronic asthma

Dependent variable	Mean (SD) (pg/mL)	Min – Max
Mild chronic ashtma	295,23 (±2,38)	292,09 – 300,91
Severe chronic asthma	294,21 (±3,24)	292,09 – 304,4

Based on distribution IL-13 according to asthma diagnosis, it seems IL-13 in mild chronic asthma was higher compared with severe chronic asthma, which was 295,23 (±2,38) pg/ml, 294,21 (±3,24) pg/ml, respectively.

To determine whether there were significant differences between levels of IL-4, IL-13, and ECP in the mild and severe chronic asthma group, a comparative test was conducted between the dependent and independent variables. Before the comparative test is carried out, the data distribution normality test is performed by using *saphiro wilk* normality test because the number of samples is < 50 people. From the results of the normality test, the results are obtained as shown in the following table.

Table 5. Normality Tests for IL-4, IL-13, and ECP levels in Mild Chronic Asthma and Severe Chronic Asthma

Variable	P Value	Transformation variable	P value
IL-4	0,000	Log IL-4	0,000
IL-13	0,000	Log IL-13	0,000
ECP	0,000	Log ECP	0,000

From Table 4.6, it is known that both IL-4, dan IL -13 do not have normal data distribution, although efforts have been made to normalize the previous data distribution because the results of the saphiro wilk test showed $p < 0.05$. Therefore the comparative test used is a non-parametric test, namely the *mann-whitney* test. The results of the comparative test of *mann-whitney* obtained the following results:

Table 6. Mann Whitney Test Results on Dependent and Independent Variables

Independent variable	Dependent variable	Average (SD)	P Value
IL-4	Mild Chronic Asthma	3.44 (± 0.73) pg / ml	0.552
	Severe Chronic Asthma	3.64 (± 1.11) pg / ml	
IL-13	Mild Chronic Asthma	295.23 (± 2.38) pg / ml	0.043
	Severe Chronic Asthma	294.21 (± 3.24) pg / ml	
ECP	Mild Chronic Asthma	27.96 (± 6.53) µg / L	0.904
	Severe Chronic Asthma	28.90 (± 2.62) µg / L	

From the table above it is found that the only variable that has a p-value < 0.05 is the IL-13 level. While the other two variables, IL-4 and ECP, have $p > 0.05$. This shows that there is a significant relationship between the degree of chronic asthma based on spirometry and IL-13 levels. Whereas for IL-4 and ECP levels, there was no significant relationship between these variables and the degree of chronic asthma based on spirometry.

The results of the bivariate analysis showed that only one independent variable, namely IL-13, was related to the degree of asthma so that multivariate analysis in this study could not be carried out.

2 Discussion

This study was conducted on 46 samples, consisting of two groups namely chronic asthma, mild and severe. The diagnosis of chronic asthma is made through history and physical examination, then the classification of the degree of asthma is performed using spirometry examination. Respondents of the study were control patients in non-infectious pulmonary disease who were newly diagnosed or had been known to have asthma before.

The mean age in patients with mild degrees of chronic asthma in this study was 24.96 years ± 7.64 years, while in the severe chronic asthma group the mean age was higher than the mild degree of 29.52 years ± 8.88 years. A comparative test between two groups was carried out to see whether there were significant differences in age between the group of mild and severe chronic asthma. The results of this comparative test obtained the results of the p-value of 0.232 ($p > 0.05$). These results indicate that there is no significant age difference between the two For sex, the number of research respondents with female sex is more than men.

The number of patients with chronic asthma female is 33 people from 46 samples of the study (72%), while men only numbered 13 people (28%). Gender distribution based on asthma group found that asthmatic patients with female gender both from the mild and severe asthma group had more numbers compared to patients with the male gender. The comparative test between the two groups got the p-value of 0.190 ($p > 0.05$) which showed that there was no significant gender difference between the mild chronic asthma and severe chronic asthma groups.

Most of the research that raised the theme of asthma had a much larger number of female respondents than men. One of the contributing factors is the association of asthma with body mass index (BMI) and fat bodies, where there is more fat in the body in women than men. Observational and cluster analysis found that gender factors were one of the factors influencing the appearance of symptoms and management of asthma in addition to other factors such as environment, race, obesity and *specific endotypes* [4].

IL-4 is released by Th2 cells that contribute to asthma immunopathogenesis. In addition to IL-4, Th2 cells that are sensitized by allergens in asthma sufferers also release IL-5. Both IL-4 and IL-5 will affect B lymphocytes to synthesize IgE. IgE that has been synthesized will bind to mast cells and there is a process of mast cell degranulation and eosinophils which will release mediators, one of which is ECP, which will cause bronchial hyperactivity which is the pathogenesis of obstruction or asthma symptoms, resulting in an asthma condition chronic acute asthma or other languages are often referred to as chronic, acute exacerbations of asthma [6].

The IL-13 number, measured in all of the study samples using ELISA method shows that the mean of IL-13 in mild chronic asthma is higher than severe asthma. The IL-4 number in mild chronic asthma has the mean of 295,23 (±2,38) pg/ml. Meanwhile the mean of IL-13 number in severe asthma in lower than mild asthma, 294,21 (±3,24) pg/ml. In another study about the IL-13 number in asthma patients has different results, and the exact number for IL-3 serum has not been set. Pukelsheim *et al* got a predictive number of IL-3 around 0,87 (0,50-1,52 pg/ml) [7]. Davoodi,s study in 2012 showed that the median number of IL-3 serum level is 40,0 pg/ml in asthma patients and 58,25 pg/ml in non-asthmatic patients (normal population), and in these two groups there is no difference between the IL-13 number [8]. After the comparative analytical study between severe asthma and mild, the result showed that there is no difference between the IL-4 serum in severe asthma or mild asthma.

A genetic factor might play a big role in asthma pathogenesis and better used to determine the severity of asthma rather than the number of cytosine serum. Ashford's study in 2007 found a correlation between gene which expressed IL-4 with IL-4 serum, but in some asthma cases that expressed IL-4 gene, the IL-4 serum still within the normal range. Not many literatures explained the difference of IL-4 number in patients with mild and severe asthma [9].

In addition to IL-4, one of the cytokines that get attention in the pathogenesis of asthma is interleukin 13 (IL-13) which is a protein in humans encoded by the IL-13 gene (Lackie J et al., 2010). IL-13 has a biological effect similar to IL-4. IL-13 is a cytokine secreted by various types of cells, especially Th2 cells which are mediators of inflammation and disease (6). Although IL-13 has functions and effects on immune cells similar to IL-4. In addition to inducing Th2 cells [10], IL-13 is thought to be a more central mediator of physiological changes caused by inflammation in many tissues [11]. IL-13 is associated primarily with the induction of airway disease and has an anti-inflammatory role. IL-13 induces a class of proteins known as matrix metalloproteinases (MMPs) in the airways. IL-13 induces MMP as part of a protective mechanism against excessive inflammation which predisposes to shortness of breath [12].

The results of the examination of IL-4 levels in the serum of asthmatics carried out in this study found that the mean level of IL-4 in mild chronic asthma was smaller than the mean of severe asthma. In mild chronic asthma levels of IL-4 with a mean of 3.44 (± 0.73) pg/ml. While the mean level of IL-4 in severe asthma is higher than the mild degree of 3.64 (± 1.11) pg/ml. No literature establishes a definite range of normal IL-4 levels found in normal people and asthma patients.

IL-13 levels measured to all study samples using the ELISA method found that the mean level of IL-13 in mild chronic asthma was higher than that of severe asthma. In mild chronic asthma levels of IL-4 obtained have a mean of 295.23 (± 2.38) pg/ml. While the mean level of

IL-13 in severe asthma is lower than the mild degree of 294.21 (± 3.24) pg/ml. Other studies examining IL-13 levels in asthma patients have different findings, and exact levels for serum IL-13 have not been established. Pukelsheim et al. research found that the predictive value of IL-13 was 0.87 (0.50-1.52 pg/ml) [6]. Research by Davoodi, 2012 found that median serum IL-13 levels were 40.0 pg/ml in asthma patients and 58.25 pg/ml in non-asthmatic patients (normal population), and there were no differences in IL-levels in these two groups. 13 meaningful between the two groups [12]. After a comparative analysis between two severe and mild asthma groups in this study, the results did not show a significant difference between IL-4 levels in mild asthma patients with severe asthma.

The non-parametric comparative test, namely the Mann-Whitney test conducted on IL-13 levels in two asthma groups in this study obtained results that IL-13 is the only variable that has a p-value <0.05. While the other two variables, IL-4 have p> 0.05. This shows that there are significant differences in IL-13 levels in the mild degree of chronic asthma and severe chronic asthma groups.

In accordance with the theory of Gour et al. which states that IL-13 is higher in inflammatory tissues than IL-4, this is caused by several things, namely higher IL-13 local production than IL-4 and other cytokines, the increasing affinity of type 2 receptor complex for IL-13 and the mechanism of inhibition induced by IL-4 through type I receptor complexes so that IL-4 levels are lower than IL-13 [12].

According to the literature, there are differences of opinion regarding the effect of high levels of IL-13 on the severity of the degree of an asthma attack. Some researchers initially agreed that IL-13 would decrease with increasing degrees of asthma. This is caused by the role of IL-13α2 receptors which are considered as decoy receptors. These receptors will bind to IL-13 and induce the process of asthma pathogenesis but the more levels of IL-13α2 and IL-13 receptors are bound, the degree of asthma will be lighter. A study of mice found that the soluble form of the IL-13α2 receptor was found in mice, the soluble form of this receptor and its administration to mice decreased the degree of inflammation that occurred in the rat's airway. Another study of rats made into IL-13α2 receptor deficiency showed higher serum IgE levels than wild rats. However, the mechanism of IL-13α2 and IL-13 receptor binding mediating the inflammatory process is still unclear. A study suggests that IL-13 will send signals through the IL-13α2 receptor to stimulate a variant of a protein activator (AP-1) which will initiate the transcription process of TGF-β1 [12].

Another thing that can be the cause of the inverse relationship between serum IL-13 levels and the degree of asthma in this study is the sample studied is a sample that has received treatment from the lung section Dr. M. Djamil Padang. Severe asthma will be treated using a low to high dose inhaled corticosteroid controller, while in mild asthma the use of corticosteroids as a controller is rarely even not recommended if an asthma attack can be overcome with Short Acting B2 inhalation agonist alone [1]. According to Gour et al., 2015, IL-13 levels will decrease in asthmatic patients who received therapeutic regimens for allergen and corticosteroids desensitization [12]. The use of long-term therapy should be a factor to be considered in assessing the relationship between cytokine levels and the degree of asthma [12]

The results of this study found that there was no proven significant relationship between the degree of chronic asthma and levels of IL-4 can be caused because researchers divided the degree of chronic asthma into two criteria: mild chronic asthma (FEV1 ≥ 80%) and severe chronic asthma (FEV1 <80%) so this difference is very narrow.

4 Conclusion

There was no correlation between the degree of chronic asthma based on spirometry and Interleukin levels 4. There was a relationship between the degree of chronic asthma based on spirometry and levels of Interleukin 13. There was no relationship between the degree of chronic asthma based on spirometry and levels of Cationic Protein Eosinophil.

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