# LEVEL OF INTERLEUKIN-6 IN STABLE COPD PATIENTS USING THE EXHALED BREATH CONDENSATE

## Kadar Interleukin-6 Pasien PPOK Stabil Menggunakan Uji Napas Kondensasi

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**ABSTRACT**

Chronic obstructive pulmonary disease (COPD) is a chronic systemic inflammatory disease that is associated with high morbidity and mortality rates. Interleukin 6 is a proinflammatory cytokine that can be used as a marker of COPD treatment. We conducted a cross-sectional study involving 83 stable COPD patients at X Jakarta Hospital, mostly male (90,4%) with mean ages was 66,07 years. Patients were interviewed using the CAT and mMRC scoring. Patients also underwent a spirometry and had their condensed breaths collected using a device made of a S-shaped glass tube that was immersed in a thermos filled with ice cubes and collected for ELISA examination. This study result was IL-6 detected in 76 patients (91,56%) with mean level of IL-6 was 0,702 pg/ml ± 0,41 pg/ml. We conclude that IL-6 levels from exhaled breaths of stable COPD patients can be collected using a condensation exhalation device and can be recommended as biomarkers of successful treatment of COPD.

**KEYWORDS:**
Exhaled Breath Condensation Test, Stable COPD, IL-6 Level

**ABSTRAK**

Penyakit paru obstruktif kronik (PPOK) merupakan penyakit inflamasi sistemik kronis yang berhubungan dengan angka morbiditas dan mortalitas yang tinggi. Interleukin 6 merupakan sitokin proinflamsi yang dapat digunakan sebagai penanda keberhasilan pengobatan PPOK. Penelitian ini menggunakan design cross sectional dengan 83 sampel pasien PPOK stabil di RS X Jakarta. Pasien diwawancarai menggunakan skor CAT dan mMRC. Pasien juga melakukan pemeriksaan spirometri dan dikumpulkan hembusan napas kondensasinya menggunakan alat yang terbuat dari tabung kaca berbentuk huruf S yang direndam pada termos berisi es batu sehingga hembusan napasnya dapat dikumpulkan untuk kemudian diperiksakan ELISA. Hasil penelitian didapatkan bahwa IL-6 terdeteksi pada 76 pasien (91,56%) dengan kadar IL-6 rata-rata 0,702 pg/ml ± 0,41 pg/ml. Kami menyimpulkan bahwa kadar IL-6 dari hembusan napas pasien PPOK stabil dapat dikumpulkan menggunakan alat hembusan napas kondensasi dan untuk selanjutnya dapat direkomendasikan untuk dijadikan biomarker keberhasilan pengobatan pasien PPOK.

**KATA KUNCI:**
Uji Hembusan Napas Kondensasi, PPOK Stabil, Kadar IL-6

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## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic disease that has become a major health problem in the world and has a high morbidity and mortality rate. The death rate due to COPD ranks 4th in the world and is estimated to increase to number 3 in 2020 (Vestbo *et al.,* 2013). The prevalence data for COPD in Indonesia in 2013 is 5.6% per million according to *Riskesdas*. The goals of COPD management are to prevent disease progression, prevent and treat exacerbations, reduce symptoms, improve exercise tolerance and improve quality of life (Vestbo *et al.,* 2013; WHO, 2018; RISKESDAS, 2013). Moraes *et al.,* in 2014 was found that IL-6 levels serum patient stable COPD was still higher than controls (de Moraes *et al.,* 2014). El-shimy et al., in 2014 using serum samples and bronchial rinses found increased levels of IL-6 in

exacerbated COPD and their levels increased with increasing degrees of COPD compared to the control group (El-shimy *et al.,* 2014). Evaluation of changes in forced expiratory volume (FEV1) within one second by spirometry in COPD patients can only evaluate therapeutic interventions with bronchodilators but is not specific, hence the use of exhaled breath condensation tests to assess levels of inflammatory cytokines in the airways of COPD patients is useful for evaluating pathway-targeted COPD treatment that was targeted in the pathway of inflammatory process (Chhabra, 2012).

Assessment of cytokines in COPD patients in previous studies used more invasive methods with blood plasma samples, sputum induction and bronchial rinses. The exhaled breath condensation test is a non-invasive method, but the use of this tool to assess levels of inflammatory cytokines in COPD patients like IL-6 has never been studied, so researchers are interested using the exhaled breath condensation test to assess cytokine levels in stable COPD patients.

**METHOD**

This research was a cross sectional study with a descriptive analytic method. The research was conducted at the Asthma-COPD Policlinic of X Jakarta Hospital and the IMERI Laboratory. This research has etical approved by Komite Etik Penelitian Kesehatan Persahabatan Hospital with register number: 08/KEPK-RSUPP/01/2019. We collected all stable COPD patients that went to the Asthma-COPD Clinic of X Jakarta Hospital in January - December 2019. Inclusion criteria are Patients ≥ 40 Years of age, stable COPD patients who were treated at the asthma-COPD clinic of *Persahabatan Hospital* both men and women who did not have exacerbations in their pass 4 weeks, COPD patients who volunteered to participate in the entire research program by agreeing to the research consent form and signing it. Exclusion criteria are COPD patients with acute exacerbation in the last 4 weeks, psychiatric patients and pregnant women.

Primary data were obtained from interviews and questionnaires that had been prepared and patients also underwent a spirometry examination (CHEST Spirometry) and their spirometry data was taken, we also collected exhaled breath condensation test samples using a S-shaped glass tube that was immersed in a thermos filled with ice cubes and then the sample obtained will be carried out with an ELISA examination in the IMERI laboratory to measure IL-6 level. The design and method of the device and collection were adjusted based on research by Vyas, et al in 2012 (Figure 1) (Vyas *et al.,* 2012).
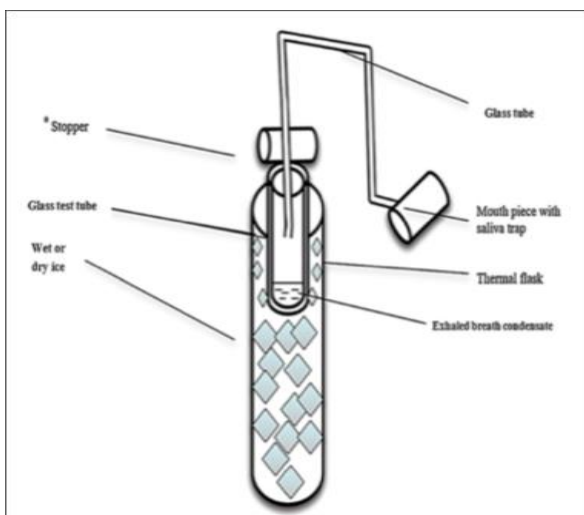
**Figure 1. Exhaled breath condensation device** (Vyas *et al.*, 2012)

**Table 1. Characteristics of Stable COPD Subjects**

| Variabel | Total (n= 83) | % |
|---|---|---|
| Age: Mean ± SD (year) | 66,07±8.44 | |
| Sex: | | |
| Male | 75 | 90,4 |
| Female | 8 | 9,6 |
| Smoking status: | | |
| Non smoker | 12 | 14,5 |
| Smoker | 71 | 85,5 |
| Brinkman Index: | | |
| Light | 13 | 15,7 |
| Medium | 20 | 24,1 |
| Severe | 38 | 45,8 |
| Exacerbation history in the past year | | |
| < 2 | 56 | 72,7 |
| ≥ 2 | 21 | 27,3 |
| CAT score | | |
| < 10 | 44 | 53 |
| ≥ 10 | 39 | 47 |
| mMRC Score | | |
| 0-1 | 38 | 45,8 |
| ≥ 2 | 45 | 54,2 |
| EBC value: Mean ± SD (mL) | 0.81566 ± 0.532 | |

Samples were obtained by means of consecutive sampling until the minimum number of samples was met. Data analysis using SPSS 18 which then a normality test was performed using the Kolmogorov-Smirnov test with p value> 0.05 and homogeneity test with Levene's test with p value <0.05, to obtain IL-6 level data was homogeneous.

**RESULT AND DISCUSSION**

During the study period from January 2019 to December 2019, the number of research subjects obtained was 85 stable COPD patients but 2 subjects was excluded due to failing maneuvers during spirometry, so there are 83 stable COPD patients from the minimum sample 60 patients. In this study, the average age of stable COPD subjects was older than 60 years of age, mostly males. The smoking status of most subjects were smokers with the most brinkman index is severe category. The basic characteristics of COPD patients in this study are shown in Table 1.

The characteristic of spirometry value in 83 patients stable COPD: VEP1 / VEP1 predicted (%) was 52,96% ± 21,83 ($p$= 0.01) with the VEP1 / KVP (%) value was 54,17% ± 10,26 ($p$= 0.08). The increase in VEP1 (%) was 11.42% ± 10.3 ($p$= 0.001). The most stable COPD degree in this study was COPD group B with the highest degree of obstruction being GOLD 3 (Figure 2).

**COPD Stabile Group**

- COPD Group A
- COPD Group B
- COPD Group C
- COPD Group D



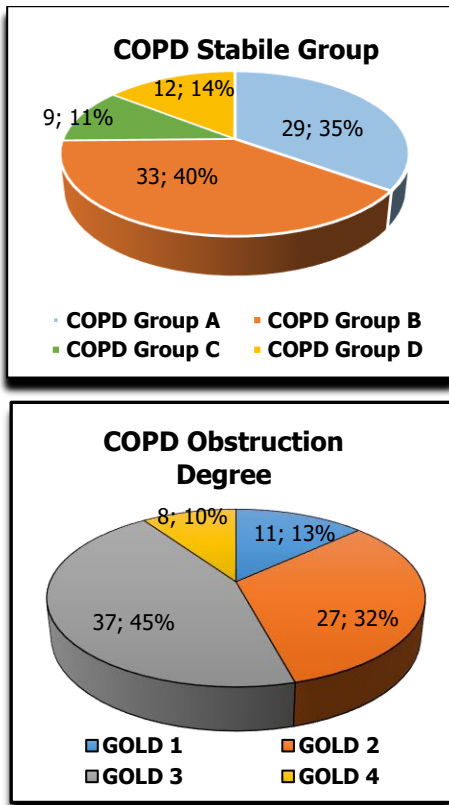**COPD Obstruction Degree**

- GOLD 1
- GOLD 2
- GOLD 3
- GOLD 4

**Figure 2.** Detectable levels of IL-6 by COPD Group in Persahabatan Hospital

In this study, of the 83 subjects, as many as 76 (91,56%) subjects was detected IL-6 levels by the ELISA method. IL-6 levels from 76 subjects, the mean level was 0.702 pg / mL ± 0.416. The mean levels of IL-6 level by COPD group showed in table 2 and IL-6 cytokine levels detected in stable COPD subjects according to the degree of COPD group (Graphic 1).

**Table 2. IL-6 Cytokine Mean Levels Detected in Stable COPD Patients According to The Degree of COPD Group**

| Stable COPD Group (n=76) | Mean levels of IL-6 (pg/mL) $\pm$ SD |
|---|---|
| COPD Group A (n=24) | 0,716 $\pm$ 0,07 |
| COPD Group B (n=31) | 0,713 $\pm$ 0,67 |
| COPD Group C (n=9) | 0,638 $\pm$ 0,22 |
| COPD Group D (n=12) | 0,693 $\pm$ 0,13 |
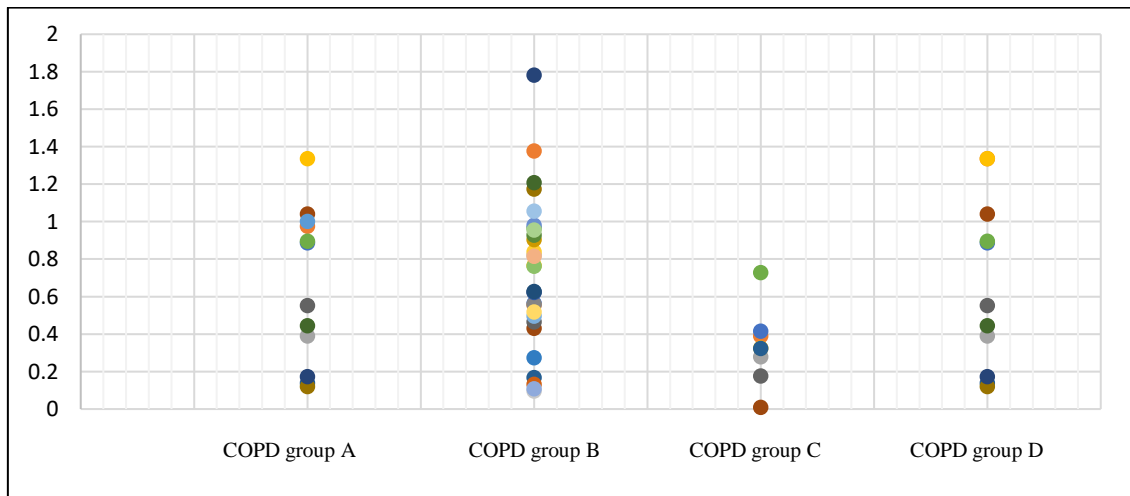


**Graphic 1. Scatter plot image of IL-6 levels by the degree of stable COPD group**

In this study, the average age of stable COPD subjects was 66,07 ± 8,844 years with the youngest age being 47 years and the oldest age was 85 years and most of the subjects were male and most were smokers with the most Brinkman index (BI) found to be severe IB. The results of this study are in line with Barnes in 2016 which states that COPD is more

common in elderly men because of the high prevalence of smoking in men, but now there has been a change, namely that the prevalence of COPD is more common in women in line with the increase in smoking habits in women (Barnes, 2016). Lung function also begins to decline progressively with age and these changes include changes in the

structure of the lungs, chest wall and respiratory muscles (Brandsma *et al.,* 2014).

El-Shimmy *et al.* in 2014 study regarding levels of IL-6, IL-8 and TNF-α from serum and fluid samples of COPD patients who were stable and healthy found that levels of IL-6, IL-8 and TNF-α were significantly increased in patients with FEV1 degree of obstruction <50% prediction than stable COPD patients with 80% degree of obstruction ≥ FEV1 ≥ 50% prediction and for healthy non smoking patients (El-shimy *et al.,* 2014).

In this study, IL-6 levels were moreable to be detected in 76 (91,56%) subjects from the exhaled breath condensation test. Serum IL-6 levels according to the study of Wei et al., 2015 were also found to be increased in stable COPD than in healthy patients (Wei *et al.,* 2015). A study by Walter et al. in 2008 and a review article by Talman et al. in 2019 stated that serum IL-6 is one of the cytokines that can predict decreased lung function (VEP1) (Walter *et al.,* 2008; Talman *et al.,* 2019).

Interleukin 6 is a proinflammatory cytokine produced in the lungs by intertisial fibroblasts, alveolar macrophages, large blood vessels and bronchial epithelial cells, added to that IL-6 can also be produced by various cells and organs including adipocyte, muscle, liver and lung cells. The IL-6 cytokine will be elevated in chronic pulmonary inflammatory conditions such as allogeneic

transplantation, bleomycin which induces fibrosis and in interstitial lung disease. Biologically, IL-6 is the main cytokine regulator of C-reactive protein and fibrinogen in the liver. Interleukin 6 plays an important role in hematopoiesis, causing excessive thrombocytosis and leukocytosis. IL-6 levels were also found to be elevated, especially in exacerbated COPD conditions, however IL-6 levels from exhaled breath condensation tests were still detectable in normal people and stable COPD, although the levels would be higher in exacerbated COPD patients (El-shimy *et al.,* 2014).

Sin *et al.* in their article states that in a stable COPD condition IL-6 plays a role in the increase in levels of CRP, fibrinogen, leukocytes and blood platelets compared to healthy control patients. Interleukin 6 plays a role in systemic inflammation in COPD. Overexpression of IL-6 also causes emphysema and airway inflammation. Therefore, IL-6 is an important biomarker in COPD (Sin and Man, 2008). This study has limitations, namely that healthy subjects who are not COPD are not included in the study, so that the baseline value of the cytokines IL-6 levels of healthy subjects were not obtained.

**CONCLUSION AND SUGGESTIONS**

From this study, the exhaled breath condensation test can be used to detect cytokine levels in stable COPD by group with ELISA examination. In this study, IL-6 levels can be used

for monitoring therapy and as a therapeutic target in stable COPD patients. Further studies are needed in healthy subjects without COPD to determine the cut off point value of cytokine IL-6 levels in healthy patients with stable COPD patients. In future studies, it should be done completely in stable COPD without comorbidities in the lungs.

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