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Detection of Volatile Organic Compounds (VOC) in COPD Patients and Its Correlation with Serum Leukotriene B4 and Neutrophil

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Abstract

Background: COPD has been estimated become the third leading cause of death throughout the world in 2030. COPD can cause extrapulmonary complications by promoting chronic systemic inflammation. Spirometry is still the diagnostic standard of COPD, but certain maneuvers and trained operator are needed. Leukotriene B4 and neutrophil are also involved in COPD pathogenesis. In last few decades, the development of non invasive method for detecting lung disease has emerged, such as Volatile Organic Compounds (VOC) examination. This study aims to analyze the difference of VOC in exhaled breath of stable COPD and control subjects, also the correlation between VOC with serum Leukotriene B4 and neutrophil.

Material and Method: A case-control study was conducted recruiting 40 stable COPD patients and 40 control subjects. Exhaled breath sample was collected for VOC detection and venous blood sample for examining Leukotriene B4 and neutrophil. Breath sample was analyzed using an arrayed sensor breath analyzer to check the concentration of 13 VOCs.

Result: There were significant difference ($p<0.05$) of CO_2, C_2H_5OH, C_3H_6O, NO_2, CO, NH_3, C_6H_6, dan C_3H_8 levels between COPD patients and control subjects. Further analysis showed that C_2H_5OH was significant correlated with Leukotriene B4. However, there was no VOC correlated with serum neutrophil in this study.

Conclusion: Several VOCs may differ COPD patients from control subjects. Furthermore, VOC is also correlated with serum Leukotriene B4 in COPD.

Keywords: Volatile Organic Compounds, Leukotriene B4, Neutrophil, COPD

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is preventive and treatable disease with persistent respiratory symptoms caused by gas or toxic particle exposure [1]. Beside affecting lung, COPD also

promotes chronic systemic inflammation contributing to extrapulmonary complications. Smoking is main risk factor of COPD [2]. According to WHO, 2.9 million people die due to COPD and it has been estimated that COPD become the third leading cause of death globally in 2030. Prevalence of COPD in Indonesia was 3,7% or around 9,2 million people in 2018 [3].

Spirometry is still diagnostic standard for COPD, but it needs certain maneuvers and trained operator. In last few decades, the development of non invasive method for detecting lung disease has emerged, such as Volatile Organic Compounds (VOC) examination [4]. VOC is compounds that evaporate easily and produced endogen or exogenously [5]. Most wide used techniques for detecting VOC are gas chromatography and electronic nose (eNose). VOC identification using breath analyzer is simple and does not need certain technique [6].

COPD is related to activation of macrophage, neutrophil, and CD8 T-lymphocyte in airway and lung parenchyma because of smoke and other toxic substances. This will stimulate production of cysteinyl leukotriene (LTB4, LTC4, LTD4, and LTE4) which can induce neutrophil recruitment, airway smooth muscle constriction, vascular permeability, and mucus hypersecretion [7]. Neutrophilia in airway can correlate with lung function decline in COPD [8].

This study aims to analyze the difference of VOC in exhaled breath of stable COPD and healthy subjects, also the correlation of VOC with serum Leukotriene B4 and neutrophil.

Material and Method:

The study design used was case-control study conducted in Saiful Anwar Hospital recruiting 40 stable COPD patients and 40 healthy subjects that fulfilled inclusion and exclusion criteria. Outpatient and inpatient were included in this study from period of March until October 2022. Exhaled breath sample was collected for VOC detection and venous blood sample for examining Leukotriene B4 and neutrophil. Breath sample was analyzed using an arrayed sensor breath analyzer to check the concentration of 13 VOCs. The breath analyzer used was developed by Physics Department of Universitas Brawijaya named "*Ubreath*". We checked concentration of LTB4 using ELISA kit.

Inclusion criterias include stable COPD patients, post ECOPD patients who were in stable condition, willing to join the study and sign informed consent form. Patients suffering from acute exacerbation symptoms of COPD were excluded. Acute exacerbation was defined by increasing dyspnea, cough, and or sputum production. The criterias for control subjects include subjects who were not diagnosed with COPD and spirometry test showed no obstructive pattern. This study has got ethical approval from Health Research Ethics Commission of Saiful Anwar Hospital Malang with number of 400/295/K.3/102.7/2022. We analyzed the data using SPSS version 26. Mann Whitney test was used for analyzing data variables between two groups. While correlation between variables was analyzed using Spearman test.

Result:

This study involved total of 80 subjects of which description can be seen at table 1.

Table 1. Sociodemographic characteristic of subjects

Characteristics		Control Subjects (n= 40)	COPD subjects (n= 40)
Gender	Male	22 (55%)	33 (82,5%)
	Female	18 (45%)	7 (17,5%)
Age	years old	31,35 (±3)	64,73 (±10,6)
Body Height	cm	164,9 (±9,0)	162,1 (±4,8)
Body Weight	kg	70,15 (±15,9)	52,4 (±8,7)
***Body Mass Index* (BMI)**	kg/m^2	25,6 (±4,2)	19,9 (±2,9)
Smoking Status	No smoker	36 (90%)	0 (0%)
	Ex smoker	0	4 (10%)
	Passive smoker	2 (5%)	10 (25%)
	Active smoker	2 (5%)	26 (65%)
Symptoms	Dyspnea	0	27 (67,5%)
	Cough	0	23 (57,5%)
	Production of sputum	0	11 (27,5%)
	Chest pain	0	2 (5%)
Comorbid	Lung cancer	0	20 (50%)
	Lung TB	0	2 (5%)
	Asthma	6 (15%)	0

There was 82,5% male subjects in COPD group vs 55% male subjects in control group. Mean BMI of control group was higher than that of COPD group (25,6 vs 19,9). All of subjects with COPD got exposure from smoke, whether as active, passive, or ex smoker. We found half of COPD group subjects had lung cancer as comorbid.

Some of blood and spirometry parameters were checked in this study. Blood parameters include leucocyte, neutrophil, and LTB4 level. Whereas, value of FEV1/FVC and FEV1 become parameters of spirometry included. Results of examination were shown in table 2.

Table 2. Laboratorium and spirometry result of subjects

Parameter		N	Mean	Std.Deviation	Minimum	Maximum	p value
Leucocyte	Control	40	7595,75	1876,9	3640	12570	
	Case	40	9981,2	3800,3	4230	20630	0,002
LTB4	Control	40	84,04	89,79	6,37	486,08	
	Case	40	244,1	186,9	28,79	546,78	<0,001
Neutrophil %	Control	40	63,03	9,099	43,1	78,9	
	Case	40	72,077	11,653	45,50	92,90	<0,001

Absolute Neutrophil	Control	40	4877,0	1730,5	2210	9920	
	Case	40	7327,8	3576,1	2270	16630	0,001
FEV1/FVC	Control	40	122,9	147,9	83,4	112,1	
	Case	40	62,06	8,08	41,1	69,8	<0,001
FEV1	Control	40	89,5	9,3	80,4	117,6	
	Case	40	46,08	9,9	20,9	66,1	<0,001

Note: significant if p value < 0,05; LTB4, Leukotriene B4; Neutrophil%, percentage of neutrophil; FEV1, *Forced Expiratory Volume 1 second*; FVC, *Forced Vital Capacity*

The level of serum leucocyte, LTB4, and neutrophil were found higher in COPD group than in control one. Normal distribution of data was found in parameters of percentage of neutrophil and FEV1, hence parametric analysis used was independent t-test. While other parameters (leucocyte, LTB4, absolute neutrophil, FEV1/FVC) used non parametric analysis i.e. Mann Whitney test.

Breath analyzer was used to check the concentration of VOC. Thirteen VOCs detected from *Ubreath* tool include CO_2, C_2H_5OH, CH_2O, C_7H_8, C_3H_6O, NH_4, C_6H_{14}, NO_2, CO, NH_3, CH_4, C_6H_6, and C_3H_8. Table 3 showed the result of VOC between control and COPD subjects.

Table 3. Level of VOCs among subjects

Parameter		N	Mean	Std, Deviation	Minimum	Maximum	P value
CO_2	Control	40	2849,58	1076,51	400	4598	
	Case	40	1577,75	1052,70	400	4404	<0,001
C_2H_5OH	Control	40	1,4	0,32	0,8371	9,6526	
	Case	40	1,85	0,7	0,8798	9,2253	<0,001
CH_2O	Control	40	0,08	0,02	0,0495	0,1350	
	Case	40	0,08	0,02	0,034	0,134	0,776
C_7H_8	Control	40	0,005	0,003	0,002	0,014	
	Case	40	0,005	0,003	0,001	0,013	0,776

Parameter		N	Mean	Std, Deviation	Minimum	Maximum	P value
C_3H_6O	Control	40	11,1	3,5	3,69	18,42	
	Case	40	4,8	2,4	0,351	14,647	<0,001
NH_4	Control	40	1,41	1,46	0,0001	3,8486	
	Case	40	0,59	1,035	0,0001	3,5605	0,776
C_6H_{14}	Control	40	0,35	0,05	0,252	0,415	
	Case	40	0,29	0,09	0,006	0,405	0,776
NO_2	Control	40	0,82	0,39	0,000	1,57	
	Case	40	0,06	0,15	0,0001	0,5725	<0,001
CO	Control	40	0,025	0,042	0,0001	0,1174	

	Case	40	0,60	0,24	0,0001	0,8368	<0,001
NH_3	Control	40	0,0001	0,0000	0,0001	1,0000	
	Case	40	0,004	0,013	0,0001	0,0453	0,022
CH_4	Control	40	0,08	0,02	0,0470	0,1088	
	Case	40	0,19	0,23	0,1029	0,9999	0,310
C_6H_6	Control	40	0,6	0,02	0,56	0,64	
	Case	40	0,6	0,05	0,53	0,72	<0,001
C_3H_8	Control	40	2,34	0,6	1,4770	3,6590	
	Case	40	3,2	1,2	1,6113	7,1166	0,001

Note: CO_2, Carbon dioxide; C_2H_5OH, Ethanol; CH_2O, Metanal; C7H8, Toluene; C_3H_6O, Acetone; NH_4, Ammonium; C_6H_{14}, Hexane; NO_2, Nitrogen dioxide; CO, Carbon monoxide; NH_3, Ammonia; CH_4, Methane; C_6H_6, Benzene; $C3H_8$, Propane.

Statistical analysis we used for these VOCs was Mann Whitney test since the distribution of data was not normal. Seven VOCs could significantly differ COPD and control subjects. Those VOCs were CO_2, C_2H_5OH, C_3H_6O, NO_2, CO, NH_3, C_6H_6, and C_3H_8. Level of CO_2, C_3H_6O, and NO_2 were lower in COPD subjects. While C_2H_5OH, CO, NH_3, and C_3H_8 levels were higher in COPD group.

Among 8 VOCs detected significant between COPD and control subjects, Spearman correlation test was done to determine the correlation with serum LTB4 and neutrophil. Analysis results were shown in table 4 and 5.

Table 4. Correlation between serum LTB4 and VOCs

Correlation between variable		**P value**	**Correlation coefficient**
Serum LTB4	CO_2	0,155	0,229
	C_2H_5OH	0,009	0,410
	C_3H_6O	0,268	-0,180
	NO_2	0,209	-0,203
	CO	0,353	-0,151
	NH_3	0,227	-0,195
	C_6H_6	0,089	-0,273
	C_3H_8	0,137	0,239

Table 5. Correlation between serum neutrophil and VOCs

Correlation between variable		**P value**	**Correlation coefficient**
Serum Neutrophil	CO_2	0,416	-0,132
	C_2H_5OH	0,882	0,024
	C_3H_6O	0,973	0,005
	NO_2	0,343	-0,154
	CO	0,275	0,177
	NH_3	0,129	0,244

	C_6H_6	0,438	0,126
	C_3H_8	0,686	-0,066

There was only one VOC correlated with serum LTB4 that is C_2H_5OH ($p = 0.009$; $r = 0.410$). Nevertheless, we found there was no VOC correlated with serum neutrophil based on Spearman test result.

Discussion:

Patients with COPD are male in majority in this study (33 subjects). Prevalence of COPD is more common in male [3]. A meta analysis study in US showed prevalence of COPD in male was 9.23% and 6.16% in female [9]. Average age of COPD patients is 64.73 years old. Older age is related to decrease of mitochondrial function that stimulates oxidative stress. This is shown by increasing Reactive Oxygen Species (ROS) and lipid peroxidation. Later is also negatively correlated with value of FEV1 [10].

Subjects with COPD have lower average of IMT than control ones. A retrospective study by Zhenchao et al. showed that IMT was positively correlated with lung function and negatively correlated with inflammation level (CRP and neutrophil level) and exacerbation severity. Overweight and obesity patients have better nutritional status and stronger respiratory muscles [11]. As many as 26 COPD subjects are active smoker and 10 others are passive smoker. Smoking is main risk factor of developing COPD. The pathogenesis of COPD involve oxidative stress, protease imbalance, and inflammatory cells (macrophage, CD8 T-lymphocyte and neutrophil) can cause lung inflammation, mucus hypersecretion, and airway remodeling [12].

There is half (50%) of COPD subjects have lung cancer as comorbid. A review study demonstrate 40-70% of lung cancer patients show airway obstruction and indicative for COPD entity. While COPD increase 2 until 7 times fold risk of getting lung cancer. The same mechanism happen both in COPD and lung cancer is oxidative stress [13].

Mean level of serum leucocyte is higher in COPD group. Study by Aini et al. found level of blood leucocyte increased in 65.7% COPD subjects in Riau General Hospital in 2017. Blood neutrophil also increased in 68.6% of COPD subjects [14]. Neutrophil is involved in COPD pathogenesis and continuing infiltration in lung tissue is related to severity of COPD [15]. Increasing of peripheral blood neutrophil could cause increasing number of exacerbation per year compared to normal amount of leucocyte (2.3 vs 1.3 exacerbation/year) [16]. Also, serum LTB4 level is higher in COPD in this study. Similar result was shown by Kazmierczak et al., that LTB4 level was higher in COPD subjects than in healthy subjects (1772,7 pg/mL vs 680,31 pg/mL) [2]. Exposure of smoke and other noxious particle can stimulate secretion of inflammatory cytokines such as LTB4, IL-6, and IL-8. LTB4 is main product of 5-lypooxygenase enzyme that has chemotactic property and prolong lifespan of neutrophil [12].

In this study, we can see that there is significant difference of 8 VOCs (CO_2, C_2H_5OH, C_3H_6O, NO_2, CO, NH_3, C_6H_6, and C_3H_8) between COPD and control group. Level of Carbon dioxide (CO_2), acetone (C_3H_6O), and Nitrogen dioxide (NO_2) are higher in control subjects. Previous study was conducted in Saiful Anwar Hospital to analyze VOC detected in lung cancer patients compared to control subjects. That study found that ethanol (C_2H_5OH), formaldehyde (CH_2O), toluene (C_7H_8) and ammonia (NH_3) were VOCs detected higher in lung cancer patients [17]. COPD patients have impairment of CO_2 exhalation caused by increase of respiratory burden such as airway resistance and hyperinflation. Decrease of respiratory muscle strength can be caused by nutritional factor and neuromyopathy effect of systemic inflammation [18]. Acetone is common substance in exhaled breath from decarboxylation of lipolysis or

amino acid degradation [19]. Patients with COPD are mostly at older age and lose skeletal muscle mass. Hence, acetone, as exhaled VOC, that is affected by age and metabolism will get impact not only by the disease itself, but also age [20]. Shahzad et al, found the mean level of exhaled acetone in healthy subjects was higher than in COPD patients (0.66 ppm vs 0.50 ppm) [21]. While sources of NO_2 are from smoke, oven, and water heater. Besides, NO2 is surrounding air pollutant that can irritate human airway. NO_2 cannot be concluded whether related with COPD or originates from surrounding air pollutions [22].

The other 5 VOCs are found higher in COPD group. Ethanol (C_2H_5OH) is product of carbohydrate and amino acid fermentation. It is found higher in active smoker than non smoker people [23]. Benzene is also found higher in smoker's exhaled breath with specificity more than 90%. While propane (C_3H_8) is aldehyde contained in smoke [19]. Increase of exhaled Carbon monoxide (CO) is due to endogenously produced from the body and from lung inflammation process. A study in 2018 demonstrate exhaled breath level of CO in COPD patients increase 3 times higher than in healthy subjects in India [24]. Ammonia (NH_3) is resulted from urea hydrolysis in nitrogen cycle. Increasing level of ammonia also mean increasing of oxidative stress [21]. Ethanol is the only VOC correlated with serum LTB4 in this study ($p = 0.009$; $r = 0.41$), whereas there is no VOC correlated with neutrophil. Allers et al. show that exhaled level of ethanol is higher in smokers than in no smokers, though this result is not significant differs COPD with healthy subjects [23]. Theoretically, oxidative stress will cause accumulation of macrophage and neutrophil that increase ROS production. LTB4 is produced from arachidonic acid metabolism in lipid membrane. But exhaled breath VOC is produced from lipid peroxidation which is not related to arachinodic acid [3].

Limitation in this study: environmental VOC surrounds location of sampling have not been checked first. Then, bias of this study can also be caused by absence of homogeneity between case and control group and lung cancer as comorbid.

Conclusion:

C_2H_5OH, C_6H_6, CO, C_3H_8, NH_3 may differ COPD patients from control subjects. Furthermore, ethanol is also correlated with serum Leukotriene B4 in COPD, nevertheless there is no VOC detected correlated with serum neutrophil.

Conflict of Interest

None.

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