ORIGINAL ARTICLE

Volatile Organic Compounds (VOC) and Serum Leukotriene B4 between COPD Patients and COPD with Lung Cancer Patients

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ABSTRACT

Introduction: Chronic obstructive pulmonary disease (COPD) is estimated to become the third leading cause of death worldwide in 2030. COPD can affect the lungs and cause chronic systemic inflammation. Leukotriene B4 (LTB4) is involved in COPD and lung cancer pathogenesis. There has been the development of non-invasive methods for detecting lung disease in the last few decades, such as the examination of volatile organic compounds (VOC). This study aimed to analyze the serum LTB4 and the difference of VOCs in exhaled breath of stable COPD patients and COPD with lung cancer patients.

Methods: This case-control study recruited 20 stable COPD patients and 20 patients with COPD and lung cancer. An exhaled breath sample was collected in Tedlar bags and analyzed using an arrayed sensor breath analyzer to check the concentration of VOCs. Meanwhile, a venous blood sample was collected to examine the level of LTB4 using an ELISA kit. Independent t-test and Mann-Whitney test were used to analyze the data.

Results: The carbon dioxide (CO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), benzene (C_6H_6), and propane (C_3H_8) levels were significantly different ($p < 0.05$) in COPD-only patients compared to COPD with lung cancer patients. Serum LTB4 increased in both groups.

Conclusion: CO_2, CO, and C_3H_8 levels increased, but the NO_2 level decreased in COPD patients with lung cancer compared to COPD-only patients. Serum LTB4 increased in COPD with lung cancer patients.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is estimated to become the third leading cause of death in 2030 globally. It is a disease with chronic respiratory symptoms because of airways and/or alveoli abnormalities.[1] It also causes chronic systemic inflammation aside from affecting the lungs. Smoking remains the leading risk factor for COPD.[2] The World Health Organization (WHO) recorded that 2.9 million people died due to COPD.[3] In 2019, the global prevalence of COPD was 10.3%, while its prevalence in Indonesia was 3.7% or around 9.2 million people.[4] Lung cancer is the fifth leading cause of death of all cancers worldwide, with the current overall 5-year survival rate of 16%, which has remained the same in the past three decades.[5]

The standard COPD diagnostic is still spirometry, but it requires certain maneuvers and trained operators. In the last few decades, non-invasive methods for detecting lung disease have been developed, such as examining volatile organic compounds (VOC).[6] VOC compounds evaporate easily and produce endogen or exogenous compounds.[7] Gas chromatography and electronic nose (eNose) are the most widely used techniques for detecting VOCs. Using a breath analyzer to identify VOCs is a simple technique.[8]

Pathogenesis of COPD is related to the activation of macrophages, neutrophils, and CD8 T-lymphocytes in the airway and lung parenchyma because of smoke and other toxic substances. This will stimulate the production of cysteinyl leukotriene (LTB4, LTC4, LTD4, and LTE4), which can induce airway smooth muscle constriction, vascular permeability, and mucus

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hypersecretion.[9] Meanwhile, lung cancer shares a similar pathogenesis with COPD, which is oxidative stress and chronic inflammation.[5]

Based on the development of VOC examination to detect the presence of COPD early and increased leukotriene B4 (LTB4) in both COPD and lung cancer patients, this study aimed to analyze the serum LTB4 and difference of VOCs in exhaled breath of stable COPD patients compared to COPD with lung cancer patients.

METHODS

This case-control study was conducted at Dr. Saiful Anwar General Hospital, Malang. The study included 20 stable COPD patients and 20 COPD patients with lung cancer who fulfilled the inclusion and exclusion criteria. Patients with outpatient and inpatient status were included from March until October 2022. Detection of VOC was performed using an exhaled breath sample, whereas a venous blood sample was collected for the LTB4 examination. Breath samples were collected in Tedlar bags from the patients and then analyzed within 15 minutes using an arrayed sensor breath analyzer to measure VOC concentration. The breath analyzer was developed by the Department of Physics, Universitas Brawijaya, Malang, named "Ubreath". The concentration of LTB4 was checked using an enzyme-linked immunosorbent assay (ELISA) kit.

Inclusion criteria included stable COPD and lung cancer patients. Meanwhile, the exclusion criteria were patients who suffered from acute exacerbation symptoms of COPD and acute deterioration clinical symptoms of lung cancer such as shortness of breath and chest pain. Acute exacerbation of COPD was defined by increasing dyspnea, cough, and/or sputum production. All of the patients signed informed consent forms before participating. This study did not describe and analyze the histopathology of lung cancer types. Ethical approval was obtained from the Health Research Ethics Commission of Dr. Saiful Anwar General Hospital, Malang, with the number 400/295/K.3/102.7/2022. Data analysis used the International Business Machines Corporation (IBM) Statistical Package for the Social Sciences (SPSS) version 26. Depending on their distribution, Mann-Whitney and independent t-tests were used to analyze the data.

RESULTS

A total of 40 patients were included in this study, of which the demographic characteristics can be seen in Table 1.

Table 1. Demographic characteristics of patients

Characteristics		COPD Patients (n = 20)	COPD + Lung Cancer Patients (n = 20)
Gender	Male	14 (70%)	18 (90%)
	Female	6 (30%)	2 (10%)
Age	Years old	59.4 (± 3)	70.05 (± 10.6)
Smoking Status	Non-smoker	0 (0%)	0 (0%)
	Ex-smoker	1 (5%)	2 (10%)
	Passive smoker	6 (30%)	3 (15%)
	Active smoker	13 (65%)	15 (75%)
Symptoms	Dyspnea	14 (70%)	17 (85%)
	Cough	10 (50%)	12 (60%)
	Production of sputum	8 (40%)	10 (50%)
	Chest pain	0 (0%)	3 (15%)

COPD: chronic obstructive pulmonary disease

This study involved 14 male patients in the COPD-only group and 18 male subjects in the COPD with lung cancer group. All of the patients were more than 40 years old. No subject had a smoke exposure history. As many as 65% of COPD-only patients were active smokers, while 75% of active smokers were found in the COPD with lung cancer group. Fifty percent or more of the patients had symptoms of dyspnea and cough with or without sputum production.

This study checked LTB4 in a venous blood sample and examined it using an ELISA kit. The result is shown in Figure 1.

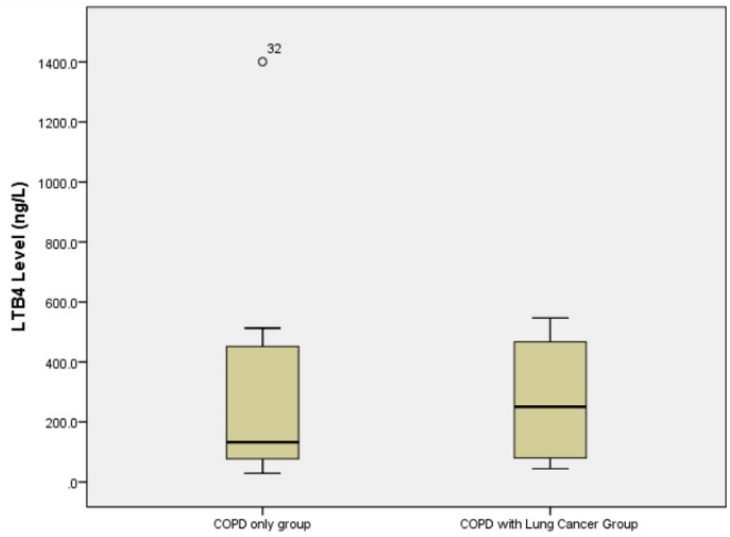

Figure 1. Serum LTB4 results of patients

The average level of LTB4 serum was slightly lower in the COPD-only group than in COPD with lung cancer group. The result was then analyzed to determine whether it was significantly different between the two groups (Table 2).

Table 2. Comparison of serum leukotriene B4 results between groups

Parameter		N	Mean	Standard Deviation	Minimum	Maximum	p-Value
LTB4 serum (ng/L)	Control	20	273.6	± 319.2	28.8	1401.0	0.5
	Case	20	277.64	± 193.97	44.4	546.8	

Note: control = COPD-only group; case = COPD + lung cancer group.
Significant if p-value < 0,05.

Table 2 shows that the comparison level of serum LTB4 between the COPD-only group and COPD with lung cancer group was not statistically significant (p >0.01). Although the data distribution was normal, nonparametric analysis, i.e. Mann-Whitney test, was performed.

The breath analyzer (Ubreath) was used to measure the concentration of VOCs. Eight VOCs were detected from that tool, including CO_2, ethanol (C_2H_5OH), acetone (C_3H_6O), NO_2, CO, ammonia (NH_3), C_6H_6, and C_3H_8. Table 3 shows the VOC results between both groups.

Table 3. Level of volatile organic compounds among patients

Parameter		N	Mean	Standard Deviation	Minimum	Maximum	p-Value
CO_2	Control	20	2353.05	± 332.13	2654	400	0.006
	Case	20	3346.1	± 1322.65	4070	4598	
C_2H_5OH	Control	20	1.21	± 0.39	0.94	2.397	0.098
	Case	20	1.43	± 0.25	0.84	1.884	
C_3H_6O	Control	20	10.28	± 3.06	4.97	14.65	0.445
	Case	20	11.95	± 3.86	3.69	18.42	
NO_2	Control	20	0.96	± 0.335	0.157	1.299	0.025
	Case	20	0.67	± 0.398	0.000	1.340	
CO	Control	20	0.00015	± 0.000671	0.000	0.003	<0.001
	Case	20	0.05085	± 0.048386	0.000	0.117	
NH_3	Control	20	0.00	± 0.00	0.00	0.00	0.145
	Case	20	0.00	± 0.00	0.00	0.00	
C_6H_6	Control	20	0.6005	± 0.0159	0.577	0.626	0.026
	Case	20	0.604	± 0.0228	0.563	0.636	
C_3H_8	Control	20	1.7161	± 0.26131	1.477	2.450	0.004
	Case	20	2.8046	± 0.52203	2.014	3.659	

Note: control = COPD-only group; case = COPD + lung cancer group.
CO_2: carbon dioxide; C_2H_5OH: ethanol; C_3H_6O: acetone; NO_2: nitrogen dioxide; CO: carbon monoxide; NH_3: ammonia; C_6H_6: benzene; C_3H_8: propane

From Table 3, the comparison of VOCs between COPD with lung cancer group and COPD-only group can be observed. Statistical analysis used was an independent t-test for C_2H_5OH, C_3H_6O, C_6H_6, and C_3H_8 since the data distribution was normal. Meanwhile, the Mann-Whitney test was used for CO_2, NO_2, CO, and NH_3 since the data distribution was not normal. Five VOCs significantly differentiated those 2 groups, i.e. CO_2, NO_2, CO, C_6H_6, and C_3H_8 ($p < 0.05$). Three compounds were detected higher in COPD with lung cancer patients than in COPD-only patients: CO_2 (3346.1 vs. 2353.05), CO (0.05085 vs. 0.00015), and C3H8 (2.8046 vs. 1.7161). Meanwhile, a lower concentration of NO_2 was found in the COPD with lung cancer group than in the COPD-only group. Both groups had relatively the same level of C_6H_6, with statistical significance.

DISCUSSION

The patients were males in the majority, both in the COPD-only group and the COPD with lung cancer group in this study (14 and 18 patients, respectively). COPD prevalence is more common in males.[3] A meta-analysis study in the United States (US) showed the prevalence of COPD in males was 9.23% and 6.16% in females.[10] Similar to COPD, lung cancer is also more prevalent in males, with approximately 1.37 million cases in 2018, with the highest incidence rates in Micronesia, Polynesia, Central and Eastern Europe, and Eastern Asia.[11] While the incidence rates of lung cancer are lower in females, that is 725,000 cases in 2018.[11]

Patients with COPD and/or lung cancer are typically older. The decrease in mitochondrial function in older age may stimulate oxidative stress. This is shown by increasing reactive oxygen species (ROS) and lipid peroxidation.[12] Lung cancer is a complex disease caused by mutations of oncogenes, which transform normal cells into malignant cells under genetic and epigenetic influences. People with COPD are at high risk of lung cancer because these two diseases share similar pathogenesis, including chronic inflammation, epigenetic changes, and impaired deoxyribonucleic acid (DNA) repair mechanism due to oxidative stress. COPD increases four- to six-fold risk of developing lung cancer regardless of age, sex, and smoking history.[5] A review study demonstrated that 40-70% of lung cancer patients showed airway obstruction indicative of COPD entity.[13]

All patients in this study had a history of smoking, whether as active, passive, or ex-smokers. Smoking is the main risk factor for COPD. Oxidative stress, protease and anti-protease imbalance, and inflammatory cells (macrophage, CD8 T-lymphocyte, and neutrophil) are involved in the pathogenesis of COPD, which can induce lung inflammation, mucus hypersecretion, and airway remodelling.[14] Smoking is not only the most common cause of COPD but also lung cancer. Tobacco smoke contains more than 7,000 chemical products, including at least 69 known carcinogens. Meanwhile, secondhand smoke has the role of indirect exposure to carcinogens resulting from the burning of tobacco products. As much as 80-90% of lung cancer diagnoses are associated with tobacco smoking in the US.[11]

The mean serum LTB4 level is slightly higher in COPD with lung cancer patients in this study. Exposure to smoke and noxious particles or gases might cause the release of inflammatory cytokines, including LTB4, IL-6, and IL-8. LTB4 is the main product of the 5-lipoxygenase enzyme, having the chemotactic properties of leucocytes, primarily neutrophils.[14] Eicosanoids, including leukotrienes and prostaglandins, are linked to tumor-promoting inflammation. The pathway of 5-lipoxygenase has a tumor-promoting effect that has been implicated in many types of cancer, including lung cancer. LTB4 has been shown to induce a pro-inflammatory environment that promotes tumor development.[15,16]

A significant difference of 5 VOCs (CO_2, NO_2, CO, C_6H_6, and C_3H_8) among 8 VOCs was detected between the COPD with lung cancer and the COPD-only groups. CO_2, CO, and C_3H_8 levels were higher in COPD with lung cancer patients. Meanwhile, the COPD-only group had a higher concentration of NO_2 but relatively the same C_6H_6 level compared to the COPD with lung cancer group. A previous study conducted at Dr. Saiful Anwar General Hospital, Malang, analyzing VOC detected in lung cancer patients compared to control patients, found that C_2H_5OH, formaldehyde (CH_2O), toluene (C_7H_8), and NH_3 were higher in lung cancer patients.[17]

COPD patients have impairment of CO_2 exhalation caused by increased respiratory burden, such as airway resistance and hyperinflation. Nutritional factors and the neuromyopathy effect of systemic inflammation can cause decreased respiratory muscle strength.[18] Smoke, ovens, and water heaters are sources of NO_2. Furthermore, NO_2 is a surrounding air pollutant that can irritate the human airway. Whether NO2 is related to COPD or originates from surrounding air pollution cannot be concluded.[19] The increase in exhaled CO is due to endogenously produced from the body and the lung inflammation process. A study conducted in 2018 demonstrated that exhaled breath levels of CO in COPD patients increased three times higher than in healthy patients in India.[20]

C_2H_5OH is a product of carbohydrate and amino acid fermentation. It is found to be higher in active

smokers than in non-smokers.[21] C_6H_6 is also higher in smokers' exhaled breath, with a more than 90% specificity. Gashimova, *et al*. (2020) found that C_6H_6 occurred in more than 50% of lung cancer patients in that study.[22] Meanwhile, C_3H_8 is an aldehyde contained in a cigarette.[22,23] Recent studies showed many VOCs were related to lung cancer, resulting from oxidative stress that can be produced by the peroxidation of DNA bases induced by cancer. C_3H_8, as an alkane derivative, can result from oxidative stress as an endogenous process. C_2H_5OH, propanol, and methanol are produced from hydrocarbon metabolism and absorbed through the gastrointestinal (GI) tract. C_6H_6 and toluene, aromatic compounds, also increase in lung cancer patients. These can result exogenously from petrol, smoking, and oil.[24,25] A study by Dragonieri, *et al*. (2009) showed several VOCs (isoprene, pentane, and C_6H_6) could differ in non-small cell lung cancer (NSCLC) from COPD patients.[26] Increased oxidative events are associated with neoplastic process irrespectively from cigarette smoking. Additionally, the induction of cytochrome p450 might increase the catabolism of VOCs, potentially altering the compounds in lung cancer patients. Meanwhile, the presence of COPD may cause an alteration of the VOC spectrum. The imbalance of proteinase-anti-proteinase and chronic airway inflammation in COPD pathogenesis led to oxidative stress and airway remodeling.[26]

CONCLUSION

The levels of exhaled breath CO_2, CO, and C_3H_8 were higher in COPD patients with lung cancer compared to COPD-only patients. VOC examination might be useful as a screening tool to detect COPD and lung cancer. Serum LTB4 increased in COPD with or without lung cancer patients, even though the difference was not statistically significant. There may be potential benefits for giving leukotriene modifiers to COPD patients. However, more research is necessary. Additional studies are needed to determine whether serum LTB4 could be a COPD and lung cancer biomarker.

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Conflict of Interest
The authors declared there is no conflict of interest.

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Authors' Contributions
Study design, data sampling, analysis, and discussion: SD, ASL, KWP, and AYPW. All authors contributed and approved the final version of the manuscript.

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