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Original Article

The Correlation between Volatile Organic Compounds (VOC) with Leukotriene B4 and Eosinophil Counts in Chronic Obstructive Pulmonary Disease Patients

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Abstract

Background : Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder characterized by increasing Leukotriene B4 (LTB4) and eosinophil counts. Volatile organic compounds (VOCs) have shown promise as non-invasive biomarkers, reflecting COPD pathophysiology. Identifying specific VOCs associated with increased LTB4 and eosinophil counts could lead to the discovery of potential biomarkers for COPD severity or progression. This study aims to investigate the correlation between VOCs and leukotriene B4 (LTB4) levels, as well as eosinophil counts counts in COPD patients.
Methods : Using an observational-analytic method with a case-control approach, 20 COPD patients and 20 controls were enrolled from the respiratory outpatient department of Dr. Saiful Anwar General Hospital, Malang. VOC levels were measured using a breath analyzer, while LTB4 levels were determined through enzyme-linked immunosorbent assays. Spearman's correlation tests examined associations between VOCs, LTB4, eosinophil counts, and comorbidity, with Mann-Whitney tests comparing results against the control group. Data significance was set at $p < 0.05$.
Results : There were 40 COPD patients and 40 controls in this study. There were significant differences between VOCs in the COPD group and the control group ($p < 0.05$). LTB4 level significantly increased in the COPD group than in the control group ($p < 0.001$), and there was no difference in the eosinophil level. There was a correlation between LTB4 and VOC level of C_2H_5OH in COPD patients ($p = 0.009$; $r = 0.410$). There was no correlation between eosinophil counts and VOCs ($p = 0.939$). The level of VOCs was significantly different between patients with only COPD and patients with COPD and comorbid lung cancer ($p < 0.05$).
Conclusion : There is a correlation between VOC and LTB4 in COPD patients.

Keywords : Volatile Organic Compounds, Leukotriene B4, Eosinophil, Chronic Obstructive Pulmonary Disease

INTRODUCTION

Chronic obstructive pulmonary disease, or known as COPD, is a disease characterized by persistent respiratory symptoms due to exposure to toxic gases or particles that cause airway or alveolar abnormalities.[1] COPD is caused by a complex interaction between genetics and the environment. The main risk factor for COPD is smoking, but other factors that can influence include age, gender, lung development, particulate matter exposure, socioeconomic status, asthma, hyperreactivity, chronic bronchitis, and infection.[2] The incidence of COPD is increasing every year. The results of the 2018 Basic Health Research (Riskesdas) show that the prevalence of COPD in Indonesia is 3.7% or around 9.2 million people.[3] According to the WHO, 2.9 million people die from COPD and it is estimated that by 2030, COPD will be the third most common cause of death in the world.[4]

The standard technique for diagnosing COPD is spirometry. However, spirometry can only show restrictions in the airway without identifying the cause of these restrictions.[5] VOC is a compound with a light molecular mass, a low boiling point, and relatively high vapor pressure; it also evaporates easily. VOC can come from endogenous or exogenous sources.[6] VOC is used as the newest modality to identify lung diseases. Analysis of the VOC can be used as a non-invasive, fast, and precise investigation to diagnose COPD.[7] There are several techniques often used to collect data and to detect and analyze VOC gases, often including gas chromatography (GC) and electronic nose (eNose) as the modalities, Mass spectrometry (GCMS) or flame ionization detection (GC-FID), proton transfer reaction mass spectrometry (PTR-MS), selective ion flow tube mass spectrometry (SIFT-MS), laser spectroscopy, colorimetric sensors with matrices and gold nanoparticles. Sensors (VNP).[8]

COPD is a disease associated with inflammation and oxidative stress. According to research, levels of LTB4 and other inflammatory markers in the body such as IL-8 will increase in COPD preogression.[9] LTB4 functions as a neutrophil and T-cell chemoattractant, which has a correlation with lung function progression.[10] In some COPD patients, eosinophils contribute to airway obstruction biomarker. One-third of stable COPD patients are known to have elevated eosinophil counts. This can determine which patients will benefit from inhaled corticosteroids and determine the prognosis of patient exacerbations.[11] Identifying specific VOCs associated with increased LTB4 and eosinophil counts could lead to the discovery of potential biomarkers for COPD severity or progression. Based on existing problems, theories, and supporting journals, the researchers were interested in analyzing VOC in COPD patients and its relationship with LTB4 and blood eosinophil levels.

METHODS

This study used an observational-analytic method with a case-control design approach to assess differences in VOC, LTB4 levels, and blood eosinophils in stable COPD patients at Dr. Saiful Anwar General Hospital, Malang. This study was conducted in AugustSeptember 2022 at the outpatient clinic of Dr. Saiful Anwar General Hospital, Malang. This research has an ethical aprroval with No 400/14/K.3/102.7/2023 from Health Research Ethics Commission of General Hospital Dr. Saiful Anwar.

The population of this study is stable COPD patients who visit the pulmonary polyclinic at Dr. Saiful Anwar General Hospital, Malang, as subjects and PPDS Pulmonology and Respiratory Medicine Specialist Medical Education Program, Faculty of Medicine, University of Brawijaya, as control subjects.

This research applies strict inclusion and exclusion criteria to ensure sample homogeneity and the validity of research outcomes. Inclusion criteria involve patients with stable COPD or those who have undergone treatment post-acute exacerbation and are currently in a stable condition. The research subjects range in age from 40 to 70 years and express willingness to participate after receiving adequate explanations and signing informed consent. On the other hand, there are exclusion criteria that encompass subjects experiencing acute COPD exacerbation symptoms, such as increased shortness of breath, heightened cough frequency, increased sputum production, and changes in sputum color. This is done to ensure that research subjects are in a stable condition and not influenced by ongoing exacerbation episodes. Furthermore, the samples were divided into two research groups, namely research subjects and controls. Data on serum levels of VOC, leukotriene B4, and eosinophil were collected from each group.

The data obtained were to be recorded on the research sheet and were then processed and analyzed. Data analysis method used in this study include the Chi-square test or Fisher's exact test for categorical data. Mann-Whitney test or independent t-test were used for the analysis of variables between the two groups. Meanwhile, the correlation between variables was analyzed using Pearson's or Spearman's correlation test with significant level of $p<0.05$. The statistical testing tool used to analyze the data is SPSS version 26.

RESULTS

The research subjects consisted of 80 subjects making the 2 research groups. The research group consisted of 40 healthy control subjects (pulmonary PPDS) and 40 subjects in the COPD case group. The sociodemographic characteristics of the research subjects can be seen in Table 1. Normality test used Shapiro-Wilk test. In this study, it is shown that age, height, platelet

TABLE 1
Sociodemographic Characteristics of the Research Subjects

Characteristics		Healthy control (n= 40)	COPD cases (n= 40)
Gender	Man	22 (55%)	33 (82.5%)
	Woman	18 (45%)	7 (17.5%)
Age	year	31.35 (± 3)	64.73 (±10.6)
Height	cm	164.9 (± 9.0)	162.1 (±4.8)
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	Do not smoke	36 (90%)	0 (0%)
	Former smoker	0	4 (10%)
	Passive smoker	2 (5%)	10 (25%)
	Active smoker	2 (5%)	26 (65%)
Complaint	Hard to breathe	0	27 (67.5%)
	Cough	0	23 (57.5%)
	Sputum production	0	11 (27.5%)
	Chest pain	0	2 (5%)
Comorbidities	Lung cancer	0	20 (50%)
	Pulmonary TB	0	2 (5%)
	Asthma	6 (15%)	0

count, and compound C6H6 had a normal distribution so the process was continued with an independent t-test analysis. Normality analysis of body weight, Hb levels, leukocytes, hematocrit, eosinophils, LTB4 levels, FEV1/FVC values, FEV1, compounds O_2, CO_2, O_3, 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, and C_3H_8 had an abnormal distribution, so a Mann-Whitney analysis was performed.

Laboratory Examination Results and Spirometry

This study showed that there were significant differences in LTB4 levels, Hb levels, hematocrit values, lymphocyte values, and spirometry results between controls and COPD patients. LTB4 levels were higher in COPD patients compared to the controls ($p = 0.020$; $p = 0.000$; $p = 0.000$, $p = 0.000$, $p = 0.000$ ($p < 0.05$)) (Table 2).

Examination Results of Levels of Volatile Organic Compounds

There were significant differences in the parameters CO_2, C_2H_5OH, C_3H_6O, NO_2, CO, NH_3, C_6H_6, and C_3H_8 between the case group and the control group. The mean of CO_2, C_3H_6O, and NO_2 were significantly lower in the case group than in the control group (2,849.58 vs 1,577.75; 11.1 vs 4.8; and 0.82 vs 0.06, respectively). Meanwhile, the mean of C_2H_5OH, CO, NH_3, and C_3H_8 were significantly higher in the case group than in the control group (1.85 vs 1.4, 0.6 vs 0.025, 0.004 vs 0.0001 and 3.2 vs. 2.34) respectively. There is no significant difference in the mean parameters CH_2O and C_7H_8 between the two groups (Table 3).

Relationship between Serum LTB4 and Serum Eosinophil and Spirometry Parameters

Statistical test results demonstrated a relationship between serum LTB4 levels and serum neutrophils and FEV1. No significant relationship was found between LTB4 and eosinophil counts. The FEV1 parameter was found to have no relationship with serum LTB4 (Table 4).

The Relationship between FEV1 and VOCs

The results of the statistical tests revealed that the CO_2 parameter has a significant relationship with FEV1 ($p = 0.049$), which was then tested using the Spearman's method with a correlation result of -0.313. The parameter C_2H_5OH shows a significant relationship with FEV1. Spearman's test showed a moderate correlation between FEV1 and C_2H_5OH (correlation coefficient of 0.410).

TABLE 2
Laboratory Data and Spirometry

Characteristics		Control	COPD	*p-values*
LTB4		84.04 ± 89.79	244.1 ± 186.9	<0.001[b]
Laboratory	Hb	14.26 ± 1.96	12.23 ± 2.17	0.000[b]
	Leukocytes	7,595.75 ± 1,876.91	9,981.25 ± 3,800.29	0.002[b]
	Hematocrit	43.65 ± 4.50	37.24 ± 6.54	0.000[b]
	Platelets	331,950.0 ± 68,313.36	318,425.0 ± 121,215.61	0.540[a]
	Eosinophils (%)	1.98 ± 1.45	2.01 ± 1.98	0.939[b]
	Eosinophils (cells)	136.5 ± 95.58	216.87 ± 230.92	0.623[b]
	Lymphocytes	27.81 ± 7.26	18.37 ± 10.11	0.000[a]
Spirometry	FEV1/FVC	122.89 ± 147.93	62.06 ± 8.08	<0.001[b]
	FEV1	89.47 ± 9.29	46.07 ± 9.89	<0.001[b]

[a]Independent t-test; [b]Mann-Whitney test

TABLE 3
Comparison of VOC Compounds in Research Subjects

VOC compounds	Control	COPD	*p-values*
CO_2	2,849.575 ± 0.324	1577,75±,1052,701	< 0.001[b]
C_2H_5OH	1.376 ± 0.324	1,855±,701	< 0.001[b]
CH_2O	0.0763 ± 0.02	0.075±.021	0.776[b]
C_7H_8	0.005 ± 0.002	0.005±.002	0.776[b]
C_3H_6O	11.115 ± 3.543	4,814±2,378	< 0.001[b]
NH_4	1.414 ± 1.457	0.594±1.035	0.776[b]
C_6H_{14}	0.35 ± 0.046	0.286±.093	0.776[b]
NO_2	0.816 ± 0.390	0.055±.148	< 0.001[b]
CO	0.025 ± 0.042	0.603±.243	< 0.001[b]
NH_3	0.0001 ± 0.00	0.004±.012	0.022[b]
CH_4	0.080 ± 0.020	0.189±.230	0.310[b]
C_6H_6	0.602 ± 0.019	0.634±.048	< 0.001[a]
C_3H_8	2.343 ± 0.619	3.195±1.253	0.001[b]

[a]Independent t-test; [b]Mann-Whitney grades

Meanwhile, the C_3H_6O and NO_2 parameters show a negative correlation with FEV1, with correlation coefficients of -0.417 and -0.316, respectively. On the other hand, the parameters CO, NH_3, C_6H_6, and C_3H_8 do not show a significant relationship with FEV1, so the Spearman's correlation test does not give significant results. (Table 5).

Relationship Between LTB4 and Serum Neutrophil Levels with VOC Levels

There is a relationship between serum LTB4 and VOC parameters. Of the 8 VOCs tested, only 1 parameter has a significant relationship with LTB4, namely C_2H_5OH ($p = 0.009$). Then, the test was continued with Spearman's test, which produced a correlation coefficient of 0.410, meaning that there is moderate strength between the two

TABLE 4
The relationship between serum LTB4 levels and eosinophil counts and spirometry parameters (FEV1)

Relationship between variables			Correlation coefficient	*p-value*
Serum LTB4	with	Eosinophil %	-0.179	0.112
	with	Absolute Eosinophils	-0.103	0.364
	with	FEV1	-0.173	0.125

*Paired t-test, p=0.05

TABLE 5
The relationship between FEV1 values and VOC levels

Relationship between variables			Correlation coefficient	*p-value*
FEV1	with	CO_2	-0.313	0.049
	with	C_2H_5OH	0.410	**0.009**
	with	C_3H_6O	-0.417	0.007
	with	NO_2	-0.316	0.047
	with	CO	0.166	0.305
	with	NH_3	0.130	0.423
	with	C_6H_6	0.294	0.066
	with	C_3H_8	-0.025	0.876

parameters. On the other hand, the remaining 7 VOC parameters have a statistically non-significant relationship with serum LTB4 (Table 6).

Relationship Between Serum Eosinophil Levels and VOC Levels

Statistical test results showed that none of the 8 VOC parameters have a significant relationship with eosinophil counts. The p-values of each VOC parameter are as follows: CO_2 (0.593), C_2H_5OH (0.295), C_3H_6O (0.933), NO_2 (0.954), CO (0.283), NH_3 (0.870), C_6H_6 (0.163), and C_3H_8 (0.990). This means that serum eosinophil levels are not related to VOC parameters (Table 7).

DISCUSSION

In this study, the age of patients with COPD is older than the age of those in the control group. COPD is 23 times more common in older people, and being in the elderly population is a significant risk factor for COPD. Aging is the progressive degeneration of tissues that affects the structure and function of vital organs. Age-related diseases arise when physiological anti-inflammatory and antioxidant mechanisms fail to protect the body from damage caused by chronic inflammation and increased reactive oxygen species (ROS). This imbalance leads to cellular and/or tissue damage that, combined with common COPD risk factors such as smoking, contributes to the development of COPD in older adults. From the third decade of life, age-related decline in lung function and structural dysfunction begins. Loss of function in the elderly may contribute to the "senile lung" phenomenon, which is characterized by alveolar enlargement in the absence of wall damage. As lung function declines and age-related structural changes occur, the lung's defense mechanisms become less effective. This increases the risk of lung infections and leads to decreased protective responses against oxidative stress and inflammation.[12]

Smoking may be an early trigger of proteostatic dysregulation in COPD. Oxidative stress caused by cigarette smoking causes protein breakdown and leads to the accumulation of misfolded proteins in the endoplasmic reticulum (ER), leading to the ER stress response. The unfolded protein response (UPR) is a compensatory cellular response to ER stress. This process attempts to restore homeostasis by reducing protein synthesis and increasing the degradation of misfolded proteins via the ubiquitin-proteasome pathway. If the UPR is unable to handle stress, the process of apoptosis occurs. In addition to protein degradation, cigarette smoking reduces proteasomal activity in alveolar epithelial cells, resulting in poor clearance of damaged proteins. Long-term accumulation of inactive proteins eventually leads to apoptosis and triggers chronic

TABLE 6
Relationship between serum LTB4 levels and VOC levels

Relationship between variables			Correlation coefficient	*p-value*
Serum LTB4	with	CO_2	0.229	0.155
	with	C_2H_5OH	0.410	**0.009**
	with	C_3H_6O	-0.180	0.268
	with	NO_2	-0.203	0.209
	with	CO	-0.151	0.353
	with	NH_3	-0.195	0.227
	with	C_6H_6	-0.273	0.089
	with	C_3H_8	0.239	0.137

TABLE 7
The relationship between serum eosinophil levels and VOC levels (Spearman's test)

Relationship between variables			Correlation coefficient	*p-value*
Eosinophil counts	with	CO_2	-0.061	0.593
	with	C_2H_5OH	0.119	0.295
	with	C_3H_6O	-0.010	0.933
	with	NO_2	0.006	0.954
	with	CO	-0.122	0.283
	with	NH_3	-0.019	0.870
	with	C_6H_6	-0.157	0.163
	with	C_3H_8	-0.001	0.990

inflammation, thereby contributing to the pathogenesis of COPD.[13] In this study, the majority of COPD patients were male. The results of this study are consistent with previous studies, which show that men make up 64% of patients with COPD.[14]

The Comparison of Laboratory Results and VOC between COPD and Controls

This study showed that there is an increase in LTB4 levels in COPD patients compared to the controls. In COPD patients, cytokine levels increase as macrophages and alveolar epithelial cells release LTB4, a chemical factor that attracts immune cells. Macrophages and alveolar cells also produce IL-8/CXCL8 and growth-related oncogene (GROα)/CXCL1, which increase the inflammatory response by recruiting more leukocytes from the blood to the site of inflammation. Other chemotactic factors such as CXCL5 and CXCL8 can also increase the migration of neutrophil cells in COPD patients.[15] Leukotriene B4 (LTB4) is a chemokine that influences the accumulation of granulocytes and macrophages at sites of inflammation. BLT1 is a highly specific receptor for LTB4 that is expressed on the surface of inflammatory and immune cells. Currently, inhibition of the LTB4/BLT1 pathway using BLT1 antagonists is used to reduce mitochondrial dysfunction in various inflammatory diseases as well as sepsis, asthma and arthritis.[16]

This study also shows that the leukocyte count of COPD patients increased when compared to the controls. Macrophages, essential for lung homeostasis and defense against inhaled threats, increase substantially in COPD lungs. However, half of COPD patients, despite elevated macrophages, show chronic bacterial colonization, indicating innate immune dysfunction. These macrophages originate from circulating monocytes recruited to the lungs, undergoing further differentiation in the tissue.[17] This study showed that the lymphocytes of COPD patients were significantly lower than those of the controls. Persistent inflammation is key in COPD

development, with lymphocytes crucial in its progression. Fibrotic conditions reveal infiltration of macrophages and T lymphocytes. Severe COPD cases, as seen in bronchoalveolar lavage tests, exhibit a notable rise in macrophages and neutrophils but a decrease in lymphocytes. Lymphopenia may signal a weakened immune system, heightening infection risk and potentially indicating a systemic response to stress.[18]

This study shows that patients with COPD experience a significant decrease in lung function compared to controls. COPD results in decreased lung function due to various factors, including oxidative stress, airway inflammation, cellular aging, and cell death induced by cigarette smoke. Cigarette smoke-generated excessive reactive oxygen species (ROS) damages the lungs, contributing to COPD pathogenesis. Smokers exhibit reduced Nrf2 levels, a key protector against oxidative stress, resulting in insufficient antioxidant production. Oxidative processes not only impact lung damage but also induce cellular senescence in epithelial and stem cells. Lung aging leads to the development of senescence-associated secretory phenotypes (SASPs), releasing inflammatory molecules and causing chronic damage to lung structures.[19] The results of this research are consistent with previous studies, which showed that patients with asthma and COPD show a decrease in FEV1, FVC, and peak expiratory flow rate compared to normal people.[20] In this study, several VOC compounds are found to be significantly different in COPD patients. VOCs that can be identified from cellular and enzymatic metabolic pathways, on the other hand, can be used to better understand the underlying complex biological processes so that they can become new therapeutic targets. Labeled VOC compounds can be used to identify specific cellular or microbial activity in respiratory tract diseases.[21]

This study shows that there are 8 compounds that have significantly different potential as markers in COPD. These compounds and their metabolites hinder alveolar macrophages (AM), causing increased airway leakage, harmful ROS generation, and disruption of the lung's antioxidant/oxidant balance. Ethanol exposure impairs pathogen clearance through reduced CBF, impaired alveolar macrophage function, and affects the adaptive immune system. This compromised CBF and weakened immune response contribute to infectious lung diseases, like bacterial pneumonia in alcoholics. Ethanol's effects on epithelial permeability and fluid clearance, through ion channel dysregulation and increased TGF-β, also contribute to lung fibrosis.[22]

This study shows that there is an increase in C3H6O or acetone compounds. Patients with COPD are older on average and tend to lose skeletal muscle mass as the disease progresses. A study by Shahzad *et al*. showed that the average exhaled acetone level of healthy patients (0.66 ppm) was higher than those with COPD (0.50 ppm). Blood plasma of COPD patients shows low concentrations of amino acids, abnormalities in glycometabolism, decreased metabolites related to immune functionincluding glutamineand increased metabolism of fats including acetoacetic acid and acetone, which are products of fatty acid catabolism.[23] Research by Van Berkel (2010) aims to evaluate the VOC profile that differentiates COPD patients from controls.[24]

Based on research by Basanta *et al*. (2012) indicates that the use of VOCs could differentiate patients with COPD from controls. The results of this study indicate that there are 11 aldehyde compounds that can be used to differentiate COPD patients from controls. However, the metabolism of this aldehyde is increased in smoking patients and can be reduced when N-acetylcysteine is administered.[5] Other studies have also stated that ketone compounds are increased in AECOPD patients compared to COPD patients. In addition, some of these VOCs are also associated with the presence of potential bacterial pathogens.[7]

The difference in exhaled benzene (C_6H_6) levels in the case group and the controls in this study is significant. A study by Filipiak *et al*. (2012) found that certain VOCs were detected significantly more frequently in the exhaled breath of smokers compared to nonsmokers ($p<0.05$), namely benzene, toluene, and hexane with specificities above 90%.[25] Propane (C_3H_8) differed significantly between the case and control groups. Propane belongs to the class of VOC pollutants commonly used in cooking, heating, and grilling foods. In addition, propane is one of the aldehydes that is a constituent of cigarette smoke.[25] There were marked increased levels of carbon monoxide (CO) in the case group compared to controls. In non-smokers, exhaled CO is increased due to endogenous production and in a condition of pulmonary inflammation. A study in India in 2018 showed that, in COPD, exhaled CO levels increased > 3 times more than in healthy individuals.[26] A significant difference in elevated carbon monoxide (CO) levels was found between the disease group and the control group. A study in India in 2018 showed that COPD levels in the exhaled air of COPD patients increased more than 3 times compared to healthy individuals.[26]

Nitrogen dioxide (NO_2) levels were higher in the control group than in the study group. Sources of NO_2 are cigarette smoke, and combustion devices such as ovens, stoves, and water heaters. Studies show exposure to NO_2 can increase the risk of exacerbation of asthma and COPD patients. In addition, NO_2 is an ambient air pollutant, which is an irritant to the airways.[2] Ammonia (NH_3) is a product of the hydrolysis of urea, which is involved in the nitrogen cycle. In exhaled air, NH_3 showed no difference between normal subjects and patients with cystic fibrosis, but it increased in patients with asthma and ARI. Increased urea levels are in line with increased oxidative stress. Brushing teeth and rinsing can reduce NH_3 levels

in exhaled air. Another research by Shahzad *et al*. in 2022 showed NH_3 in exhaled air can be a useful biomarker in COPD because it has a positive relationship with FEV1 values.[28]

Relationship between FEV1 and VOCs

There are four VOC parameters that showed a significant relationship with FEV1 values, namely CO_2, C_3H_6O, NO_2, and C_2H_5OH. There is a negative correlation between the CO_2 parameter and the FEV1 value. CO_2 retention can be used as an indicator of the severity of obstruction in COPD patients. This retention is associated with decreased ventilation function, respiratory muscle weakness, worsening of respiratory symptoms, lung damage, and respiratory failure. The study conducted by Wei *et al*. showed that CO_2 retention was associated with worse FEV1 values in acute exacerbation of COPD.[29] The negative correlation results of this study may be due to the stable condition of COPD patients with the use of bronchodilators thereby demonstrating better CO_2 exhalation.

In this study, it was found that there is a negative correlation between FEV1 values with C_3H_6O and NO_2; that is, the lower the FEV1 value, the higher the levels of C_3H_6O and NO_2 in the exhaled air. A study also indicated that C_3H_6O levels in exhaled air were higher in smokers with lung cancer compared to healthy active smokers. A positive correlation was found between FEV1 and C_2H_5OH. Shahzad *et al*. in their study showed that the ethanol (C_2H_5OH) levels of COPD patients were almost the same as those of healthy patients (0.96 ppm vs 1.09 ppm). In addition, exhaled ethanol levels measured based on the degree of COPD obstruction were also relatively the same (mild: 0.92 ppm; moderate: 1.07 ppm; severe: 0.93 ppm).[28]

The Relationship between LTB4 and Eosinophils with VOC

The research findings indicated that there is a correlation between serum LTB4 and ethanol, from the eight VOC parameters tested. An increase in serum LTB4 will cause an increase in ethanol levels in exhaled air. Research by Allers *et al*. showed higher levels of ethanol in exhaled air in smokers compared to non-smokers; although, this difference was not significant in the COPD group when compared to the healthy group.[30] None of the VOC parameters showed a significant association with eosinophil counts. Until now, no study has analyzed the correlation between LTB4 and eosinophil counts with VOC parameters in COPD cases. In theory, oxidative stress in the airways and lungs can result in the accumulation of inflammatory cells, including macrophages and neutrophils, which increase ROS production. LTB4 is produced from the metabolism of arachidonic acid in lipid membranes in response to increased ROS. LTB4 acts as a chemoattractant for neutrophils, which plays an significant role in airway inflammation in COPD patients and contributes to lung tissue damage. Meanwhile, the increased VOC of expired air in COPD cases is a product of lipid peroxidation in lipid membranes that is not related to arachidonic acid metabolism, which plays an significant role in the airway inflammation process in COPD patients and contributes to lung tissue damage. Meanwhile, the increased VOC of expired air in COPD cases is a product of lipid peroxidation in lipid membranes that is not related to arachidonic acid metabolism.[4]

Role of Eosinophils in COPD and VOC

This research has revealed that there are no distinctions in eosinophil levels between the COPD group and the control group. Furthermore, eosinophils do not appear to be associated with all types of volatile organic compounds (VOCs). Eosinophils play a role in the inflammatory response seen in COPD. In specific situations, inflammation attracts eosinophils to the lungs, where they release various chemicals, including chemokines (such as CCL5, CCL11, CCL13), cytokines (such as interleukin (IL)-2, IL-3, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-25), and cytotoxic granular substances (such as major basic proteins, eosinophil cationic proteins, eosinophil peroxidases, eosinophil-derived neurotoxins), all contributing to the inflammatory process. In a state of equilibrium, eosinophils circulate in the bloodstream and adhere to the bronchial vascular endothelium when necessary. Eosinophil infiltration into the airways occurs only when inflammatory signals induce the expression and/or activation of specific adhesion molecules on the endothelium and bronchial vascular epithelium. This recruitment to the airway is regulated by chemokines like CCL5, 7, 11, 13, 15, 24, and 26, along with their corresponding receptors, such as CCR1, CCR2, and CCR3. This interaction between chemokines and receptors, in conjunction with chemoattractant receptor homologous molecules found on type 2 helper T cells and their ligand, prostaglandin D2, plays a crucial role in this process.[31]

In a study conducted by Nishimura *et al*. (2012), it was demonstrated that a rapid decline in lung function is indicative of more severe emphysema and lower levels of circulating eosinophils. This indirectly suggests a possible connection between emphysema and blood eosinophil counts in COPD.[32] Another study by Papaioannou *et al*. (2017), involving 98 COPD patients, reported that individuals with significant emphysema, affecting ≥15% of lung parenchyma, had lower blood eosinophil counts compared to those without emphysema.[33] These findings are consistent with our observations of longer survival in COPD patients with higher eosinophil counts.

This study also highlights the presence of several VOCs that significantly differ between COPD patients

and healthy controls. Additionally, certain VOCs can differentiate between COPD patients with and without comorbidities. However, it's important to note that this study has limitations, including the consideration of pollutant exposure locations and the absence of analysis regarding other comorbidities such as diabetes, hypertension, and oral diseases. These various factors are believed to influence the VOC profiles of COPD patients.

CONCLUSION

Based on the results and discussion above, it can be concluded that there are differences in the profile of volatile organic compounds (VOC) and levels of leukotriene B4 in COPD patients and the control group. There is no difference in eosinophil counts in COPD patients and the control group. There is a relationship between serum leukotriene B4 and VOC for the C2H5OH component in COPD patients. There is no relationship between differences in volatile organic compound (VOC) profiles and eosinophil serum levels in COPD patients and control patients. The researchers suggest that it is necessary to check the VOC levels in ambient air to prove whether or not there is an environmental effect on the research results and to take homogeneous samples between COPD case groups and healthy controls.

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