Original Article

Change in Exhaled Volatile Organic Compounds (VOC) Profile and Interleukin-17 Serum in Lung Cancer Patient

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Abstract

Background: In recent years, there have been studies regarding biomarkers for early detection of lung cancer. The expansion of tumor is accompanied by distinct metabolic process product, which results in identifiable changes in the volatile organic compounds (VOC) emission profile. The content of such molecules differs between healthy and lung cancer patients. Furthermore, the expression of Interleukin-17 (IL-17) was linked to the clinical and pathological aspects of lung cancer patients. The aim of this study is to profile the exhaled VOC and the level of IL-17 in the serum of lung cancer patient.

Methods: Fourty patients with confirmed lung cancer and 42 healthy subjects as control were gathered for this study. VOC was measured using breath analyzer and sensor array, while IL-17 was measured by ELISA. Statistical analysis was conducted using Kruskal-Wallis test and Spearman correlation test with $P<0.05$ considered significant.

Results: We examined 15 VOCs and found that ethanol (C_2H_5OH), formaldehyde (CH_2O), toluene (C_7H_8) and ammonia (NH_3) in lung cancer patient were increased significantly compared to control ($P<0.05$; $P<0.05$; $P<0.05$ and $P=0.001$ respectively). However, the level of IL-17 in control subjects was higher ($P=0.299$) than patients with lung cancer.

Conclusion: Ethanol, formaldehyde, toluene and ammonia can potentially be used as biomarkers for lung cancer. However, the role of IL-17 in lung cancer screening still needs further investigations.

Keywords: interleukin-17, lung cancer, VOC

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Submitted: April 25th, 2022
Accepted: December 12th, 2022
Published: January 28th, 2023

J Respirol Indones. 2023
Vol. 43 No. 1: 9–14
https://doi.org/10.36497/jri.v43i1.316

INTRODUCTION

In recent years, lung cancer has become one of the main causes of cancer deaths globally. Data from GLOBOCAN 2018 stated that there is an estimation of 2.09 million new cases and 1.76 million deaths from lung cancer.[1] Lung cancer is predicted to be the main cause of cancer death in men and women in the next 20 years as well.[2] All lung malignancies, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) combined, have a 5-year relative survival rate of 19%, and NSCLC has higher 5-year survival rate (23%) than SCLC (6%).[3] One of numerous factors that contributes to the poor outcome in lung cancer is that the disease is frequently only diagnosed at an advanced stage after the patient has developed symptoms. This highlights the importance of early detection of lung cancer in order to improve the patients' survival.[4]

The one method that's currently approved for lung cancer screening is low-dose computerized tomography scan (LDCT). Studies show that this method lowers lung cancer mortality by 20% and is indicated for high-risk individuals. However, there is a risk of radiation exposure, high expense and high likelihood of false positives when employing LDCT as a screening approach.[4,5]

Therefore, several other methods have been developed as screening tools, namely the analysis of volatile organic compounds (VOCs) or specific genomic approaches. VOCs referred to volatile organic compounds that can be found in the human body. In patients with lung cancer, VOCs are metabolized differently and its profile is changed. However, there are currently no consistent VOC biomarkers for lung cancer and the VOC set used in investigations also varies. Therefore, breath analysis is still in an early stage of clinical application.[6]

T-helper 17 (Th17) cells are the principal producers of interleukin-17 (IL-17), a proinflammatory cytokine. IL-17 and IL-17-expressing cells have lately been investigated in a variety of cancers, including NSCLC. In NSCLC patients, high level of IL-17 in serum was found to be associated with late stage of the disease, overall survival (OS) and disease-free survival (DFS). IL-17 expression was also significantly elevated in human NSCLC tissues and associated with clinical and pathological characteristics of patients, such as TNM staging, OS and DFS. Furthermore, in NSCLC patients, the frequency of IL-17-producing T cells have been observed to be dysregulated.[7]

In spite of both VOCs and IL-17 increase in lung cancer, the clear explanation for the correlation remains unclear. In this study, we aim to profile exhaled VOC and serum IL-17 in lung cancer patients.

METHODS

This study was conducted in RSUD dr. Saiful Anwar Malang, East Java, Java, Indonesia from October 2021 to January 2022. Patients with lung cancer found in outpatient clinics and wards were enrolled in the study. The inclusion criteria were patients with primary lung cancer who were in stable condition and consented to take part in the study. Subjects with secondary lung cancer and in acute or unstable condition were excluded. The control group consisted of healthy subjects without lung cancer based on anamnesis, physical examination and radiological finding. Minimal number of samples from each group is 31. Samples were obtained by consecutive sampling. Eighty-two subjects who met inclusion and exclusion criteria were measured for their exhaled VOC and IL-17 serum.

Exhaled VOCs were measured using a breath analyzer. The breath analyzer was developed by Universitas Brawijaya, Malang (Ubreath) which does analysis and measurement using sensor array. Samples of VOCs were collected using breath apparatus and connected to Ubreath. Data was automatically entered and collected in the computer. Serum was obtained by phlebotomy and the samples were sent to biomolecular laboratory to have the IL-17 level measured by ELISA.

Data were logarithm-transformed as necessary to meet normality and homoscedasticity criteria. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to find any significant changes in VOC profile and IL-17 levels between lung cancer and control group. To examine the difference in VOC profiles in lung cancer patients depending on the cancer type, stage and management, researchers used repeated ANOVA. The Spearman correlation test was used to find the association between VOC profile and IL-17 in the two groups. With value of $P<0.05$, differences were measured using IBM SPSS software version 25.0.

RESULTS

Among the 82 subjects, 40 were lung cancer patients and 42 healthy controls (Table 1). The median age for lung cancer group was 56.6 years old, which is older than the control group. There were more male than female subjects, with percentage of 60% vs 40% in lung cancer group and 57.14% vs 42.86% in control group. Most subjects in lung cancer group were smokers (55% vs 45%), while the opposite was found in control group in which 97.7% were non-smokers.

Table 1. Demography of Study Subject

Characteristic	Lung Cancer (n=40)	Control (n=42)
Age, range (mean)	40-72 (56.6)	25-38 (30.8)
Sex		
Male	24 (60.00%)	24 (57.14%)
Female	16 (40.00%)	18 (42.86%)
Smoking		
Smoker	22 (55.00%)	1 (2.30%)
Non-smoker	18 (45.00%)	41 (97.70%)
Histological type		
Adenocarcinoma	27 (67.50%)	-
Adenosquamous cell carcinoma	4 (10.00%)	-
Squamous cell carcinoma	5 (12.50%)	-
Small cell lung cancer	4 (10.00%)	-
Stage		
IIIB	2 (5.00%)	-
IVA	15 (37.50%)	-
IVB	23 (57.50%)	-
Chemotherapy		
Chemotherapy	34 (85.00%)	-
Targeted therapy	6 (15.00%)	-

In lung cancer group (n=40), the most prevalent type of lung cancer was adenocarcinoma (67.50%), followed by squamous cell carcinoma (12.50%), adenosquamous cell carcinoma (10.00%) and small cell carcinoma (10.00%). All patients were found at late stage of the disease, with 57.50% in stage IVB, 37.50% IVA and 5.00% IIIB. All lung cancer patients had received chemotherapy (85.00%) or targeted therapy by tyrosine kinase inhibitor (15.00%).

We evaluated the concentrations of 15 distinct VOCs in exhaled air between lung cancer patients and healthy controls, using value of $P<0.05$ to account for multiple comparisons (Table 2). Ethanol, toluene, formaldehyde and ammonia concentrations in the exhaled air of lung cancer patients were considerably higher ($P<0.05$; $P<0.05$; $P<0.05$ and $P=0.001$ respectively) than in the subject control group. Thus, the researcher focused on those four compounds in this study.

Table 2. Profile of Volatile Organic Compound Subject

VOCs	Lung Cancer (Mean) ppm	Control (Mean) ppm	*P*
Oxygen (O_2)	21.22254	20.7988	0.282
Ozone (O_3)	58.14644	110.8965	0.0001
Carbon Dioxide (CO_2) (1)	1470.21055	701.1388	0.188
Carbon Dioxide (CO_2) (2)	1496.00533	714.5339	0.204
Ethanol (C_2H_5OH)	1.24580	0.8148	0.0001
Formaldehyde (CH_2O)	0.51899	0.0453	0.0001
Toluene (C_7H_8)	0.61858	0.0167	0.0001
Acetone (C_3H_6O)	0.08536	0.2279	0.0001
Ammonium (NH_4)	0.44946	0.9996	0.0001
Hexane (C_6H_{14})	0.41880	0.4589	0.0001
Nitrogen (NO_2)	0.98333	1.5615	0.001
Carbon Monoxide (CO)	0.00009	0.0000	0.306
Ammonia (NH_3)	0.90681	0.6637	0.001
Methane (CH_4)	0.47846	0.5175	0.0001
Sulphur Dioxide (SO_2)	2.59492	2.5316	0.133

Furthermore, we investigated the differences between histological types of lung cancer, stage and therapy with the VOCs (Table 3). The concentration of ethanol, toluene, formaldehyde and ammonia in the exhaled air of lung cancer patients did not differ significantly between each histological type of lung cancer ($P=0.404$; $P=0.978$; $P=0.967$ and $P=0.535$ respectively), neither did they with the cancer stages ($P=0.298$; $P=0.086$; $P=0.086$ and $P=0.107$ respectively) nor the therapy ($P=0.570$; $P=0.081$; $P=0.081$; $P=0.130$ respectively).

Table 3. The differences between the histological types, stage and therapy of lung cancer with VOCs and IL-17

VOCs	Ethanol (*P*)	Formaldehyde (*P*)	Toluene (*P*)	Ammonia (*P*)	IL-17 (*P*)
Histological type	0.404	0.967	0.978	0.535	0.751
Stage	0.298	0.084	0.086	0.107	0.342
Therapy	0.570	0.081	0.081	0.130	0.363

While comparing the IL-17 level between both groups, we found the level of IL-17 in control group was higher ($P=0.299$) than lung cancer group (Figure 1) with no significant difference between those two groups.

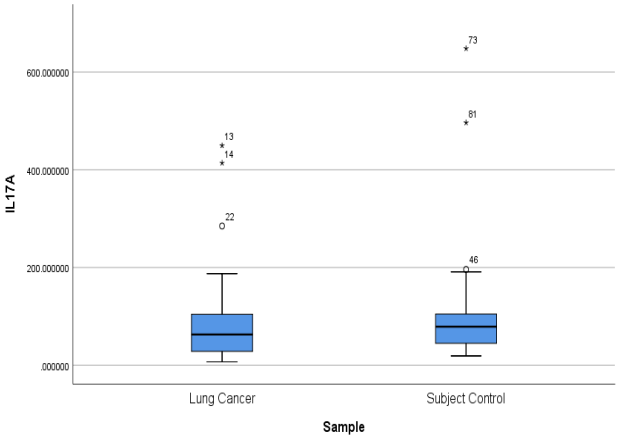

Figure 1. IL-17 comparison between lung cancer and control groups

We also examined the level of IL-17 on patient with lung cancer, according to the lung cancer type, stage and therapy. No significant differences were found with $P=0.751$; $P=0.342$ and $P=0.363$ respectively (Table 3). There wasn't any correlation either between the concentration of those four VOCs and the level of IL-17 statistically ($P=0.277$; $P=0.477$; $P=0.412$ and $P=0.269$ respectively) (Table 4).

Table 4. Association between VOC and IL-17

VOCs	IL-17 (*P*)
Ethanol	0.277
Formaldehyde	0.477
Toluene	0.412
Ammonia	0.269

DISCUSSION

The goal of this study is to determine whether VOCs and IL-17 may be used for early detection of

lung cancer. Lung cancer is a disease that's mostly asymptomatic in the early stages but fatal in an advanced stage. Radiological imaging can be used for lung cancer screening while a definitive diagnosis is determined by histopathology examination. VOCs from exhaled air may reflect metabolic changes caused by the disease and play a role as biomarkers for lung cancer.[8]

We identified ethanol, toluene, formaldehyde and ammonia as possible biomarkers for lung cancer, especially in advanced stages. Several studies have been conducted to identify components of VOC compounds. One study conducted by Oguma et al. concluded that ethanol and toluene concentration had shown an increase in lung cancer compared to the control group.[9]

Differences in VOC concentrations caught on the sensor devices are influenced by metabolic activity in cancer cells. Cancer cells directly undergo changes in their metabolic products since they need large amount of energy to support its uncontrolled proliferation. The Warburg effect is a cancer metabolism process in which the activation of aerobic glycolysis occurs as the main pathway for obtaining energy. Changes in cellular metabolism result in metabolic changes that accelerate the growth of cancer cells and also change the profile of respiratory VOCs.[10] The increased ethanol concentrations in the study were most likely caused by the Warburg effect on the cancer patient group.

The concentration of formaldehyde was also found to elevate in this study. Endogenous formaldehyde may increase in malignancy. In studies involving patients with breast and prostate cancer, it has been reported that there's an increase of the endogenous formaldehyde concentration in the urine. In vivo studies also show abnormally elevated formaldehyde inside cancer cell tissue. Endogenous formaldehyde is produced through a number of biochemical pathways in cells through the enzymatic reaction process of oxidative demethylation. Other factors such as cigarette smoke, electronic cigarettes and aspartame sweeteners can also cause increase in the amount of formaldehyde.[11]

The increase of ammonia concentration found in this study can be explained by cancer cells' need to absorb and process high amount of glutamine as a supplement for nucleotide biosynthesis. Glutamine is a non-essential amino acid that can be synthesized by cells through glutamine synthetase and is present in the blood in the form of free amino acids. Ammonia is formed as a result of the breakdown of glutamine into glutamate.[12] This is similar to Spinelli's research (2018) which stated that ammonia is a cellular metabolism product that is mainly excreted by proliferating cells, for example cancer cells. Ammonia accumulates in tumor microenvironment 10 times higher compared to healthy tissue.[13]

Toluene has been researched as biomarker for detecting lung cancer. Toluene is a metabolite product of cancer cells, hence its concentration tends to elevate in lung cancer. However, the mechanism remains unclear.[14]

The global incidence of lung cancer shows that NSCLC accounts for 80-85 percent of all lung cancer. According to Chen et al, the cytokine IL-17 is vital in the process of microangiogenesis in the tumor microenvironment, stimulates cell proliferation and has a role in the metastatic process. Wu et al. also found that IL-17 promotes tumor angiogenesis and cell proliferation while also inhibits apoptosis via inflammatory activation pathways.[15,16]

Several studies have been conducted to investigate the expression of IL-17 cytokines in the peripheral blood of NSCLC patients. Dutkowska et al. revealed that IL-17 is a proinflammatory cytokine that plays a role in chronic inflammation, autoimmunity and malignancies associated with inflammation. They further claim that IL-17 plays a direct or indirect function in lung cancer spread and progression, boosting tumor angiogenesis and cell proliferation while blocking apoptosis. Hence, higher level of IL-17 expression was linked to earlier stages of cancer.[17]

In our study, the average levels of IL-17 in lung cancer were smaller than those of healthy subjects (87.77 and 101.03 pg/mL) with a $p > 0.05$. This result is not in line with the study conducted by Chen et al which found that IL-17 expression in lung cancer was

higher compared to the control group. Since Chen et al's study used subjects with untreated lung cancer, we assumed that these differing results happened because our patients have been treated by chemotherapy that might affect the concentration of IL-17. This is consistent with the study by Xiang et al which revealed that there was a decrease in serum IL-17 levels in breast cancer patients who received chemotherapy and radiotherapy compared to the control group, though the underlying mechanism remains unclear.[16,18]

Another study by Wang et al explored the association between lung cancer prognosis and IL-17 level, which shows that the increase in IL-17 expression was closely related to poor clinical output in lung cancer patients. Our study didn't find any significant differences between IL-17 levels to lung cancer stadium and types.[19]

LIMITATION

Our research has a number of limitations. Firstly, our study used patients who were receiving chemotherapy at the time of enrollment, which might have influenced the concentration of inhaled VOCs and IL-17. Secondly, due to the small number of early-stage patients, the current investigation was underpowered to discover exhaled VOCs relevant for early diagnosis of lung cancer. To continue and complete the VOC and IL-17 profile that is useful in evaluating patients with suspected lung cancer, a prospective study is required.

CONCLUSION

Some exhaled VOCs such as ethanol (C_2H_5OH), formaldehyde (CH_2O), toluene (C_7H_8) and ammonia (NH_3) in lung cancer patients were higher compared to control group, hence their potential as biomarkers of lung cancer. However, the level of IL-17 in this study was higher in control group instead. IL-17 has weak correlation with VOCs.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

None.

FUNDING

None.

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