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Volatile Organic Compounds (VOCs) and Interleukin-23 Levels in Lung Cancer: A Future Biomarker

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ABSTRACT

Introduction: Lung cancer (LC) is the world's second leading cause of death due to malignancy. In Indonesia, LC is one of the top three malignancies. Volatile organic compounds (VOCs) from the respiratory reflect changes in metabolism caused by disease and may be a biomarker of LC. Interleukin-23 (IL-23) has been known as a pro-inflammatory cytokine in the development and progression of cancer. This study aimed to identify levels of IL-23 and VOCs in LC patients.

Methods: This study involved 40 LC patients and 42 controls. VOCs were taken by the subject exhaling their third deep breath into the sample bag, which was immediately analyzed using an E-nose-based device. As for the IL-23, the cytokine was taken from the blood serum and then analyzed using the ELISA method. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test data normality. Mann-Whitney and Kruskal Wallis tests were conducted for variables. Spearman correlation and heat map were used to find the correlation between the observed gases and IL-23.

Results: The concentration of ozone ($p = 0.000$), ethanol ($p = 0.000$), formaldehyde ($p = 0.000$), toluene ($p = 0.000$), acetone ($p = 0.000$), ammonia ($p = 0.000$), ammonium ($p = 0.001$), nitrogen ($p = 0.001$) and methane ($p = 0.000$) in LC group differed with controls. The same outcome was also observed in comparing LC patients and control groups of IL-23 ($p = 0.000$). Spearman correlation analysis revealed a positive correlation between serum IL-23 with formaldehyde ($p = 0.029$), toluene ($p = 0.014$), and ammonia ($p = 0.028$) and a negative correlation with nitrogen ($p = 0.011$). Compared to the control group, all types of LC were observed to have higher levels of IL-23. A weak positive correlation was found in formaldehyde (Cv = 0.23), toluene (Cv = 0.23), and ammonia (Cv = 0.13). A weak negative correlation was obtained in acetone (Cv = -0.12), ammonium (Cv = -0.11), and nitrogen dioxide (Cv = 0.23).

Conclusion: Weak linear correlations were obtained between the cytokine and formaldehyde, toluene, ammonia, ammonium, and nitrogen dioxide. A higher IL-23 concentration was observed in the LC group than in the control group. The volatile concentration was significantly different between LC and control groups.

INTRODUCTION

Cancer is the second most deadly disease worldwide. In the case of lung cancer (LC), the disease is placed in the second rank after breast cancer. In Indonesia, cancer is ranked third, with a death rate of around 11/100,000 male citizens. The number of LC cases was reported to reach 360 in Dr. Saiful Anwar General Hospital, Malang, only in 2018. Most cases were stated at a terminal level due to late diagnosis.[1–3]

Interleukin-23 (IL-23) is known to have the capability to induce LC proliferation. The cytokine influences LC persistence, life cycle, and cancer growth. IL-23 induces the differentiation of naive T cell CD-4 and maintenance of T-helper cell 17 (Th-17). IL-23 also stimulates interleukin 17 (IL-17) downstream signaling

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pathway via Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3). The expression IL-23R was not reported in lung squamous cell carcinoma but in adenocarcinoma and small-cell carcinoma. Furthermore, IL-23R polymorphism is associated with breast cancer, nasopharyngeal carcinoma, and LC risk.

Cancer cells are reported to alternate the metabolism process to support uncontrolled proliferation. The Warburg effect plays a role as the main pathway to obtain energy. Changes in cellular metabolism lead to the acceleration of cancer cell growth and the respiratory volatile organic compounds (VOCs) profile.[4,5] Many studies have linked various VOC types with cancer, especially LC.[6–8] The presence of propane, isobutane, acetonitrile, acetone, carbon disulfide, 2-propanol, dimethyl sulfide, methyl acetate, isoprene, 2-methyl-2-butene, 1.4-pentadiene, 1-pentene, methyl vinyl ketone, 2.3-butanedione, 2-methylfuran, ethyl acetate, hexane, cyclohexane, 2-pentanone, hexanal, 2-methylheptane, cyclohexanone, ethylbenzene, p-xylene, o-xylene, 4-heptanone, 2.4-dimethylheptane, 2.3.4-trimethylhexane, 4-methyloctane, nonane, ß-pinene, 4.7-dimethylundecane, limonene, and dodecane were reported to have a potential to be used as a biomarker in LC.[9,10] However, all those studies were only focused on the difference between LC VOCs and the control group statistically. In this study, we strengthened the correlation by providing the correlation between the VOCs and IL-23.

A breath analyzer has been developed to detect the growth of LC. The device works by analyzing the breath exhalation content and evaluating the volatolomics content from breath.[11] The cancer cell releases the volatolomics content in the breath, blood, urine, sweat, etc. The content concentration depends on the tissues, blood, and the partition coefficient of blood/air.[3,12] A breath analyzer has a good potential to be used as a diagnosing system due to its non-invasive procedure. This study evaluated the correlation between VOC content in patients' breath with IL-23 cytokine. The result of this study is expected to support the knowledge about the correlation between cytokine and breath content.

METHODS

Subject

A case-control selection, cross-sectional study (two-gate design) was chosen for this study. Forty LC patients were selected to participate based on inclusion factors of their ability to blow their third deep breath into an airbag sample. Forty-three healthy subjects were chosen as the control. The procedure was conducted at Dr. Saiful Anwar General Hospital, Malang. All procedures were approved by ethical clearance no. 400/201/K.3/102.7/2022.

VOCs extraction

Thirteen gases consisting of nine volatile and four non-volatile compounds were observed in this study. The volatile compounds were ethanol, formaldehyde, toluene, acetone, methane, ammonium, nitrogen dioxide, ammonia, and sulfur dioxide. The non-volatile group comprised oxygen, ozone, carbon dioxide, and carbon monoxide. The volatile gas selection was based on the previous study about the potential of exhaled gas as an LC biomarker.[2,8,13,14] As for the non-volatile gas, the selection was grounded on the potential of the gases as breath-print.[14,15] The gases were collected by asking the participant to blow their third deep breath into a 500 ml sample bag.[16] Then, the air sample was injected into an E-nose device for measurement. The measurement data were saved as a CSV file for further analytical procedure.

IL-23 extraction

The blood serum was collected from the patient by using venipuncture sampling. A 3.0 ml blood sample was taken from the participant for IL-23 extraction. The cytokine extraction followed the procedure of ELISA analysis for IL-23.[17]

Statistical approach

IL-23 and VOC content were analyzed using SPSS 26. One sample, Kolmogorov-Smirnov and Shapiro-Wilk tests, were used for a normal test. The variable was evaluated by using Mann-Whitney and Kruskal Wallis. IL-23 and VOCs gases were assessed using the Spearman correlation test with a p-value < 0.05. The correlation was presented in the form of a heatmap analysis.

RESULTS

Forty-two healthy subjects aged 25-38 years old participated in this study as a control group. The control group consisted of 24 male subjects and 18 female subjects. One control subject was an active smoker, while the other 41 were non-smokers. Then, as a group of LC patients, 40 LC patients were selected, consisting of 24 men and 16 women. Twenty-two subjects were active smokers, while 18 subjects were non-smokers. In the LC group, 27 patients were diagnosed with bronchogenic adenocarcinoma, four with bronchogenic adenosquamous carcinoma, five with bronchogenic squamous cell carcinoma, and five with bronchogenic small cell carcinoma. For the LC stage, two patients

were at level III-B, 15 were on IV-A, and 23 were on IV-B. Subject information is presented in Table 1.

Table 1. Subject information

Categories	LC	N	Control	N	p-value
Age (years old)	40-72	40	25-38	42	0.000
Gender	Male	24	Male	24	0.623
	Female	16	Female	18	
Smoking history	Active smoker	22	Active smoker	1	0.000
	Non-Smoker	18	Non-smoker	41	
Cancer cell types	Adenocarcinoma bronchogenic	27			
	Adenosquamous carcinoma bronchogenic	4			
	Squamous cell carcinoma bronchogenic	5			
	Small cell carcinoma bronchogenic	5			
Stadium	III B	2			
	IVA	15			
	IVB	23			
Treatment	Chemotherapy	34			
	Targeted therapy	6			

As presented in Table 1, the age distribution was different between LC and control groups. However, age was reported not to influence VOC concentration.[18]

IL-23 level

We found that the data were not distributed normally in evaluating IL-23 by using Kolomogorov-Smirnov for one sample and Saphiro-Wilk methods. Furthermore, the Mann-Whitney approach resulted in p-values < 0.05, indicating the data came from a different distribution. The observation of IL-23 is presented in Table 2. In the table, it can be seen that the mean level for the control group was 248.33 pg/ml. However, this level was raised significantly to 957.87 pg/ml in the cancer group, as shown in Figure 1. A similar result was also observed on the median at 174.74 pg/ml for the control group and 417.21 pg/ml for LC patients. In our observation, IL-23 level for LC patients ranged from 37.22 pg/ml to 4978.50 pg/ml. On the other hand, the control group ranged from 34.26 pg/ml to 1174.34 pg/ml.

Table 2. IL-23 level in the control group and LC patients

Group	IL-23 (pg/mL)		
	Median	Min	Max
Control	417.21	34.26	1174.34
LC	174.74	37.23	4978.50

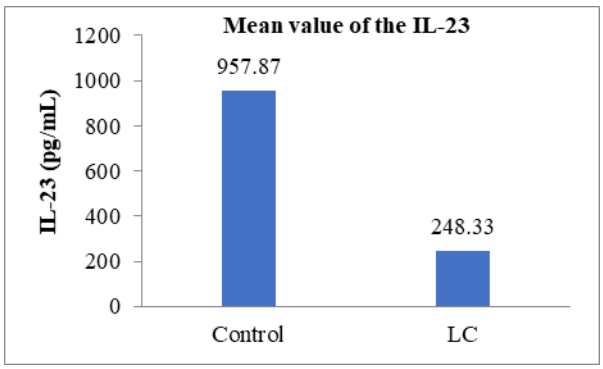

Figure 1. The comparison of IL-23 levels between LC and control groups

Furthermore, we evaluated IL-23 levels regarding LC cell types. We obtained the cytokine level was significantly different with p-value = 0.001. As shown in Table 3, the IL-23 difference between adenosquamous carcinoma bronchogenic and small cell carcinoma bronchogenic was distinguishable. We obtained an IL-23 level of 1458.26 pg/ml for adenosquamous carcinoma bronchogenic and 2602.87 pg/ml for small cell carcinoma bronchogenic. Both types of cells were significantly different compared to adenocarcinoma bronchogenic and squamous cell carcinoma bronchogenic. As shown in the table, IL-23 level for adenocarcinoma bronchogenic and squamous cell carcinoma bronchogenic was observed at similar levels (688.37 pg/ml and 696.87 pg/ml, respectively, for both types of cells). Compared to the control group, all types of LC were observed to have higher levels of IL-23. More observation on the influence of the treatment found no alteration in the level of IL-23 between chemotherapy and targeted therapy. However, further analysis must be conducted to support this conclusion since the number of samples between both treatments was significantly different.

Table 3. The median of IL-23 level on various types of the LC cell

LC cell type	IL-23 (pg/ml)	p-value
Control	340.45	
Adenocarcinoma bronchogenic	1171.61	
Adenosquamous carcinoma bronchogenic	364.71	0.001*
Squamous cell carcinoma bronchogenic	2475.15	
Small cell carcinoma bronchogenic	174.74	

*significant with p-value < 0.05

Evaluation of VOC

In this study, we observed at least five VOC and seven non-VOC gases. In VOC, we measured the concentration of ethanol, formaldehyde, toluene, acetone, and methane. We observed the concentration of ethanol, formaldehyde, and toluene higher than the

control group. For example, ethanol concentration in cancer patients was measured at 1.26 ppm. This concentration was higher than the control group at 0.81 ppm. As for formaldehyde, the concentration was 0.53 ppm higher than the control group. Toluene concentrations were 0.60 ppm higher in the cancer group than in the control group.

Furthermore, the control group was higher than the cancer group in the concentration of acetone and methane. The concentration of the cancer group was quantified at 0.09 ppm and 0.48 pm for acetone and methane. The measurement is presented in Table 4.

Table 4. The median of volatile and non-volatile compounds concentration on LC and control group

Gas	LC (ppm)	Adenocarcinoma bronchogenic	Adenosquamous carcinoma bronchogenic	Small cell carcinoma bronchogenic	Squamous cell carcinoma bronchogenic	Control	p-value
Volatile compounds							
Ethanol	1.13	1.23	1.26	1.23	1.04	0.83	0
Formaldehyde	0.32	0.11	0.53	0.68	0.59	0.01	0
Toluene	0.14	0	0.4	0.66	0.43	0	0
Acetone	0.06	0	0	0	0	0.23	0
Methane	0.48	0.48	0.48	0.47	0.49	0.52	0
Ammonium	0.34	0.29	0	0.18	0.29	1.05	0
Nitrogen dioxide	0.77	0.72	0.6	1.03	0.59	1.54	0
Ammonia	0.97	0.92	1.11	0.91	1.15	0	0
Sulfur dioxide	2.58	2.57	2.5	2.7	2.6	2.56	0.13
Non-volatile compounds							
Oxygen	21	21	21	22	21	20.8	0
Ozon	34	20	20	20	20	76	0
Carbon dioxide	1487	1707	1060	787	1198	715	0
Carbon monoxide	0	0	0	0	0	0	0.31

*significant with p-value < 0.05

As for non-volatile gas, a higher concentration was obtained on oxygen, carbon dioxide, and ammonia. All three gases were measured at 21.28 ppm, 1496.00 ppm, and 0.91 ppm in the cancer group. In the control group, the three gases were obtained at 20.77 ppm, 715.85 ppm, and 0.00 ppm. As for ozone, ammonium, and nitrogen dioxide, the cancer patient produced less concentration than the control group. The cancer patient produced only 58.15 ppm, while the control group released 75.90. In the case of ammonium and nitrogen dioxide, the cancer patient produced 0.45 ppm and 0.98 ppm of gases. In the control group, ammonium and nitrogen concentrations increased to 1.05 ppm and 1.54 ppm, respectively. As for carbon monoxide and SO_2, the alteration was not significant.

Based on the normality test, the gas concentration data for both groups were non-normally distributed. However, a non-parameter test for the entire data found a p-value < 0.05 for ethanol, formaldehyde, toluene, acetone, and methane. The same outcome was obtained on ozone, ammonium, nitrogen dioxide, and ammonia. P-value < 0.05 indicated that the data significantly differed between the control and other patient groups. Further observation on the influence of cell type and treatment found no difference between the control and LC patient group (p-value > 0.05).

The correlation between VOCs and IL-23

The calculated correlation value (Cv) of VOCs and IL-23 is revealed in Figure 2. As shown in the figure, there was no strong linear correlation between the cytokine and VOCs. We observed the strongest linear correlation on the weak level obtained in the analysis of IL-23 with formaldehyde, toluene, acetone, ammonium, nitrogen dioxide, and ammonia (Cv < 0.1 or > 0.1). A weak positive linear correlation was shown on formaldehyde (Cv = 0.23), toluene (Cv = 0.23), and ammonia (Cv = 0.13). A negative correlation was found between cytokine and acetone (Cv = -0.12), ammonium (Cv = -0.11), and nitrogen dioxide (Cv = 0.23). In the case of ethanol, methanol, sulfur dioxide, oxygen, ozone, and carbon dioxide, we found no correlation (-0.1 > Cv < 0.1). As for carbon monoxide, the concentration was observed at 0 ppm for LC and control groups.

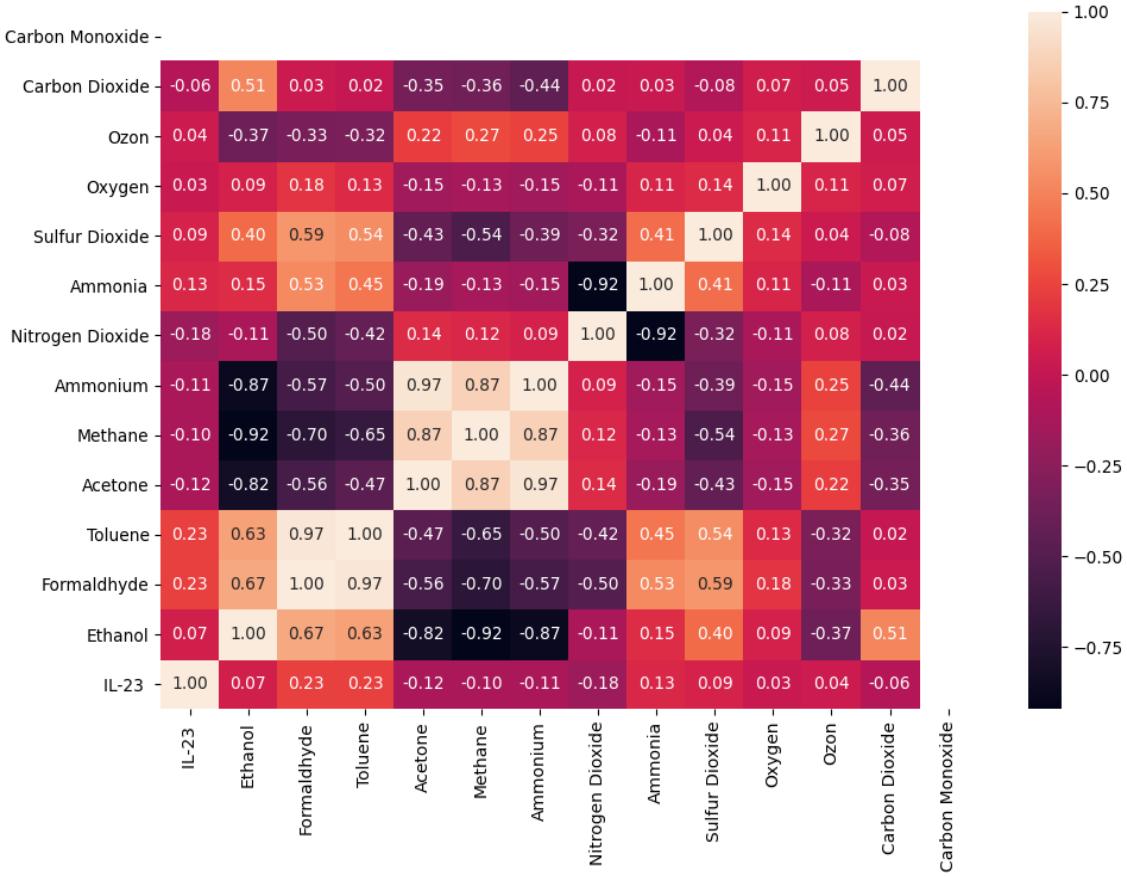

Figure 2. Heatmap analysis between the observed gases and IL-23

DISCUSSION

IL-23 has been known to influence the carcinogenesis process. The cytokine can induce LC proliferation and has a significant role in cancer persistence.[19] In previous studies, the expression of IL-23 was found to induce tumor cells.[20] IL-23 was reported to elevate in the case of LC, particularly the small cell type.[17] Since the increase of IL-23 was linked with LC severity, understanding the correlation with the exhaled volatile compound may provide additional evidence for the compound as an LC biomarker. Our finding reconfirmed the higher cytokine concentration in LC patients compared to the control group.[20–23]

Meanwhile, in the case of ethanol, formaldehyde, toluene, and ammonia, LC was obtained to have a higher concentration than the control group. In previous studies, the formaldehyde concentration was reported to increase in LC patients. This mechanism produces endogenous formaldehyde from several biochemical pathways through oxidative demethylation enzymatic reactions. Endogenous formaldehyde comes from a single carbon intermediate cycle (1C) after the enzymatic division of serine to produce glycine.[24] The formaldehyde molecule condensed with tetrahydrofolate (THF), releasing 5,10-methylene-TH. Next, the endogen formaldehyde altered to be other folates before it was taken by single carbon into a biosynthetic pathway and methylation reaction.[25] In the oxidative phase, folate is degraded into two main components: pterin moiety and p-amino benzoyl glutamate (p-ABG). Meanwhile, the methylene bridge that connected these two units is released as formaldehyde. As a result, several folate derivatives are genotoxic to the cell with alcohol dehydrogenase 5 (*Adh-5*) deficiency.[25,26] In breast and prostate cancer, formaldehyde and ethanol were detected in the urine.[27] A similar mechanism was also reported on toluene.[27,28] Toluene increases mice's phosphorylation of the p-53 gene. Toluene causes a mutation and deletion in the tumor suppressor gene. As a result, cell proliferation was uncontrollable in the cancer cell.[24,29]

As for ammonia, the gas was related to the metabolism product on the proliferation cell. The gas was 10 times higher in the microtumor than in healthy tissues.[29,30] Since IL-23 is responsible for cancer cell proliferation, the increase in ammonia concentration was strongly associated with the cytokine. Ammonia was formed as the final result of glutamine breakdown. Glutamine is a non-essential amino acid synthesized by cells via glutamine synthetase. Glutamine is found in the blood in the form of free amino acids. As a result, the ammonia concentration is associated with the cancer cell since the cells absorb and process a large amount of glutamine for nucleotide biosynthesis.[29] Nitrogen dioxide is essential for the de novo synthesis of various biomolecules, especially nucleotides and amino acids.

Amino acids and nucleotides are the main nitrogen source in cells and can quickly support the growth of cancer cells.[31,32]

Nitrogen compounds are mainly produced from amino acid reduction reactions. Amino acids, such as glutamine, arginine, aspartate, alanine, glisten, and serin, are largely responsible for cell proliferation and are the main reservoir for cellular nitrogen in the microtumor.[33,34] In their correlation with IL-23, the concentration of nitrogen dioxide is supposed to be linked with the increase of cytokine activity. However, this study found low nitrogen levels in the LC group. The inverse relationship between H_2O_2 and atmospheric nitrogen caused the contrary result.[35] However, the reduction in nitrogen dioxide level was reported after three cycles of chemotherapy.[36]

In the correlation between IL-23 and the volatile compound, we found a weak correlation between cytokine and formaldehyde, toluene, acetone, ammonium, nitrogen dioxide, and ammonia. We found formaldehyde, toluene, and ammonia to have a positive correlation. In the concentration, formaldehyde, toluene, and ammonia were higher than the control. This outcome is similar to IL-23, which also has a higher concentration in LC. In the result, the correlation of the cytokine was positive. Meanwhile, the correlation was negatively weak since the concentration of acetone, ammonium, and nitrogen dioxide was higher in the control group. Since we only compared LC and control group, it was impossible to determine the regression correlation. To find the whole correlation picture, further study is needed by observing the IL-23 and volatile compound concentrations in early-stage cancer.

CONCLUSION

This study reconfirmed the higher concentration of IL-23 on LC than control group. A similar outcome was also found in ethanol, formaldehyde, toluene, and ammonia. Although the concentrations were significantly different, the concentration of acetone, methane, ammonium, and nitrogen dioxide was higher in the control group. Weak linear correlations were obtained between the cytokine and formaldehyde, toluene, ammonia, ammonium, and nitrogen dioxide. A higher IL-23 concentration was observed in the LC group than in the control group. The volatile concentration was significantly different between LC and control group. Based on the result, formaldehyde, toluene, ammonia, ammonium, and nitrogen dioxide has the potential to be used as LC biomarker. However, further observation of the volatile compounds and the IL-23 may be needed by observing both on the early-stage LC.

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Conflict of Interest

The authors declared there is no conflict of interest.

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Authors' Contributions

Conceiving and designing the research protocol, collecting data, conducting research, providing research materials, organizing: RDWL, UAS and AYPW. Interpretating data and analyzing: UAS, TWA, SDJ, ASL and AYPW. Writing initial and final draft of the article and providing logistic support: RDWL, UAS, TWA, SDJ and AYPW. All authors critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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