Original Article

Analysis of Volatile Organic Compounds in the Exhaled Breath of COVID-19 Patients

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Abstract

Background: It has been more than 2 years since COVID-19's first cases were reported in 2019. Rapid diagnosis of COVID-19 is necessary to prevent its spread. A sample for COVID-19 testing is collected by a naso-oro-pharyngeal swab. This procedure is often uncomfortable and requires a trained examiner. Exhaled breath contains thousands of volatile organic compounds (VOC), which are likely to change during infection. This study aimed to analyze the difference in VOC in the exhaled breath between COVID-19 and healthy subjects.

Methods: A cross-sectional study was carried out, recruiting 90 confirmed cases of COVID-19 and 42 healthy subjects. A sample of exhaled breath was collected by using a 500-mL airbag in both groups. The sample was analyzed using an arrayed sensor breath analyzer to quantify the concentration of CO_2, C_7H_8, C_6H_{14}, CH_2O, NH_4, TVOC, NO_2, PM1.0, CO, NH_3 and Acetone.

Results: The medians of CO_2, NH_4, TVOC, NO_2, and acetone were significantly lower in COVID-19 patients compared to healthy subjects (respectively 607.3 vs 1175.1; 0.0 vs 1.05; 0.05 vs 146.6; 0.04 vs 1.55; 0.0 vs 0.23) while C_7H_8, CH_2O, CO, and NH_3 were significantly higher (respectively 0.92 vs 0.0; 0.55 vs 0.01; 0.24 vs 0.0; 1.99 vs 0.67; all with $P<0.05$.). Furthermore, we found that NH_4, acetone, NH_3, and CO were positively correlated with the severity of COVID-19, while CO_2 and TVOC were negatively correlated.

Conclusion: COVID-19 patients emit distinctive VOC profiles in comparison with healthy subjects, and this is related to the severity of the disease.

Keywords: COVID-19, diagnosis of COVID-19, volatile organic compounds

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INTRODUCTION

Back on December 31, 2019, the World Health Organization (WHO) China Country Office reported several cases of pneumonia with unknown causes, later identified as Coronavirus disease 2019 (COVID-19) cases.[1] COVID-19 is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2). This virus is the third in the Corona family that causes epidemic and continually becomes pandemic.[2] Until now, it has already infected more than 600 million people in the world and caused more than 6 million deaths.[3]

Rapid diagnosis is one of the key means of controlling the pandemic situation. Standard confirmation of acute SARS-CoV-2 infection is based on the detection of unique viral sequences by nucleic acid amplification tests (NAATs), such as reverse-transcription polymerase chain reaction (RT-PCR).[4] The procedure is not widely available, requires a long processing time, and is expensive in many countries. Therefore, developing a test that is inexpensive yet rapid and reliable to diagnose COVID-19 is needed. The rapid diagnostic test or RDT, which is an antigen-detection diagnostic test designed to directly detect SARS-CoV-2 proteins, was widely developed.[5]

Both of the procedures involve testing the sample obtained through respiratory specimens. Testing combined nasopharyngeal and oropharyngeal swabs from one individual has been shown to increase sensitivity and improve reliability.[4] Although obtaining the sample is generally considered safe, it requires semi-skilled staff and is

often uncomfortable, and several complications have been reported, with the possibility of more incidents escaping systemic recording. Several complications of pharyngeal swabs include a break of the swab's tip, foreign body sensation, epistaxis, dislocation of the temporomandibular joint, and leakage of the cerebrospinal fluid.[6] Although only a small number of complications are reported, raising awareness is needed. Moreover, inventing a new testing procedure with fewer complications while remaining reliable is a better alternative.

Volatile organic compounds (VOCs) are organic compounds that evaporate easily at room temperature. VOCs can be derived from the environment (exogenous), taken through inhalation or ingestion, or produced within the body.[7] Recent studies have shown at least 1765 VOCs can be detected in humans. Physiological metabolism, products of metabolic processes from microbial pathogens, and host response to pathological processes such as inflammation and infection can affect the VOCs. Thus, VOCs emanating from exhaled breath may provide a deep insight into various biochemical processes in the human body.[7,8]

Previous studies have shown bacterial pneumonia, reactive oxygen species (ROS), inflammation, septic condition, the use of ventilators, and viral infection can emit different VOC profiles.[7,9] The infection with SARS-CoV-2 is also believed to have a distinguished VOC profile. Analysis of VOCs in the exhaled breath has the potential to become a diagnostic test that is not only quick but also non-invasive, reliable, and widely available.[7]

METHODS

A cross-sectional study was conducted at Saiful Anwar General Hospital, East Java Province, Indonesia, and Idjen Boulevard Field Hospital, Malang, Indonesia. Confirmed cases of COVID-19 patients, regardless of their severity, who were admitted to one of these hospitals were randomly selected and provided informed consent. Subjects with acute deterioration, using invasive ventilation, or being unable to provide exhaled breath samples were excluded. Healthcare professionals with no respiratory symptoms and negative results of RDT or RT-PCR for SARS-CoV-2 were also recruited as healthy subjects for a comparison group. Subjects in the COVID-19 group were further divided into subgroups based on the severity of the disease.

The severity of the disease is classified based on the national guidelines for COVID-19 management in Indonesia. Those without any symptoms were classified as asymptomatic. Mild degree is defined as a patient with symptoms such as cough, fatigue, fever, anorexia, shortness of breath, myalgia, sore throat, nasal congestion, headache, diarrhea, nausea and vomiting, anosmia, and ageusia, without any sign of pneumonia. A moderate degree is marked by pneumonia with room air saturation equal to or above 93%. If the saturation drops below 93%, then it is classified as severe. The critical degree is defined as those with acute respiratory distress syndrome (ARDS), septic shock, or sepsis.

Subjects in both groups were asked to exhale into 500 mL of a sealed airbag. The valve of the airbag is then opened and connected by a tube to the breath analyzer device. This device is equipped with an arrayed sensor to detect the concentration of CO_2, C_7H_8, CH_2O, NH_4, TVOC, NO_2, NH_3, CO, and acetone. The results were obtained within less than 30 minutes, recorded in a customized program, and quantified for further analysis. The concentration of VOCs is compared between COVID-19 and control groups. Further comparisons were also done in the COVID-19 group based on its severity. This study also analyzed the correlation between VOCs and the severity of COVID-19.

RESULTS

A total of 132 participants were included, divided into two groups. Group 1 was the COVID-19 group with 90 confirmed cases of COVID-19 patients. The second group consisted of 42 healthy subjects, defined as the control group.

Table 1. Characteristics of subjects

Characteristics	Group 1 (n= 90)	Group 2 (n= 42)	*P*
Gender			
Male	61 (67.8%)	23 (54.8%)	0.148
Female	29 (32.2%)	19 (45.2%)	
Age			
18-29	27 (30%)	18 (42.9%)	
30-39	9 (10%)	24 (57.1%)	<0.001*
40-49	11 (12.2%)	0 (0%)	
>49	43 (47.8%)	0 (0%)	
Smoking Status			
Never smoker	55 (61.1%)	39 (92.8%)	
Ex-smoker	28 (31.1%)	1 (2.4%)	<0.001*
Active smoker	7 (7.8%)	2 (4.8%)	
Comorbid			
Diabetes Mellitus	14 (15.5%)	1 (2.4%)	0.026*
Cardiovascular Disease	20 (22.2%)	3 (7.1%)	0.033*
Asthma	4 (4.4%)	6 (14.3%)	0.047*
Chronic Obstructive Pulmonary Disease	0 (0%)	0 (0%)	-
Active Tuberculosis	0 (0%)	1 (2.4%)	0.142
Obesity	11 (12.2%)	6 (14.3%)	0.742
Malignancies	0 (0%)	0 (0%)	---
None	55 (61.1%)	27 (64.3%)	0.726

Table 2. Comparison between VOCs of exhaled breath between healthy subjects and COVID-19 subjects

Parameter		N	Mean	SD	Median	*P*
CO_2	Healthy	42	1278.5	610.9472	1175.14	
	COVID-19	90	711.3599	348.57465	607.27	0.0001
	Total	132	891.8136	519.30829	891.21	
C_7H_8	Healthy	42	0.0167	0.06872	0	
	COVID-19	90	0.8791	0.67732	0.93	0.0001
	Total	132	0.6047	0.68973	0.47	
CH_2O	Healthy	42	0.0453	0.10214	0.01	
	COVID-19	90	1.7664	2.01069	0.55	0.0001
	Total	132	1.2188	1.84324	0.28	
NH_4	Healthy	42	0.9996	0.61911	1.05	
	COVID-19	90	1.1749	2.00002	0	0.001
	Total	132	1.1191	1.68651	0.52	
TVOC	Healthy	42	0.4158	0.59951	0.1466	
	COVID-19	90	0.1313	0.20685	0.05	0.0001
	Total	132	0.2218	387.62139	0.080	
NO_2	Healthy	42	1.5615	0.76288	1.54	
	COVID-19	90	0.0441	0.02336	0.04	0.0001
	Total	132	0.5269	0.82817	0.79	
CO	Healthy	42	0	0	0	
	COVID-19	90	0.2298	0.07332	0.24	0.0001
	Total	132	0.1567	0.12326	0.12	
NH_3	Healthy	42	0.6637	0.32482	0.66	
	COVID-19	90	2.08	1.3989	1.99	0.0001
	Total	132	1.6294	1.34202	1.32	
ACET	Healthy	42	0.2279	0.1536	0.23	
	COVID-19	90	1.0751	1.99449	0	0.001
	Total	132	0.8055	1.69319	0.11	

Note: CO_2=carbon dioxide; C_7H_8=Toluene; CH_2O=Formaldehyde; NH_4=Ammonium; TVOC=Total Volatile Organic Compounds; NO_2=Nitrogen dioxide; CO=Carbon monoxide; NH_3=Ammonia; ACET=Acetone

Table 3. Correlation of VOCs between subgroups based on disease severity in COVID-19 group

VOCs	Degree of severity	Median (Min-Max)	Mean±SD	*P*
CO_2	Asymptomatic	603.4 (462.67-1673.9)	---	
	Mild	711.8 (400-1876.5)	---	
	Moderate	612.83 (400-747.73)	---	0.002*
	Severe	548.27 (400-1443.4)	---	
	Critically ill	431.03 (400-1246.2)	---	
TVOC	Asymptomatic	0.09 (0.02-0.836)	---	
	Mild	0.11 (0-0.709)	---	
	Moderate	0.02 (0-0.15)	---	0.0001*
	Severe	0.02 (0-0.194)	---	
	Critically ill	0.01 (0-0.071)	---	
CO	Asymptomatic	---	0.14±0.06	
	Mild	---	0.20±0.07	
	Moderate	---	0.28±0.03	0.0001*
	Severe	---	0.27±0.05	
	Critically ill	---	0.28±0.04	
NH_3	Asymptomatic	---	1178.89±634.10	
	Mild	---	971.46±644.57	
	Moderate	---	1398.91±289.86	0.043*
	Severe	---	1232.86±428.48	
	Critically ill	---	1250.63±392.80	
NH_4	Asymptomatic	0 (0-10.19)	---	
	Mild	0 (0-3.43)	---	
	Moderate	0.12 (0-3.94)	---	0.0001*
	Severe	1.43 (0-6.64)	---	
	Critically ill	1.625 (0-5.8)	---	
ACET	Asymptomatic	0 (0-8.17)	---	
	Mild	0.00 (0-1.64)	---	
	Moderate	0.27 (0-5.30)	---	0.002*
	Severe	0.47 (0-6.35)	---	
	Critically ill	0.96 (0-6.52)	---	
NO_2	Asymptomatic	---	0.06±0.02	
	Mild	---	0.04±0.02	
	Moderate	---	0.04±0.03	0.275
	Severe	---	0.04±0.03	
	Critically ill	---	0.04±0.02	
C_7H_8	Asymptomatic	1.525 (0.09-2.54)	---	
	Mild	0.16 (0.01-1.72)	---	
	Moderate	1.105 (0-1.82)	---	0.243
	Severe	0.92 (0-2.14)	---	
	Critically ill	1.015 (0-1.59)	---	
CH_2O	Asymptomatic	4.4 (0-7.16)	---	
	Mild	0.02 (0-5.14)	---	
	Moderate	0.27 (0-3.02)	---	0.100
	Severe	1.49 (0-4.64)	---	
	Critically ill	2.245 (0-4.71)	---	

Note: CO_2 and TVOC show a negative correlation; CO, NH_3, NH_4, and ACET show a positive correlation; NO_2, C_7H_8, and CH_2O show no correlation

Group 1 was further divided based on its severity, 12 (13.3%) subjects were asymptomatic, 33 (36.7%) subjects had mild disease, 10 (11.1%) subjects had moderate disease, 23 (25.6%) subjects had severe disease, 12 (13.3%) subjects were critically ill. Nine parameters of VOCs from exhaled breath samples were compared between group 1 and group 2. In COVID-19 groups, we obtained a

significantly higher concentration of C_7H_8, CH_2O, CO, and NH_3 when compared to the control group (respectively 0.92 vs 0.0; 0.55 vs 0.01; 0.24 vs 0.0; 1.99 vs 0.67; all with a P<0.05). While for the other markers, the concentration was significantly lower, including CO_2, NH_4, TVOC, NO_2, and acetone, in COVID-19 groups (respectively 607.3 vs 1175.1; 0.0 vs 1.05; 0.05 vs 146.6; 0.04 vs 1.55; 0.0 vs 0.23; all with a P<0.05).

Further comparisons in the COVID-19 group were carried out to analyze the difference between VOCs concentration based on the severity of the diseases. Only six markers showed differences. CO (P=0.0001), CO_2 (P=0.002), NH_4 (P=0.0001), NH_3 (P=0.043), Acetone (P=0.002), and TVOC (P=0.0001) were significantly different between subgroups based on disease severity. The severity of the disease was also correlated with the concentration of those markers. The positive correlations were observed in NH_4, Acetone, CO, and NH_3 with correlation coefficients of 0.476, 0.358, 0.645, and 0.236, respectively, while the negative correlations were seen in CO_2 and TVOC with correlation coefficients of -0.407 and -0.574.

DISCUSSION

Although studies related to the content of human breath have been conducted a long time ago, with the first study recorded in the period 1777-1783 by Lavoisier, research in this medical field has not developed widely.[9] Using one of the VOCs as a biomarker of disease is generally insufficient because of its complexity and heterogeneity, including environmental exposures and the presence of chronic diseases. Detecting VOCs in exhaled breath has the potential to be used as a diagnostic tool or a large-scale screening modality. Rather than detecting one VOC as a marker for one disease, using a set of VOCs and finding its pattern to create a “fingerprint” or “breath-print” is the preferred approach.[10] This study found that a set of VOCs consisting of C_7H_8, CH_2O, CO, NH_3, CO_2, NH_4, TVOC, NO_2, and acetone was able to differentiate between COVID-19 patients and healthy subjects.

Continuous monitoring of exhaled CO_2 is a method to ensure adequate ventilation during mechanical ventilation. The volume of CO_2 excreted by the cardiorespiratory system is a sensitive indicator of not only ventilation efficiency but also pulmonary perfusion and cardiac output.[11] Based on the Enghoff-Bohr equation, a lower concentration of CO_2 can reflect the condition of increased dead space, ventilation–perfusion mismatch, and ARDS. Those conditions usually occur in COVID-19 and are usually associated with its severity.[12] The low concentration of CO_2 in our research may be caused by those conditions. Using a metabolic analyzer and volumetric capnography are preferable methods to measure CO_2 concentration and predict partial pressure of the mean expired CO_2.[11]

Higher concentrations of NH_3 and lower concentrations of NH_4 in COVID-19 patients, as well as a positive correlation with the degree of severity, may occur as a result of lower pH in the respiratory airway, acidification by gastric fluid, and influence of nitrite or nitrate metabolism by respiratory or gastrointestinal bacteria.[13,14] Previous studies of the airway pH of patients with ARDS failed to document acidification, although these patients had acidopnea.[13] Lower pH in the esophagus showed a higher expression of angiotensin-converting enzyme 2 (ACE2).[15] Higher expression of ACE2 may be responsible for the increased severity of COVID-19 and the risk of death from COVID-19.[15,16]

The cytochrome P450 (CYP) expression and activity are greatly affected by an immune response and altered during COVID-19 infection.[17] The altered activity of CYP may lower the metabolism of C_7H_8 (toluene), causing higher excretion of toluene in exhaled breath.[18] Intracellular pro-oxidant/antioxidant imbalance leads to oxidative stress, resulting in lipid peroxidation.[19] Oxidative stress occurred during

COVID-19, and increased levels of activated neutrophils increased the production of CH_2O.[20,21] Oxidative stress also induces heme oxygenase-1 (HO-1) activity in the airway, nasal epithelium, alveolar macrophages, endothelial cells, and other lung cell types, thus endogenously producing CO.[22] The concentration of CO also increases during infection, neutrophilic inflammation, and other critical conditions requiring mechanical ventilation.[23] Similar to CO, acetone may also increase as a result of infection, septicemia, and critically ill conditions.[24] Treatment given during COVID-19 may also affect the concentration of VOCs. Antioxidants such as vitamin E and vitamin C, which are given in all COVID-19 subjects, are known to reduce the level of NO_2.[25]

LIMITATION

This study has some limitations. The first limitation is that a sensor-based analyzer can only calculate one type of VOC concentration per sensor. Thus, targeted VOCs are already predetermined, and other VOCs that may be pathognomonic for a disease remain undetectable. The second limitation is many things can affect the results of research related to VOC. Variations of the sampling process and environmental influences such as exogenous VOCs, humidity, and temperature cannot be fully controlled. This may cause differences in results between one study and another.

CONCLUSION

COVID-19 affects many aspects of the human body and causes an alteration in the composition of VOCs in exhaled breath. Our study shows there are differences in several VOC concentrations that can differentiate between COVID-19 patients and healthy subjects. Those distinctive profiles can be used as a method of diagnosing COVID-19 that is fast, reliable, and without risk of complications. Disease severity is also known to affect changes in VOC concentrations. With this discovery, VOC concentration may be expected to be a prognostic biomarker in the future, as it is known that the severity of the disease in COVID-19 affects the patient's prognosis, although further research is required.

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CONFLICT OF INTEREST

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REFFERENCE

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