Volatile Organic Compounds as Biomarkers

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Abstract

Cancer is one of the leading causes of death worldwide. Mortality rate from cancer can be reduced when the cases are diagnosed early. Due to poor prognosis and lack of any method for early detection of cancer, volatile organic compounds (VOCs) have been used as a non-invasive and screening tool for detection of cancer. An online literature search was conducted to identify studies reporting VOCs in various body fluids like breath, urine and blood.

Results: VOCs are specific for each cancer type with limited cross over. The types of cancer which have been considered are urinary bladder, prostate, colorectal, breast. TP53 gene has been shown to be the most mutated gene.

Conclusion: Various studies have shown that VOCs in different fluids can be detected by non-invasive method by using breath analysis. However, large clinical trials are required for better pharmacological intervention.

Keywords: VOCs: Volatile Organic Compounds**,** WHO: World Health Organization**,** CBC: complete blood count**,** GC-MS: Gas chromatography–mass spectrometry.

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INTRODUCTION

Cancer is one of the leading causes of death in the world.[1] With the mortality rate being estimated at around 7,70,230 in the year 2020, the number has increased with the years passing by, reaching 7,89,202 in the year 2021 and 8,08,558 in the year 2022.[2] The main reason for the current high mortality rate in India is the dearth in the ability of current diagnostic tools to detect cancer in its early stages, leading to high mortality rate. With the diagnosis of cancer in its early phases and with better prognosis, the mortality rate can be decreased.

Going by the data by WHO, 1 in 10 Indians is diagnosed with cancer and 1 in 15 die due to it.[2] Tobacco use, alcohol consumption, unhealthy diet, physical inactivity and air pollution are some of the factors causing high probability of cancer.

Some of the cancer screening methods are as follows:
1) Blood chemistry test
2) Complete blood count (CBC)
3) Cytogenetic analysis
4) Immunophenotyping
5) Liquid biopsy
6) Tumour marker tests
7) Volatile organic compound analysis [3]

What are volatile organic compounds?
Volatile organic compounds are the compounds that are produced and discharged through the metabolism of cancer cells or the body's immune system. [4] Therefore, they are used as cancer biomarkers for early detection of cancer.

Collection of these volatile organic compound samples can be done through various sites such as: -
i. Breath ii Blood iii. Urine iv. Saliva [5,6]
The VOCs can be detected through non-invasive diagnostic technique based on sensing metabolic volatile organic compounds related to the disease that helps in early diagnosis.[5] Few of these compounds have been traced in the breath, and analysis of urine for VOCs aids in the early detection of cancer. All these tests are invasive and have not been helpful for early detection, except for volatile organic compound analysis in breath.

EVALUATION OF VOCs

The evaluation of volatile organic compounds in exhaled breath is becoming a fast and non-invasive diagnostic tool to assess emitted endogenous VOCs resulting from oxidative stress. Due to the non-invasive nature of test, it is convenient for the patients.[7]

In human breath, VOC profiles may be distinct to an individual, influenced by one's unique personal microbiome, external exposures and immunological response.[8] VOCs are used for the diagnosis of bladder cancer, prostate cancer, lung cancer and various other types of cancer.

Some of the VOCs emitted from the human body as depicted in various studies were:

1. VOCs in blood: -
Blood directly reflects the internal environment of the body, including the nutritional, metabolic and immune status of an individual. The disease-specific VOCs in the blood have been useful in diagnosing diseases such as ovarian cancer, colorectal cancer, lung cancer, and hepatic encephalopathy. Some of the metabolic biomarkers which were found at significantly higher or lower levels in cancer patients were:

- Cyclohexanone
- 1-hexanol
- 2-ethyl-butylated hydroxytoluene
- 2,2,4-trimethyl-3-hydroxy-isobutyl ester.
- p-xylene [9]

1. VOCs in breath:
Exhaled breath contains VOC that can be contributed to either exogenous or endogenous volatile compounds. Collecting breath samples is relatively simple, painless and non-invasive. Breath VOCs can act as diagnostic markers for lung cancer. The lung cancer cells may produce these volatile organic compounds which are detectable in breath. VOCs in breath samples such as pentane, ethane and aldehydes [10] were observed in patients with lung cancer and lung diseases.

3. VOCs in urine: -
VOCs in urine may be the intermediate products or end products of metabolic pathways and have been found to have various structural motifs such as ketone, alcohol, furan, pyrrole and sulfide with a specific odour. Some studies have linked

urinary VOCs to infectious diseases and different types of cancer including prostate cancer, renal cancer, bladder cancer. Urinary VOC patterns in cancer patients are often different from the pattern in urine samples from control subjects, although the difference depends on cancer types and even cancer stages. [11]

BREATH ANALYSIS OF VOLATILE ORGANIC COMPOUNDS

The detection of cancer at an early stage increases the chances for successful treatment of the disease. Detecting and measuring breath VOCs become a very optimistic approach for the early detection and screening of various cancers, as well as other diseases.[12]]

Tumour cells have been reported to produce volatile organic compounds (VOC) which are unique and can reflect the disease status. The detection and analysis of VOC biomarkers from exhaled breath has been recognised as a new frontier in the early detection of cancer due to its rapid, non-invasive and inexpensive cancer screening tool.[13] To detect specific VOCs of low concentrations from exhaled breath, and to enhance the accuracy of early diagnosis, many breaths collection and analysis approaches have been developed.

VOCs are detected and analysed by using analytical instrument GC-MS. [14] Studies have shown that GC-MS provide outstanding sensitivity at part per billion and parts per trillion levels in VOC analysis with the pre-concentration steps. [14,15,16]

The most accurate method for identifying VOCs is a combination of gas chromatography–mass spectrometry (GC-MS) which is highly accurate and allows for the selective detection of individual VOCs. Previous studies that used GC-MS have identified potentially disease-specific VOCs as indicators of several malignant tumours. However, it is both time-consuming and expensive and trained experts are required to perform the technique.[17]

A recent technique which has been used in various studies is detecting VOCs by using electronic noses or e-noses which is an easy and inexpensive method. [18] Electronic noses are devices that allow detection and identification of various organic compounds or complex odours based on gas sensor arrays to simulate the function of human olfactory system. Electronic nose is an instrument which consists of various electronic chemical sensors with partial specificity and pattern-recognition system, which is capable of recognizing simple or complex odours. E-nose technology can use exhaled breath VOC pattern analysis for the early detection and screening of cancers.[19]

The analysis of volatile organic compounds In exhaled breath samples is a valuable tool in the early detection of cancer. It is a non-invasive and potentially inexpensive way to detect various pathological conditions including cancer.[20] Clinical trials with spectrometry and spectroscopy techniques, the standard volatile-compound detection methods, have shown the potential for diagnosing illnesses like cancer, multiple sclerosis, Parkinson's disease, tuberculosis and diabetes using breath tests.[13]

Breath analysis is a non-invasive method and has the potential to detect cancer at an earlier stage by analysing volatile biomarkers in exhaled breath. [20, 21,22] The breath-borne VOCs have also been studied in covid -19 screening. [23]

DETECTION OF VOCs IN URINE

Urine odour (smell or scent) analysis by sensorial techniques, maybe one of the most antique methods for the detection of disease conditions. Urine is a complex organic fluid from the chemical point of view, due to large number of constituents and variable composition, it is proved that urine composition can vary depending on factors such as gender, age, hormonal status, food habits, physical activity and underlying pathological conditions. Bladder cancer emits specific volatile organic compounds (VOCs) in urine headspace that can be detected by an electronic nose.

Head space solid phase microextraction (SPME) was applied for the extraction of volatile organic compounds from urine samples, and gas chromatography time of flight mass spectrometry (GC×GC TOF MS) was used for the separation and detection of urinary volatiles.[24]

Cystoscopy remains the gold standard in bladder cancer diagnosis, but it is invasive and

uncomfortable.[24] Urinary cytology and radiology play an important role in follow-up but have some limitations in the diagnostic setting and still remains operator sensitive. Hence, many efforts are focusing on the development of accurate non-invasive diagnostic tests for bladder cancer.[24]

Monitoring of volatile organic compounds (VOCs) in exhaled breath shows great potential as a non-invasive method for assessing haemodialysis efficiency.[25] Commercial electronic noses based on electro-resistive sensing technologies, such as metal oxides and conducting polymers, have previously been used to detect lung cancers from healthy controls.

Urine headspace has also been shown to be a suitable medium to measure by electronic nose for distinguishing prostate cancer. Faecal immunochemical tests (FITs) are currently the most commonly used non-invasive screening tests for colorectal cancer and adenomas with restricted sensitivity. Faecal volatile organic compounds (VOCs) may serve as a diagnostic biomarker of colorectal cancer and adenomas. [26,27]

A total of 188 different urinary VOCs were reported as prostate cancer biomarkers largely comprising of ketones, aldehydes, carboxylic acids, nitrogen and sulphur. Hexenal is a possible biomarker related to seven types of cancer. [11] The data from various studies have shown hexenal to be one of the main markers in lung cancer and the levels are significantly higher in different biological matrices such as blood and breath when compared to cancer patients and controls.

Dimethyl disulphide was presented as a potential biomarker in 7 out of 12 cancers in different studies. This substance is a sulfide compound that is present in urine and is one of the main substances. Volatile sulfide metabolites are produced mainly by incomplete metabolism of methionine. Dimethyl disulphide was present in lower levels in the bladder, breast colorectal leukaemia and lymphoma and in only one (head and neck).

Phenol is one of the major chemical families identified in urine from the oncological group.[15,27] Phenol was the most reported biomarker related to cancer of breast, colon, gastroesophageal, head and neck, renal and prostrate.

Table 1: Metabolic alterations and VOCs associated with cancer of urinary bladder, prostrate, colorectal and breast:

Cancer	**Main metabolic alteration**	**VOCs**	**Most mutated genes**
Urinary bladder	• Glycolysis mitochondrial • Mitochondrial dysfunctional	Hexanal sulfide dimethyl 4-heptanone 2-butanone	FGFR3 HRAS TSC1 TP53[28]
Prostrate	Pentose phosphate pathway	Hexanal phenol 2-butanone furan	TP53 ELAC2 CDKN1B [29]
Colorectal	Glycolysis glutaminolysis fatty acid synthesis	Hexanal dimethyl disulfide 3-heptanone P-cymene 2-methyl-3-phenyl-2-propenal-1,2,4trimethyl benzene	APC TP53 BRAF [30]
Breast	• Glucose metabolism • Amino acid metabolism	• Furan • Dimethyl disulfide • Phenol • 3-heptanone • 4- heptanone	BRCA1 BRCA2 TP53[31]

RESULTS

VOCs are also known impart certain odour which can be found in urine, saliva, blood and other body fluids. These VOCs are the end products of cell metabolic activities.[32] The activities of cancerous cells differ from the normal cell activities and hence different VOCs are produced than normal cells. The normal cells become cancerous by various factors but, major factor aiding this change is said to be the mutation of genes. Four different types of cancer have been included with different gene mutations, except TP53 gene which was found to be common in all these cancers as depicted in table 1. Therefore, TP53 gene has been shown to be the most mutated gene.

CONCLUSION

Various cancer studies and have detected volatile organic compounds by using electronic noses or e-noses which is an easy and inexpensive method; however, there are limitations. Further research needs to be done as a specific VOC as a biomarker of a particular cancer type.[32] Studies of the exhaled breath and other matrices investigating potential biomarkers of cancer is still in its progression. Some of these compounds appear in more than one cancer, while some are unique compounds. These VOCs can be used to differentiate patients from healthy individuals [3] Large-scale screening studies and normal profiles of VOCs in all matrices being studied are needed. But the use of urinary VOCs has demonstrated a potential application in cancer diagnosis as it has shown an easy, non-invasive test for early detection of disease. Although the pathways affecting VOC production are yet to be fully understood, the VOCs which have been identified to be related to specific cancers may provide valuable information to study the pathways of VOCs production in the various cancers. Therefore, further clinical trials are required which should be followed-up for long term so as to know the potential and predictive VOCs and early detection of cancer.

REFRENCES

1. Ma, X., & Yu, H. Global burden of cancer. The Yale journal of biology and medicine,2006; 79(3-4), 85–94. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1994799
2. http://cancerindia.org.in
3. https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis
4. Janfaza, S., Khorsand, B., Nikkhah, M., & Zahiri, J. Digging deeper into volatile organic compounds associated with cancer. Biology Methods Protoc. 2019; Nov 27; 4(1), bpz014. https://doi.org/10.1093/biomethods/bpz014
5. Agarwal SM, Sharma M, Fatima S**.** 2016**;** VOCC: a database of volatile organic compounds for cancer. RSC Adv**.** 6, 114783-114789. https://pubs.rsc.org/en/content/articlelanding/2016/RA/C6RA24414A
6. Arasaradnam RP, Wicaksono A, O'Brien H, Kocher HM, Covington JA, Crnogorac-Jurcevic T. Noninvasive Diagnosis of Pancreatic Cancer Through Detection of Volatile Organic Compounds in Urine. Gastroenterology. 2018; Feb;154(3):485-487.e1. doi: 10.1053/j.gastro.2017.09.054. Epub 2017 Nov 10. PMID: 29129714. https://pubmed.ncbi.nlm.nih.gov/29129714/
7. Ratiu IA, Ligor T, Bocos-Bintintan V, Mayhew CA, Buszewski B. Volatile Organic Compounds in Exhaled Breath as Fingerprints of Lung Cancer, Asthma and COPD. J Clin Med. 2020; Dec 24;10(1):32. doi: 10.3390/jcm10010032. PMID: 33374433; PMCID: PMC7796324. https://doi.org/10.3390/jcm10010032
8. Resse, K.L., Rasley, A., Avila, J.R. et al. Metabolic Profiling of Volatile Organic Compounds (VOCs) Emitted by the Pathogens Francisella tularensis and Bacillus anthracis in Liquid Culture. Sci Rep 2020; 10, 9333. https://doi.org/10.1038/s41598-020-66136-0
9. Ashley, D. L., Bonin, M. A., Cardinali, F. L., McCraw, J. M., & Wooten, J. V. Measurement of volatile organic compounds in human blood. Environmental health perspectives. 1996; 104 Suppl 5(Suppl 5), 871–877. https://doi.org/10.1289/ehp.96104s5871
10. Mazzone PJ. Analysis of volatile organic compounds in the exhaled breath for the diagnosis of lung cancer. J Thorac Oncol. 2008 Jul;3(7):774-80. doi:

10.1097/JTO.0b013e31817c7439. PMID: 18594325. https://pubmed.ncbi.nlm.nih.gov/18594325/

11. da Costa BRB, De Martinis BS. Analysis of urinary VOCs using mass spectrometric methods to diagnose cancer: A review. Clin Mass Spectrom. 2020; Oct 31; 18:27-37. doi: 10.1016/j.clinms.2020.10.004. PMID: 34820523; PMCID: PMC8600992. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8600992/
12. Kabir, E., Raza, N., Kumar, V., Singh, J., Tsang, Y. F., Lim, D. K., Szulejko, J. E., & Kim, K. H. 2019; Recent Advances in Nanomaterial-Based Human Breath Analytical Technology for Clinical Diagnosis and the Way Forward. *Chem*, *5*(12), 3020-3057. https://doi.org/10.1016/j.chempr.2019.08.004
13. Sun, X., Shao, K. & Wang, T. Detection of volatile organic compounds (VOCs) from exhaled breath as noninvasive methods for cancer diagnosis.2016; Anal Bioanal Chem 408, 2759–2780. https://doi.org/10.1007/s00216-015-9200-6.
14. Lett, L., George, M., Slater, R. et al. Investigation of urinary volatile organic compounds as novel diagnostic and surveillance biomarkers of bladder cancer. Br J Cancer 127, 329–336 (2022). https://doi.org/10.1038/s41416-022-01785-8
15. Ligor T, Ligor M, Amann A, et al. The analysis of healthy volunteers' exhaled breath by the use of solid phase microextraction and GC-MS. J Breath Res. 2008; 2(4):046006. https://iopscience.iop.org/article/10.1088/1752-7155/2/4/046006
16. Altomare DF, Di Lena M, Porcelli F, Trizio L, Travaglio E, Tutino M, Dragonieri S, Memeo V, de Gennaro G. Exhaled volatile organic compounds identify patients with colorectal cancer. Br J Surg. 2013 Jan;100(1):144-50. doi: 10.1002/bjs.8942. PMID: 23212621. https://pubmed.ncbi.nlm.nih.gov/23212621/
17. Wen Q, Boshier P, Myridakis A, Belluomo I, Hanna GB. Urinary Volatile Organic Compound Analysis for the Diagnosis of Cancer: A Systematic Literature Review and Quality Assessment. Metabolites. 2021;11(1):17. https://doi.org/10.3390/metabo11010017
18. Scheepers, M. H. M. C., Al-Difaie, Z., Brandts, L., Peeters, A., van Grinsven, B., & Bouvy, N. D. 2022; Diagnostic Performance of Electronic Noses in Cancer Diagnoses Using Exhaled Breath: A Systematic Review and Meta-analysis. JAMA network open, 5(6), e2219372. https://doi.org/10.1001/jamanetworkopen.2022.19372
19. Škapars R, Gašenko E, Broza YY, Sīviņš A, Poļaka I, Bogdanova I, Pčolkins A, Veliks V, Folkmanis V, Lesčinska A, et al. Breath Volatile Organic Compounds in Surveillance of Gastric Cancer Patients following Radical Surgical Management. Diagnostics. 2023; 13(10):1670. https://doi.org/10.3390/diagnostics13101670
20. Mohamed N, van de Goor R, El-Sheikh M, Elrayah O, Osman T, Nginamau ES, Johannessen AC, Suleiman A, Costea DE, Kross KW. Feasibility of a Portable Electronic Nose for Detection of Oral Squamous Cell Carcinoma in Sudan. *Healthcare*. 2021; 9(5):534. https://doi.org/10.3390/healthcare9050534
21. Queralto N, Berliner AN, Goldsmith B, Martino R, Rhodes P, Lim SH. Detecting cancer by breath volatile organic compound analysis: a review of array-based sensors. J Breath Res. 2014 Jun;8(2):027112. doi: 10.1088/1752-155/8/2/027112. Epub 2014 May27. PMID: 24862241 https://pubmed.ncbi.nlm.nih.gov/24862241/
22. Pham YL, Beauchamp J. Breath Biomarkers in Diagnostic Applications. *Molecules*. 2021; 26(18):5514. https://doi.org/10.3390/molecules26185514
23. Jing Li, Ami Hannon, George Yu, Luke A. Idziak, Adwait Sahasrabhojanee et al Electronic Nose Development and Preliminary Human Breath Testing for Rapid, Non-Invasive COVID-19 Detection. 2023; ACS sens, 8(6), 2309–2318. https://doi.org/10.1021/acssensors.3c00367
24. Bassi P, Di Gianfrancesco L, Salmaso L, Ragonese M, Palermo G, Sacco E,

Giancristofaro RA, Ceccato R, Racioppi M. Improved Non-Invasive Diagnosis of Bladder Cancer with an Electronic Nose: A Large Pilot Study. *Journal of Clinical Medicine*. 2021; 10(21):4984. https://doi.org/10.3390/jcm10214984

25. Mochalski, P., King, J., Haas, M. et al. Blood and breath profiles of volatile organic compounds in patients with end-stage renal disease. BMC Nephrol 15, 43 (2014). https://doi.org/10.1186/1471-2369-15-43
26. Westenbrink E, Arasaradnam RP, O'Connell N, Bailey C, Nwokolo C, Bardhan KD, Covington JA. Development and application of a new electronic nose instrument for the detection of colorectal cancer. Biosens Bioelectron. 2015 May 15;67:733-8. doi: 10.1016/j.bios.2014.10.044. Epub 2014 Oct 23. PMID: 25465796. https://pubmed.ncbi.nlm.nih.gov/25465796/
27. de Meij TG, Larbi IB, van der Schee MP, Lentferink YE, Paff T, Terhaar Sive Droste JS, Mulder CJ, van Bodegraven AA, de Boer NK. Electronic nose can discriminate colorectal carcinoma and advanced adenomas by fecal volatile biomarker analysis: proof of principle study. Int J Cancer. 2014 Mar 1;134(5):1132-8. doi: 10.1002/ijc.28446. Epub 2013 Sep 2. PMID: 23959518.
28. Zhang, X., Zhang, Y. Bladder Cancer and Genetic Mutations. Cell Biochem Biophys 73, 65–69 (2015). https://doi.org/10.1007/s12013-015-0574-z
29. Dong JT. Prevalent mutations in prostate cancer. J Cell Biochem. 2006 Feb 15;97(3):433-47. doi: 10.1002/jcb.20696. PMID: 16267836. https://pubmed.ncbi.nlm.nih.gov/16267836/
30. Sameer AS (2013) Colorectal Cancer: Molecular Mutations and Polymorphisms. Front. Oncol. 3:114. doi: 10.3389/fonc.2013.00114 https://doi.org/10.3389/fonc.2013.00114
31. Shiovitz S, Korde LA. Genetics of breast cancer: a topic in evolution. Annals of oncology: official journal of the European Society for Medical Oncology, 2015; 26(7),1291–1299. https://doi.org/10.1093/annonc/mdv022
32. Schmidt K, Podmore I. Current Challenges in Volatile Organic Compounds Analysis as Potential Biomarkers of Cancer. J Biomark. 2015; 2015:981458. doi: 10.1155/2015/981458. Epub 2015 Mar 30. PMID: 26317039; PMCID: PMC4437398. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4437398/