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Systematic Review

Investigating the Potential of Non-Invasive Breath Test Analysis for Early Detection of Oral Cancer: A Systematic Review

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ABSTRACT

Oral cancer is the 6th most common type of human cancer with a survival rate of around 50% originates in the squamous cells of the mouth and throat. Early diagnosis of oral cancer remains the cornerstone to enhance treatment outcomes as many cases are still diagnosed at advanced stages. The exhaled-breath-analysis tests identifying novel Volatile Organic Compounds (VOC) as biomarkers for oral cancer provide an emerging alternative as a non-invasive diagnostic tool. **Objective:** To investigate the potential of non-invasive exhaled breath test analysis using VOCs as biomarkers for the early detection of oral cancer. **Methods:** Epidemiological studies published from twenty years (2004-2024) were included from PubMed, Google Scholar, Sci-hub and Science Direct databases using preferred reporting items for systematic reviews and meta-analyses guidelines. **Results:** According to this systematic review breath analysis tests coupled with other methods could serve as a feasible supplemental tool with high sensitivity, specificity, and accuracy for identifying oral cancer. The cancer-associated 40 novel VOC biomarkers identified in this review mostly belong to groups including, Alkanes, aldehydes, Ketones, and alcohols. **Conclusions:** Exhaled breath analysis techniques including Gas-Chromatography (GC), Mass-Spectrometry (MS), Selected-Ion-Flow-Tube (SIFT) and Polymer-based e-nose identified 40 novel VOC biomarkers belonging to Alkane, Aldehyde, Ketone, and Alcohol Groups. The results indicate that the exhaled breath analysis tests could serve as a feasible, non-invasive diagnostic tool to supplement the traditional diagnostic procedures like biopsy and assist in generating results with high sensitivity, specificity, and accuracy for identifying oral cancer at an early stage.

INTRODUCTION

Oral cancer is a malignant neoplasm that develops on the lip or oral cavity and poses a significant challenge to global public health. It is typically defined as Oral Squamous Cell Carcinoma (OSCC) because in dental settings higher proportion of cancers stem from oral squamous cells [1]. It exhibits varying levels of differentiation and inclination for lymph node metastasis [2]. Oral cancer comprises 48% of Head and Neck Cancer Cases (HNSCC) [3]. Whereas Oral Squamous Cell Carcinoma (OSCC) accounts for 90% of oral cases based on histological diagnosis [4]. OSCC is a major type of HNSCC that specifically affects squamous cells originating in the oral cavity whereas Head and neck cancer is a broader term encompassing various anatomical sites and originates from the epithelial lining of the oral cavity, hypopharynx, oropharynx and larynx [5]. According to the incidence of cancers, oral cancer resides within the top 10

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rankings and despite the advancement in research and therapeutic interventions, significant improvements in survival outcomes have not been obtained in the recent years indicating a global health challenge for all. [1]. Oral cancers pose a challenge to the treatment regimen [4]. The progression of oral cancers involves multiple steps accompanied by changes in normal mucosa and persists until the development of invasive cancer and metastasis [6]. Oral cancer is a multifactorial lesion that involves various risk factors including tobacco and alcohol, ultraviolet radiation (lip cancer), genetic predisposition, immunosuppression, smoking and Human Papillomavirus Virus (HPV). Among them, alcohol consumption and smoking are deemed as primary factors in the development of malignancy in the oral cavity [7]. According to worldwide reports cancers across all the regions of the oral cavity and pharynx are collectively grouped, representing the sixth most common cancer globally [1]. Approximately 389,485 new cases and 188,230 death cases related to oral cancer were reported in 2022 and it is estimated that these cases will continue to increase globally. Asia accounts for around 50% of all reported cancers [8]. A global study of 195 countries spanning 28 years found the Age-Standardized Rate of Incidence (ASRI) of oral cancer to be highest in Pakistan (27.03/100,000, 95% CI = 22.13-32.75/100,000). In terms of national distribution figures, oral cancer is the second leading cancer (9.6%) in Pakistan [9]. Early cancer detection is crucial for increasing patient survival rate, improving effective therapy, and decreasing mortality rates. There is a need for reliable non-invasive diagnostic tools to attain this objective [10, 11]. Traditionally, procedures like Biopsy and cytology have served as gold standards for diagnosing oral cancer. However, they are invasive for patients, time-consuming, and require longer recovery. Moreover, by the time these procedures are performed, the cancers have spread and advanced to higher stages. Therefore, the main emphasis of recent research endeavours is centred on early detection and prevention of oral cancer, and new diagnostic procedures which are reliable, simpler, non-invasive, and cost-effective alternatives [12]. Recently, exhaled breath analysis has been employed to detect various cancers including gastrointestinal cancer, lung cancer, breast cancer and oral cancer at early stages [13-16]. It is relatively a reliable, simple, and non-invasive method based on principle for conducting a potential investigation of Volatile Organic Compounds (VOCs) as biomarkers in the human body [17]. Volatile organic compounds (carbon-based) produced through the metabolism of cells are released into the blood, reach the lung, get diffused and expelled through the exhaled breath and can provide useful information about the metabolic state of an organism [18]. VOCs generated and emitted through the cellular metabolism of cancer cells are considered innovative cancer-associated biomarkers for diagnostic application. Exhale breath analysis usually involves profiling VOCs present in the breath samples of cancer groups and healthy controls [19]. The specific oral cancer-associated VOCs can be detected within the breath which are emitted by normal cellular metabolism and distinguished from cancer cells [17]. The breath analysis technique and discovery of cancer biomarkers opened up a new domain for the possibilities of early detection of oral cancer [18].

Therefore, this comprehensive systematic review aimed to investigate the potential and performance of non-invasive breath tests in the early detection of oral cancer and to address the identified OSCC and HNSCC-associated VOC biomarkers. This involves compiling data from multiple studies concerning breath tests in oral cancer settings to establish a comprehensive overview of the potential of diagnostic tests. Volatile organic compound: Procedures and working.

METHODS

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed to write this systematic review. The data of last twenty years 2004-2024 was collected using several databases (PubMed, Google Scholar, Sci-hub and Science Direct) using Boolean logic "AND" and "OR", Medical Subject Headings (MeSH Terms) and keywords. Different terminologies were used to explore the literature "Squamous cell carcinoma", "Head and Neck cancer", "Oral cancer", "breath analysis", and "Volatile organic compounds". A total of 90 articles were retrieved from the included databases. Out of all these articles. 15 articles were considered eligible after applying inclusion/exclusion criteria and deleting the duplicates and irrelevant articles. Five articles were case-control studies with four articles having cohort design 2, having feasibility, study design, and 2 having cross-sectional studies whereas the other two had prospective controlled and pilot studies (Figure 1).

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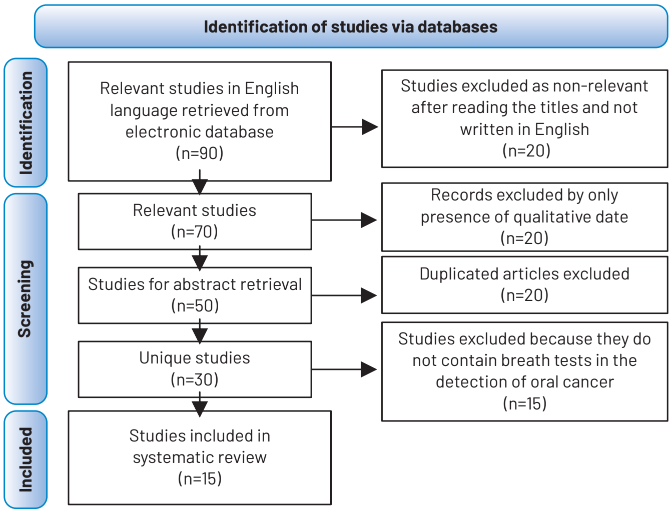

Figure 1: Prisma Flowchart Depicting the Study Selection Process

RESULTS

Figure 2 summarized the different factors this study found to be involved in the progression of oral cancer. It includes tobacco and alcohol, ultraviolet radiation, genetic predisposition, immunosuppression, smoking and Human Papillomavirus Virus(HPV).

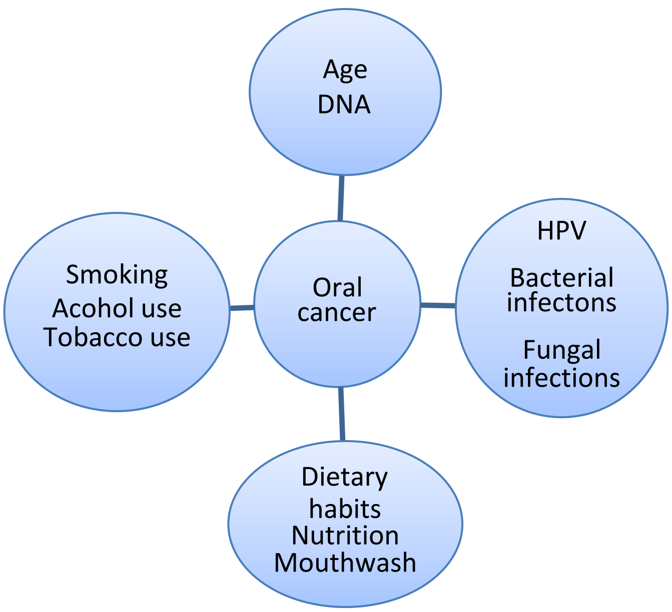

Figure 2: Factors Involves in the Progression of Oral Cancer

The studies included in this systematic review performed different exhaled breath analysis tests using VOCs as biomarkers for the early detection of oral cancer. The first step in the EBA procedures involved collecting breath samples from the cancer patients and healthy controls in an airbag like Tedler-Bag followed by different analysis techniques to analyze the VOC composition of samples. The most common techniques included Solid Phase Microextraction (SPME) used to collect and concentrate VOCs against a fiber coated with an absorbent material from exhaled breath for subsequent analysis using Gas-Chromatography (GC) which analysed the chemical properties to separate the VOCs, Mass-Spectrometry (MS) which used mass-to-charge ratio to distinguish the specific molecules and Selected-Ion-Flow-Tube (SIFT) in which pre-selected reactant ions were used to ionise the VOCs. Polymer-based e-nose is a relatively new technique, a specific type of e-nose that utilizes conducting polymers, organic materials with electrical conductivity that changes upon exposure to VOCs, as its sensor material. Following sample collection, chemical reactivity was used to separate VOCs, and based on the unique reaction patterns, the VOCs were recognized. To detect VOCs present in the breath samples studies used pure samples of hexadecane, nonanal, decanal, undecane, and 1-octene hydrocarbons as reference points. This assisted the researchers in comparing the retention times of unknown compounds in the samples to the retention times of the known standards, resulting in the recognition of VOCs present in the breath. SIMCA (Soft Independent Modelling of Class Analogy) technique was used to investigate samples in cancer and control groups and to classify them based on VOC profiles. Nonparametric tests like Kruskal-Wallis and Mann-Whitney were applied ($p \leq 0.05$) and zones with a higher average class prediction accuracy (threshold value above 0.65) were considered more likely to contain VOCs that could potentially be used as biomarkers for oral cancer. To determine the association of VOC biomarkers with oral cancer, only those studies with specificity and sensitivity mandated in their inclusion criteria were included in this systematic review. This included studies that compared the differential abundance VOC profiles between patients of oral cancer and healthy controls and utilized established diagnostic methods including biopsy and clinical examination for confirming oral cancer diagnosis. Studies also used cancer-free individuals having digestive issues as control by using the QuinTron Breath-Tracker (QBT), in conjunction with other techniques, which is commonly used to measure H_2, CH_4, and CO_2, often linked to digestive issues as this ensured that the identified VOCs are specific to oral cancer. Studies also assessed the potential biological plausibility between specific VOCs and the metabolic processes in oral cancer development. As per previous studies, the increased levels of aldehydes are linked to enhanced lipid peroxidation, a process associated with inflammation and tissue damage in cancer. Moreover, elevated ketones indicate altered energy metabolism in cancer cells. Lastly, consistent findings across multiple studies further validated the VOCs for oral cancer. The data assessed through this systematic review indicates that non-invasive breath tests have the potential to diagnose and discriminate oral cancer patients from healthy controls based on VOC biomarkers (Table 1).

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Table 1: Summary of Extracted Data on the Potential of Non-Invasive Breath Analysis Techniques Based On Voc Profile

S.No.	Cases	Control	Oral Cancer Type	Breath Analysis Technique	Key Outcomes	Study	References
1	22	19	HNSCC	GC/MS	Clearly Distinguish HNSCC from Controls and Benign Tumor	Feasibility	Gruber *et al.*, 2014 [20]
	21		Benign		Cost-Effective/Reliable Screening Tool		
2	50	50	HNSCC	SIFT-MS	Early Detection / Accurate / Practical Sensitivity 80% and Specificity 86%	Cohort	Dharmawardana *et al.*, 2020 [21]
3	22	40	HNC	GC/MS and E-NOSE	Distinguish HNC from H Cost Effective/Reliable Screening Tool	Cross-Sectional	Hakim *et al.*, 2011 [22]
4	26	26	OSCC	GC/MS	Clearly Distinguish OSCC from H Based on VOC	Cross-Sectional	Bouza *et al.*, 2017 [12]
5	50	50	OSCC	GC	Effective Accessory Non-Invasive Sensitivity 68.0% and Specificity 72.0%	Case-Control	Kwon *et al.*, 2022 [16]
6	10	40	SCC	GC/MS	Feasible Test Approach Based on Absence of Cancer Associated VOC after Therapy	Prospective Cohort	Hartwig *et al.*, 2017 [23]
7	49	35	OSCC	E-nose	Sensitivity 88% and Specificity 71%	Cohort	Mohamed *et al.*, 2021 [24]
8	35	50	OSCC	GC/IMS	Average Accuracy 80-90%/reliable	Prospective Controlled	Mentel *et al.*, 2021 [25]
9	91	72	HNSCC	E-nose	Sensitivity 72% and Specificity 79% Portable/non-invasive	Feasibility	Van de Goor *et al.*, 2020 [26]
10	23	21	HNSCC	SIFT-MS	Sensitivity 91% and Specificity 76%	Pilot	Chandran *et al.*, 2019 [27]
11	15	15	HNC	Polymer base e-nose	Not Identified Specific VOC /Cost-Effective	Case-Control	Anzivino *et al.*, 2022 [28]
12	74	61	HNSCC	Quintron Breath-Tracker and SIFT-MS	Novel-Non-Invasive Can Assess Gut Fermentation Activity Linked to Cancer	Case-Control	Dharmawardana *et al.*, 2020 [21]
13	11	20	Laryngeal Carcinoma	SPME/GCMS	Discriminated Cancer Group from Healthy Control Based on VOC Pattern	Case-Control	García *et al.*, 2014 [29]
14	42	13	OSCC/ Bronchial/ Laryngeal	E-Nose Cyranose 320	Sensitivity 100% and Specificity 80% Distinguish Cancer from Controls by VOC Analysis	Case-Control	Fielding *et al.*, 2020 [30]
15	36	23	HNSCC	E-Nose	Sensitivity 90% and Specificity 80 %	Cohort	Leunis *et al.*, 2014 [31]

Abbreviations: GC-MS, gas chromatography-mass spectrometry; HNSCC, Head and Neck squamous cell; OSCC, Oral squamous cell carcinoma; e-nose, electronic nose; SPME; solid phase microextraction, VOC; Volatile organic compound; SIFT; selected ion flow-tube. Ten studies used different methodologies and breath analysis techniques to collect and analyze the VOC composition of exhaled breath air from the cancer patients' group and healthy controls [20,23,24,12,16,25-29]. The remaining five studies used e-nose technology based on portable e-nose devices [30-34]. The samples in these studies were analyzed using Gas Chromatography-Mass spectrometry [GC-MS; 20, 12, 25, 23, 24, 26], Solid phase microextraction preconcentration followed by (SPME/GC-MS; 29), Selected Ion Flow-Tube-Mass spectrometry (SIFT-MS; 27, 23, 28]. Whereas in the study conducted by Kwon IJ *et al.*, a new simple GC system was employed for the diagnoses of OSCC [16]. An overview of clinical characteristics of the study group (cancer group and healthy controls) including age, gender, smoking/alcohol status and cancer stage is given in the supplementary information in table 2 titled Clinical characteristics of the study groups attached as a supplementary file. Due to heterogeneity in extracted data, clinical features were not correlated with oral cancer. However, smoking and alcohol are regarded as primary factors found in 90% of cases of oral cancer [35, 36].

Novel VOC Biomarkers for HNSCC and OSCC

Nine studies identified 40 novel VOCs potential biomarkers within the group encompassing alkanes, ketones, aldehydes, alcohols and thiols for the detection of oral cancers. Sixteen VOC biomarkers were identified by Bouza M *et al.*, for HNSCC [12]. Of these compounds benzaldehyde and 3, 7-dimethyl undecane exhibited a significant correlation ($p < 0.05$) with tumor size and recurrence. There was a significant correlation observed between Butyl acetate and the histological degree of differentiation.

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Markar SR *et al.*, identified three VOC biomarkers for HNSCC [33]. Removed Garcia RA *et al.*, also found ethanol and 2-butanone as significant biomarkers for the identification of laryngeal OSCC [29]. In the study by Chandran D et al., the level of HCN biomarker was observed to be significantly different between the HNSCC group and healthy controls[27](Table 3).

Table 3: VOCs Identified as Potential Biomarkers for HNSCC and OSCC

S. No.	Cancer Biomarkers / VOC Profiling	Group	Ora Cancer Type	References
1	Undecane	Alkane	HNSCC	Gruber *et al.*, 2014 [20]
	2-Propenenitrile	Nitrile		
	Ethanol	Alcohol		
2	Formaldehyde	Aldehyde	HNSCC	Dharmawardana *et al.*, 2020 [21]
	Methyl Mercaptan	Thiol		
3	4,6-Dimethyl-Dodecane	Alkane	HNC	Hakim *et al.*, 2011 [22] And Bouza *et al.*, 2017 [12]
	2 ,6 Dimethyl Propanoic Acid			
	5-Methyl-3-Hexanone	Ketone		
	2,2-Dimethyl-Decane	Alkane		
	Limonene	Terpene		
4	2,2,3-Erimethyl-, exobicyclo [2.2.1]heptane	Ketone	OSCC	
	Udecance	Alkane		
	Dodecane	Alkane		
	Decanal	Aldehyde		
	Benzaldehyde			
	3,7-Dimethyl Undecane	Alkane		
	4,5-Dimethyl Nonane	Alkane		
	1-Octene	Alkene		
	Butyl Acetate	Ester		
	Hexadecane	Alkane		
	Styrene	Alkene		
	Benzyl Alcohol	Alcohol		
	2-ethyl-1-Hexanol	-		
	Toluene	Benzene		
	Dodecanal	Aldehyde		
	Nonanal	Aldehyde		
5	Ch3SH	Thiol	OSCC	Kwon *et al.*, 2022 [16]
	H2S	Thiol		
6	Dimethyl Disulfide (DDS)	Disulfides	OSCC	Hartwig *et al.*, 2017 [23]
	Decamethylcyclo-pentasiloxane	Cyclic Siloxanes		
	P-Xylene (PX	Xylene		
	N-Heptane	Alkane		
	Methyl Ethyl Ketone	Ketone		
	Toluene	Benzene		
	1-Heptene	Alkene		
	Dibutylhydroxytoluene	-		
7	HCN		HNSCC	Chandran *et al.*, 2019 [27]
8	CH4:H2	Alkane hydrogen	HNSCC	Dharmawardana *et al.*, 2020 [21]
9	Ethanol	Alcohol	Laryngeal Carcinoma	García *et al.*, 2014 [29]
	2-Butanone	Ketone		

Abbreviations: HNSCC, Head and Neck Squamous Cell; OSCC, Oral Squamous Cell Carcinoma; T3, Tumor Stage 3

DISCUSSION

In this systematic review, we present a comprehensive overview of different studies concerning the diagnostic performance of exhale breath tests based on volatile organic compounds in the detection of oral cancer [28]. As per the analysis of the results, the exhaled breath analysis tests could serve as feasible tools to supplement the traditional diagnostic procedures like biopsy and assist in generating results with high sensitivity, specificity, and accuracy for early identifying oral cancer. The cancer-associated 40 novel VOC biomarkers identified in this review mostly belong to groups including, Alkanes, aldehydes, Ketones, and alcohols. These VOCs hold promise as an efficient non-invasive diagnostic approach and also underscore the importance of further research in the identification of other novel oral cancer-associated VOC biomarkers. Based on evident studies, in an initial effort to analyze the exhaled breath for the diagnoses and distinction of HNSCC and benign tumor from the healthy controls Gruber M *et al.*, used a breath test with Gas Chromatography/Mass Spectrometry (GC/MS) and in their analysis, found undecane, 2-propenenitrile and ethanol as potential biomarkers of these cancers [20]. To ascertain whether the breath profile can be used for discrimination of patients with or without HNSCC, Markar SR *et al.*, used an ion flow-tube mass spectrometer to analyze the breath for VOCs and concluded that the diagnostic approach is feasible (higher Sensitivity of 80% and Specificity of 86%) for early detection and distinction of HNSCC from controls [31]. They identified Formaldehyde and Methyl mercaptan as potential VOC biomarkers related to cancer. Various methods have been applied and reported in different studies up to now. Using GC/MS Bouza M *et al.*, identified several VOCs such as Undecane, dodecane, decanal, benzaldehyde, 3,7-dimethyl, undecane 4,5-dimethyl nonane, 1-octene as biomarkers for the diagnoses of OSCC and concluded that existing of aldehydes within the oral cavity may constitutes potential biomarkers [12]. In another study carried out by Hakim M *et al.*, electronic nose containing nanoparticle-based sensors was used to analyze the exhaled breath to diagnose HNS patients from healthy controls and concluded the method was cost-effective and reliable [22]. To assess the viability of detecting VOC biomarkers in the breath of patients, GC/MS employed by Hartwig S *et al.*, involved comparing the presence of VOCs in the breath sample before and after therapy [23]. The study results confirmed the absence of three cancer-associated VOCs (Dimethyl disulfide, Decamethyl-cyclopentasiloxane, p-xylene) in the breath samples after the therapy for HNSCC ensuring the feasibility of the diagnostic method. In a study performed by Kwon IJ *et al.*, comparative analysis of exhaled breath was conducted between patients with Oral Squamous Cell

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Carcinoma OSCC and healthy controls via using simple Gas chromatography [16]. The study aimed to investigate whether the exhaled breath test can serve as a novel non-invasive and effective diagnostic test for oral squamous carcinoma. The study results demonstrated significantly elevated concentrations of hydrogen sulfide and methyl mercaptan in the OSCC group than in the healthy controls affirming the non-invasive method. However, in comparison to other studies, breath test conducted in this study exhibited low sensitivity 68.0% and specificity 72.0% indicating that breathe analysis through a simple GC system requires refinement in clinical practice. Dharmawardana N *et al.,* conducted a study using an ion flow-tube mass spectrometer and a Quintron BreathTracker to discriminate breath samples of the HNSCC group from healthy controls based on methane and hydrogen ratio increased with tumor stage [21]. In another study conducted by Mohamed N *et al.*, 12 VOC was extracted from the saliva of OSCC patients as potential OSCC biomarkers. Using e- nose base technology study was conducted by Shield KD *et al.*, in Sudan to distinguish the OSCC group from the healthy control [24, 36]. A portable e-nose device was used to collect and analyze the breath samples. Observed diagnostic accuracy of the test was 81% with good sensitivity at 88% and specificity of 71% concluded that this diagnostic strategy is cost-effective and efficient with limited resources to confront the burden posed by OSCC. Another study using e-nose technology was performed by van de Goor RM *et al.*, and found an average accuracy of 72% at a sensitivity of 79% and specificity of 63% indicating that the diagnostic method correctly discriminated the HNSCC group from healthy controls [26]. Leunis N *et al.*, employed an e-nose with metal oxide-based sensors and verified that the VOCs pattern was different between the HNSCC group and a control group with a sensitivity of 90% and a specificity of 80% [31]. In comparison, studies on the use of breath tests in diagnoses of other cancers including breast and lung, have revealed similar findings with higher levels of sensitivity and specificity [37, 38]. In comparison to the non-invasive diagnosis technique, the biopsy method is invasive and could spread the disease [32, 39]. If future research can provide stronger evidence for the utilization of breath test methods, we believe that these tests could be used in screening initiative that may improve the rates of diagnoses of oral cancer at earlier stages and improve disease prognosis [40]. Oral cancer is one of the leading causes of mortality and a global burden due to its late diagnosis at early stages. Exploring the potential of non-invasive breath test methods and VOC compounds facilitates the healthcare community by providing insights into the accuracy, feasibility and reliability of these methods for the early detection of oral cancer [41]. Furthermore, it provides information on the specific oral cancer-associated (HNSCC and OSCC) VOC biomarkers aiding in the advancement of diagnostic tools and contributing to the early detection and monitoring of condition of oral cancers. By assessing the evidence base for breath tests the systematic review assists the clinicians in making informed decisions regarding the integration of these diagnostic approaches in oral cancer settings ultimately, improving patients care and outcomes. While EBA shows promise for non-invasive oral cancer detection, overcoming the following limitations is crucial. The technology for EBA is still evolving, and widespread availability and standardization remain concerns. Limited research conducted so far has resulted in insufficient data for many potential VOC markers which might need further validation in larger clinical trials to confirm their effectiveness for routine clinical use. Moreover, early-stage oral cancers might not produce significant changes in VOC profiles, potentially leading to missed diagnoses. Lastly, natural variations in breath VOCs due to genetics can affect the test's sensitivity.

CONCLUSIONS

The systematic review provides a comprehensive summary of the exhaled breath analysis techniques like Gas-Chromatography (GC), Mass-Spectrometry (MS), Selected-Ion-Flow-Tube (SIFT) and Polymer-based e-nose which identified 40 novel VOC biomarkers belonging to Alkane, Aldehyde, Ketone, and Alcohol Groups. The results indicate that the exhaled breath analysis tests could serve as a feasible, non-invasive diagnostic tool to supplement traditional diagnostic procedures like biopsy and assist in generating results with high sensitivity, specificity and accuracy for identifying oral cancer at an early stage.

Authors Contribution

Conceptualization: MRT

Methodology: MM, NI, SAT, SPS

Formal analysis: ATA

Writing, review and editing: SPS, SA, MRT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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