Review Article
Salivary Biomarkers in Lung Cancer

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A very low percentage of lung cancer (LC) cases are discovered at an early and treatable stage of the disease, leading to an abysmally low 5-year survival rate. This underscores the immediate necessity for improved diagnostic, prognostic, and predictive biomarkers for LC. Biopsied lung tissue, blood, and plasma are common sources used for LC diagnosis and monitoring of the disease. A growing number of studies have reported saliva to be a useful biological sample for early and noninvasive detection of oral and systemic diseases. Nevertheless, salivary biomarker discovery remains underresearched. Here, we have compiled the available literature to provide an overview of the current understanding of salivary markers for LC detection and provided perspectives for future clinical significance. Valuable markers with diagnostic and prognostic potentials in LC have been discovered in saliva, including metabolic (catalase activity, triene conjugates, and Schiff bases), inflammatory (interleukin 10, C-X-C motif chemokine ligand 10), proteomic (haptoglobin, zinc-α-2-glycoprotein, and calprotectin), genomic (epidermal growth factor receptor), and microbial candidates (Veillonella and Streptococcus). In combination, with each other and with other established screening methods, these salivary markers could be useful for improving early detection of the disease and ultimately improve the survival odds of LC patients. The existing literature suggests that saliva is a promising biological sample for identification and validation of biomarkers in LC, but how saliva can be utilized most effectively in a clinical setting for LC management is still under investigation.

1. Introduction

Lung cancer (LC) is the leading cause of cancer-related deaths globally [1]. The two main subtypes of LC are non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), which account for 84% and 13% of LC, respectively [2–4]. Tobacco smoke is the single greatest risk factor of LC, though other less common risk factors include asbestos, radon, second-hand smoke, alcohol, arsenic, chromium, nickel, and polycyclic aromatic hydrocarbons [5, 6]. NSCLC can be further divided into adenocarcinoma (AC), squamous cell carcinoma (SCC), and large cell carcinoma (LCC). NSCLC has a poor five-year survival rate of 25%, often related to diagnosis of the disease at a late stage with frequent distant metastasis [4, 7]. There are two subtypes of SCLC, oat cell carcinoma, and combined-SCLC. The latter subtype is defined as SCLC with components of NSCLC [5]. SCLC has an exceptionally low five-year survival rate of less than 7% [4, 8] associated with its aggressive growth and high metastatic potential [9]. Early stages of SCLC may be treated by chemotherapy and radiotherapy, while NSCLC in its early stages may be treated successfully by surgical resection [10]. Indeed, if LC is diagnosed at an early and localised stage, the 5-year survival rate increases to 59%. Unfortunately, only 17% of all LC cases are diagnosed at this stage [4]. In order to improve treatment success in terms of reduced morbidity and mortality, early diagnosis of the disease is crucial.

Both low-dose computerized tomography (LDCT) and chest radiographs have been investigated as methods of LC-screening. In a randomized clinical trial comprising at least 53,000 heavy smokers, former and active, LDCT resulted in a 20% decrease in the LC mortality rate, as compared to the chest radiographs [11]. Consequently, several medical associations recommended LC-screening using LDCT for heavy active and former smokers [12–14]. However, LDCT-screening is not completely free from limitations, and it can result in false positive and negative results.
and can cause a radiation hazard. The false positive results can lead to unnecessary further testing and invasive procedures, while the false negative results can delay necessary treatment [15, 16]. As a consequence of these limitations associated with LDCT, the development of complementary screening methods is highly coveted [17]. In this regard, molecular biomarkers are increasingly recognised as key knowledge not only to better understand LC biology but also to provide earlier and more precise diagnosis and to assign patients to the best targeted treatment available so that ineffective overtreatment is avoided.

Accordingly, several tumor markers such as mRNA [18, 19], microRNAs [20], cytokines [21], antioxidant enzymes [22], and fatty acids [23] spanning across several sample types such as blood/plasma [24, 25], sputum [26], and expired air [27, 28] have been investigated in LC. Although bodily fluids, such as blood, serum, urine, and sputum, have been extensively examined as liquid biopsy for diagnostic, prognostic, and predictive markers in LC, limited data exist on saliva as a potential liquid biopsy in LC [29].

Human saliva has been investigated as a biological fluid for diagnosis of diseases, including human malignancies. Saliva is a preferred biological sample as saliva collection is noninvasive and the procedure is quicker, cheaper, and more convenient for the patient as compared to invasive processes such as blood collection [30]. Importantly, saliva consists of a pool of biomolecules such as proteins, mRNA, miRNA, enzymes, and immunoglobulins coming from different sources, such as the salivary glands themselves [31, 32], secretions from nasal cavity and lower respiratory tract [33], gingival crevicular fluid [33, 34], and blood plasma as an ultrafiltrate [35] (Figure 1). Systemic diseases, including LC, may influence the salivary glands’ function and subsequently the quantity and composition of saliva [36, 37]. In a lung cancer mouse model, a significant alteration of biomarkers in the saliva was observed. These observations suggest that tumors, even if not in close proximity, may release mediators affecting the salivary gland function and subsequently the composition of saliva [38]. In addition, saliva contains several types of bacteria, fungi, and virus species [39]. Change in the profile of these biomolecules and the microbiota in saliva in disease conditions forms the basis for the use of saliva in diagnosis and prognosis of human diseases.

The usefulness of salivary markers in both oral and systemic diseases has been investigated [40], though how markers of extraoral pathologies, like lung cancer, are found in saliva is not fully understood, and represents an important research area. This review is aimed at offering an overview of diagnostic and prognostic biomarkers in human saliva for LC (Table 1).

2. Methods

A literature search using the databases of PubMed and Google Scholar was performed. The search words involved the combination of the following terms from the Medical Subject Headings (MeSH): “lung cancer,” “biomarkers,” and “saliva.” The systematic search yielded 27 articles, in the timespan from 2011 to the 31st of December 2020. The inclusion criteria were as follows: (a) type of studies (human clinical studies) and (b) studies with full-text availability. The exclusion criteria were articles not related to LC and salivary biomarkers and/or articles for which full texts were not available in English. Additionally, individual articles retrieved manually from the reference list of the relevant papers were also included.

3. Metabolic and Inflammatory Biomarkers

Altered cellular metabolism has been identified as an emerging hallmark of cancer [41], opening an opportunity for biomarker discovery. Salivary metabolomics is a relatively new field, and accordingly, few studies have addressed the question of altered metabolic markers in saliva in cancer versus normal controls. It has, logically enough, primarily been applied to oral cancer but is increasingly expanding to more systemic diseases. The most frequently used techniques are 1H+13C NMR and mass spectrometry [42]. Bel’skaya et al. performed a comprehensive biochemical analysis of unstimulated saliva from 425 LC patients (with no prior treatment) (consisting of AC, SCC, mixed ADC+SCC, neuroendocrine, and undifferentiated LC), 168 noncancerous lung disease patients, and 550 healthy controls [43]. A major shift in salivary metabolite composition, specifically those involved in lipid peroxidation and protein metabolism, as well as metabolic enzyme activity (increased alanine aminotransferase (ALT), decreased aspartate aminotransferase (AST), and decreased AST/ALT coefficient), was observed in LC as compared to healthy controls. The change in metabolic enzyme activity was also explored previously by Bel’skaya and Kosenok [44]. Although the histological subtypes were found to have similar metabolic enzyme activities, a significant difference was observed between LC (all subtypes) and healthy controls.

Bel’skaya et al. further investigated the value of additional markers for their diagnostic utility; however, none of the investigated biochemical salivary markers could be independently used in the early diagnosis of LC. The most informative biochemical parameters were catalase activity, level of triene conjugates and Schiff bases, pH, sialic acid, alkaline phosphatase, and chloride ion concentration in saliva. This panel of seven parameters could be used to diagnose LC with 69.5% and 87.5% sensitivity and specificity, respectively. Among these parameters, Bel’skaya suggested catalase activity to be the most important parameter for LC diagnosis [43]. In addition, Fourier transform infrared spectroscopy has been examined for its utility in detecting differences in biochemical salivary parameters between LC patients and healthy subjects. Most notably in the advanced stages of LC, a significant difference was evident at infrared spectra of 1070–1240 cm⁻¹ [45].

The prognostic value of salivary biochemical markers was also investigated by Bel’skaya et al. [43]. An increased concentration of lactate dehydrogenase (LDH) activity and lower imidazole (IC) concentrations were found to be significantly associated with favourable prognosis of LC. A LDH concentration of more than 1133 U/L and less than
0.311 mmol/L of IC, combined, could effectively predict a favourable outcome. Compared to patients with poor prognosis, the favourable outcomes were 1.4 (46.8% to 77.0%), 1.9 (27.1% to 47.5%), and 2.0 (18.0% to 43.3%) times more likely to survive at one, three, and five years. This was further studied in specific subtypes, and it was found that high LDH and low IC were favourable for SCC, but not for AC or neuroendocrine LC [46]. Instead, low IC levels combined with high seromucoids and uric acid were favourable for the prognosis of AC patients, and a combination of high NO, urea, and ALP was favourable for neuroendocrine tumors, as these values tend to decrease with the progression of the disease. As a predictive marker, C-reactive protein (CRP) may be of value as its concentration increases with tumor size and regional metastasis, especially in NSCLC [47].

Inflammation is well known as both a cause and effect of tumor development. Chronic inflammation can lead to DNA damage and promote carcinogenesis. The inflammatory tumor microenvironment fosters invasion and metastatic potential of cancer cells [49, 50]. This makes inflammatory markers promising targets not only as diagnostic biomarkers but also valuable tools for determining prognosis. Several inflammation-related cytokines have been identified as significantly deregulated in NSCLC compared to healthy controls [47, 51]. Both proinflammatory and anti-inflammatory cytokines were overexpressed in the saliva of LC patients, including interleukin-1β (IL-1β), ILIRN, and IL-10.
### Table 1: Summary of putative salivary markers in lung cancer.

| Author, year | Histological type | Sample size (LC/control) | Markers | Collection | Category | Sensitivity/specificity |
|--------------|-------------------|--------------------------|---------|------------|----------|------------------------|
| **Metabolic** |                   |                          |         |            |          |                        |
| Bel’skaya, 2020 [43] | AC, SCC, AC +SCC, NEC | 425/550* | Catalase activity, triene conjugates, Schiff bases, pH, sialic acid, alkaline phosphatase, chloride | Unstimulated WMS | Amino acids, biochemical | 69.5%/87.5% |
| Bel’skaya, 2017 [44] | AC, SCC, NEC | 286/573 | ALT, AST/ALT, ALP, GGT, β-amylase | Unstimulated WMS | Biochemical | n/a |
| **Inflammatory** |                   |                          |         |            |          |                        |
| Koizumi, 2018 [48] | NSCLC | 35/35 | IL-1β, ILIRN, IL7, IL10, CCL11, CXCL10, PDGF-BB, TNF | Unstimulated WMS | Protein | 60.6%/80.8% ** |
| **Proteomic** |                   |                          |         |            |          |                        |
| Xiao, 2012 [53] | Not specified | 26/26 | Haptoglobin, zinc-α2-glycoprotein, calprotectin | Unstimulated WMS | Protein | 88.5%/92.3% |
| Zhang, 2012 [56] | NSCLC, SCLC | 32/64 | CCNI, EGFR, FGFl9, FRS2, GREB1 | Unstimulated WMS | mRNA | 93.75%/82.81% |
| Wei, 2014 [64] | NSCLC | 40/n/a | EGFR 19-del, EGFR 21-L858R | Unstimulated WMS | DNA | n/a |
| Pu, 2016 [65] | NSCLC other, AC, SCC | 17/n/a | EGFR 19-del, EGFR 21-L858R | Not specified | DNA | n/a |
| Ding, 2019 [66] | NSCLC other, AC, SCC | 78/26*** | EGFR 19-del, EGFR 21-L858R | Unstimulated WMS | scfDNA † | n/a |
| **Transcription** |                   |                          |         |            |          |                        |
| Zhang, 2019 [90] | AC, SCC | 39/20 | Veillonella, Streptococcus | Unstimulated WMS | 16S rRNA | n/a |
| Yan, 2015 | AC, SCC | 61/25 | Capnocytophaga, Veillonella | Not specified | 16S rDNA | 84.6%/86.7%-78.6%/80.0% ¥ |
| Yang, 2018 [92] | NSCLC | 75/172 | Sphingomonas, Blastomonas, Acinetobacter, Streptococcus | Unstimulated WMS | 16S rRNA | n/a |

*Also included 168 nonmalignant lung disease cases; **IL10 and CXCL10 only; ***also included 15 nonmalignant lung disease cases; †saliva circulating free DNA; ¥for SCC and AC, respectively. AC: adenocarcinoma; LC: lung cancer; n/a: not applicable; NEC: neuroendocrine cancer; NSCLC: non-small-cell lung cancer; SCC: squamous cell carcinoma; SCLC: small cell lung cancer; WMS: whole mouth saliva.
IL7, IL10, C-C motif chemokine 11 (CCL11), C-X-C motif chemokine ligand 10 (CXCL10), platelet-derived growth factor-BB (PDGF-BB), and tumor necrosis factor (TNF-α). Of these, the combination of IL10 and CXCL10 had the greatest diagnostic potential, with a sensitivity of 60.6% and specificity of 80.8%. The proinflammatory cytokines IL-6, IL-8, IL-18, and TNF-α have also been implicated in advanced LC [47].

4. Proteomic Biomarkers

Proteomic techniques have been predominantly used to analyse blood but have recently been adopted in salivary samples. Among such techniques are iTRAQ [52, 53] and two-dimensional gel electrophoresis (2-DE) [54, 55], which have been widely used to analyse the proteome of a number of LC subtypes. The salivary proteome has most often been profiled by two-dimensional gel electrophoresis with mass spectrometry (2DE-MS), though new techniques are being adapted to salivary proteomics as well. 2DE-MS was used in an investigation of 16 potential proteins as salivary biomarkers for early LC detection. Seventy-two subjects were enrolled in the study. The three proteins haptoglobin, zinc-2-glycoprotein, and calprotectin, combined, reached a sensitivity of 88.5% and specificity of 92.3% for diagnosis of LC [56]. Therefore, the combination of haptoglobin, zinc-α-2-glycoprotein and calprotectin represents a promising saliva-based diagnostic tool for LC.

Another mode of entry for biomolecules present in saliva is exosomal transport. These circulating exosomes contain lipids, proteins, and nucleic acids produced by tumor cells and can be transported in the blood as encapsulated membranes, the content of which resembles that of their parent tumor cells [57]. Sun and collaborators established a standardised method of exosome-isolation from saliva to compare their proteomic profiles. In saliva, 319 exosomal proteins were identified, along with 994 in serum, by liquid chromatography tandem mass spectrometry. Eleven exosomal proteins were discovered in saliva and plasma of LC patients that were not present in healthy subjects. This finding raises the possibility for the potential use of salivary exosomes as diagnostic biomarkers in LC [58].

5. Transcriptomic and Genomic Biomarkers

Several salivary transcriptomic and genomic biomarkers have received attention as molecules with diagnostic and prognostic potential. Among these are five mRNA candidates: CCND1 (encoding for cyclin D1), EGF (encoding for epidermal growth factor receptor), FGF19 (encoding for fibroblast growth factor 19), FRS2 (encoding for fibroblast growth factor receptor substrate 2), and GREB1 (growth regulation by estrogen in breast cancer 1). The transcriptome signature of these genes was able to distinguish both NSCLC and SCLC from control subjects with a sensitivity of 93.75% and a specificity of 82.81% [59].

Currently, one of the most researched genetic markers for LC diagnostics is EGFR. EGFR-testing has traditionally been performed on surgically biopsied tissues. However, at the stage of biopsy taking, the LC has in most cases already progressed too far and frequent biopsies for monitoring EGFR mutations are impractical for these patients [60–62]. Therefore, the detection of EGFR by other means is highly sought after. EGFR is a membrane receptor frequently expressed in NSCLC that influences proliferation, angiogenesis, and chemoresistance, as well as inhibits apoptosis and promotes metastasis of NSCLC cells [63]. Identifying the presence and type of EGFR mutations is crucial in NSCLC patients as the common mutations, exon 19 deletion (19del) and exon point mutation 21-L858R (L858R) [64], are treatable by tyrosine kinase inhibitors such as erlotinib, gefitinib and osimertinib [65].

Electric Field-Induced Release and Measurement (EFIRM) has recently been introduced for the detection of mutations in EGFR. This method allows for cell-free DNA analysis using specific mutation-detecting probes, with improved sensitivity and specificity over PCR-based methods in NSCLC patients. Blood, urine, or saliva can be used as biological samples for EFIRM [66]. Two clinical studies, blinded, using EFIRM with saliva as a sample, identified the EGFR mutations exon 19del and L858R in NSCLC patients. The similarity between EFIRM-results and the gold standard of biopsy genotyping was high, 96-100% [67, 68]. Despite the promising results, the studies were of a small scale and need for large scale studies is evident, to explore the rate of false-positive and false-negative results [67, 68]. The method of EGFR detection by EFIRM fulfills many of the clinical requirements for successful and efficient detection and may become a clinical method in the future, on its own or with supplementary analysis of biopsies [67]. Another potential method of detecting EGFR mutations includes droplet digital PCR analysis of saliva-derived plasma cell-free DNA (plasma-cfDNA) and saliva cell-free DNA (saliva-cfDNA). No significant differences in the quantification or in concentrations of scfDNA were found between NSCLC, healthy or patients with benign lung lesions. However, the concordance rate of EGFR mutations between plasma-cfDNA and saliva-cfDNA was 83.78%[69]. Interestingly, a study by Li et al. [70] compared the concordance in detection of EGFR mutations of EFIRM and droplet digital PCR. The study involved thirteen patients with NSCLC, who donated plasma and saliva samples. Both EGFR mutations, exon 19del and L858R, were detected in both saliva and plasma samples with a sensitivity of 100%, while droplet digital PCR showed a sensitivity of 85.6% in plasma and 15.4% in saliva. The EFIRM-method was able to detect ultrashort (40-60 bp) circulating tumor-DNA fragments in saliva and plasma. This presents yet another promising and novel target for LC diagnosis in the earliest stages of the disease. In general, EGFR identification by EFIRM based on a simple saliva test provides high sensitivity. The method may be proven to be a great diagnostic supplement in the clinical setting.

6. Microbial Biomarkers

Bacterial homeostasis is important for normal bodily function, including in the oral cavity. The complex interaction
involved in homeostasis of normal oral flora is considered to minimize the growth of foreign microbial invaders and opportunistic microorganisms [71]. At least 700 unique bacterial species inhabit oral cavity, though more than half are currently impossible to culture [72]. When secreted, saliva is initially sterile [73] but is quickly contaminated by bacteria shed from the surfaces of tonsils, tongue, throat, and other oral surfaces [74, 75]. The normal oral microbiome, mainly comprised of the salivary microbiome and nonshedding bacteria on supra- and subgingival dental surfaces, has largely been characterised [76, 77]. The microbial profile of saliva mirrors the composition of microbiota on oral mucosa and on dental surfaces [76, 77]. The composition of oral or salivary microbiota has been suggested to reflect oral and general health status [78].

Bacterial dysbiosis is linked to the development of a number of diseases, not only at the site of bacterial imbalance but also at distant organs. Recently, there has been a growing interest in exploring the link between salivary bacteria and the incidence and severity of respiratory infections, including COVID-19 [79, 80]. An association between periodontal disease, an inflammatory condition in the gingiva and supporting structures of teeth induced by bacteria, and several respiratory infectious conditions has been reported previously [81]. It has been suggested that oral periodontopathic bacteria can be aspirated into the lung leading to pneumonia [82, 83]. Furthermore, microbial dysbiosis at different organs and in the body fluids including that of saliva has been linked to several types of cancer, such as oral, oesophageal, colorectal and lung cancer [77, 84–88]. As an example, in colorectal cancer specimens, significantly higher levels of Prevotella, Escherichia coli, Bacteroides fragilis, Streptococcus gallolyticus, Enterococcus faecalis, and Streptococcus bovis have been detected as compared to normal colon tissues [88–90]. Similarly, in another study, significantly higher levels of Fusobacterium nucleatum (F. nucleatum) and Clostridium difficile were observed in patients with colorectal cancer as compared to control subjects [91]. Interestingly, enrichment of some of these bacteria such as Prevotella and F. nucleatum has been shown in oral squamous cell carcinoma specimens [87].

Several studies, using 16S rRNA sequencing technology, have reported a differential profile of salivary microbiota in LC patient as compared to the control specimens. Zhang and collaborators, using 16S rRNA sequencing technology, reported a higher richness and lower diversity of salivary microbiota in NSCLC patients as compared to that of healthy subjects [92]. The authors also reported an increase in Veillonella and Streptococcus and a simultaneous decrease of Fusobacterium, Prevotella, Bacteroides, and Faecalibacterium genera in NSCLC patients compared to the control subjects [92]. Similar findings have been reported by Yu and coworkers in NSCLC specimens [93]. In parallel to the above observation in LC specimens, Yan et al. found increased abundance of Veillonella and Capnocytophaga in saliva from LC patients (SCC and AC) as compared to that of normal controls [94]. Of note, the enrichment profile of Veillonella and Capnocytophaga in saliva was able to distinguish control subjects from lung SCC with a specificity of 86.7% and sensitivity of 84.6%, and from AC with 80% and 78.6%, respectively [94]. Interestingly, saliva from non-smoking female LC patients was reported to be enriched with Sphingomonas and Blastomonas and diminished with Acinetobacter and Streptococcus as compared to normal controls [95]. These observations, although different from other studies using saliva from LC patients, could be related to that fact that LC in non-smokers is considered to be a different disease compared to smoking related LC [54, 96–98]. Overall, these observations indicate that LC might be associated with microbiome dysbiosis in saliva and profiling of salivary microbiome might have a diagnostic value in LC.

Despite the association between microbial profile in saliva and LC as described above, the possible contribution of salivary microbiota to LC carcinogenesis is not understood. Nevertheless, salivary microbiota has been shown to influence p53 and apoptosis signalling pathways in LC tumor cells [95]. In addition, salivary microbiota has been shown to influence systemic inflammatory status in LC patients [92]. A positive correlation between Veillonella in saliva from NSCLC and neutrophil-lymphocyte ratio and a negative correlation between Streptococcus and lymphocyte-monocyte ratio have been reported. The same authors reported a decrease in folate biosynthesis and an increase in xenobiotics and amino acid metabolism in salivary metabolites from NSCLC patients [92]. Given the association between inflammation and metabolic deregulation and LC [49, 50, 92, 99, 100], the above observations indicate a possible link between dysbiotic salivary microbiota and LC carcinogenesis. However, larger and longitudinal studies are needed to clarify these suggestions.

The potential use of salivary microbiota as a diagnostic biomarker has several limitations. The microbiota is dynamic, and it continuously changes to local and systemic conditions. Moreover, its composition depends on the host’s age, ethnicity, diet, oral hygiene, dental status, antibiotic use, and smoking habit [101–105]. Most of the studies so far on this topic have been conducted in Chinese population. As a result, a standardised set of microbial diagnostic markers for LC is still in its infancy.

7. Conclusions

Saliva as a biological sample offers several advantages. Saliva collection is a noninvasive procedure, is quicker, cheaper and more convenient for the patient as compared to invasive procedures such as blood collection. Importantly, saliva consists of a pool of biomolecules coming from different sources, such as the salivary glands themselves, secretions from nasal cavity, and lower respiratory tract. The composition of saliva is suggested to reflect local and systemic health and disease conditions. In line with this, several studies have supported a link between LC and qualitative and quantitative changes in salivary composition. Accordingly, there is a growing interest in the identification of saliva-based biomarkers in LC. Recent studies have identified a number of saliva-based protein, genomic and transcriptomic, and microbial biomarkers with diagnostic and prognostic value in LC. Among them, mutation status in EGFR in saliva from
LC patients has emerged as potential diagnostic/prognostic marker in LC. Additionally, the salivary microbiome is a growing and fresh research area which may provide identification of microbiome-based markers in LC. However, the diagnostic and prognostic value of individual salivary markers for LC seems limited. This supports the need for identification of panel of markers, preferably combining inflammatory, genomic, transcriptomic, and microbial markers in saliva. At present, studies exploring the use of salivary diagnostic biomarkers for LC are limited to small-scale studies. Studies with larger patient groups are needed to assess salivary biomarkers’ diagnostic reliability in larger and more diverse populations.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] K. Nie, Y. Jia, and X. Zhang, “Cell-free circulating tumor DNA in plasma/serum of non-small cell lung cancer,” *Tumor Biology*, vol. 36, no. 1, pp. 7–19, 2015.

[2] Lung Cancer-Non-Small Cell: Statistics, 2021, April 2021, https://www.cancer.net/cancer-types/lung-cancer-non-small-cell/statistics.

[3] Lung Cancer-Small Cell: Statistics, 2021, April 2021, https://www.cancer.net/cancer-types/lung-cancer-small-cell/statistics.

[4] Society, AC, *Cancer Facts & Figures 2021*, American Cancer Society, Atlanta, 2021.

[5] N. Basumallik, *A.M. Small Cell Lung Cancer*, 2021.

[6] S. B. Clark, *A.S. Non Small Cell Lung Cancer*, 2021, April 2021, https://www.ncbi.nlm.nih.gov/books/NBK562307/?report=classic.

[7] A. Jemal, R. Siegel, E. Ward et al., “Cancer statistics, 2006,” *CA: A Cancer Journal for Clinicians*, vol. 56, no. 2, pp. 106–130, 2006.

[8] N. Tsoukalas, E. Aravantinou-Fatorou, P. Baxevaros et al., “Advanced small cell lung cancer (SCLC): new challenges and new expectations,” *Annals of translational medicine*, vol. 6, no. 8, p. 145, 2018.

[9] L. A. Byers and C. M. Rudin, “Small cell lung cancer: where do we go from here?,” *Cancer*, vol. 121, no. 5, pp. 664–672, 2015.

[10] S. Wang, S. Zimmermann, K. Parikh, A. S. Mansfield, and A. A. Adjei, “Current diagnosis and management of small-cell lung cancer,” *Mayo Clinic Proceedings*, vol. 94, no. 8, pp. 1599–1622, 2019.

[11] D. Aberle and C. Berg, “The National Lung Screening Trial: overview and study design,” *Radiology*, vol. 258, no. 1, pp. 243–253, 2011.

[12] R. A. Smith, D. Brooks, V. Cokkinides, D. Saslow, and O. W. Brawley, “Cancer screening in the United States, 2013,” *CA: a Cancer Journal for Clinicians*, vol. 63, no. 2, pp. 87–105, 2013.

[13] F. C. Dettorbeck, P. J. Mazzone, D. P. Naidich, and P. B. Bach, “Screening for lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines,” *Chest*, vol. 143, no. 5, pp. e78S–e92S, 2013.

[14] J. Vansteenkiste, L. Crinò, C. Dooms et al., “2nd ESMO Consensus Conference on Lung Cancer: early-stage non-small-cell lung cancer consensus on diagnosis, treatment and follow-up,” *Annals of Oncology*, vol. 25, no. 8, pp. 1462–1474, 2014.

[15] D. R. Aberle, F. Abtin, and K. Brown, “Computed tomography screening for lung cancer: has it finally arrived? Implications of the national lung screening trial,” *Journal of Clinical Oncology*, vol. 31, no. 8, pp. 1002–1008, 2013.

[16] P. B. Bach, J. N. Mirkin, T. K. Oliver et al., “Benefits and harms of CT screening for lung cancer: a systematic review,” *JAMA*, vol. 307, no. 22, pp. 2418–2429, 2012.

[17] G. Sozzi and M. Boeri, “Potential biomarkers for lung cancer screening,” *Translational lung cancer research*, vol. 3, no. 3, pp. 139–148, 2014.

[18] A. Zhang, H. Sun, and X. Wang, “Saliva metabolomics opens door to biomarker discovery, disease diagnosis, and treatment,” *Applied Biochemistry and Biotechnology*, vol. 168, no. 6, pp. 1718–1727, 2012.

[19] X. Shang, H. Zi, Y. Li et al., “Combined use of salivary biomarkers and carcinoembryonic antigen for lung cancer detection in a Chinese population,” *Medicine*, vol. 98, no. 31, pp. e16511–e16511, 2019.

[20] I. Vannini, F. Fanini, and M. Fabbri, “MicroRNAs as lung cancer biomarkers and key players in lung carcinogenesis,” *Clinical Biochemistry*, vol. 46, no. 10-11, pp. 918–925, 2013.

[21] M. Schapher, O. Wendler, and M. Gröschl, “Salivary cytokines in cell proliferation and cancer,” *Clinica Chimica Acta*, vol. 412, no. 19-20, pp. 1740–1748, 2011.

[22] Y. Soini, R. Kaartenahko-Wiik, P. Pääkkö, and V. Kinnula, “Expression of antioxidant enzymes in bronchial metaplastic and dysplastic epithelium,” *Lung Cancer*, vol. 39, no. 1, pp. 15–22, 2003.

[23] J. de Castro, M. C. Rodriguez, V. S. Martinez-Zorzano, M. Llanillo, and J. Sánchez-Yagüe, “Platelet linoleic acid is a potential biomarker of advanced non-small cell lung cancer,” *Experimental and Molecular Pathology*, vol. 87, no. 3, pp. 226–233, 2009.

[24] I. Hoseok and J. Y. Cho, “Chapter Three-Lung Cancer Biomarkers,” in *Advances in Clinical Chemistry*, G. S. Makowski, Ed., pp. 107–170, Elsevier, 2015.

[25] Y. Wu, Y. Wu, J. Wang et al., “An optimal tumor marker group-coupled artificial neural network for diagnosis of lung cancer,” *Expert Systems with Applications*, vol. 38, no. 9, pp. 11329–11334, 2011.

[26] S. J. Cameron, K. E. Lewis, M. Beckmann et al., “The metabolomic detection of lung cancer biomarkers in sputum,” *Lung Cancer*, vol. 94, pp. 88–95, 2016.

[27] Y. Saalberg and M. Wolff, “VOC breath biomarkers in lung cancer,” *Clinica Chimica Acta*, vol. 459, pp. 5–9, 2016.

[28] H. P. Chan, C. Lewis, and P. S. Thomas, “Exhaled breath analysis: novel approach for early detection of lung cancer,” *Lung Cancer*, vol. 63, no. 2, pp. 164–168, 2009.
Mediators of Inflammation

[29] J. J. Underwood, R. S. Quadri, S. P. Kalva et al., “Liquid biopsy for cancer: review and implications for the radiologist,” Radiology, vol. 294, no. 1, pp. 5–17, 2020.

[30] A. Zhang, H. Sun, P. Wang, and X. Wang, "Salivary proteomics in biomedical research," Clinica Chimica Acta, vol. 415, pp. 261–265, 2013.

[31] B. J. Baum, "Principles of saliva secretion," Annals of the New York Academy of Sciences, vol. 694, no. 1 Saliva as a D, pp. 17–23, 1993.

[32] J. Ekström, N. Khosravani, M. Castagnola, and I. Messana, “Saliva and the control of its secretion,” in Dysphagia: Diagnosis and Treatment, O. Ekberg, Ed., pp. 21–57, Springer International Publishing, Cham, 2017.

[33] E. Kaufman and I. B. Lamster, “The diagnostic applications of saliva—a review,” Critical Reviews in Oral Biology & Medicine, vol. 13, no. 2, pp. 197–212, 2002.

[34] J. Adeoye and P. Thomson, “The Double-Edged Sword - an hypothesis for COVID-19-induced salivary biomarkers," Medical Hypotheses, vol. 143, p. 110124, 2020.

[35] C.-Z. Zhang, X. Q. Cheng, J. Y. Li et al., "Saliva in the diagnosis of diseases," International Journal of Oral Science, vol. 8, no. 3, pp. 133–137, 2016.

[36] S. Hu, Y. Yen, D. Ann, and D. T. Wong, "Implications of salivary proteomics in drug discovery and development: a focus on cancer drug discovery," Drug Discovery Today, vol. 12, no. 21–22, pp. 911–916, 2007.

[37] S. Hu, J. A. Loo, and D. T. Wong, “Human saliva proteome analysis and disease biomarker discovery," Expert Review of Proteomics, vol. 4, no. 4, pp. 531–538, 2007.

[38] K. Gao, H. Zhou, L. Zhang et al., “Systemic disease-induced salivary biomarker profiles in mouse models of melanoma and non-small cell lung cancer," PLoS One, vol. 4, no. 6, article e5875, 2009.

[39] W. G. Wade, “The oral microbiome in health and disease," Pharmacological Research, vol. 69, no. 1, pp. 137–143, 2013.

[40] A. Roi, L. C. Rusu, C. I. Roi, R. E. Luca, S. Boia, and R. I. Munteanu, “A new approach for the diagnosis of systemic and oral diseases based on salivary biomolecules," Disease Markers, vol. 2019, Article ID 8761869, 11 pages, 2019.

[41] D. Hanahan and R. A. Weinberg, “Hallmarks of cancer: the next generation," Cell, vol. 144, no. 5, pp. 646–674, 2011.

[42] A. Gardner, G. Carpenter, and P. W. So, “Salivary metabolomics: from diagnostic biomarker discovery to investigating biological function," Metabolites, vol. 10, no. 2, p. 47, 2020.

[43] L. V. Bel’skaya, E. A. Sarf, V. K. Kosenok, and I. A. Gundyrev, “Biochemical markers of saliva in lung cancer: diagnostic and prognostic perspectives," Diagnostics, vol. 10, no. 4, p. 186, 2020.

[44] L. Bel’skaya and V. Kosenok, “The activity of metabolic enzymes in the saliva of lung cancer patients," National Journal of Physiology, Pharmacy and Pharmacology, vol. 7, no. 6, p. 1, 2017.

[45] L. Bel’skaya, E. Sarf, and I. Gundyrev, “Study of the IR spectra of the saliva of cancer patients," Journal of Applied Spectroscopy, vol. 85, no. 6, pp. 1076–1084, 2019.

[46] L. Bel’skaya and V. Kosenok, “A new field of application of saliva tests for prognostic purpose: focus on lung cancer," Biomedical Chemistry: Research and Methods, vol. 3, no. 3, article e01133, 2020.

[47] L. V. Bel’skaya, V. K. Kosenok, G. Massard, and E. E. Orlova, “Salivary cytokine levels in lung cancer with distant and regional Metastases," Biomedical Chemistry: Research and Methods, vol. 1, no. 2, article e00012, 2018.

[48] Y. M. Goh, S. S. Antonowicz, P. Boshier, and G. B. Hanna, “Metabolic biomarkers of squamous cell carcinoma of the aerodigestive tract: a systematic review and quality assessment," Oxidative Medicine and Cellular Longevity, vol. 2020, Article ID 2930347, 13 pages, 2020.

[49] L. M. Coussens and Z. Werb, “Inflammation and cancer," Nature, vol. 420, no. 6917, pp. 860–867, 2002.

[50] F. R. Greten and S. I. Grivennikov, “Inflammation and cancer: triggers, mechanisms, and consequences," Immunity, vol. 51, no. 1, pp. 27–41, 2019.

[51] T. Koizumi, V. Shetty, and M. Yamaguchi, “Salivary cytokine panel indicative of non-small cell lung cancer," The Journal of International Medical Research, vol. 46, no. 9, pp. 3570–3582, 2018.

[52] X. L. Zhang, Z. Z. Wu, Y. Xu et al., “Saliva proteomic analysis reveals possible biomarkers of renal cell carcinoma," Open Chemistry, vol. 18, no. 1, pp. 918–926, 2020.

[53] M. Pernemalm, L. de Petris, H. Eriksson et al., “Use of narrow-range peptide IEF to improve detection of lung adenocarcinoma markers in plasma and pleural effusion," Proteomics, vol. 9, no. 13, pp. 3414–3424, 2009.

[54] K. Jessie, W. W. Pang, Z. Haji, A. Rahim, and O. H. Hashim, “Proteomic analysis of whole human saliva detects enhanced expression of interleukin-1 receptor antagonist, thioredoxin and lipocalin-1 in cigarette smokers compared to nonsmokers," International Journal of Molecular Sciences, vol. 11, no. 11, pp. 4488–4505, 2010.

[55] X. Lou, T. Xiao, K. Zhao et al., “Cathepsin D is secreted from M-BE cells: its potential role as a biomarker of lung cancer," Journal of Proteome Research, vol. 6, no. 3, pp. 1083–1092, 2007.

[56] H. Xiao, L. Zhang, H. Zhou, J. M. Lee, E. B.aron, and D. T. W. Wong, “Proteomic analysis of human saliva from lung cancer patients using two- dimensional difference gel electrophoresis and mass spectrometry," Molecular & cellular proteomics: MCP, vol. 11, no. 2, pp. M111.021121–M111.021121, 2012.

[57] W. Sun, J. D. Luo, H. Jiang, and D. D. Duan, “Tumor exosomes: a double-edged sword in cancer therapy," Acta Pharmacologica Sinica, vol. 39, no. 4, pp. 534–541, 2018.

[58] Y. Sun, S. Liu, Z. Qiao et al., “Systematic comparison of exosomal proteomes from human saliva and serum for the detection of lung cancer," Analytica Chimica Acta, vol. 982, pp. 84–95, 2017.

[59] L. Zhang, H. Xiao, H. Zhou et al., “Development of transcriptomic biomarker signature in human saliva to detect lung cancer," Cellular and Molecular Life Sciences: CMLS, vol. 69, no. 19, pp. 3341–3350, 2012.

[60] J. Uchida, K. Kato, Y. Kukita et al., “Diagnostic accuracy of noninvasive genotyping of EGFR in lung cancer patients by deep sequencing of plasma cell-free DNA," Clinical Chemistry, vol. 61, no. 9, pp. 1191–1196, 2015.

[61] A. Kawahara, C. Fukumitsu, T. Taira et al., “Epidermal growth factor receptor mutation status in cell-free DNA supernatant of bronchial washings and brushings," Cancer Cytopathology, vol. 123, no. 10, pp. 620–628, 2015.

[62] N. Sueoka-Aragane, N. Katakami, M. Satauchi et al., “Monitoring EGFR T790M with plasma DNA from lung cancer patients in a prospective observational study," Cancer Science, vol. 107, no. 2, pp. 162–167, 2016.
[63] G. da Cunha Santos, F. A. Shepherd, and M. S. Tsao, “EGFR Mutations and Lung Cancer,” *Annual Review of Pathology: Mechanisms of Disease*, vol. 6, no. 1, pp. 49–69, 2011.

[64] W. Zhou and D. C. Christiani, “East meets West: ethnic differences in epidemiology and clinical behaviors of lung cancer between East Asians and Caucasians,” *Chinese Journal of Cancer*, vol. 30, no. 5, pp. 287–292, 2011.

[65] B.-C. Liao, C.-C. Lin, and J. C.-H. Yang, “Second and third-generation epidermal growth factor receptor tyrosine kinase inhibitors in advanced nonsmall cell lung cancer,” *Current Opinion in Oncology*, vol. 27, no. 2, pp. 94–101, 2015.

[66] C.-C. Lin, W. L. Huang, F. Wei, W. C. Su, and D. T. Wong, “Emerging platforms using liquid biopsy to detect EGFR mutations in lung cancer,” *Expert Review of Molecular Diagnostics*, vol. 15, no. 11, pp. 1427–1440, 2015.

[67] F. Wei, C. C. Lin, A. Joon et al., “Noninvasive saliva-based EGFR gene mutation detection in patients with lung cancer,” *American Journal of Respiratory and Critical Care Medicine*, vol. 190, no. 10, pp. 1117–1126, 2014.

[68] D. Pu, H. Liang, F. Wei et al., “Evaluation of a novel saliva-based epidermal growth factor receptor mutation detection for lung cancer: a pilot study,” *Thoracic cancer*, vol. 7, no. 4, pp. 428–436, 2016.

[69] S. Ding, X. Song, X. Geng et al., “Saliva-derived cdDNA is applicable for EGFR mutation detection but not for quantitation analysis in non-small cell lung cancer,” *Thoracic Cancer*, vol. 10, no. 10, pp. 1973–1983, 2019.

[70] F. Li, F. Wei, W. L. Huang et al., “Ultra-short circulating tumor DNA (ucctDNA) in plasma and saliva of non-small cell lung cancer (NSCLC) patients,” *Cancers (Basel)*, vol. 12, no. 8, p. 2041, 2020.

[71] X. He, J. S. McLean, L. Guo, R. Luz, and W. Shi, “The social structure of microbial community involved in colonization resistance,” *The ISME Journal*, vol. 8, no. 3, pp. 564–574, 2014.

[72] J. A. Aas, B. J. Paster, L. N. Stokes, I. Olsen, and F. E. Dewhirst, “Defining the normal bacterial flora of the oral cavity,” *Journal of Clinical Microbiology*, vol. 43, no. 11, pp. 5721–5732, 2005.

[73] S. A. Schröder, A. Bardow, S. Eickhardt-Dalbøge, H. K. Johansen, and P. Homøe, “Is parotid saliva sterile on entry to the oral cavity?” *Acta Oto-Laryngologica*, vol. 137, no. 7, pp. 762–764, 2017.

[74] N. A. Hasan, B. A. Young, A. T. Minard-Smith et al., “Microbial community profiling of human saliva using shotgun metagenomic sequencing,” *PLoS One*, vol. 9, no. 5, article e97699, 2014.

[75] N. Segata, S. Haake, P. Mannon et al., “Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples,” *Genome biology*, vol. 13, no. 6, p. R42, 2012.

[76] P. Lif Holgersson, A. Esberg, A. Sjödin, C. E. West, and I. Johansson, “A longitudinal study of the development of the saliva microbiome in infants 2 days to 5 years compared to the microbiome in adolescents,” *Scientific Reports*, vol. 10, no. 1, p. 9629, 2020.

[77] E. Zaura, B. J. F. Keijser, S. M. Huse, and W. Crieland, “Defining the healthy core microbiome of oral microbial communities,” *BMC Microbiology*, vol. 9, no. 1, p. 259, 2009.

[78] D. Belstrøm, “The salivary microbiota in health and disease,” *Journal of Oral Microbiology*, vol. 12, no. 1, pp. 1723975–1723975, 2020.

[79] Y. Takahashi, N. Watanabe, N. Kamio, R. Kobayashi, T. Iinuma, and K. Imai, “Aspiration of periodontopathic bacteria due to poor oral hygiene potentially contributes to the aggravation of COVID-19,” *Journal of Oral Science*, vol. 63, no. 1, pp. 1–3, 2021.

[80] N. Marouf, W. Cai, K. N. Said et al., “Association between periodontitis and severity of COVID-19 infection: a case-control study,” *Journal of Clinical Periodontology*, vol. 48, no. 4, pp. 483–491, 2021.

[81] F. A. Scannapieco, “Role of oral bacteria in respiratory infection,” *Journal of Periodontology*, vol. 70, no. 7, pp. 793–802, 1999.

[82] L.-C. Yang, Y. J. Suen, Y. H. Wang, T. C. Lin, H. C. Yu, and Y. C. Chang, “The association of periodontal treatment and decreased pneumonia: a Nationwide population-based cohort study,” *International Journal of Environmental Research and Public Health*, vol. 17, no. 1, p. 356, 2020.

[83] L. S. Jerónimo, L. G. Abreu, F. A. Cunha, and R. P. Esteves Lima, “Association between periodontitis and nosocomial pneumonia: a systematic review and meta-analysis of observational studies,” *Oral Health & Preventive Dentistry*, vol. 18, no. 1, pp. 11–17, 2020.

[84] P. Gholizadeh, H. Eslami, M. Yousef, M. Asgharzadeh, M. Aghazadeh, and H. S. Kafil, “Role of oral microbiome on oral cancers, a review,” *Biomedicine & Pharmacotherapy*, vol. 84, pp. 552–558, 2016.

[85] S. H. Lee, J. Y. Sung, D. Yong et al., “Characterization of microbiome in bronchoalveolar lavage fluid of patients with lung cancer comparing with benign mass like lesions,” *Lung Cancer*, vol. 102, pp. 89–95, 2016.

[86] P. K. Mukherjee, H. Wang, M. Retuerto et al., “Bacteriome and mycobacteriome associations in oral tongue cancer,” *Oncotarget*, vol. 8, no. 57, pp. 97273–97289, 2017.

[87] L. Zhang, Y. Liu, H. J. Zheng, and C. P. Zhang, “The oral microbiota may have influence on oral cancer,” *Frontiers in Cellular and Infection Microbiology*, vol. 9, no. 476, 2020.

[88] I. Sobhani, J. Tap, F. Roudot-Thoraval et al., “Microbial dysbiosis in colorectal cancer (CRC) patients,” *PloS One*, vol. 6, no. 1, article e16393, 2011.

[89] J. C. Arthur, E. Perez-Chanona, M. Mühlbauer et al., “Intestinal inflammation targets cancer-inducing activity of the microbiota,” *Science*, vol. 338, no. 6103, pp. 120–123, 2012.

[90] S. Krishnan and G. Eslick, “Streptococcus bovis infection and colorectal neoplasia: a meta-analysis,” *Colorectal Disease*, vol. 16, no. 9, pp. 672–680, 2014.

[91] M. H. Fukugaiti, A. Ignacio, M. R. Fernandes, U. Ribeiro Júnior, V. Nakano, and M. J. Avila-Campos, “High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients,” *Brazilian Journal of Microbiology*, vol. 46, no. 4, pp. 1135–1140, 2015.

[92] W. Zhang, J. Luo, X. Dong et al., “Salivary microbial dysbiosis is associated with systemic inflammatory markers and predicted oral metabolites in non-small cell lung cancer patients,” *Journal of Cancer*, vol. 10, no. 7, pp. 1651–1662, 2019.

[93] G. Yu, M. H. Gail, D. Consonni et al., “Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features,” *Genome Biology*, vol. 17, no. 1, p. 163, 2016.

[94] X. Yan, M. Yang, J. Liu et al., “Discovery and validation of potential bacterial biomarkers for lung cancer,” *American
Journal of Cancer Research, vol. 5, no. 10, pp. 3111–3122, 2015.

[95] J. Yang, X. Mu, Y. Wang et al., “Dysbiosis of the salivary microbiome is associated with non-smoking female lung cancer and correlated with immunocytochemistry markers,” Frontiers in Oncology, vol. 8, p. 8(520), 2018.

[96] S. Sun, J. H. Schiller, and A. F. Gazdar, “Lung cancer in never smokers – a different disease,” Nature Reviews Cancer, vol. 7, no. 10, pp. 778–790, 2007.

[97] S. Saito, F. Espinoza-Mercado, H. Liu, N. Sata, X. Cui, and H. J. Soukiasian, “Current status of research and treatment for non-small cell lung cancer in never-smoking females,” Cancer Biology & Therapy, vol. 18, no. 6, pp. 359–368, 2017.

[98] Q. Lan, C. A. Hsiung, K. Matsuo et al., “Genome-wide association analysis identifies new lung cancer susceptibility loci in never-smoking women in Asia,” Nature Genetics, vol. 44, no. 12, pp. 1330–1335, 2012.

[99] Y. Zhang, B. Chen, L. Wang, R. Wang, and X. Yang, “Systemic immune-inflammation index is a promising noninvasive marker to predict survival of lung cancer,” Medicine, vol. 98, no. 3, pp. e13788–e13788, 2019.

[100] M. Gomes, A. L. Teixeira, A. Coelho, A. Araújo, and R. Medeiros, “The role of inflammation in lung cancer,” in Inflammation and Cancer, B. B. Aggarwal, B. Sung, and S. C. Gupta, Eds., pp. 1–23, Springer Basel, Basel, 2014.

[101] R. K. Gazdeck, S. R. Fruscione, G. R. Adami, Y. Zhou, L. F. Cooper, and J. L. Schwartz, “Diversity of the oral microbiome between dentate and edentulous individuals,” Oral Diseases, vol. 25, no. 3, pp. 911–918, 2019.

[102] M. Dzidic, M. C. Collado, T. Abrahamsson et al., “Oral microbiome development during childhood: an ecological succession influenced by postnatal factors and associated with tooth decay,” The ISME Journal, vol. 12, no. 9, pp. 2292–2306, 2018.

[103] J. Wu, B. A. Peters, C. Dominianni et al., “Cigarette smoking and the oral microbiome in a large study of American adults,” The ISME Journal, vol. 10, no. 10, pp. 2435–2446, 2016.

[104] M. R. Mason, H. N. Nagaraja, T. Camerlengo, V. Joshi, and P. S. Kumar, “Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome,” PLoS One, vol. 8, no. 10, article e77287, 2013.

[105] K. Chaouachi and K. M. Sajid, “A critique of recent hypotheses on oral (and lung) cancer induced by water pipe (hookah, shisha, narghile) tobacco smoking,” Medical Hypotheses, vol. 74, no. 5, pp. 843–846, 2010.