

Review of Literature**Salivary Chemical Factors in Relation with Oral Cancer in Smokers and Non-Smokers: a Literature Review**

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KEY WORDS

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ABSTRACT

Squamous cell carcinoma of oral cavity is of malignant tumors, which causes cancerous complications. DNA damage, mainly because of products of oxidative stress like reactive oxygen species, is a frequent mutagenic that triggers carcinoma. Smoking increases the probability of cancer incidence. Saliva is the first biological medium to interact with external compounds, especially smoking substances. The present study overviews the salivary level of some remarkable compounds in relation with smoking and squamous cell carcinoma.

To collect data, English literature was searched in databases including PubMed, ScienceDirect and Google Scholar. The keywords used for search were as follows: 'Carcinoma, Squamous Cell', 'Smoking', 'Saliva', and 'Biomarkers'. The inclusion criteria were the presence of salivary chemical factors in relation with oral cancer and influence by smoking. Out of 239 found articles, only 56 were selected.

Our results demonstrated the potential role of salivary biochemistry to predict and/or treat complications with cancer in both smoker and non-smoker individuals.

Changes in concentrations of salivary chemicals including antioxidants, total antioxidant, glutathione and uric acid, epithelial growth factor, cytokine biomarkers, superoxide dismutase activity, and transcriptome were related to squamous cell carcinoma and could be used as potential biomarkers for cancer prognosis; moreover, enhancement of antioxidant level might be a potential treatment.

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Introduction

Squamous cell carcinomas (SCCs) of the oral cavity, pharynx, and larynx are different types of head and neck squamous cell carcinoma (HNSCC) and represent about 3% of all malignant tumors in the United States. [1] Smoking and alcohol intake are the most important recognized risk factors for HNSCC. [2] Moreover, oxidative stress plays a pivotal role in the pathogenesis of aging and several degenerative diseases, such as atherosclerosis, cardiovascular disease, type 2 diabetes and cancer. [3]

The most frequent potentially mutagenic spontaneous event is DNA damage, which mainly occurs as a

result of chemical attack. Chemical attack in large part is a product of oxidative metabolism and reactive oxygen species (ROS) are particular products of this phenomenon. [4-6] Reactive oxygen generating systems can promote tumor progression, and reactive ROS has been hypothesized to have a role in cigarette smoke-associated carcinogens. [7-9]

Presently, smoking health problem has been proven throughout the world. Estimations reveal that by 2020, smoking will be the cause of one in every three deaths. [10] Many studies have shown that cigarette, and recently hookah, have been the major etiologic factors for oral SCC. [11] Based on the World Health Or-

ganization (WHO) declaration, tobacco consumption, especially hookah and cigarette smoking, is a universal threat to health. [12] Cigarette smoke comprises several materials including carbon monoxide, nitrogen, nicotine, and free radicals such as superoxide, hydrogen peroxide, hydroxyl and reactive oxygen (O^2). These compounds increase the probability of cancer incidence in different parts of body including the oral cavity. [13-15]

Smoking might affect the level of salivary cortisol and IgA in patients with oral lichen planus. [16-17] It is shown that smoking could reduce the value of total protein, calcium (Ca) and lead (Pb) of saliva. [18] In another study, it was shown that long-term smoking would significantly decrease the salivary flow rate and raises oral and dental disorders related to dry mouth. [19] According to studies, all types of tobacco are considered as risk factors for oral cancer, but snuff habits as present in Scandinavia carry lower risks of severe health threats such as oral cancer. Alcohol synergizes with tobacco as a risk factor for all upper aerodigestive tract SCC. [20]

Saliva is the first biological medium confronted by external materials, which covers and protects mucosa of the upper digestive tract at the same time, and in particular the oral cavity and pharynx. Various agents cause the carcinogen effect through alterations of chemical composition of human saliva. Human saliva has a total antioxidant capacity higher than blood plasma. [21] In addition, saliva contains polypeptides including immunoglobulin and enzymes such as lactoferrin, lysozyme and histamine. These polypeptides play a crucial role in defense mechanisms against free radicals (oxidative stress) and thereby against oral cancer occurrence. [22-23]

Since saliva is in direct contact with the oral mucosa and cancerous lesions, its molecular assessment is easy, efficient and non-invasive. Therefore, saliva is potential for widespread screening and useful for detection of early lesions. [24]

Based on statistics, the number of tobacco users will increase to 1.4 billion individuals worldwide until 2020; therefore, it is possible for everyone to be exposed to tobacco smoke. [25-26] Those who are exposed to smoking (passive smokers) also suffer from the complications of tobacco smoke indirectly. In recent years, reports have shown passive smoking as a strong

risk factor for the incidence of cardiovascular diseases. [27] Due to the increasing threat of SCC, this study aimed to overview the biochemistry of saliva among smokers and non-smokers and those suffered from different kinds of head and neck SCC, which is especially useful to recognize potential biomarkers, prognostic and therapeutic cues.

Search Strategy

To collect data, English literature was searched in databases including PubMed, ScienceDirect and Google Scholar. The keywords searched were 'Carcinoma, Squamous Cell', 'Smoking', 'Saliva', and 'Biomarkers'. The inclusion criteria were salivary chemical factors in relation with oral cancer and influence by smoking. Out of the 239 found articles, only 56 were proper. The published articles from 1965 to 2016 were evaluated. The inclusion criterion was the articles that evaluated salivary chemical factors in relation with oral cancer in smokers and non-smokers. The exclusion criteria were articles that did not evaluate the salivary chemical factors amongst smokers and those not related to oral cancer. The references of the articles were also evaluated to find articles that are more proper. Titles and abstracts of all the selected articles were evaluated and if they were suitable, the full text was reviewed. All types of studies, which were related to our goal, including the salivary levels of some remarkable compounds in relation to smoking and SCC, were selected and evaluated.

Antioxidant Status

It has been known that antioxidant status of saliva, and in particular glutathione levels, is influenced by tobacco. [28-29] Salivary antioxidant system includes various mainly water-soluble molecules and enzymes, such as uric acid, peroxidase, glutathione peroxidase, catalase, lactic dehydrogenase, glutathione reductase, and aspartate aminotransferase. [30] Numerous studies have shown changes in the activity of salivary antioxidants system in smokers and in patients with SCC compared with control group, but there are some differences between them. [21, 31-33]

Total Antioxidant

In a cross-sectional study, on saliva of 50 smokers and 50 non-smokers, the total antioxidant capacity of their saliva was evaluated by ferric-reducing antioxidant power method. It was clarified that the total antioxidant

capacity of saliva was significantly lower in smokers compared to non-smokers' group; however, the antioxidant activity was not evaluated. It was concluded that smoking might chronically affect the immune system of salivary glands. [16] In another study on 30 male smokers and 30 male non-smokers, the total antioxidant capacity was evaluated using antioxidant assay kit and a significantly higher total salivary antioxidant capacity was clarified in non-smokers than in smokers. [34] Albeit alteration of the anti-oxidative capability of saliva resulted by tobacco smoking, there is controversy over the exact cause of these changes between the studies. [35-36] In the study of Arathi *et al.* the total antioxidant activity significantly decreased in oral SCC patients and smokers when compared with control subjects. [37]

Glutathione and uric acid

Low-molecular-weight antioxidants like glutathione and uric acid, in particular glutathione, can directly scavenge free radicals or act as a substrate for enzymes such as glutathione S-transferases and glutathione peroxidases during the detoxification of hydrogen peroxide, lipid hydroperoxides, and electrophilic compounds. [38-40] In a study performed on 50 untreated patients with primary HNSCC, patients with oral or pharyngeal SCC had significantly higher salivary levels of glutathione than both controls and patients with laryngeal SCC, while there was no significant difference for salivary levels of uric acid. Moreover, there is a significant correlation between salivary uric acid level and patient's gender, uric acid levels being significantly higher in males. [30] Rise in salivary glutathione levels may precede cancer development, since higher salivary glutathione levels in smokers compared with non-smokers is probably a response to the increased oxidative stress. [29]

Epidermal Growth Factor (EGF)

Epidermal growth factor (EGF) is a small polypeptide (53 amino acids), originally isolated from mouse salivary glands. In humans, salivary glands are mainly responsible for the synthesis of EGF, making saliva a potential source for EGF in the oral cavity, whereas kidneys are responsible for systemic EGF production. [41] Binding of EGF to its receptor determines its biological activity, which is involved in activating pathways that promote cellular proliferation, survival, migration, and differentiation in most epithelial tissues, fi-

broblasts and endothelial cells. [41-42]

A study on 46 patients with oral SCC revealed that they had a significantly lower EGF level than the healthy group. [43] Different levels of EGF in tongue and floor of mouth indicates higher permeability of oral mucosa to carcinogens in these sites. [44] In addition, no association was found between salivary levels of EGF and the intensity of EGF immunohistochemical expression in tumor tissues. [43]

Cytokine biomarkers

Sensitive and specific biomarkers for SCC have the potential to help to decrease the morbidity and mortality of the disease. Elevated levels of cytokines have been found in saliva of patients with oral SCC. [45-47] Furthermore, in a collaboration study, we identified significantly enhanced levels of IL-8 protein and mRNA in saliva of patients with oral SCC as well as increased IL-6 in serums of the same group. [45] Some cytokines' role in the angiogenesis cascade has been clarified and they potentially serve as important angiogenic factors in the development of SCC. [48] Assessments have revealed higher salivary IL-1 α , IL-6, TNF- α , and VEGF- α levels in both endophytic and exophytic groups of patients with SCC of tongue than the control groups, while a higher level for IL-8 was observed just in the endophytic group. [24] In addition, smoker and smoker-drinker control groups revealed higher levels of IL-8 and VEGF- α than the healthy controls. These cytokines are rational and potential candidates for salivary biomarkers because of their established role in angiogenesis, inflammation and disease progression.

Cotinine

Cotinine is an alkaloid in tobacco and is generated as one of the metabolites of nicotine. It is stable in body fluids, has a long half-life of 15-40 hours, and is able to bind to low plasma protein. It is directly proportional to the quantity of nicotine absorbed and dose-independent disposition kinetics. [49] It is most commonly used as a marker to distinguish between tobacco smokers and non-smokers because of its higher sensitivity and specificity than other biochemical tests. [50-51] It can be measured with different techniques such as immunoassay, radioimmunoassay, fluid gas chromatography, enzyme-linked immunosorbent assay (ELISA), colorimetric assays, and NicAlert™ Saliva tests. [25, 52]

It has been observed that there are variant salivary

Table 1: Summary of saliva biochemistry in relation with smoking

Chemical	Level in SCC patients that healthy individuals	Level in smokers than non-smoker group	Comment
Antioxidant status			
Total antioxidant	Lower	Lower	Smoking affects immune system of salivary glands chronically; use of antioxidant agents, like fruits, might decrease the incidence of oral cancers among smokers
Glutathione	Higher	Higher	Inconstant concentration; widely range to be effectively used as diagnostic marker; index of oxidative stress
Uric acid	No difference	No difference	significantly higher in males
Epidermal growth factor (EGF)	lower	Lower	In relation with site of tumor; smokers showed lower EGF salivary levels; Cigarette smoking reduces EGF salivary levels in a dose-dependent manner and impairs the function of oral EGF receptor
Salivary cytokine protein			
Interleukin-1	Higher	No difference	
Interleukin-6	Higher	No difference	
Interleukin-8	Higher	Higher	Just for endophytic group; smoking and smoking–drinking controls showed higher levels
TNF-a	Higher	No difference	
VEGF-a	higher	Higher	smoking and smoking–drinking controls showed higher levels
Cotinine	N/A*	higher	Passive smokers show higher cotinine than non-smokers
HPV	N/A	Higher	In small samples no difference was observed
Enzyme activity			
Salivary peroxidase	lower	lower	exposure to oxidative stress and DNA damage
* Not applicable			

cotinine levels in smokers, passive smokers and non-smokers. In a study conducted on hookah smokers, individual sex posed to tobacco or cigarette smoke and non-smoker subjects, the level of cotinine was assayed using ELISA, and the cotinine level in hookah smokers and passive smokers was significantly higher than non-smokers (20.24 ± 5.26 and 16.098 ± 3.51 vs. 0.66 ± 0.26) and beyond the cut-off point. [53] In addition, an increase in salivary cotinine levels was observed because of increase in the use of tobacco. Another study demonstrated that saliva cotinine significantly reduced in relation with maternal age and significantly increased with number of cigarettes smoked per day, secondhand smoke exposure and number of previous full-term pregnancies. [10] Significant difference between non-smokers and passive smokers indicated the importance of this problem and the threat environments that might expose individuals to tobacco smoke.

Enzymatic activity

Superoxide dismutase (SOD) activity was reported significantly higher in the smoking group, while no detectable activity level was found in non-smokers. [31] In a study, there was a decrease of salivary peroxidase ac-

tivity in patients with oral cavity cancer than healthy control group. [54] Saggu *et al.* [55] analyzed the unstimulated saliva of 100 smokers by measuring the activity of salivary SOD and glutathione peroxidase and showed a meaningfully higher SOD activity among smokers, while the levels of GSH-Px activity were significantly higher in the nonsmoking group.

Salivary peroxidase is by far the most important antioxidant enzyme in saliva and superoxide dismutase has a secondary role. [33] The lower antioxidant status of salivary peroxidase in the cancerous group might have been one of the causes of malignant transformation. [54]

Salivary Transcriptome

Salivary transcriptomes (RNA molecules) were stable in saliva. They included mRNA molecules that cells use to convey the instructions carried by DNA for subsequent protein production. Li *et al.* discovered that RNA molecules increased in oral cancer tissues were also elevated in saliva. [56]

Conclusion

This study summarizes the salivary levels of some criti-

cal chemicals and proteins (Table 1) in patients with different types of SCC and in relation with smoking. Changes in concentration of salivary chemicals including antioxidants, total antioxidant, glutathione and uric acid, EGF, cytokine biomarkers, superoxide dismutase activity, and transcriptome is related with SCC and could be used as potential biomarkers for cancer prognosis detection, and enhancement of antioxidant level might be a potential treatment.

Conflict of Interest

The authors disclose no potential conflicts of interest.

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