The Possible Link Between Oral Microflorae and Oral Cancer Development: A Literature Review

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Abstract
Oral squamous cell carcinoma (OSCC) research is still inconclusive due to methodological differences and constraints. The study aimed to review the function of oral microflora in the progression of oral cancer and to highlight the need for good oral hygiene practices for various reasons beyond only avoiding dental problems such as cavities and gum disease. Oral carcinoma is a rapidly increasing cancer with a high mortality rate, particularly in adolescents and young adults. Despite the progress of chemotherapy and radiation therapy, the percentage of people who will be alive is less than 50% after 5 years. Oral cancer has a terrible prognosis and can spread if it is not detected early; thus, researchers should focus on developing biomarkers that might detect the disease at an earlier stage. SCC has a complex set of causes. Factors and conditions predisposing to oral cancer include tobacco, alcohol, infections (e.g., candidiasis), viruses (human immunodeficiency virus, herpes simplex virus, and human papillomavirus), and systemic conditions (iron deficiency anemia, malnutrition, and vitamin A deficiency). Changes in the structure of oral bacteria are caused by two primary risk factors for oral cancer, including smoking and alcohol consumption. These microorganisms produce carcinogenic products such as acetaldehyde, which are associated with oral cancer. The oral cavity is host to a wide variety of microorganisms, including fungi, bacteria, viruses, and other microorganisms, as one of the most abundant microbial habitats in the human body. Recent epidemiological research has linked specific periodontitis microorganisms to an increased risk of developing oral premalignant and neoplastic lesions. Clinicians have long noted a correlation among dental state, poor oral hygiene, and oral cancer, which may be independent of tobacco and alcohol use. Based on the results, more research is required to determine the precise results and the nature of the correlation between oral microbiota and oral cancer, considering the findings of the previous studies.

Keywords: Cancer, Oral cancer, Oral microbiome, Inflammation, Infection

Background
Oral cavity cancer, specifically oral carcinoma, ranks among the top ten killers in the world.1 Prevalence and mortality rates, on average, vary substantially between genders, ethnic backgrounds, and age groups.2 There have been 53,260 new cases of oral cancer detected in the US in 2020, with over 10,000 deaths attributable to the disease.3

Approximately 90% of oral cancers are squamous cell carcinomas (SCC), while the other 10% are made up of various forms, such as malignant odontogenic tumors, minor salivary gland malignancies, sarcomas, melanoma, and lymphoma.4

An increasing number of younger people are being diagnosed with deadly oral SCC (OSCC). People over the age of 40 make up 95% of those diagnosed with oral SCC, with the typical patient age at diagnosis being around 60. The oropharynx, floor of the mouth, and tongue are common sites affected by oral cancer. Unfortunately, most individuals are first detected when the disease has progressed significantly and symptoms have appeared. At the time of diagnosis, discomfort is present in 85% of cases, making it the leading symptom, leading patients to seek out specialized therapy. In advanced disease, symptoms such as dysphagia, odynophagia, otalgia, reduced mobility, oral hemorrhage, neck lumps, and weight loss may be present.1

SCC caused by oral mucosal epithelium remains a disfiguring disease due to tumor invasion, destruction of the mouth and face, cervical lymph node metastasis, and final diffusion through the blood.1

Despite the progress of chemotherapy and radiation therapy, the percentage of people who will be alive is less than 50% after five years,5 and patients often suffer from secondary and recurrent tumors. Alterations in the structure of oral bacteria are due to
two initial risk factors for oral cancer, namely, alcohol consumption and smoking. These microorganisms produce carcinogenic products such as acetaldehyde (ALD), which are related to oral cancer.7

This disease is usually not diagnosed on time, and patients are in the advanced stages of cancer during diagnosis.

Recently, the idea of developing early diagnostic biomarkers has been studied in light of the fact that a delay in diagnosing oral cancer leads to metastases and a poor prognosis.

**Oral Cancer Risk Factors**

SCC has many potential causes (Figure 1). No carcinogen has been isolated definitively.8

There is a correlation between age and the development of oral cancer because of the time it takes for genetic changes to accumulate, as well as the time it takes to be exposed to potential carcinogens (e.g., viruses, chemical and physical stimuli, and hormonal effects).1

Factors and conditions predisposing to oral cancer include viruses (human immunodeficiency virus [HIV], herpes simplex virus [HSV], and human papillomavirus [HPV]), alcohol, tobacco, betel, infections (e.g., candidiasis), and systemic conditions (iron deficiency anemia, malnutrition, and vitamin A deficiency), immune system defects, genetic abnormalities, and sunlight (in the case of vermilion lip cancer).2,8-11

The main causes of mouth and oropharynx cancer are tobacco and alcohol use.1,8,10 Many medical professionals view chronic hyperplastic candidiasis as a potentially malignant condition. Without the involvement of any other factor, certain species of *Candida albicans* can induce hyperkeratotic lesions on the tongue’s dorsal surface.2

Although viruses are suspected to play a role in a wide variety of cancers, no virus has been linked to the development of oral cancer. Adenoviruses, herpes simplex viruses, retroviruses, and human papillomaviruses have all been implicated in the development of oral cancer in the past. Today, it seems that HPV is the only type that is involved not only in oral cancer but also in the carcinomas of the tonsils, larynx, esophagus, cervix, and male and female genitalia. Subgroups 16, 18, 31, and 33 of HPV are closely related to dysplasia and SCC.2,12

Cellular proteins E6 and E7 are produced in response to HPV infection and make a combination with the results of tumor suppressor genes (TSGs) to block the impact of these genes, putting the cellular DNA repair process at risk and ultimately increasing the rate at which cell nuclei divide.13

In addition to cigarettes, alcohol, and HPV, HSV-1, and HSV-2 may also increase the chance of developing cancer of the head and neck. There is not enough data to conclude that HSV causes oral cancer at this time.1

Oral cancer risk may increase when the immune system is compromised.7 Without a strong immune system, cancerous cells cannot be detected and eliminated in their preliminary stages.2,10

SCC, along with other head and neck malignancies, is more likely among people with acquired immunodeficiency syndrome or patients undergoing immunosuppressive medications due to the treatment of malignancies or getting transplants, especially when smoking and alcohol use are present.14,15

Carcinoma is associated with a variety of molecular indications and markers, including low E-cadherin expression, P53 gene mutation, catenin gene disruption, high collagenase 3 and P27 expression, and HPV infection.

Gene expression analysis suggests that cyclin D1 has a role in prognosticating metastasis. Tongue cancer (with or without metastases) has been linked to several chromosomal abnormalities, including loss of heterozygosity.1,16,17

Proto-oncogenes become active oncogenes as a result of viral activities, radiation, or chemical, physical, and microbial carcinogens in certain malignancies. Activated oncogenes may greatly stimulate the production of new genetic products. Numerous neoplasms, including SCC, are thought to have an oncogene component in their development and spread.16,18 Proto-oncogenes related to head and neck SCC include int-2, bcl-1, bcl-2, erb-b, myc, cyclin-D1, ras, CK8, and CK19,19,20

On the other hand, when TSGs are inactivated or mutated, they indirectly allow tumor growth. TSGs related to head and neck SCC are P53, Rb, and P16INK4A. The other cases of these TSGs include FHIT, APC, VHL, DOC1, and TGF-R-II.

Oral carcinomas have been shown to harbor both oncogenes and tumor suppressors, although a definitive link between the two has to be established yet.

Most studies state that the accumulation of several genetic abnormalities is necessary before the appearance
Microorganisms and Their Association With Oral Cancer

The relationship between Helicobacter pylori bacteria and intestine cancer, which had been ignored for decades, was finally investigated in 1990. According to the research, microorganisms are responsible for 16.1% of malignancies. Even though only a few bacteria have an anti-tumor effect, a sizable population of tumor-related malignancies. The oral cavity houses a wide variety of microflora, including bacteria, fungi, viruses, and other microbes, and is thus one of the body’s most abundant microbiological resources. Recently, metagenomic research has helped identify various bacterial strains that have a role in the development of oral malignancies. The bacteria present in the oral cavity are among the carcinogenic agents and are effective in the processes of tumorigenesis. On the other hand, these biomarkers can help in the early detection of oral cavity cancers. Today, some periodontal organisms are known as key factors in causing cancers of the oral cavity.

Types of Bacteria Found in Various Parts of the Oral Cavity

More than 700 different bacterial strains can be found in the oral cavity. These bacteria can be further classified as either aerobic or anaerobic. The temperature of 37 °C and pH of about 6.5-7.5 have turned the oral cavity into a suitable environment for the survival and growth of these bacteria. On the other hand, saliva provides moisture and nutrients needed by these bacteria.

The oral cavity and oropharynx include different live bacterial strains. Table 1 presents the bacterial strains in various areas.

Potentially Oncogenic Oral Bacteria

Among the microflora of the oral cavity, some bacteria are more effective than others in causing cancer. Bacteria belonging to the genera Veillonella, Fusobacterium, Prevotella, Porphyromonas, Actinomyces, Clostridium, Haemophilus, Streptococcus, and Enterobacteriaceae are the most common culprits in the development of cancer of the oral cavity. Table 2 summarizes a list of types of oral cancer and the bacteria that contribute to their development.

The Effect of Oral Microbial Flora on Cancer Development

There are three main ways in which the oral microbiota might lead to cancer:

1. **Chronic inflammation**: Inflammatory reactions and mediators are induced by bacteria in the mouth, and some of these mediators affect the carcinogenesis process. These mediators can cause cell proliferation and mutations, which activate some oncogenes to help the tumor angiogenesis process.
2. **Anti-apoptotic activity**: The anti-apoptotic activities of bacteria in the mouth are mediated by their effect on nuclear factor kappa B.
3. **Production of carcinogenic products**: Bacteria produce carcinogenic substances during their metabolic processes.

The Mechanism of Chronic Inflammation

Several bacteria cause periodontal disease within the oral cavity, including Porphyromonas, Prevotella, and Fusobacterium. As a result of chronic inflammation caused by these bacteria, some inflammatory mediators accumulate in the mouth. The extracellular matrix, as well as epithelial and endothelial cells, are affected by these mediators. Interleukin-1 (IL-1), IL-6, IL-17, IL-23, tumor necrosis factor- (TNF-), matrix metalloproteinases MMP-8, and MMP-9 are all examples of inflammatory mediators.

Lipopolysaccharide (LPS) is a component of the cell wall of Gram-negative bacteria. When exposed to LPS from bacteria, monocytes, macrophages, neutrophils, fibroblasts, and mast cells release a cytokine. During the inflammatory process of periodontitis, IL-1 helps create osteoclasts and resorb bone. Phospholipase A2, prostaglandins (PG), acute-phase proteins, IL-6, TNF, and numerous MMPs are all secreted in response to this

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**Table 1. Different Bacterial Strains in Various Areas of Oropharynx and Oral Cavity**

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Different Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyromonas gingivalis, Veillonella atypica, Aggregatibacter, Prevotella intermedia, Selenomonas subspecies, Actinomyces odontolyticus, Veillonella parvula, Leptotrichia buccalis</td>
<td>Tongue</td>
</tr>
<tr>
<td>Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus para influenzae, Haemophilus influenzae, Streptococcus anginosus, Streptococcus mutans, Streptococcus salivarius</td>
<td>Oropharynx</td>
</tr>
<tr>
<td>Actinomyces, Streptococcus mutans, Peptostreptococcus, Eubacterium</td>
<td>Tooth surface</td>
</tr>
<tr>
<td>Prevotella, Fusobacterium, Streptococcus mitis, Porphyromonas, Propionibacterium acnes, Streptococcus sanguinis, Actinomyces</td>
<td>Gingival crevice</td>
</tr>
<tr>
<td>Haemophilus influenzae, Streptococcus viridans, Staphylococcus, Neisseria species</td>
<td>Tonsil</td>
</tr>
<tr>
<td>Rothia, Actinomyces, Mycobacterium, Corynebacterium, Microbacterium, Bifidobacterium, Propionibacterium</td>
<td>Dental plaque</td>
</tr>
</tbody>
</table>
cytokine.\textsuperscript{38,39} Furthermore, vascular endothelial growth factor and other proangiogenic factors are produced by epithelial cells in response to this cytokine’s stimulation. Inflammation triggers the release of factors necessary for angiogenesis and cancer.\textsuperscript{40} Excessive IL-1 release is typically linked to tumor invasiveness, tumor migration, and an aggressive tumor phenotype.\textsuperscript{41,42}

LPS also triggers the creation of other significant cytokines, including IL-6. During invasion and metastasis, IL-6 influences MMP secretion.\textsuperscript{43} In addition, these cytokines cause the secretion of endothelial cell-leukocyte adhesion molecules and intercellular adhesion molecule-1, which are essential for tumor attachment to endothelial cells and can promote tumor growth.\textsuperscript{44}

TNF-α, produced by monocytes, macrophages, neutrophils, fibroblasts, and mast cells in response to various stimuli, including bacterial LPS, is another crucial cytokine in inflammatory processes.\textsuperscript{45} The number of osteogenic cells and fibroblasts considerably decreases as a result of TNF-α’s production of reactive oxygen species (ROS), leukotrienes, PG, and MMPs.\textsuperscript{46}

Tumor-destroying activities are triggered by exposure to high concentrations of this cytokine, while tumorigenic processes are stimulated by exposure to low amounts.\textsuperscript{47} On the other hand, TNF-α is involved in a destruction and damage by producing active oxygen compounds and free radicals \textsuperscript{48} by inducing the expression of MMPs in the process of motility and invasion.\textsuperscript{49}

### Anti-apoptotic Activity

Some bacteria, including the normal microflora of the oral cavity, prevent the process of apoptosis, or programmed cell death, by affecting the cells. \textit{Porphyromonas gingivalis} and \textit{Fusobacterium nucleatum} bacteria are investigated in the following paragraphs.

1. \textit{Porphyromonas gingivalis}: This bacterium can activate Jak1/Akt/Stat3 anti-apoptotic signals, which control the spontaneous mitochondrial apoptotic activity.\textsuperscript{30,31} This bacterium affects the s phase of the cell cycle by reducing the expression level of the p53 TSG and activating cyclin/cyclin-dependent kinase, increasing the mitosis and tumorigenesis rates.\textsuperscript{34} \textit{P. gingivalis} inhibits the apoptosis of gingival epithelial cells through the adenosine triphosphate ligation of the purinergic receptor P2X7, which significantly affects cell growth, neovascularization, metastasis, and inflammatory cytokine secretion.\textsuperscript{35}

2. \textit{Fusobacterium nucleatum}: LPS and FadA of this bacterium bind to E-CADHERIN and activate the β-catenin signal, which begins the transcription of the \textit{Wnt} gene, activates oncogenes and pro-inflammatory cytokines, and stimulates the proliferation of cancer cells. This process plays a key role in the carcinogenesis process of this bacterium.\textsuperscript{52}

### Carcinogenic Products

Currently, little is known about bacteria-produced carcinogens, and the carcinogenic products of oral cavity bacteria identified up to now are ROS, reactive nitrogen species (RNS), volatile sulfur compounds (VSCs), and organic acids. In addition, several microorganisms contribute to cancer development by metabolizing alcohol to ALD.

#### Reactive Oxygen Species and Reactive Nitrogen Species

During inflammatory processes, TNF-α, IL-6, and TGF-β stimulate the production of ROS and RNS and reactive nitrogen intermediates (RNIs) by the epithelium and immune cells\textsuperscript{53,54}; these products react with nicotinamide adenine dinucleotide phosphate oxidase and nitric oxide...
synthase produced by bacteria such as Streptococcus oralis, S. mitis, S. sanguinis, S. gordonii. These results, along with others showing a connection between free radicals, chronic inflammation, carcinogenesis, and malignant processes, provide strong evidence for the function of bacteria in the etiology of oral cavity cancer.

**Volatile Sulfur Compounds**

Hydrogen sulfide (H2S), methyl mercaptan (CH3SH), dimethyl sulfide (CH32S), and dimethyl disulfide (CH3SSCH3) are all VSCs that are produced by certain bacteria in the oral cavity. These bacteria include Prevotella intermedia, P. gingivalis, Fusobacterium nucleatum, and Aggregatibacter actinomycetemcomitans. There is evidence that VSCs are toxic even in low concentrations and are associated with periodontitis and chronic inflammation. As a genotoxic, H2S can cause genomic instability and cumulative mutations. A high expression of enzymes that produce hydrogen sulfide has been observed in colon and ovarian cancers.

Increasing H2S concentration can enhance tumor growth, proliferation, migration, and the production of invasion pathway factors, as well as angiogenesis.

Some bacteria, including Lactobacillus, Lactococcus, Bifidobacterium, Streptococcus, Leuconostoc, and Pedicoccus, produce lactic acid during their metabolism. These acidogenic bacteria cause a decrease in the pH of the environment due to their production of lactic acid. Metastasis can be accelerated by acidic products that create an acidic and hypoxic microenvironment for tumors.

Alcohol is metabolized to the carcinogenic by-product ALD in the mouth by bacteria such as S. mitis, S. gordonii, S. salivarius, S. oralis, S. sanguinis, and Candida. Studies have shown that the ability of Neisseria bacteria to produce ALD is one hundred times higher than that of other bacteria in the oral cavity, making it a major risk factor for cancer.

**Discussion**

The study of the link between the body’s microflora and cancer is currently a hot topic in many scientific disciplines. The mouth is home to a diverse and abundant microflora. The mouth cavity is home to several species of bacteria and other microbes. However, oral cancer occurs frequently. Although researchers have found some evidence linking oral microbiota and oral malignancies, more study is required before drawing definitive conclusions.

Microflora can overgrow, and the species balance can shift if a person does not practice good oral hygiene. Clinicians have long noted a correlation among poor dental conditions, poor oral hygiene, and oral cancer, and this correlation may be independent of tobacco and alcohol use.

Confounding factors such as socioeconomic status, tobacco use, alcohol intake, nutrition, and others linked to cancer risk make it difficult to establish a direct causal relationship.

Some epidemiological research, however, has linked tooth loss to an increased risk of oral cancer. Additionally, more regular dental checkups and brushings were linked to a reduced risk.

Bone loss and tissue death surrounding teeth are symptoms of periodontitis, an infectious disease caused by the interplay of the host immune system with bacterial plaque. Gram-negative anaerobic bacteria are the primary cause of periodontitis. A certain group of these microorganisms, including F. nucleatum, P. gingivalis, and P. intermedia, have been linked to oral cancer.

Microorganisms linked to periodontitis degrade collagen, triggering inflammation and tissue damage. Fimbriae adhesins, LPSs, peptidoglycans, and lipoteichoic acids are all examples of virulence factors produced by periodontal bacteria that stimulate pro-inflammatory cytokine production.

Premalignant and neoplastic oral lesions that progress to oropharyngeal SCC have been linked to periodontitis in epidemiological studies.

The latest cancer research has uncovered novel insights into the molecular and cellular mechanisms behind carcinogenesis. Although no one marker has been identified as the primary marker, a group of markers can be recognized over time that anticipates the behavior and prognosis of the tumor and improves patient survival. Finding these early biological biomarkers can be helpful in the early diagnosis of oral cancer.

Although the oral cavity is simple to examine and cancer responds well to treatment, the majority of cases are detected at an advanced stage, when invasion and metastasis have already occurred, making treatment difficult or even impossible.

It is possible that doctors and dentists are not well-versed in spotting oral cancer in its earliest stages due to a lack of patient education.

No evidence suggests that commensal oral bacteria always have a protective function in oral dysplasia, according to a number of studies. However, oral care is necessary to prevent the growth of oral pathogens and reduce the risk of infection. Recent studies demonstrate that the oral microbiota is an important biomarker for oncological clinical outcomes. For the oral microbiome to attain the stage of being used as a tool in the oncological context, more studies are necessary.

As a result, it is essential to give oral cancer the serious and engaging treatment it deserves in educational events and retraining courses, as well as to provide the required training linked to the avoidance and identification of mouth cancer. In addition, social media can be used to educate the public about oral cancer and its risk factors.
References


