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Association of microbiota to oral squamous cell carcinoma: A short review

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Abstract:

Squamous cell carcinoma is commonly occurring cancer of the oral cavity and it is the major cause of morbidity and mortality. Apart from risk factors like tobacco habit, alcohol consumption and betel quid chewing, certain microorganisms are also implicated in the etiology of oral cancer. The oral microbiota contributes significantly in the human oral disease, systemic diseases and in health. The imbalances between microbes and their hosts mostly caused by bacteria leads to cancer. This review presents the literature on oral microbiota involved in causing oral squamous cell carcinoma. Articles were searched in databases such as PubMed, Google Scholar, Scopus, EBSCO, E-Journals and Science Direct until 2018 with the following search terms: "Association of oral microbiota with oral cancer Squamous cell carcinoma". Initially, 40 full text articles were identified and after taking into consideration of inclusion/exclusion criteria, 17 were excluded and finally, 23 articles were included in the review. The reports of 23 articles revealed that microbiota involving *Fusobacterium nucleatum, Porphyromonas gingivalis* were most commonly involved in oral cancers. As oral bacteria are found to be associated with OSCC, they can be certain biomarkers in the early diagnosis of carcinoma.

Key words:

Carcinogenesis, microbiome, oral bacteria, oral microbiota, oral squamous cell carcinoma,

Introduction:

Oral squamous cell carcinoma (OSCC) is the main cause of death compared to other oral cancers. This tumor develops from the oral mucosa with recognized 350,000 new diagnoses and 175,000 deaths across the world in 2018 (1). Risk factors such as chemicals, fibers, heavy metals & pesticides are causing OSCC by inducing prooncogenic genetic and epigenetic alterations (2-6).There are some other factors like oral injuries, inflammatory diseases, infections, and bacterial dysbiosis which are now considered as risk factors for cancer development (7-10).

Microorganisms present in the oral cavity can move to neighboring sites by spreading on contiguous epithelial surfaces. Oral cavity microorganisms are causing oral infectious diseases like dental caries, periodontitis, endodontic infections, alveolar osteitis (dry socket), and tonsillitis. Oral bacteria are also linked to cause a number of systemic diseases (11), including cardiovascular disease (12, 13), stroke (14), preterm birth (15), diabetes (16), and pneumonia (17).

Oral microbiota changes are able to cause disease by changing the link between oral bacteria and humans (18,19). Oral microbiota seems to regulate OSCC by the carcinogenetic modulation of cell metabolism and thus stimulating the formation of different cytokines associated in several pathological conditions (20–24). It is presented that the bacteria induce carcinogenesis by interfering with signal pathways and the cell cycle or by causing chronic inflammation. Cancer cells show

quick and unrestricted division, high metabolic rates and cellular morphology difference compared to normal cells. This abnormal regulation involves deficiency in cellular programs like demarcation, expansion, senescence and apoptosis. (25,26)

The present review is aimed at identifying specific microbiota association with OSCC. This review was achieved to know the association between oral microbiota and OSCC with the key question: "Does oral microbiota contribute to the development and progression of OSCC?"

Methods and Materials

Data sources and search strategy

Original research articles that focused on microbiota association to human OSCC were included in the study. Articles that have not assessed microbiota as an etiology for oral cancer, articles that were not original research and studies with insufficient data were excluded

Articles were searched in databases such as Google Scholar, PubMed, Scopus, EBSCO, E-Journals and Science Direct using keywords such as "Association of oral micriobiota to Oral squamous cell carcinoma". Articles which were published between 1998 to 2018 were included. The chosen articles references were also searched for the relevant information.

Study selection and data collection

The study was done in two stages. Firstly, the articles were examined and then the specific microbiota involved in each study was listed. In the second stage, different techniques used and results were presented.

Results

Search results

During searching, Initially 40 full-text articles were considered. After taking into consideration of inclusion/exclusion criteria, 17 were excluded and finally, 23 articles were included in the review. A total of 23 articles are listed in Table 1. 23 original research articles in which oral microbiota causing oral cancer were chosen for this review. After reviewing the articles, it was found that oral cancers are linked with altered microbial profiles. In the majority of searched articles it was found that, bacteria such as *Fusobacterium nucleatum, Porphyromonas gingivalis* in oral cancer exhibited shifts from normal health to cancer in terms of its abundance.

Discussion

The human body microbiome includes a wide variety of microorganisms like virus, bacteria, fungi & protozoa. The microbiome occupies in few parts of the host body and provides a niche for the commensal symbionts and pathobionts. Largest microbial population in the human body is present in the gastrointestinal tract, followed by the oral cavity. The emergence of innovative molecular techniques has helped in identifying nearly 700 microorganisms within the oral cavity. Currently, the concept of microorganisms has changed. They are now observed only as pathogens instead of partners of the healthy human body (27-29).

Many studies have found the interaction between microbiome and cancer. The microbiome influence carcinogenesis by various mechanisms which are not regulated by the immune system and inflammation. However, the link between the microbiome and cancer is through the immune system.

In the present review, a total of 23 original research articles reporting the association of microbiota to carcinogenesis were selected. By way of chronic infections and toxin production, most of the microorganisms interrupt the cell cycle and modify cell growth. Chronic infections result in the intracellular accretion of the pathogen which leads to the decline of apoptosis largely. (30,31) In this way, partially transformed cells are allowed to escape the self-destructive process and move to a further level of transformation, finally becoming carcinogenic. An alternative method is by the synthesis of substances that are carcinogenic by the bacteria. (32-34).

As Cancer sustains for longer duration, it is connected with changes in the body environment. So, it is understood that changes happening during cancer development will also influence the normal microbiome (35). In cancer, because of inflammation and oxidative stress, imbalance of normal flora occurs leading to the formation of nitric oxide synthetase (NOS2), reactive nitrogen species (RNS), and reactive oxygen species and an increase in cytokines such as interleukin-17 and tumor necrosis factor-alpha. As a consequence, various cellular responses occur leading to the formation of carcinogens. All these result in a shift from commensal microflora to the pathogenic state (30).

In the present review, it was identified that oral cancer is being associated with altered microbiota and the most commonly reported bacterial species were *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*

Common bacterial species and cancer development

In this review, it is found that *F. nucleatum* and *P. gingivalis* are the common bacteria in causing cancer. *P. gingivalis,* a bacterium which was isolated, plays an important role in cancer through cellular invasion. The bacterial infection activates pro-matrix metalloproteinase (MMP)-9 expression. MMP-9 disintegrates the basement membrane and extracellular matrix, promoting tumor cell migration and invasion. This makes the tumor cells to reach the lymphatic system and blood vessels leading to metastasis. In this way, *P. gingivalis* may lead to the development and progression of cancer (33-41).

F. nucleatum is another bacterium which was found to play a role in carcinogenesis. It may induce cell proliferation and migration by targeting signaling molecules such as kinases involved in cell cycle control, causing cell proliferation and migration to increase. Moreover, the bacterium secretes MMP-9 and MMP-13 (collagenase 3) by triggering p38. It also plays an important role in tumor invasion and metastasis.(42,43). It is very well established that *F. nucleatum* and *P. gingivalis* has a role in periodontitis. In an unpredicted manner, the significance of these bacteria in the cause and development of oral cancer is less understood. Chronic infection of oral cavity promote carcinogenesis (42).

Conclusion:

After reviewing the articles, it is concluded that oral microbiota has been found to be associated with carcinogenesis. The predominant bacterial species associated with oral cancer was found to be *F. nucleatum and P. gingivalis*. The results have shown the association between bacterial species and oral cancer.

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Author & year	Microbiota	Sample	Site	Methodology & validation	Conclusion
Table:1					
Lim et al. [2018] , (44)	*Corynebacterium, Paludibacter, Porphyromonas*	Oral rinse	Mouth	16S rRNA (V6–8) amplicon sequencing (Miseq)	data indicate that the oral microbiome is able to predict the presence of OSCC and OPC with sensitivity and specificity of 100 and 90%, respectively
Yang CY *et al* (45)	*F. periodonticum* increaseand decrease of S. mitis and *Porphyromonas. pasteri*	Oral rinse	mouth	16S rRNA Sequencing	Change of oral microbiota as the cancer progresses from stage 1 to stage 4. upregulated *F. periodonticum* and down-regulated *S. mitis* and *Porphyromonas. pasteri* forms a bacterial biomarker.
Wolf *et al.* [2017] (46)	Salivary microbiota	samples (collected at baseline)	Saliva	16SrRNAamplicon sequencing (454)	Changes were found in the salivary microbiome of oral and oropharyngeal SCC patients and healthy controls. These changes may be promising biomarkers for SCC tumorigenesis, disease detection and the effectiveness of potential therapeutic interventions
Shin et al. (47) [2017],	*Fusobacterium*	Tissue	Mouth	16S rRNA (V4) amplicon sequencing	an increase in *Fusobacterium* and *Parvimonas* associated with OSCC
Wang et al.(48) [2017] ,	*Parvimonas*	Tissue	Mouth	16S rRNA clone sequencing	Genus *Parvimonas* only associated with OSCC
	Fusobacterium nucleatum	Tissue	Mouth	16S rRNA (V4) amplicon sequencing	*Fusobacterium nucleatum* associated with OSCC
Al-Hebshi et al (2017) (49)	*Fusobacterium,Porphyromonas gingivalis*	Saliva	Mouth	16S rRNA (V4) amplicon sequencing	the dominant bacterial species belonging to one of five phyla: *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria* are associated with OSCC
Lee WH etal (2017) (50)	*Fusobacterium,Leptrotrichia,& Campylobacter*	Tissue	Mouth	16S rRNA (V1-V2) amplicon sequencing	*Fusobacterium,Leptrotrichia,& Campyloabacter are associated with OSCC*
Amer et al (2017)(51)					

Ghosh *et al.* [2014] (32)	Viable aerobic bacteria	Tissue	Lesional site	Histological grading, culture method	Viable aerobic bacteria were more abundant in the deeper tissues of OSCC than closer to the surface
Zaki *et al.* [2014] (41)	*Streptococcus mitis*	Saliva	Whole mouth	Culture & Gram staining, validated by the sugar fermentation test and the catalase test	Increase in the number of *Streptococcus mitis* in saliva of oral and digestive cancer patients act as an early diagnostic marker
Schimdt et al. (2014) (52)	*Fusobacterium*	Tissue	Mouth	16S rRNA (V4) amplicon sequencing	*Fusobacterium* associated with OSCC
Metgud *et al.* [2014] (38)	Aerobic and facultative anaerobic	Saliva	Mucosa, whole mouth	Culture method	Higher degree of total number of microbial colony forming unit (CFUs)/mL was found in carcinoma site and saliva
Cankovic *et al.* [2013](36)	*Streptococcus alpha-haemoliticus*	Saliva	Lesional site	Culture method	Presence of microbial flora on the irregular oral carcinoma surface contributes to chronic inflammation
Sonalika *et al.* [2012](53)	Aerobes, anaerobes, coliforms, candida and gram negative, anaerobic bacilli	Saliva	Whole mouth	Culture method	An appropriate antimicrobial protocol at the stage of diagnosis OSCC is mandatory
				PCR Immunohistochemical	*P. gingivalis* is abundantly present in malignant oral epithelium suggesting a potential

Joseph katz et al [2011] (54)	*Porphyromonas gingivalis*	tissue	Mouth	staining	association of the bacteria with gingival squamous cell carcinoma.
Pushalkar et al., 2011 (55)	*Porphyromonas gingivalis*	Saliva	Mouth	*16S rRNA* pyrosequencing	Relative abundance increased in OSCC samples compared to healthy samples
Kullander *et al.* [2009] (56)	*Staphylococcus aureus*	Tissue and swab	Lesional and normal area	Multiple displacement amplification and PCR	A strong association between *Staphylococcus aureus* and SCC was found which was found to be greater than HPV and SCC
Kang *et al.* [2009] (57)	Cariogenic bacteria, periodontopathic bacteria,	Saliva	Whole mouth	PCR	periodontopathic bacteria was significantly more prevalent in the oncological patients than in the healthy groups
Saini *et al.* [2009](58)	*Streptococcus viridians*, *Pseudomonas aeruginosa*, *Klebsiella*, *Candida albicans* & *Leptotrichia*	Saliva	Lesional site	Culture & gram staining method	Hundred percent reduction in the normal microbial flora in oral cancer was observed
Kurkivuori *et al.* [2007] (59)	Oral *Streptococci* group	Strains	Bacterial and clinical	Culture method, Fluorescence analysis & gas chromatography PCR	Oral streptococci play a pivotal role in fluctuation of salivary acetaldehyde levels after alcohol consumption and increases the risk of oral cancer development
Sasaki et al [2005] (60)	*Streptococcus anginosus*	Tissue	mouth		Authors concluded *S. anginosus* was associated with OSCC
Mager et al[2005](61)	*Capnocytophaga gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis*	Saliva	mouth	Checkerboard DNA-DNA hybridization	High salivary counts of *C. gingivalis*, *P. melaninogenica* and *S. mitis* may be diagnostic indicators of OSCC
Tateda et al [2000] (62)	*Streptococcus anginosus*	Tissue	Whole mouth	PCR	Authors concluded *S. sanginosus* was associated with OSCC It was concluded that human oral carcinoma surface biofilms harbour significantly increased numbers of aerobes and anaerobes as compared with the healthy mucosal surface of the same

Nagy et al. [1998] (63)	*Fusobacterium*	Culture method	patient	Tissue	Whole mouth