Variant identification in human periodontal ligament stem cells associated to proto-oncogenes: A review

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ABSTRACT
An oncogene is a gene that has the potential to cause cancer. Dental caries and periodontitis cause enormous health care costs and morbidity, but their genetic basis remains largely unknown. Clinical laboratories implement a variety of measures to classify somatic and sequence variants to identify clinically relevant variants to facilitate implementation of precision medicine. We developed a semi-automated tool called Variant Interpretation for Periodontal Cancer (VIC) to speed up the interpretation process and minimize individual risk biases. VIC loads pre-annotated files and automatically classifies sequence variants based on multiple criteria, with the ability for users to include additional evidence to streamline interpretation for clinical implications. Although the VIC cannot replace human examiners, it will speed up the process of interpreting variants. VIC can also be adapted by clinical laboratories into their analytical pipelines to ease the tedious process of variant interpretation.

Introduction
Oral cancer, primarily oral squamous cell carcinoma deriving from the oral mucosa, is a disease that arises from both host genetics and environmental factors; tobacco and alcohol consumption, betel quid chewing, and human papillomavirus infection are well-known risk factors (Wang et al., 2019). The incidence of oral cancer is increasing, and this disease continues to be a major global health problem. Furthermore, approximately 15% of oral cancer cases cannot be attributed to the aforementioned major risk factors, resulting in the need to explore other potential risk factors (Perera et al., 2016).

Periodontal disease is an inflammatory disease caused by microorganisms that colonize around the teeth (Loesche, 1996). Inflamed and damaged tissue, including the gingival epithelium and periodontal connective tissue, produce various inflammatory cytokines (Cekici et al., 2000). These inflammatory cytokines break down the homeostasis of periodontal tissues, activate fibroblasts and osteoclasts, and finally induce loss of connective tissue attachment and alveolar bone. A lot of studies support the concept of a synergistic microbial community acting as the modulator of systemic health. A disruption in the balance of symbiotic microbiota in the small intestine can lead to several diseases including diabetes, rheumatoid arthritis, and inflammatory bowel disease (Round and Mazmanian, 2009). Based on this theory, (Hajishengallis et al., 2015) proposed a new pathogenesis model of periodontal disease and established a novel ligature-induced periodontitis model in mice. This model enables the local longitudinal accumulation of anaerobic bacteria.

The human body is inhabited by over 10 trillion microbial cells living in symbiosis with their host. Bacteria at certain body sites have long been believed to...
be involved in immune modulation, disease development, and health maintenance. With the advent of high-throughput, next-generation sequencing (NGS), there has been a surge of interest in studying the human microbiome in the context of disease. In contrast to traditional views, recent analyses suggest the involvement of a consortium of microbes, rather than a single species, as causing disease, a phenomenon that has been well characterized for periodontal diseases.

Previous studies have proved that periodontitis is an independent risk factor of oral carcinoma epidemiologically. Along with the important role of microbiota in the cancer process and the specific functional position. Our study will be explored the microbiota and functions in periodontitis will be showing how variation of oral microbiota will be associated with human periodontal ligament stem cells link to proto-oncogenes and will be diagnostic biomarkers of human periodontal cancer shown in figure 1.

Fig 1. The oral microbiome as a reliable diagnostic tool has emerged as an important option in the early detection of periodontal treatment.

Review of literature

Normal human body function requires a healthy bacterial microbiota and balance between the human host and microbiome (Gilbert et al., 2014). The microbiome interacts with various human bodily processes. It can, for example, impact immunological responses, alter food intake, influence hunger, participate in vitamin production, protect people from external infections, and create certain anti-microbial chemicals (Wang et al., 2017). These consequences are thought to be beneficial, and life without microbes would be different - and maybe impossible (Gilbert et al., 2014). Disease may develop if the microbiota’s normal equilibrium is disrupted and some harmful bacteria become more common. The terms dysbiosis refers to aberrations that develop in otherwise balanced systems. However, as a result of internal and environmental stimuli, the microbiota is constantly changing. As a result, investigating and comprehending the intricate nature of microbiota-host interactions and disease triggers in the human body is difficult. Despite this, variations in microbiome composition have been linked to a variety of disorders. It has been proposed that changes in the composition of the bacterial microbiota may influence the emergence of inflammatory bowel disease, type 1 and type 2 diabetes, obesity, allergies, asthma, autism, and Alzheimer’s disease (Huttenhower et al., 2014). The connection between a person’s microbiota and the environment is fluid. Individually, there are usually no significant changes in microbiota composition over time (Ursell et al., 2012). Individual microbiota composition varies with age, gender, and diet in health and disease, as well as geographical variation. (Huttenhower et al., 2012 and Lloyd-Price et al., 2016).

The human microbiota begins to grow gradually before and after birth (Collado et al., 2016; Jiménez et al., 2008 and Tuominen et al., 2019). After a few months of life baby grows, microbial communities evolve and increase in microbial diversity in the oral cavity (Lif Holgerson et al., 2015). Bacterial dysbiosis in the adult oral cavity can result in gingivitis, periodontitis, dental caries (tooth decay), and endodontic abscesses. Nonetheless, due to the ongoing interaction between microbiota and the human host’s immune response, acute infections in the mouth cavity are rather uncommon, despite the extensive microbial colonisation (Zaura et al., 2014; Dewhirst et al., 2010; Kilian et al., 2016; Sender Kilian et al., 2016). Oral germs have also been linked to a variety of systemic disorders. Bacterial species present in the oral cavity have been associated to bacterial endocarditis, aspiration pneumonia, osteomyelitis, rheumatoid arthritis, and cardiovascular disease (Damgaard et al., 2017; Koziel et al., 2014; Aas et al., 2005 and Janket et al., 2003). Furthermore, periodontitis and poor dental hygiene have been linked to a higher risk of oral squamous cell cancer (OSCC) (Petersen et al., 2009). The frequency of OSCC is growing, however it has been discovered to be very diverse according to gender, behaviour (etiological variables), and region (Bray et al., 2018). While therapy has improved in recent years, an early diagnosis is still critical for a better prognosis (Mroueh et al., 2017). Unfortunately, individuals who have their initial main OSCC treated are at a greater risk of getting recurrent or subsequent primary OSCC (Vázquez-Mahía et al., 2012). This has been explained well by field cancerization, which is induced by long-term cigarette and/or excessive alcohol usage (Van Oijen et al., 2000). Another reason might be the nature of the oral cavity bacterial flora, which could have contributed to the tumorigenesis in the first place. Although OSCC seldom causes serious symptoms, it is easily identifiable by
physical inspection. As a result, it is critical that the oral cavity mucosa be checked on a regular basis by an experienced (dental) expert, especially in patients who exhibit dangerous behaviour (Kantola et al., 2001 and Rogers et al., 2011). OSCC is a fast-growing carcinoma that spreads quickly to the lymph nodes in the neck. (Abu-Ghanem et al., 2016; Ding et al., 2019 and Jerjes et al., 2010). One of most common clinical lesions is a symptom free nonhealing ulcer (Rogers et al., 2011). Pain is a symptom that is identified over longer time periods (Kantola et al., 2001 and Rogers et al., 2011). Tobacco and alcohol use are established risk factors for OSCC, accounting for 74% of the population-attributable risk (Petersen et al., 2009). It is believed that 15% of OSCC are of uncertain origin, such as bacterial microbiota involvement (Chocolatewala et al., 2010). We ruled out oropharynx-related disorders as well as other cancer entities such as salivary gland tumours, lymphatic tissue malignancies, and metastatic forms. In addition, the architecture of the oral cavity and the healthy oral bacterial flora are discussed. We also discuss several major oral disorders and syndromes that may precede the advancement of OSCC.

The Anatomy of the Oral Cavity

The mucosa of the lips forms the beginning of the oral cavity. The hard palate corresponds to the oral cavity, whereas the soft palate belongs to the pharynx. Similarly, the oral cavity includes the movable (oral) tongue, and the pharynx includes the base of the tongue behind the vallate and foliates papillae. When compared to other human body regions, the architecture of the mouth cavity is unusual. Hard tissue, or teeth that protrude through the mucosa and cover a large portion of the oral cavity, is a distinguishing trait. Teeth provide nonshedding mucosa and cover a large portion of the oral cavity. This one-of-a-kind structure can be found nowhere else in the human body. Babies are born toothless, and the first primary (deciduous) teeth normally appear around the age of six months. The shift to permanent teeth begins at the age of seven and lasts into the early twenties (wisdom teeth). The gingival sulcus is located between the teeth and the mucosal gingiva and is an essential anatomical location for the production of bacterial biofilms, i.e., plaque (Koliarakis et al., 2019 and Könönen et al., 2019). The production of saliva is an important part of oral cavity health. Saliva is generated by the main and minor salivary glands. The principal salivary gland openings are found on the floor of the mouth (i.e., sublingual caruncles) and in the buccal mucosa (the Stensen duct). Every day, around 1-2 L of saliva is generated and swallowed (Könönen et al., 2019).

Saliva is mostly consisting of water, electrolytes, mucus, antibacterial substances (such as IgA), and enzymes that aid in digestion and the killing of germs (Van’T Hof et al., 2014 and Marsh et al., 2016). Saliva function is vital for maintaining dental health. Without saliva, the prevalence of oral bacterial illnesses (such as dental caries, gingivitis, and periodontitis) soars (Van’T Hof et al., 2014 and Marsh et al., 2016).

The Healthy Oral Microbiota

According to the Human Oral Microbiome Database (HOMD), the most common phyla in the adult human oral cavity are Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria (Dewhirst et al., 2010; Huse et al., 2012 and Wade et al., 2013). Another research has also confirmed this (Aas et al., 2005 and Chattopadhyay et al., 2019). The HOMD reported 784 distinct bacterial taxa and 1,567 genomes discovered in the human oral microbiome taxonomic hierarchy in March 2020. (HOMD, www.homd.org). The bulk of them are members of the Firmicutes phylum (266 taxa and 588 genomes) and the Streptococcaceae family (38 taxa and 200 genomes). Oral bacteria have a total volume of roughly 1011 germs/mL (Dewhirst et al., 2010; Kilian et al., 2016 and Sender et al., 2016). Streptococcus is the most common genus in the oral cavity, followed by Haemophilus, Leptotrichia, Porphyromonas, Prevotella, Propionibacterium, Staphylococcus, Veillonella, and Treponema (Dewhirst et al., 2010; Aas et al., 2005; Wade et al., 2013, Chattopadhyay et al., 2019). Nevertheless, certain bacterial species are more sites specific, and others may thrive in numerous areas at the same time (Aas et al., 2005 and Huse et al., 2012). Oral microbiotas have the lowest β diversity but the largest diversity when compared to other body regions, and there are just a few differences in the makeup of oral cavity bacteria across different
individuals (Koliarakis et al., 2019 and Huse et al., 2012). As the mouth cavity is constantly exposed to external bacteria during eating, drinking, and breathing, it can be difficult to establish which species are indigenous and which are simply temporary (Dewhirst et al., 2010). Furthermore, oral microbiome differs by age, gender, and even educational level (Gao et al., 2018). However, once formed, oral microbes are relatively very persistent (Do et al., 2013). The HOMD also offers information on the bacterial makeup of the oral anatomical location. (Fig. 2) (Dewhirst et al., 2010 and Aas et al., 2005). Still, saliva allows oral germs to move easily from subgingival locations to other environments (Könönen et al., 2007).

As previously stated, the focus of this study is on bacterial microbiome and the known relationships between bacteria and cancer development. In addition, microorganisms other than bacteria can contribute to the development of cancer. Chronic oral fungal infections (mostly with Candida spp.), persistent high-risk human papillomavirus (HPV) infection, and Epstein-Barr virus (EBV) infection, for example, can all be implicated in the creation of oncogenic mutations in the oral cavity, leading to the development of OSCC (Kudo et al., 2016; Gholizadeh et al., 2016 and Syrjänen et al., 2005). High-risk HPV DNA has already been found in infants (Tuominen et al., 2018), however, it is unknown how early HPV oral positive affects health later in life. These many microorganisms may interact. We discovered changes in the makeup of bacterial microbiota in the oral cavity between oral-HPV-positive mothers and neonates and HPV-negative people (Tuominen et al., 2018).

Inflammation of the Oral Cavity and its association with local Microbiota

Oral health is first and foremost dependent on a proper balance of the diverse microbial populations that comprise the oral microbiota; when this balance is disrupted, a dysbiosis state is developed. Dysbiosis develops as a result of the disproportionate development of some types of pathogenic microbes, and it can be triggered by a variety of factors including antimicrobial drugs, ageing, hormonal changes, poor dental hygiene, smoking, or it may be promoted by other bodily illnesses. Dysbiosis can result in the development of a variety of oral disorders, including carious pathology, periodontal disease, and gingivitis (Gao et al., 2018). Inflammation is present in both periodontitis and gingivitis, although periodontitis is a more severe disease than gingivitis. The inflammation in the latter is restricted to the soft tissues around the teeth, whereas periodontitis is a chronic illness that causes the breakdown of tooth supporting structures and is regarded as the major cause of tooth loss in the adult population of industrialised countries. Periodontal disease is classified as a chronic inflammatory illness, and the bacteria that cause it can survive immune responses, especially if the condition is not treated. Inflammation is the body’s initial line of defence against infections. A proper inflammatory response ensures that the inflammation is resolved correctly, but when the inflammatory reactions are insufficient and chronic over time, substantial harm can occur both locally and systemically. Immune responses are complexly regulated as a result of interactions between different types of immunocompetent cells, which communicate with one another via cytokines, which have the role of controlling, inhibiting, or increasing inflammatory processes. Periodontitis invariably causes an increase in proinflammatory cytokines such as interleukin- (IL-) 1, IL-1, tumour necrosis α (Alpha factor) (TNF), and IL-6. IL-8 is a chemokine that aids in the recruitment of defence cells to places where their presence is required. IL-17 appears to have a role in the aetiology of periodontal disease as well. It’s a proinflammatory cytokine that promotes the development of other mediators like IL-6 and matrix metalloproteinases (MMPs). The inflammatory process is further aided by prostaglandin E2 (PGE2), which are vasoactive amines generated from arachidonic acid. They stimulate the formation of MMPs in fibroblasts and osteoclasts. Both humoral and cell-mediated immune responses are regulated by helper T cells.

Th2 cells, which generate IL-4, IL-5, IL-10, and IL-13, increase the humoral immune response. Th1 cells produce IL-2, interferon-gamma (IFN-γ), which boosts cell-mediated responses. The connection between inflammation and cancer is not novel. A large number of researches have recently addressed this problem (Song et al., 2020). Many cancers develop from sites of infection and inflammation as part of the normal host response. Indeed, there is a growing body of evidence suggesting many cancers are caused by infections; infections are responsible for up to 15% of all cancers globally, accounting for 1.2 million cases every year (Coussens et al., 2002 and Hoare et al., 2019). P53 mutations are found at rates comparable to those found in tumours associated with chronic inflammatory illnesses such as rheumatoid arthritis and inflammatory bowel disease (Coussens et al., 2002).

Undoubtedly, inflammation plays a significant part in carcinogenesis, and different oral bacteria have been demonstrated to stimulate inflammatory pathways linked with various phases of cellular change. Pathogenic bacteria can support the formation of malignant tumours, and the tumour microenvironment can specifically enhance the growth of bacteria (Zhang et al., 2019). The strong link between bacteria, chronic inflammation, and tumours has been established: the functionally...
proinflammatory bacteriome in the tumour’s body is linked to mouth cancer (Perera et al., 2018).

Infected gingival epithelial cells can activate antiapoptotic pathways such as JAK/STAT and phosphatidylinositol 3-kinase (PI3K)/Akt (GECs). GECs can internalise Porphyromonas gingivalis, a periodontitis-causing bacteria. Both routes have been linked to inflammation. Some cytokines, such as IL-6, TNF-α, and IFN-γ, work via the JAK/STAT pathway; moreover, the JAK/STAT pathway promotes NF-B and boosts TNF-production. In contrast, the PI3K/Akt pathway is implicated in the upregulation of Toll-Like receptor-4 (TLR4) mRNA in response to bacterial lipopolysaccharide (LPS). Ultimately, Akt phosphorylation and activation activates NF-B, which enhances the production of antiapoptotic genes (Hoare et al., 2019). Fusobacterium nucleatum and Porphyromonas gingivalis cause non-synergic virulence with a larger inflammatory response triggered by raised levels of TNF-α, NF-B, and interleukin IL-1, as well as higher levels of attachment and invasion into host cells (Hoare et al., 2019).

Interestingly MMPs, a family of zinc-dependent endopeptidases with over 20 distinct members, perform another intriguing function. Matrix metalloproteinases are essential for the breakdown of extracellular matrix and basement membrane. By promoting tissue breakdown, this feature promotes periodontal tissue degeneration as well as cancer growth and metastasis. (Nwizu et al., 2020). Several studies have concluded that individuals with chronic periodontitis had greater MMP-9 concentrations than controls. MMP-9 is a well-studied MMP that plays a role in cancer cell invasion, metastasis, angiogenesis, and endothelial-mesenchymal transition (EMT). It is also implicated in tumour microenvironment mediation and tumour-associated inflammation modulation via cytokines and their receptors. MMP-9 overexpression has been reported in a variety of malignant cancers (Huang et al., 2018; Silosi et al., 2015 and Parks et al., 2004).

The generation of free radical species plays a significant role in the relationship between bacteria-inflammation and cancerogenesis. Several studies have linked periodontitis to high levels of reactive oxygen species (ROS) or oxidative damage (Díaz et al., 2020). Leukocytes and other phagocytic cells can cause DNA damage or interfere with DNA repair systems by producing reactive oxygen and nitrogen species, which are typically generated by these cells to fight infection. These organisms are triggered by the creation of peroxynitrite, a mutagenic agent. The resulting compounds have a high attraction for additional inflammatory cells, prolonging the vicious cycle. Many proinflammatory cytokines (such as IFN-γ, IL1 and others) promote the stimulation of the inducible isoform of nitric oxide (NO) synthase (NOS), and hence may contribute to excessive NO generation. NO increases vascular permeability, which accelerates nutrient delivery to tumour tissue and so maintains the tumor’s fast development. This research suggests that pathogen-induced inflammatory responses might hasten mutagenesis and tissue damage (Coussens et al., 2002 and Maeda et al., 1998). Bacteria and host might interact according to the plaque ecological hypothesis (Marsh et al., 1994). The build-up of supragingival and subgingival biofilms causes inflammation, which promotes changes in physiological microbial composition. Through greater upregulation of virulence factor expression, such accumulation boosts the oral pathogen's competitiveness at the expense of oral health-associated species. As a result, a positive feedback loop is created. The majority of published studies on periodontitis and cancer show a favourable relationship. Inflammation plays an important role in this connection, but additional research is needed. Sustained cell proliferation in an environment rich in inflammatory cells, active stroma, and DNA-damage-promoting chemicals almost likely increases the risk of cancer. It is unknown if inflammatory mediators are necessary for tumour genesis and growth or whether they create a favourable environment for cancer advancement. Furthermore, a vast number of studies demonstrate that treating periodontitis significantly reduces inflammatory indicators, but more research is needed to determine how improved periodontal disease prevention and management measures may affect cancer risk (Coussens et al., 2002 and Nwizu et al., 2020).

Microbiota and Pathogenetic Mechanisms Underlying Oral Squamous Cell Carcinoma (OSCC)

Head and neck cancers account for 5% of all tumours, with half occurring primarily in the mouth cavity (Kademani et al., 2007). Mouth squamous cell carcinoma (OSCC) is a kind of head and neck squamous cell carcinoma that accounts for more than 90% of all oral cancers (Tandon et al., 2017). Despite breakthroughs in surgical methods, adjuvant radiation, and chemotherapy, the global prevalence of OSCC appears to be rising, and the 5-year overall survival rate remains poor, at around 50-60%. The biggest risk factors for oral cancer include smoking, drinking, and chewing betel (Lin et al., 2011). Other risk factors include viral infection, fungal infection, and chronic periodontitis; whereas other instances cannot be explained by any recognised risk factors (Sanjaya et al., 2011; Shaikh et al., 2015 and Hübbers et al., 2015). Oral carcinogenesis is also associated with bacteria (Khajuria et al., 2015 and Al-Hebshi et al., 2017). The
proper balance of commensal microorganisms and the host is required for physiological equilibrium, responsiveness to environmental changes, and survival. Host genetics, particularly polymorphisms in immune-related genes, as well as environmental variables such as lifestyle and diet, influence the makeup of the microbiota at distinct anatomical locations. The microbiota has crucial local effects such as barrier fortification and mucosal immunity establishment, but it also has systemic impacts such as regulation of metabolism, inflammation, and immunity. The makeup of the microbiota and the abundance of certain species at epithelial barrier surfaces influence inflammation and immunology, as well as the homeostasis of epithelial and stromal cells (Dzutsev et al., 2017). Human body surfaces are constantly subjected to environmental stress and harm. Infections, trauma, nutritional variables, and germline mutations can all lead to mucosal barrier breakdown. In most people, barrier breaches heal quickly and tissue homeostasis is re-established. Inadequate host or microbial resilience contributes to chronic barrier breach and inability to restore equilibrium. The microbiota may affect carcinogenesis (fig. 2) in these situations by modifying host cell proliferation and death, disrupting immune system function, and regulating metabolism inside a host (Garrett et al., 2015).

Fig 2. Different microbial agents classified as human carcinogens.

Burkitt lymphoma (BL), Hodgkin lymphoma (HL), post-transplant lymphoproliferative diseases (PTLD), diffuse large B cell lymphoma (DLBCL), NK/T cell lymphoma, nasopharyngeal carcinoma (NPC), and EBV-positive gastric cancer (EBV-GC) are all linked to the herpesvirus Epstein-Barr virus (EBV) (Yin et al., 2019). HBV and HCV are linked to hepatocellular carcinoma (HCC); they cause a persistent liver infection that leads to HCC in 80% of cases. The gut microbiota plays an important role in controlling liver disease and development to HCC in mice; curiously, young animals, like new-borns or young children, fail to clear HBV infection in a hydrodynamic transfection model until an adult-like gut microbiota is developed. (Dzutsev et al., 2017). Helicobacter pylori are closely linked to gastric cancer; however, this infection usually results in gastritis. HPV strains (most notably HPV16 and HPV18) have also been linked to anogenital malignancies, a subgroup of head and neck cancers, and skin cancers. Several investigations have revealed that oncogenic viruses require inflammation to cause carcinogenesis (Dzutsev et al., 2017 and de Martel et al., 2017). HPV and EBV have both been identified as oncoviruses that interact with the local microbiome to induce host inflammatory carcinogenesis in head and neck tumors (McKeon et al., 2022). Fusobacterium nucleatum is a gram-negative anaerobic bacterium that has been linked to periodontitis, preterm birth, inflammatory bowel disease, and colon cancer. Several researches on the relationship between Fusobacterium and colic carcinogenesis have found that this bacteria impacts the formation of a pro-inflammatory tumour environment. Fusobacterium nucleatum may have a causal role in cancer genesis, illness progression, and treatment resistance, according to mounting data (Zhao et al., 2022). All of these associations indicate the significance of inflammation and bacteria in carcinogenesis. Microbial proliferation, bacterial toxins, β-catenin signalling changes, and inflammation are the methods through which microbes affect cancer formation and progression. Bacterial toxins can cause direct damage to host DNA. Bacteria also cause indirect DNA damage via reactive oxygen and nitrogen species generated by the host. Cell death or cancer-enabling mutations arise when DNA damage surpasses the host cell's repair capabilities. Changes in β-catenin signalling are a common target of cancer-associated microorganisms. β-catenin signalling activates genes that regulate cell survival and proliferation. Fusobacterium nucleatum, for example, is a part of the oral microbiota and has been linked to human colorectal cancer. The binding of the Fusobacterium nucleatum FadA adhesin to E-cadherin promotes -catenin signalling, resulting in the activation of genes that govern cell survival and proliferation. When a tumour's mucosal barrier is breached, proinflammatory pathways are activated. The loss of host-microbe barriers activates pattern recognition receptors and associated signalling.
cascades. Chronic inflammatory feedforward loops mediated by NF-B and STAT3 signalling drive tumour carcinogenesis in both transforming and non-neoplastic cells (Garrett et al., 2015). It is critical to understand that the concentration of a microorganism at a tumour location does not imply that the germ is directly related to the illness. In the tumour microenvironment, certain bacteria can find favourable circumstances for survival. Lifestyle risk factors cause oral dysbiosis, which in turn influences the inflammatory and genotoxic processes (Healy et al., 2019). External pressures cause dysbiosis by disrupting the balanced equilibrium of the bacterial ecosystems in the human microbiome; in this regard, tobacco smoking and chewing, psychological stressors, and diet all have an impact on the oral microbiome and the onset and progression of periodontal diseases (Buduneli et al., 2021). In this vein, lack of oral hygiene, alcohol intake, betel quid usage, and hereditary variables all have an impact on the link between periodontal-pathogenic bacteria and oral cancer (Hsiao et al., 2018). When compared to non-smokers, smokers' oral microbiota included significantly more Veillonella dispar, Leptotrichia spp., and Prevotella pleuritidis. Heavy smokers reported higher levels of Fusobacterium nucleatum, which has significant sequence similarities with Fusobacterium nucleatum and Prevotella bivia (Al Bataineh et al., 2020). Smoking may have an impact on oral health by affecting the linkages between oral bacteria and the microbiota and their metabolic function. Smoker-enriched bacteria can raise the acidity of the oral cavity, boosting the release of amino acid-related enzymes as well as the metabolism of amino sugars and nucleotide sugars (Jia et al., 2021). Overall, lifestyle risk factors disrupt the holobiont's tuned homeostasis, causing periodontal disease and dysbiosis in the oral cavity, facilitating the initiation and advancement of oral cancer. Once these and other variables have resulted in the formation of a tumour environment, the inflammation and hypoxic niche modify the oral microbiota, resulting in a vicious cycle. Without taking into consideration environmental variables such as cigarettes and alcohol, changes in the balance of bacteria and human hosts increase the risk of oral cancer. Understanding changes in the oral microbial flora can aid in the development of antimicrobial medicines for the prevention of OSCC.

**Bacterial Communities Associated with OSCC**

Studies have shown that compared to healthy subjects, periodontitis-correlated taxa were significantly increased in the microbiota of the OSCC (Zhao et al., 2017), including Porphyromonas gingivalis, Fusobacterium nucleatum, Pseudomonas aeruginosa (Al-Hebshi et al., 2017), Fusobacterium periodonticum, Aggregatibacter segnis, Campylobacter rectus (Cruz et al., 2015), Campylobacter showae (Lugonja et al., 2016), Peptostreptococcus stomatis (Zhang et al., 2019 and Pushalkar et al., 2012), Peptostreptococcus micros, and Catonella morbi (Zhang et al., 2019), while Streptococcus, Veillonella, and Rothia were significantly decreased in cancer tissue (Hooper et al., 2006). Loss of dental elements and the presence of disease have been associated with reduced richness and low diversity in the microbiome (Gazdeck et al., 2019). Most of the pathogenic periodontal bacteria are obligate or facultative anaerobes, and their abundance changed significantly in the hypoxic tumour microenvironment. In addition, the microbiota within the tumorous mucosa was saccharolytic and aciduric species. Host proteins may also be metabolized or fermented into sulfides and nitrosamines by Firmicutes and Bacteroides, potentiating cell mutations (Pang et al., 2018). The microbiota composition appeared different depending on the type of sampling and depending on the stage of OSCC. Within the OSCC tissue, Solobacterium moorei, hydrogen sulfide producer Fusobacterium naviforme, and Neisseria flavescens were significantly increased (Haraszthy et al., 2008). These bacteria can promote invasion across the basement membrane in OSCC; this is possible because (1) the volatile sulphur compounds produced can increase ROS release by inhibiting the enzyme superoxide dismutase and (2) methyl mercaptan promotes degradation of type 4 collagen (Yaegaki et al., 2008). Fusobacterium periodonticum could potentially cooperate with Fusobacterium nucleatum in tumour progression within the tissue (Zhao et al., 2017 and Al-Hebshi et al., 2015). Fusobacterium nucleatum has the ability to coagulate with a diverse range of bacteria. In an aerobic environment it can coagulate with Porphyromonas gingivalis in cultures containing Actinomyces oris and Veillonella sp. In addition, Fusobacterium nucleatum helps generate a reducing and capnophilic environment that is necessary for the growth of Porphyromonas gingivalis (Diaz et al., 2002). The interaction between bacteria may or may not be favourable for the proliferation and survival of the species. For example, interaction of Porphyromonas gingivalis with Streptococcus gordonii may not be favourable because Porphyromonas gingivalis upregulates a series of genes involved in reduction of adhesion and signalling, and thus decrease its ability to form a biofilm (Simionato et al., 2006). There is also a negative correlation between the Porphyromonas gingivalis and S. oralis, S. crista tus, S. intermedius, or S. mutans because Porphyromonas gingivalis can reduce the production of extracellular arginine deiminase proteins by Streptococcus cristatus and Staphylococcus intermedius (Wang et al., 2009 and...
Christopher et al., 2010). According to the “key pathogen” hypothesis (Hajishengallis et al., 2012), low-abundance oral bacteria may interact through multiple interspecies pathways, such as heterotactic fermentation, which may influence the growth of some bacteria and lead to dysbiosis of the oral microbiota. Dysbiosis of the microbiota promotes the development of OSCC. Bacteria contribute to the maintenance of homeostasis of the oral microenvironment. There was a different composition of the microbiota of the tissue biopsy samples depending on the location of the tumor, for example Capnocytophaga gingivalis, Rothia mucilaginosa, and P. intermedia were significantly enriched in the lining mucosa, tongue, and gingiva, respectively (Yang et al., 2021). These bacteria can secrete peptidases in tumour sites that are activated through proteinase-activated receptors (PARs) (Van Spaendonk et al., 2017 and Eftekhar et al., 2018). In this way, these protease producing bacteria can degrade host tissue like extracellular matrix (ECM), destruct host physical barriers, and modulate host immune response, finally contributing to the onset and progression of tumors (Alfano et al., 2016). Microbiome analysis of tumour tissues versus normal buccal mucosa of OSCC patients using the 16S rDNA sequencing revealed an increase of genes related to cell motility in tumour sites, such as bacterial chemotaxis and flagellar assembly, genes associated with proinflammatory bacterial component, such as lipopolysaccharide biosynthesis, and genes involved in metabolism of cofactors and vitamins (Zhang et al., 2019). Inter-sample variation of OSCC oral microbiome was significantly associated with site of sampling, i.e., tumour site or buccal site far distant from the tumour site, in addition to the increase and decrease of oral bacterial species, some low-abundance oral bacteria might contribute to development of OSCC. A lower abundance of Streptococcus genera was observed in patients with OSCC, associated with an oral health condition. There were significantly higher abundance of Streptococcus infantis in smokeless tobacco non-consumers compared to that in smokeless tobacco consumers and contralateral buccal site of OSCC samples compared to that in the OSCC tumour site (Saxena et al., 2022). Streptococcus sanguinis, associated with periodontal health (Griffen et al., 2012), could reduce the colonization of soft tissue surfaces by A. actinomycetemcomitans (Sliepen et al., 2009). However, it is not clear whether Streptococcus genera actually promotes oral health or survives exclusively in healthy microenvironments (Hayes et al., 2018). Streptococcus sanguinis, despite promoting the adhesion of Fusobacterium nucleatum, reduces its production of H2O2 and its killing effect (He et al., 2012). Other members of the oral microbiome that have been associated with a reduced risk of OSCC development included Corynebacterium, Kingella, Leptotrichia, Neisseria, Parvimonas micra, and Haemophilus parainfluenza. Their presence was suggested to be cancer protective (Schmidt et al., 2014). However, in cancer, development metabolism and the functionally specialized role of the bacterial community are more relevant than composition (Lamont et al., 2022). While Capnocytophaga gingivalis, Prevotella melanogenica, Streptococcus mitis, Fusobacterium periodonticum, Prevotella tannerae, and Prevotella intermedia were significantly enriched in unstimulated saliva samples of OSCC patients (Hsiao et al., 2018; Yang et al., 2021 and Pushalkar et al., 2011). Capnocytophaga gingivalis, Prevotella melanogenica, and Streptococcus mitis are significantly elevated in the saliva of patients with OSCC with a diagnostic sensitivity and specificity of 80% and 82%, respectively (Mager et al., 2005). Neisseria species, which are numerous in saliva samples, could play an important role in alcohol-related carcinogenesis because they produce acetaldehyde (Muto et al., 2000).

The bacteriome of saliva from patients with OSCC differed significantly from tumour tissue microbiota in terms of community structure, however, remained similar at taxonomic and metabolic levels except for elevated abundances of Streptococcus, Lactobacillus, and Bacteroides, and acetoin-biosynthesis, respectively (Gopinath et al., 2021). Overabundance of Porphyromonas gingivalis in saliva was associated with advanced pathologic staging but lower recurrence rate of OSCC (Chen et al., 2021). The origin of Porphyromonas gingivalis in OSCC tissue might be from the salivary microbial reservoir. Increased abundance of Fusobacteria species in oral tongue samples of OSCC patients was associated with significantly increased programmed death-ligand 1 (PD-L1) expression and—along with reduced abundance of Rothia and Streptococcus species—with lower alpha diversity (Michikawa et al., 2021).

Multiple studies have found that the microbiota predicts disease severity and treatment result in malignancies such as lung, pancreatic, colorectal, oral, breast, prostate, and liver cancers. Several research published in the last five years have found a link between Fusobacterium nucleatum and clinical outcomes in human malignancies. Differences in the flora of various intestinal segments, for example, in the colon, may result in a varied prognosis for colorectal cancer. Fusobacterium nucleatum is one of the possible infections connected to a poor prognosis, while high Fusobacterium nucleatum levels have been linked to a better stage. (Pignatelli et al., 2021 and Mima et al., 2016). Other studies found that adjuvant chemotherapy was more successful in patients with low Fusobacterium nucleatum levels than in those with
greater *Fusobacterium nucleatum* levels in terms of survival outcomes (disease-free survival and/or overall survival-OS-time) (Flanagan et al., 2014 and Yan et al., 2017). Intriguingly, since the prognosis of colon cancers differs depending on the tumour position (left vs. right) and various bacteria are present throughout the gut, this suggests that the bacteria impact the prognosis (Petrelli et al., 2017 and Mima et al., 2016). In the instance of esophageal cancer, *Fusobacterium nucleatum*-positive patients had considerably lower cancer-specific survival and OS, as well as greater cancer-specific death, as compared to *Fusobacterium nucleatum*-negative cases. They also discovered an increase in the CCL20 chemokine and suggested that *Fusobacterium nucleatum* contributes to the development of aggressive tumour behaviour via activating chemokines. *Streptococcus anginosus*, in particular, has been linked to esophageal cancer rather than mouth cancer (Yamamura et al., 2016 and Morita et al., 2003). The microbiome has recently been shown to have an impact on breast tissue. Dysbiosis may contribute to the development of breast cancer via pathways related to immune modulation and the establishment of a tumour microenvironment, but most notably to oestrogen metabolism and the estrobolome (Jarman et al., 2020), a phrase referring to a group of bacteria capable of regulating oestrogen circulation in the enterohepatic tract. The oestrogen can then be deconjugated by the enzyme -glucuronidase, which is released by gut flora. This active, unbound oestrogen is reabsorbed into the circulation and binds to oestrogen receptors, causing a variety of physiological reactions that affect reproductive and cardiovascular health. Variations or changes in the microbiota result in increased circulating oestrogen levels and an increased risk of breast cancer (Shapira et al., 2013). The gut microbiome of breast cancer patients differs from that of healthy persons, and certain species contain members capable of causing double-stranded DNA breaks in breast cancer cells. (Chen et al., 2019 and Eslami-S et al., 2020). Furthermore, a link has been shown between microbiome makeup and cancer grade, implying a link between the microbiome and breast cancer development and progression. Finally, balancing a dysbiotic microbiota with antibiotic medication, which often raises the risk of breast cancer, can be beneficial before or after a cancer diagnosis (Bard et al., 2015 and Jarman et al., 2020). Different bacteria, including *Fusobacterium nucleatum* and *periodonticum*, *Streptococcus salivarius*, *Porphyromonas*, and various *Lactobacillus* subspecies, have been linked to the diagnosis of HNSCC. Because they start in and *Neisseria meningitidis*, the periodontal pathogens *Fusobacterium nucleatum* (subspecies polymorphum), *Campylobacter subspesies*, *P. aeruginosa*, and *Porphyromonas* are called "mobile microbiomes." Furthermore, altering the pH microenvironment, as well as using probiotic bacteria like *Streptococcus dentisani* or *Streptococcus A12* and particular antimicrobial peptides, are potential options for combating oral cancer as a multifactorial illness. (Chattopadhyay et al., 2019). Oral antibiotics should be studied more closely since they may affect the gut microbiota, boosting immunological dysbiosis and decreasing the quantity of probiotic bacteria, favouring the development of OSCC (Wei et al., 2022)

**Limitations and Perspectives**

The challenge of comparing research with varied techniques is the review's limitation. Indeed, the choice of analytic technique and sample type has a significant impact on the results and prevents us from doing a quantitative study. To evaluate the etiopathogenic connections between microbiota and OSCC, multi-centre longitudinal association studies using third-generation sequencing technology are required. As a result, we recommend more study in this emerging field, which might lead to the identification of useful prognostic targets and possibly therapeutics for OSCC patients. Understanding the interplay of bacteria in the oral biofilm requires the use of metagenomics and transcriptomics.

**Periodontal Disease**

Periodontitis is classified as an advanced inflammatory gingival disease caused by bacterial dysbiosis and eventually it can lead to tooth loss (Hajishengallis et al., 2014 and Michaud et al., 2018). It starts as gingival bleeding in response to inflammation to bacterial biofilm accumulation (plaque) around the tooth marginal gingival surfaces (Michaud et al., 2017). Periodontitis develops over years when dental plaque continues to accumulate, leading to the formation of periodontal pockets and tissue destruction, and it is therefore classified as a chronic infection (Michaud et al., 2017). If this chronic disease is left untreated, it maintains low-level inflammation and increases blood CRP levels (Paraskevas et al., 2008 and Ebersole et al., 1997). Periodontitis is a relatively common oral disease; it is estimated that the majority of adults (50–70%) present some clinical symptoms of periodontal disease (Eke et al., 2012). Known periodontal pathogens, such as *Tannerella forsythia, P. gingivalis*, and *Treponema denticola*, are not usually detected in oral cavities of healthy humans. The literature in fact presents a unique set of so-called periodontal pathogens. This unit consists of *Aggregatibacter actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, Prevotella intermedia, P. nigrescens, Parvimonas micra, Campylobacter rectus, and Fusobacterium nucleatum* (Wang et al., 2016 and Carrillo et al., 2010). But, as these
species sometimes can be detected in healthy individuals it has become obvious that the dysbiosis and the relative abundance of pathological species is the main trigger of disease onset (Abusleme et al., 2013; Diaz et al., 2016 and Hajishengallis et al., 2011). Furthermore, multiple bacteria, rather than a single bacterium, work synergistically. Patients suffering from periodontitis have a 2–5 times higher risk of acquiring any cancer compared to healthy controls, even among patients who have never smoked (Michaud et al., 2018 and Corbella et al., 2018). The correlation to OSCC in particular seems to be consistent (Michaud et al., 2017 and Fitzpatrick et al., 2010). Thus, an increased number of missing teeth, as a sign of periodontitis, has been linked to a higher OSCC prevalence (Bundgaard et al., 1995). The elevated cancer risk of periodontitis patients is thought to be associated with differences in the oral bacterial microbiota composition. These periodontopathogenic bacteria (especially *P. gingivalis, P. intermedia, and F. nucleatum*) enable and maintain constant chronic infection and the systemic inflammation response (Li et al., 2000). The bacterial impacts are mainly indirect changes observed based on increased levels of leukocytes and cytokines after the initial inflammatory response (Hoare et al., 2019 and Herrero et al., 2018). Some periodontal pathogens can also directly affect specific intracellular pathways, promote cell survival, activate oncogenic pathways, reduce proapoptotic protein expression, and increase cell migration and invasion, in addition to enhancing metastasis (Hoare et al., 2019). *P. gingivalis* and *F. nucleatum* can additionally activate cell transformation (Yilmaz et al., 2004). Other periodontal pathogens, i.e., *A. actinomycetemcomitans, T. forsythia, and T. denticola*, can produce virulence factors that induce the release of proinflammatory cytokines (Chattopadhyay et al., 2019).

All these changes in chronic periodontal disease maintain the chronic inflammation process and destruction of periodontal tissue. These alterations at the cellular level can furthermore induce permanent genetic alterations in epithelial cells (Chattopadhyay et al., 2019). After several years this continuous exposure to cell metabolites can trigger abnormal cell divisions and eventually even carcinoma development (Michaud et al., 2018).

**Mucosal Lesions**

The World Health Organization has classified a number of oral lesions as potentially malignant (precursor) lesions for OSCC (Warnakulasuriya et al., 2007). Lichen planus, leukoplakia, and erythroplakia are the most often encountered clinical manifestations in the human oral cavity, and these patients that are known to have a somewhat higher risk of malignant transformation compared to their healthy counterparts (Chattopadhyay et al., 2019). Oral Lichen Planus Oral (OLP) is a chronic inflammatory mucosal disease of unknown origin (Reichart et al., 2016). Some 0.1–4.0% of the population is estimated to have OLP lesions (females more than males), and most of the lesions are asymptomatic (Lodi et al., 2005 and Mortazavi et al., 2014). OLP is known to be potentially malignant, and around 1.0–3.0% of the lesions progress to OSCC (Zhang et al., 2000). It is important to regularly check OLP patients in order to detect suspicious changes early. Some OLP patients do not require any treatment, while others benefit from local corticosteroid treatment. Occasionally, some patients experience symptom relief and diminished clinical lesions with chlorhexidine mouthwash alone, which suggests a bacterial contribution to the disease (Stashenko et al., 2019). As a matter of fact, oral bacterial dysbiosis has been detected in OLP patients (Wang et al., 2016 and Klimesova et al., 2018). Higher levels of *Porphyromonas* and *Solobacterium* and *P. melaninogena* have been observed in OLP patients, with a significantly lower abundance of *Haemophilus, Corynebacterium, Cellulosimicrobium, and Campylobacter* compared to the healthy control group (Wang et al., 2016). Furthermore, another study detected more abundant *Fusobacterium, Leptotrichia, and Lautotropia* in OLP lesions, while *Streptococcus* was detected more in healthy patients (Klimesova et al., 2018). *Porphyromonas* has been correlated with the severity of OLP (Wang et al., 2016). Nevertheless, the possible causative role of bacteria in OLP and/or malignant transformation has not yet been elucidated (Flemer et al., 2018).

**Leukoplakia and Erythroplasia**

Leukoplakia is defined as a whitish lesion on the oral mucosa that is not related to any other specific disease and is mainly asymptomatic (Mortazavi et al., 2014 and Fan et al., 2018). Erythroplakia, on the other hand, is a similar red lesion of the oral mucosa (Mortazavi et al., 2014). Both leukoplakia and erythroplakia are clinical terms and the diagnosis must be confirmed by biopsy and histopathological analysis. The prevalence of leukoplakia is around 1.0–20.0% and that of erythroplakia is 0.01–0.2% (Mortazavi et al., 2014; Mascitti et al., 2019 and Ahn et al., 2012). OSCC development is observed in 15.6–39.2% of cases with leukoplakias, while the rate is 51.0% in erythroplakias (Mortazavi et al., 2014). Leukoplakia has been detected to harbor more *Haemophilus, Leptotrichia, Campylobacter, Rothia mucilaginosa*, and *Fusobacteria*, with lower levels of *Firmicutes* (Sheu et al., 2008 and Kato et al., 2016). There is various literature is currently available describing the potential bacterial microbiota changes observed in
Tobacco and Ethanol
Cigarette smoke contains hundreds of toxic chemicals. Regular smoking is known to increase individuals’ risk of OSCC and other cancers, as well as chronic obstructive pulmonary disease, cardiovascular disease, and periodontitis (Yang et al., 2019 and Michaud et al., 2007). In addition, smoking directly affects oral mucosal sites and therefore also the oral bacterial composition. Smoking has been detected to reduce bacterial diversity (α diversity), especially in buccal mucosa and by changing the bacterial composition favoring R. mucilaginosa, Streptococcus salivarius, and S. mitis (Michaud et al., 2007 and Farrell et al., 2012). Furthermore, higher levels of Prevotella, Veillonella, and Leptotrichia have been observed in current smokers (Torres et al., 2015). On the other hand, lower levels of F. nucleatum and Leptotrichia have been detected in patients who smoke and have OLP (Sheu et al., 2008). Nonetheless, the levels of S. mutans and Lactobacillus have been observed to be unchanged even with regular smoking (Ahn et al., 2012). Elevated levels of R. mucilaginosa, Veillonella, Streptococcus, and Leptotrichia have been connected to OSCC independently, without the presence of smoking (Healy et al., 2019).

Ethanol is not carcinogenic on its own, but its metabolites acetaldehyde, hydroxyl ethyl radicals, and hydroxyl radicals are (Hayes et al., 2018). Acetaldehyde has the potential to cause chromatic changes and point mutations to DNA and hyperproliferation of the epithelium (Mascitti et al., 2019 and Multhoff et al., 2012). Some known oral pathogens, such as R. mucilaginosa, Neisseria spp., and S. mitis, have been detected to be able to transform ethanol to acetaldehyde (Grivennikov et al., 2010). Also the endogenous production of acetaldehyde by oral bacteria is higher with poor oral hygiene (Katz et al., 2011).

Oral Microbiota and OSCC
The decrease in Firmicutes and increased levels of Fusobacteria has been linked to OSCC (Peters et al., 2017 and Healy et al., 2019). Significantly higher levels of Peptostreptococcus, Fusobacterium, Prevotella (especially P. melaninogenica), Porphyromonas, Veillonella (mainly Veillonella parvula), Haemophilus, Rothia, and Streptococcus have been detected in OSCC samples (Narikiyo et al., 2004). OSCC can be divided into different disease stages by the TNM (tumor, node, and metastasis) classification (Cummins et al., 2013). These TNM stages of OSCC have been observed in significantly different oral bacterial microbiota compositions. Yang et al. (Healy et al., 2019) detected those levels of Streptococcus, Haemophilus, Porphyromonas, and Actinomyces decreased in carcinoma progression while F. periodonticum, P. micra, S. constellatus, Haemophilus influenza, and Filifactor alocis were associated with OSCC and their levels increased along with disease severity with the TNM classification. Furthermore, the most severe OSCC at stage 4 represented significantly more complex microbiota than those at lower stages. Differences have also been detected between precursor lesions and OSCC (Torres et al., 2015 and Healy et al., 2019). Severe dysplasia, before the onset of OSCC, has been associated with elevated levels of Leptotrichia spp. and C. concisus (Sheu et al., 2008) Zhang et al. (Gaonkar et al., 2018) suggested 3 possible mechanisms behind these changes. Bacteria might influence tumorigenesis by: (1) stimulating chronic inflammation, (2) acting as an antiapoptotic agent, or (3) producing carcinogenic substances (Gaonkar et al., 2018). Strong evidence of two potentially carcinogenic oral bacteria has accumulated in recent years with in vitro and in animal models. P. gingivalis and F. nucleatum have both been shown to be able to induce the production of inflammatory cytokines, as well as cell proliferation and cellular invasion, in OSCC with various different mechanisms (Cisek et al., 2017). P. gingivalis was responsible for induction of the production of interleukins, tumor necrosis factor (TNF)-α, and matrix metalloproteinases (MMP) and for inhibition of apoptosis (Xu et al., 2020). It also prevented the activity of the p53 tumor suppressor gene (Chattopadhyay et al., 2019 and Xu et al., 2020). Continued exposure to P. gingivalis has also been demonstrated to increase the invasiveness of OSCC (Xu et al., 2020). F. nucleatum, on the other hand, was responsible for the promotion of cell proliferation and for the increase in the production of interleukins and other MMP that drive tumor invasion and metastasis (Chattopadhyay et al., 2019 and Xu et al., 2020). All of these changes resulted in an elevated transcriptionsal activity of oncogenes and proinflammatory cytokines (Xu et al., 2020). Both of these bacteria, i.e., P. gingivalis and F. nucleatum, were able to release endotoxins, such as lipopolysaccharides, which in turn can activate inflammation-associated cytokine production. Inflammation-associated cytokine production is the major factor in bacteria-induced inflammation and a contributor to carcinogenesis (Lodi et al., 2005 and Starzyńska et al., 2014). The previous studies are observational studies or in vitro findings. However, there is also evidence of a bacterial contribution to tumorigenesis with an animal model. Stashenko et al. (Bäckman et al., 2007) colonized germ-free mice with different oral microbiomes and exposed them to a 4-NQO carcinogenic agent. Mice with
oral microbiome and 4-NQO had more and larger OSCC compared to controls with only 4-NQO treatment.

**Oral Microbiota and Cancers of Other Body Sites**
Several specific oral pathogenic bacteria have been linked to cancers of other body sites, in addition to OSCC development. Since the oral cavity is the starting point of the digestive system, pathogenic bacteria may, e.g., disseminate via saliva from the oral cavity and have an impact on distant organs. It has been estimated that, on a daily basis, 1011 oral bacteria make their way through the digestive system (He et al., 2017). Oral dysbiosis have been connected mainly to patients with tumors of the gastrointestinal tract and esophageal, gastric, pancreatic, and colorectal cancers (Yanik et al., 2015). As previously discussed, bacteria, such as the periodontal pathogens P. gingivalis and F. nucleatum, have several different mechanisms via which they can interfere with cell signaling and cause tumorigenesis (Cisek et al., 2017 and Amer et al., 2017). These specific bacteria, in addition to Rothia, T. denticola, and P. intermedia, have been detected in colorectal cancer specimens where they are thought to cause disruption in the intestinal microbiota, ultimately causing dysbiosis (Stämpfli et al., 2009). Colorectal cancer has also been connected to Streptococcus and Prevotella, both oral bacterial In addition, a higher prevalence of the periodontopathogens P. gingivalis, Porphyromonas, S. mitis, and A. actinomycetemcomitans in the oral cavity has been found to increase the risk of pancreatic cancer (Bewley et al., 2017). Periodontal disease itself, in general, elevates the risk of acquiring pancreatic cancer (Grine et al., 2019). On the other hand, Fusobacteria showed protective association (Bewley et al., 2017). Inconsistent results have been detected with Leptotrichia according to different studies in pancreatic cancer patients’ oral cavity (Bewley et al., 2017 and Lee et al., 2017). Furthermore, higher levels of circulating P. gingivalis antibodies have been linked to a higher risk of pancreatic cancer (Johnson et al., 2000 and Pushalkar et al., 2011). Peters et al. (Schmidt et al., 2014) found the oral periodontal pathogens T. forsythia and P. gingivalis to be more abundant in esophageal cancer. In addition, levels of T. denticola, S. mitis, and S. anginosus have also been detected to be increased in esophageal cancer patients (Nagy et al., 1998).

**Oral squamous cell carcinoma**
Oral cancers rank eleventh among the common malignancies globally. 40% affected are in developing regions such as South-east Asia. Ninety percent of all oral cancers are squamous cell carcinoma originating from the mucosal epithelium. If detected during its early stages, the 5-year survival rate of oral cancer is 60-80% . The etiology of Oral Squamous Cell Carcinoma (OSCC) is multifactorial and a combination of environmental risk factors and genetic predisposition. The risk factors can be grouped as established, strongly suggestive, possible and speculative factors based on the available global evidence. Tobacco along with alcohol and betel quid usage is the most important etiological factors in South East Asia. Risk of oral cancer due to tobacco and alcohol is estimated to be more than 80% . The average delay time in diagnosing and treating oral cancers is about 2 to 5 months. Delayed detection may account for high morbidity rate of OSCC patients. Early detection and diagnosis lead to a greater survival rate and play a significant role in successful treatment of the disease. Recently, factors such as the oral microbiome are being explored for their role as significant risk factors. (Borse et al., 2020)

**Microbiome**
Microbiome refers to “the totality of microbes, their genetic information, and the milieu in which they interact”. Microbiome” is a terminology coined by Joshua Lederberg to signify the ecological community of commensal, symbiotic and pathogenic microorganisms that share our body space. These microbial organisms that contribute to microbiome are termed as microbiota. The human cells are out numbered by the microbes that occupy the body by several folds, thus earning humans the name of supraorganisms. The microbiota s composition can vary according to the environmental sites and the host status. In health, the microbiome is in a state of homeostasis wherein the majority of the microorganisms act as commensals or symbiotics. When this relatively stable state of microbial homeostasis is disrupted, dysbiosis takes place. The anatomical location is a primary determinant for community composition: interpersonal variation is substantial and is higher than the temporal variation seen at most sites in a single individual. Also, there are greater interpersonal similarities than a snap shot view indicates since the microbial system is dynamic in nature. Diet inventories and 16S rDNA sequencing characterization of 98 fecal samples have shown that the fecal communities are clustered into enterotypes distinguished primarily by levels of Bacteroides and Prevotella. Enterotypes are strongly associated with long-term diets, particularly protein and animal fat (Bacteroides) versus carbohydrates (Prevotella). The substantial intestinal metagenomic changes is caused by dietary changes and the enterotypes are known to cluster based on dietary abundance of animal protein or carbohydrate. Characterization of nasopharyngeal microbiota of 96 healthy children was
done in 2011 by barcoded pyrosequencing of the V5-V6 hypervariable region of the 16S-rRNA gene, and compared microbiota composition between children sampled in winter/fall with children sampled in spring. The approximately 1000000 sequences generated represented 13 taxonomic phyla and approximately 250 species-level phyla types (OTUs). Microbiota profiles varied strongly with season, with in fall/winter a predominance of Proteobacteria (relative abundance (% of all sequences): 75% versus 51% in spring) and Fusobacteria (absolute abundance (% of children): 14% versus 2% in spring), and in spring a predominance of Bacteroidetes (relative abundance: 19% versus 3% in fall/winter, absolute abundance: 91% versus 54% in fall/winter), and Firmicutes. This study reveals that there is seasonal variation of nasopharyngeal microbiota in young children which is independent of antibiotic use or viral co-infection. The vaginal bacterial communities of 396 asymptomatic North American women who represented four ethnic groups (white, black, Hispanic, and Asian) and the species composition was characterized by pyrosequencing of barcoded 16S rRNA genes. The communities were clustered into five groups: four were dominated by Lactobacillus iners, L. crispatus, L. gasseri, or L. jensenii, whereas the fifth had lower proportions of lactic acid bacteria and higher proportions of strictly anaerobic organisms, indicating that a potential key ecological function, the production of lactic acid, seems to be conserved in all communities. The proportions of each community group varied among the four ethnic groups, and these differences were statistically significant [P < 0.0001]. Moreover, the vaginal pH of women in different ethnic groups also differed and was higher in Hispanic (pH 5.0 ± 0.59) and black (pH 4.7 ± 1.04) women as compared with Asian (pH 4.4 ± 0.59) and white (pH 4.2 ± 0.3) women. A microarray was designed to detect and quantify the small subunit ribosomal RNA (SSU rRNA) gene sequences of most currently recognized species and taxonomic groups of bacteria. They used this microarray, along with sequencing of cloned libraries of PCR-amplified SSU rDNA, to profile the microbial communities in an average of 26 stool samples each from 14 healthy, full-term human infants, including a pair of dizygotic twins, beginning with the first stool after birth and continuing at defined intervals throughout the first year of life. To investigate possible origins of the infant microbiota, they also profiled vaginal and milk samples from most of the mothers, and stool samples from all of the mothers, most of the fathers, and two siblings. Most of the breast milk and maternal vaginal samples clustered perfectly by anatomic site of origin. The composition and temporal patterns of the microbial communities varied widely from baby to baby. Despite considerable temporal variation, the distinct features of each baby’s microbial community were recognizable for intervals of weeks to months. The strikingly parallel temporal patterns of the twins suggested that incidental environmental exposures play a major role in determining the distinctive characteristics of the microbial community in each baby. By the end of the first year of life, the idiosyncratic microbial ecosystems in each baby, although still distinct, had converged toward a profile characteristic of the adult gastrointestinal tract. The similarity of the microbial community profiles of stool samples from babies 1 year of age and older, to each other and to those of the adult stool samples suggested that the infant gastrointestinal communities converged over time toward a generalized “adult-like” microbiota. The infants’ gastrointestinal microbiota was not significantly more similar to that of their parents than to that of other adults. The transition to an “adult-like” profile was found to often follow the introduction of solid foods. The shift in gut microbial communities was studied following antibiotic therapy using a mouse model to control the host genotype, diet, and other possible influences on the microbiota. They employed a tag-seq sequencing strategy targeting the V6 hypervariable region of the bacterial small-subunit (16S) rRNA combined with massively parallel sequencing to determine the community structure of the gut microbiota. Inbred mice in a controlled environment harbored a reproducible baseline community that was significantly impacted by antibiotic administration. The ability of the gut microbial community to recover to baseline following the cessation of antibiotic administration differed according to the antibiotic regimen administered. Severe antibiotic pressure resulted in reproducible, long-lasting alterations in the gut microbial community, including a decrease in overall diversity. Thus, according to the review on microbiota by Cho and Blaser et al, each human over a lifetime develops a densely populated microbiome that is recapitulated in every individual and in every generation (Cho and Blaser 2012).

*Helicobacter pylori* presence is strongly associated with particular diseases and important age-related differences. Its presence increases the risk for developing peptic ulcer disease, gastric Mucosa Associated Lymphoid Tissue (MALT) tumors, and gastric adenocarcinoma but also is associated with decreased reflux esophagitis and childhood-onset asthma; demonstrating the complex biological interactions with microbiota. Colon microbiome: Inflammatory Bowel Disease susceptibility is associated with host polymorphisms in bacterial sensor genes such as nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and toll-like receptor 4 (TLR4). Early childhood antibiotic exposure has been associated with increased risk for Crohn’s disease and...
significantly diminished microbial diversity has been seen. Crohn’s disease patients have over-representation of *E. faecium* and of several *Proteobacteria* compared to controls. Gut microbiome associated pathology: Liver: Gut microbiota may be involved in hepatologic conditions, including Non-Alcoholic Fatty Liver Disease (NAFLD), alcoholic steatosis and hepatocellular carcinoma. Patients with cirrhosis have community-wide changes at multiple taxonomic levels, with enrichment of *Proteobacteria* and *Fusobacteria* (phyla), and *Enterobacteriaceae*, *Veillonellaceae*, and *Streptococcaceae* (family). Obesity: In humans, obesity is associated with decreased *Bacteroidetes* and diminished bacterial diversity (Ley et al, 2006). Antibiotic use in human infants, before the age of 6 months was related to obesity development while perinatal administration of a Lactobacillus rhamnosus GG-based probiotic decreased excessive weight gain during childhood. Rheumatoid arthritis: Dysbiosis within gut lumen can cause dysregulation of host immune responses (local expansion of Th17 cells that activate B cells to produce antibodies) leading to increased antibody production against joints. The complexity of dysbiosis and disease is best defined by Hills criteria which states that “The criteria include the strength of association, its consistency, specificity, temporality, and biological plausibility, and whether biological gradients are present, experimental support exists, and support can be extrapolated from known causal relationships.

**Oral microbiome**

In humans, oral microbiome is one of the most complex microbiomes. It is highly diverse, and includes bacteria, virus, fungi, archaea and protozoa. More than 600 bacterial species have been detected, of which 50% have not been cultivated. A majority of 96% of bacteria belong to the phylum *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochaetes* and *Fusobacteria*; while the remaining 4% belong to *Euryarchaeota*, *Chlamydia*, *Chloroflexi*, *Synergistetes*, *Tenericutes* and *candidate phyla*. (Divisions SR1 AND TM7). A candidate phylum is a lineage of prokaryotic organism for which until recently no cultured representatives have been found. Due to the continuum of the oral cavity with the external environment, the oral bacterial flora undergoes dynamic changes in immeasurable rates. This diversity varies from birth to adulthood due to various external and internal influences. Throughout childhood, the oral microbial load is found to increase but the microbial diversity seems to decrease. The initial colonizers depend on: Type of delivery: Babies born by vaginal delivery have bacterial communities quite similar to the mothers vagina – predominantly *Lactobacillus*, *Prevotella*, and *Sneathia spp* but babies born by cesarean section have bacteria similar to those present in the mother’s skin – predominantly *Staphylococcus*, *Corynebacterium*, and *Propionibacterium spp*. Personal relationships: The infants show microflora according to the frequency of contact with the surrounding adults and children, domestic animals. Hygiene habits and diet: Presence of *Streptococcus* species in edentulous children have been demonstrated thus disproving the fact that these species colonize only during the eruption of teeth. Hence oral hygiene practices become even more important right from birth. An increased diet of fermentable carbohydrates can favour the growth of acidogenic and acidic species. Development of teeth: Primary dentition: Higher prevalence of bacteria belonging to the class Gamma *proteobacteria* (*Pseudomonacea*, *Moraxellacea*, *Enterobacteriaceae*, and *Pateurellaceae*) are present. Permanent dentition: Higher prevalence of bacteria belonging to *Veillonellaceae* family and *Prevotella* are seen. Other factors that can influence oral microbiome composition are genetics, host defences, microbial interactions (Quorum Sensing), and receptors for attachment, temperature, atmosphere, pH, and salivary flow. (Deo and Deshmukh 2019).

Genetics: Genetic polymorphisms associated with interleukin (IL)-1, or other cytokines, can increase the likelihood of detecting certain key periodontal pathogens, and pre-dispose individuals to periodontitis. Host-defences and microbial cross-talk: The host defence system is actively engaged in cross talk with its resident microbiota in order to effectively maintain a constructive relationship. Host cell pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) are strategically deployed in tissues to sample the extracellular and intra-cellular environments and recognize microbe-associated molecular patterns (MAMPs), such as lipopolysaccharide, lipoteichoic acid, nucleic acid. They activate multiple signalling pathways many of which converge on nuclear factor κB (NF-κB). MAMPs are present on, or are released from, all microbial cells. The host has evolved systems to enable them to tolerate resident microorganisms without initiating a damaging inflammatory response, while also being able to mount an efficient defence against pathogens. Environmental factors: Nutrients such as amino acids, proteins, and glycoproteins are obtained from endogenous supplies, and mainly from saliva, although gingival crevicular fluid (GCF) is another potential source. Saliva contains amino acids, peptides, proteins, and glycoproteins, vitamins and gases, and it also provides the main buffering capacity for the mouth. The catabolism of the more complex host molecules, such as host
glycoproteins, requires the sequential or concerted action of consortia of bacteria, in which their metabolic capabilities are combined. Importantly for the stability of the microbial consortium, the metabolism of these substrates leads to only minor and slow changes to the local pH, which are well tolerated by the normal resident microbiota. In contrast the main impact of diet is the provision of fermentable carbohydrates that leads to ecologically devastating falls in pH, which if repeated frequently enough, lead to the selection of acidogenic and acid-tolerating bacteria and a greater risk of dental caries. Even a small change in pH can alter the growth rate and pattern of gene expression in subgingival bacteria, for example, the expression of proteases by *P. gingivalis* increases at alkaline pH, and thereby can increase the competitiveness of some of the putative pathogens. This could favour the growth of periodontal pathogens, such as *P. intermedia*, *P. gingivalis*, and *A. actinomycetemcomitans* that have alkaline pH optima for growth. If sustained, the combined selective pressures of the environmental factors will lead to a re-arrangement of community structure and an enrichment of the proportions of the anaerobic and proteolytic component of the microbiota. As the child develops into an adult there is a shift in the bacterial population from aerobic or facultative gram-positive cocci to anaerobic fastidious gram negative bacteria i.e.; from a greater proportion of bacteria from phyla *Firmicutes* and *Actinobacteria* to *Bacteroidetes*, *Fusobacteria*, *Spirochaetes*, and *Candidatus Saccharibacteria*.

**Clinical significance**

When microbial homeostasis is disrupted by external or internal factors, oral diseases such as dental caries, pulpal disease, periapical disease, and oral cancer may occur. Dental caries: When there is an increased dietary carbohydrate intake, bacteria that ferment the carbohydrates such as *Streptococcus mutans*, *Lactobacilli*, and *Streptococcus sobrinus* adhere to the tooth surface and increase the acidity of the biofilm. This in turn increases the load of these acidogenic bacteria and outcompetes the resident flora such as *Streptococcus sanguis* and *Streptococcus gordonii*. Recent studies have shown that *Firmicutes*, *Actinobacteria*, and *Proteobacteria* are the 3 most abundant phyla in patients with caries using Next Generation Sequencing. The difference in oral microbial diversity between children with severe early-childhood caries (S-ECC) and caries-free (CF) controls was evaluated in a study by means of a cultivation-independent approach called denaturing gradient gel electrophoresis (DGGE). Pooled dental plaque samples were collected from 20 children aged 2 to 8 years. Differences in DGGE profiles were distinguished on the basis of a cluster analysis. The microbial diversity and complexity of the microbial biota in dental plaque were found to be significantly less in S-ECC children than in CF children. Periodontitis: A dysbiotic microenvironment has been observed in periodontal inflammation, which is triggered mainly by *Porphyromonas gingivalis*. This bacteria exerts a keystone effect via host modulation to breakdown homeostasis by remodeling the regular microbiome into a disease-provoking one. Endodontic disease: (i) Pulpal disease: *P. micra*, *F. nucleatum* and *Viellonella* species have been implicated in endodontic pulpititis while Atopio genomo species *C1, P. alactolyticus*, *Streptococcus* species were found in deep dental caries. Rocaset et al noted this shift in microbial population suggesting the change in environment as the cause. (ii) Periapical disease: Periapical disease includes apical periodontitis and apical abscess. Gram negative saccharolytic rods such as *Fusobacterium* or *Bacteroides* are predominantly found in root canal spaces associated with periapical disease. Microbes such as *F. nucleatum*, *Spirochaetes* (especially *Treponema*), *Actinomyces*, *Lactobacillus*, *Enterococcus faecalis*, *Dialister* species have been implicated in the periapical diseases by recent studies so far which degrade the nitrogenous compounds into short chain fatty acids, ammonia, sulfur compounds, and indole that induce tissue inflammation by modulating immune response and promote apoptosis.

**Role of Oral Microbiome in Inflammation**

Inflammation due to infections, environmental factors, and therapy induces angiogenesis, tumor progression, and metastasis (Chattopadhyay et al., 2019). Bacterial infection induces initiation and progression of oncogenic processes. Host cells have PRRs such as TLR family, which recognize pathogen-associated molecular patterns or DAMPs that activate the innate immune response. Bacterial endotoxins (LPS), metabolic byproducts of bacterial infection, and increased enzymatic activity because of bacterial infection can induce somatic mutations in host genomes and alters the signaling pathway. Activation of transcription factor nuclear factor KB (NF-kB) is an essential feature of bacteria-associated tumor development. During infection, gram-negative bacteria release endotoxins such as LPS from their outer membrane. Bacterial LPS binds highly sensitive PRRs such as TLRs, particularly TLR4, which in turn activates inflammatory-associated cytokine production via NF-KB signaling pathway. This signaling event is one of the major factors in bacteria-induced inflammation as Technology in Cancer Research & Treatment well as the contributor to carcinogenesis. Lipopolysaccharide from a potential pathogen such as *P gingivalis* and *F nucleatum* is responsible for the activation immune system at the
cellular level in periodontal diseases. Bacterial endotoxin enhances the production of tumor necrosis factor a (TNF-a) from macrophages. Inflammatory cytokines such as interleukin (IL)-1b, IL-6, and TNF-a are responsible for periodontal tissue damage. Interleukin-1b may be involved in bone resorption and the attachment loss that are characteristic properties of periodontitis. TNF-a is responsible for the generation of free radicals during sepsis. Bacterial products such as endotoxins (LPS), enzymes (eg, proteases, collagenases, fibrinolisin, and phospholipase), and metabolic by-products (eg, H2S, ammonia, and fatty acids) may induce permanent genetic alterations in epithelial cells of the host that drive proliferation and/or survival of epithelial cells. Microorganisms induce inflammation by activating neutrophils, macrophages, monocytes, lymphocytes, fibroblasts, and other cells that drive secretion of cytokines and matrix metalloproteases. Bacteria generate reactive oxygen species (eg, hydrogen peroxide and oxygen radicals), reactive nitrogen species (nitric oxides), reactive lipids, and metabolites (eg, malondialdehyde, 4-hydroxy-2-nonenal) in epithelial cells that drive DNA damage in epithelial cells contributing to disease phenotype. Bacterial flagella were considered as key inflammatory structures in regulating OSCC-related inflammation. It has been reported that F. nucleatum subspecies polymorphum, Campylobacter subspecies, and P. aeruginosa showed significant association with OSCC, whereas S. mitis, R. mucilaginosa, and H. parainfluenzae were the most significantly abundant genus in the healthy individuals. Genes involved in bacterial mobility, flagellar assembly, bacterial chemotaxis, and LPS synthesis were significantly associated with OSCC. Functional prediction also revealed that genes involved in DNA repair and combination, purine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, ribosome biogenesis, and glycolysis/gluconeogenesis were enriched in healthy individuals. Pseudomonas aeruginosa induces DNA breaks in epithelial cells that drive chromosomal instability. Lipopolysaccharide, flagella, and cytotoxins (eg, ExoU) of P. aeruginosa have potent inflammatory activity that drives carcinogenesis. This activates NF-kB signaling pathway through the recruitment of neutrophils. LasI factor, secreted from P. aeruginosa, downregulates the expression of E-cadherin that induces invasion and metastasis. The periodontal pathogens Fusobacterium, Porphyromonas, and Campylobacter (common in GI infections) are considered as “mobile microbiome” that originates in the OC but also associated with extra-oral infections and inflammation. Rothia, Streptococcus, and Prevotella produce oral ALD that promote oral carcinogenesis. (Chattopadhyay et al., 2019)

Variant analysis and cancer

With the rapid development of massively parallel next-generation sequencing (NGS) technologies, a large number of cancer genomes, exomes, or gene panels are being sequenced around the world for both biomedical research and clinical diagnosis. DNA sequencing has become an important component in cancer diagnosis and treatment, which facilitates the implementation of precision medicine. However, determining the clinical impacts of somatic variants in cancer presents a different set of challenges from those for germline variants.

Various tools and databases have been developed by different laboratories and institutes, in combination with experts’ opinions, for the interpretation of clinical significance on sequence variants. Annotation tools, such as ANNOVAR (Wang et al., 2010) and Spif (Cingolani et al., 2012), as well as many computational prediction algorithms, such as SIFT (Ng et al., 2001), PolyPhen-2 (Adzhubei et al., 2010), MutationAssessor (Reva et al., 2011), MutationTaster (Schwarz et al., 2010), and PROVEAN (Choi et al., 2012 and Choi et al., 2015), can annotate variants with respect to transcript structure or predicted functional importance; however, they mostly focus on germline variants. Several cancer-specific variant databases have gathered and curated unstructured information on the effectiveness of therapies targeting specific cancer drivers, such as the Catalogue of Somatic Mutations In Cancer (COSMIC) (Forbes et al., 2017), My Cancer Genome (https://www.my cancergenome.org), Clinical Interpretations of Variants in Cancer (CIViC) (Griffith et al., 2017), OncoKB (Chakravarty et al., 2017), the Precision Medicine Knowledge Base (PMKB) (Huang et al., 2017), and Cancer Genome Interpreter (CGI) (Tamborero et al., 2018). However, these databases have varying data formats and can often only interpret well-known hotspot somatic variants. Additionally, these databases should be used with caution because they compile information from heterogeneous sources, and many submitted variants lack clinical-grade curation or may only be discovered in exploratory research studies. Therefore, how to comprehensively annotate and interpret the clinical significance of somatic variants is an important yet unresolved challenge.

To standardize the clinical interpretation of cancer genomes, the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and College of American Pathologists (CAP) published standards and guidelines for the interpretation and reporting of sequence variants in cancer in 2017 (Li et al., 2017). The AMP-ASCO-CAP guidelines proposed to categorize somatic variants into a four-tiered categorization system based on their clinical significances, namely strong clinical significance,
potential clinical significance, unknown clinical significance, and benign or likely benign. The guidelines also present primary resources for evidence needed to effectively assess the clinical significance of a particular variant. In addition, ClinGen Cancer Somatic Working Group suggested the standards of the interpretation of cancer variants and developed the Minimal Variant Level Data (MVLD) framework to interpret and report clinically actionable drug-associated somatic variants (Madhavan et al., 2018 and Ritter et al., 2016).

**Conclusion**

This review confirms the association of oral pathogens with oral cancer and illustrates which bacteria can be used as biomarkers in the early detection of oral cancer. Microbial dysbiosis is evident in patients with oral infection. Several types of bacteria appear to be involved in the progression, metastasis and recurrence of oral cancer. The human microbiota can determine the response to periodontal treatment through various mechanisms and thus influence it positively or negatively however, oral infection as a risk factor for the development and progression of oral cancer requires further elucidation. Even the genera, species, or combinations of bacteria involved have not yet been fully characterized. Only studies performed with Next Generation Sequencing (NGS) were analysed as it requires a small sample volume, eliminating PCR errors. The significantly increased number of bacteria in the saliva samples was not the same as in the tissues of oral cancer patients. Using the oral microbiome as a reliable diagnostic tool has emerged as an important non-invasive option in the early detection of periodontal treatment.

**Conflict of interest**

The authors have no conflicts of interest to declare.

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