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Exploring the role of oral microbiome dysbiosis in cardiometabolic syndrome and smoking

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ABSTRACT

Oral microbiome research has gained significant interest in recent years due to its potential impact on overall health. Smoking has been identified as a significant modulator of the oral microbiome composition, leading to dysbiosis and possible health consequences. Research has primarily focused on the association between smoking and oral microbiome, as well as smoking's association with cardiometabolic syndrome (CMS). This narrative review presents an overview of the recent findings and current knowledge on the oral microbiome and its role in CMS, including the effects of smoking and ethnicity. We discussed the development and composition of the oral microbiome and the association of periodontitis with diabetes and cardiovascular diseases. Furthermore, we highlighted the correlations between oral microbiome and CMS factors, such as diabetes, hypertension, dyslipidemia, and obesity. There is a need for further research in this area to better understand the mechanisms underlying the impact of smoking on oral microbiome dysbiosis and the development of CMS. Interestingly, geographic location and ethnicity have been shown to impact the oral microbiome profiles across populations. This knowledge will help develop personalized disease prevention and treatment approaches considering individual differences in oral microbiome composition. Understanding the complex interplay between oral microbiome, smoking, and CMS is essential for developing effective prevention and treatment strategies for a wide range of diseases.

Abbreviations: CMS: cardiometabolic syndrome; T2D: type 2 diabetes; CVD: cardiovascular disease; HMP: human microbiome project; NIH: National Institute of Health; IHMC: International Human Microbiota Consortium; DM: diabetes mellitus; BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; IR: Insulin resistance

Background

The human microbiome is a complex ecosystem comprising 10 to 100 trillion symbiotic microbial cells, including bacteria, viruses, fungi, and protozoa.[1] Microbiome projects worldwide have been dedicated to understanding the role of microorganisms in health and disease. The human microbiome project, for instance, was committed from 2007 to 2016 and focused on determining the microorganisms that make up the microbiome in five body sites, including the skin, oral cavity, nasal cavity, gastrointestinal tract, and urogenital tract.[2] One key area of interest is the oral microbiome, which is critical in maintaining oral health and preventing diseases.

Recent studies have indicated that the oral microbiome is highly dynamic and can be affected by several factors, such as cardiometabolic syndrome (CMS). CMS describes a cluster of metabolic disorders that includes insulin resistance (IR), dyslipidemia, hypertension, and obesity, which could lead to the development of type 2 diabetes (T2D) and cardiovascular disease (CVD). CMS is mostly preventable and treatable; however, it is one of the highest causes of mortality worldwide, with 22.4 million deaths.[3,4] Alarmingly, almost half of these deaths were categorized as

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"premature deaths," occurring before the age of 60 and often leading to a significant reduction in the quality of life for affected individuals.[3,4]

There has been emerging evidence that the oral microbiome may be involved in the pathogenesis of CMS factors and may be influenced by lifestyle factors such as smoking. Smoking is one of the habits worldwide that has significantly impacted the oral microbiome. Several studies have studied the role of smoking on the oral microbiome in different populations.[5–7] However, the mechanisms between oral microbiome, smoking, and CMS are largely unexplored. The physiological effects of numerous chemicals found in smoking have not received sufficient research attention, and there is even less understanding of how the oral microbiome might influence the interactions between cigarette smoke and human physiology. The circulatory system serves as a pathway through which oral microbiome bacteria can potentially impact the risk of CMS. The blood supply associated with each tooth allows metabolites or endotoxins produced during oral bacterial metabolism to enter the bloodstream.[8] Consequently, this process can lead to systemic inflammation, affecting various other parts of the body.[8] A recent study conducted in China found that smoking was associated with enrichment of four bacterial genera in the oral microbiome (*Anaeroglobus, Megasphaera, Actinomyces, and Rothia*), which were linked to elevated triglyceride levels.[9] Additionally, *Anaerglobus* was negatively associated with HDL-C levels.[9] Overall, there is still a lack of evidence on the role of oral microbiome in CMS and the effects of smoking in different populations. This narrative review aims to provide an overview of the current knowledge.

Oral microbiome overview

Microorganisms residing in the oral cavity are referred to as the oral microbiome. It includes various distinct habitats, which include teeth, gums, tongue, hard palate, soft palate, cheeks, and lips. Adjacent anatomical structures such as tonsils, pharynx, middle ear, trachea, esophagus, eustachian tube, nasal passages, and sinuses are included in the human oral microbiome. The

human oral microbiome is defined as all microorganisms present on or within the oral cavity and its contagious extension, except for the distal esophagus. However, most studies and samples have been from the oral cavity itself.[10]

The healthy oral microbiome harbors approximately 50–100 million bacteria belonging to 700 species and is the second most abundant and diverse microbiome in the human body, following the gut microbiome [11–13] The oral cavity region provides an optimal environment for microorganism growth with an average temperature of 37 °C and a saliva pH consistently between 6.5–7.5, allowing bacteria to thrive in a stable environment.[14] Moreover, saliva serves as a source of hydration for microorganisms and functions as a transport medium for nutrients to microorganisms. The oral microbiome and its host have a mutually dependent and evolutionary relationship characterized by continuous communication.[15] The oral microbiome plays diverse roles, including physiological, immunological, metabolic, mucosal protection, nutritional, and detoxifying functions.[15,16]

The development of the oral microbiome starts at a very early stage of life. During delivery, the newborn encounters the microflora of the mother's uterus and vagina and later with the other microorganisms in the atmosphere during delivery.[13] Initially, the newborn's oral cavity is usually sterile, but it becomes inoculated with microorganisms from the first feeding, and the residential oral microflora acquisition process starts.[17] *Streptococcus salivarius* and *Streptococcus mitis* are pioneer species that inhabit the oral cavity at the early stages after birth.[18] In the first year after birth, the oral cavity is mainly invaded by aerobic bacteria, including *Streptococcus, Lactobacillus, Actinomyces, Neisseria, and Veillonella*.[19] Later, after teeth eruption, anaerobic microorganisms, such as *Prevotella*, *Fusarium*, and more, dominate the environment that exists between the gums and teeth.[18] *Streptococcus* species, including *Streptococcus parasanguis* and *Streptococcus mutans,* can grow on enamel and colonize gingival epithelial surfaces.[19]

The Human Microbiome Project (HMP) was initiated in 2007 as a collaborative effort between the National Institute of Health (NIH) and the

International Human Microbiota Consortium (IHMC).[20] The main objective was to comprehensively characterize the human microbiota on a large scale and explore their significance in relation to human health and disease.[20] According to the expanded Human Oral Microbiome Database (eHOMD) (https://homd.org/, accessed on 16 April 2023), there are 774 oral bacterial species, 58% are named, 16% have been cultivated but unnamed, and 26% have been identified through DNA analysis but yet to be cultivated; 96% of the total oral microbiome belongs to six broad phyla: Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Bacteroidetes, and Spirochetes.[21] These oral microorganisms significantly impact various aspects of human health, ranging from the host metabolic process to immune responses.[22]

Microbial dysbiosis is an imbalance in the microorganisms of the host. Indeed, oral microbiome dysbiosis has a high chance of spreading into adjacent epithelial surfaces, leading to potentially infectious diseases. Moreover, studies have linked oral bacteria dysbiosis to several systematic diseases, including CVD and diabetes.[23–28] By understanding the role of the oral microbiome in health and disease, researchers can develop better treatments and strategies to promote oral health.

Oral microbiome and periodontitis

Periodontitis is a bacterially induced chronic inflammatory disease.[29] The disease is characterized by bone and connective tissue loss, tooth mobility, and, ultimately, tooth loss.[30] The host's immunological response is responsible for 80% of tissue destruction, while oral bacteria account for the remaining 20%.[29] Poor oral hygiene practices lead to plaque accumulation, triggering a chronic inflammatory response and affecting the gingival tissues. Gingivitis is the earliest periodontal disease stage and is reversible with simple oral hygiene practices.[29,31] However, if left untreated, it can proceed into periodontitis due to persistent plaque accumulation and the patient's response to the bacterial challenge.[29] Periodontitis is commonly associated with anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and Spirochetes such as *Treponema denticola*.[32–34]

Oral bacteria can enter the bloodstream through various activities, including eating, flossing, and tooth brushing, leading to bacteremia.[35] Studies have shown that periodontitis is linked to CVD due to the pathogenic oral bacteria found in atherothrombotic mice tissue.[36,37] The local inflammatory response triggers pro-inflammatory cytokines, which can also enter the bloodstream and initiate an acute inflammatory response.[38] Eventually, it results in a local inflammatory response and triggers pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα), which can also enter the bloodstream and initiate an acute inflammatory response.[38] Chronic systemic inflammation caused by these cytokines can increase the risk of developing CVD, hypertension, and T2D.[35,38] Additionally, the release of reactive protein can activate cytokines and oxidative stress response, further exacerbating chronic systemic inflammation.[38]

Inflammation in periodontitis also drives dysbiosis by promoting the growth of pathogenic bacteria that can utilize nutrients released from the destruction of periodontal tissues, such as collagen fragments and heme-containing compounds.[39,40] These bacteria, including *Porphyromonas gingivalis,* can thrive in low-oxygen environments created by inflammation and have an increased ability to cause inflammation.[41] They are known as "inflammophilic pathobionts" and can upregulate virulence-associated genes in response to certain nutrients.[40] The overgrowth of these bacteria is a major contributor to the development of periodontitis.[42]

A meta-analysis has identified individuals with periodontitis to have a moderate to increased risk of developing coronary heart disease, ischemic stroke, and CVD.[43–46] Another meta-analysis has also resulted in the correlation of periodontitis increasing the risk of hypertension.[47] Evidence shows periodontitis treatment decreased CVD markers, consisting of C-reactive protein and circulating lipid.[36,48] Moreover, diabetic patients had a 24% risk of developing cardiometabolic syndrome (CMS); periodontitis patients had a 26% risk of developing diabetes.[49]

Porphyromonas gingivalis, Treponema denticola, and *Tannerella forsythia* are common bacteria

found in the subgingival biofilms that contribute to the development of periodontitis.[50] Recent studies have found an association between *Treponema* and *Corynebacterium* bacteria for the development of periodontitis and CMS in subgingival plaque and saliva samples.[51] Furthermore, *Filifactor alocis* and *Fretibacterium fastidiosum* were found to be dominant in subgingival plaque of periodontitis patients who smoke.[52]

Treatment for periodontitis has been shown to improve CVD markers, such as C-reactive protein and circulating lipids.[53] Furthermore, diabetic and periodontitis patients are at an increased risk of developing CMS and diabetes. Therefore, early detection and management of periodontitis is essential and can help in reducing the risk of developing CMS.

Oral microbiome influence on cardiometabolic syndrome

CMS is also known as metabolic syndrome x. As previously mentioned, it is a combination of metabolic diseases that includes a combination of diabetes mellitus (DM), hypertension, central obesity, and dyslipidemia. Studies have shown a strong link of CMS in developing atherosclerotic CVD, peripheral vascular disease, coronary artery disease, myocardial infarction, cerebrovascular Diabetes Mellitus disease, stroke, and DM.[54–56] An essential factor of CMS is obesity, which is on the rise globally where it is estimated that 1.1 billion adults are overweight and 312 million are obese.[57] Although obesity is well-recognized risk of developing CMS, a study revealed middle-aged men with CMS are at an increased risk of developing CVD and related deaths regardless of their Body mass index (BMI).[58]

There are various internationally recognized definitions of CMS used. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), CMS is defined as the presence of 3 or more clinical abnormalities, which include: dyslipidemia, central obesity, systemic arterial hypertension, and hyperglycemia.[59] European Group for Study of Insulin Resistance (EGIR), the American Association for Clinical Endocrinology (AACE), and the International Diabetes Federation (IDF)

are international organizations and have slightly different criteria for CMS; however, all include visceral obesity and insulin resistance.[60,61] However, the World Health Organization (WHO) and ATPIII diagnostic criteria are more widely used. The WHO defines CMS as the presence of DM and IR as the primary factors and other risk factors such as obesity, high triglycerides, reduced high-density lipoprotein (HDL), hypertension, or micro-albuminuria.[61] EGIR and ATPIII defined obesity in CMS as visceral obesity rather than total obesity or overweight.[57] The effects of hyperglycemia, hypertension, dyslipidemia, and obesity on the oral microbiome will be discussed below. Figure 1 summarizes the oral microbiome of the different CMS.

Hyperglycemia

Hyperglycemia is the increase of glucose in the bloodstream. The hemoglobin A1c (HbA1c) test is used to evaluate the percentage of the hemoglobin glycosylation and a person's level of glucose control.[62] A level below 5.7% HbA1c is considered normal, 5.7 to 6.4% HbA1c is considered as pre-diabetic and a level higher than 6.7% HbA1c is considered diabetic.[62] Diabetes is a chronic metabolic disorder, it is one of the top 10 causes of mortality worldwide; in 2019, 463 million individuals were living with diabetes, which is expected to increase by 2045 to 700 million.[63]

Studies have revealed a strong association between diabetes and both the gut and oral microbiome. In particular, diabetes is bidirectionally linked to periodontitis.[31,64–66] This association is believed to be due to several factors, including increased glucose levels in gingival crevicular fluid and saliva, which provides a favorable environment for the growth of pathogenic bacteria. In addition, pH changes and reduced saliva flow associated with diabetes can cause alterations in the oral microbiome.

Interestingly, studies have suggested that individuals with periodontitis are at a higher risk of developing diabetes, and individuals with diabetes are more likely to develop periodontitis.[67] For example, a study conducted on Hispanic patients with T2D and periodontitis found that the most frequently isolated microorganisms from
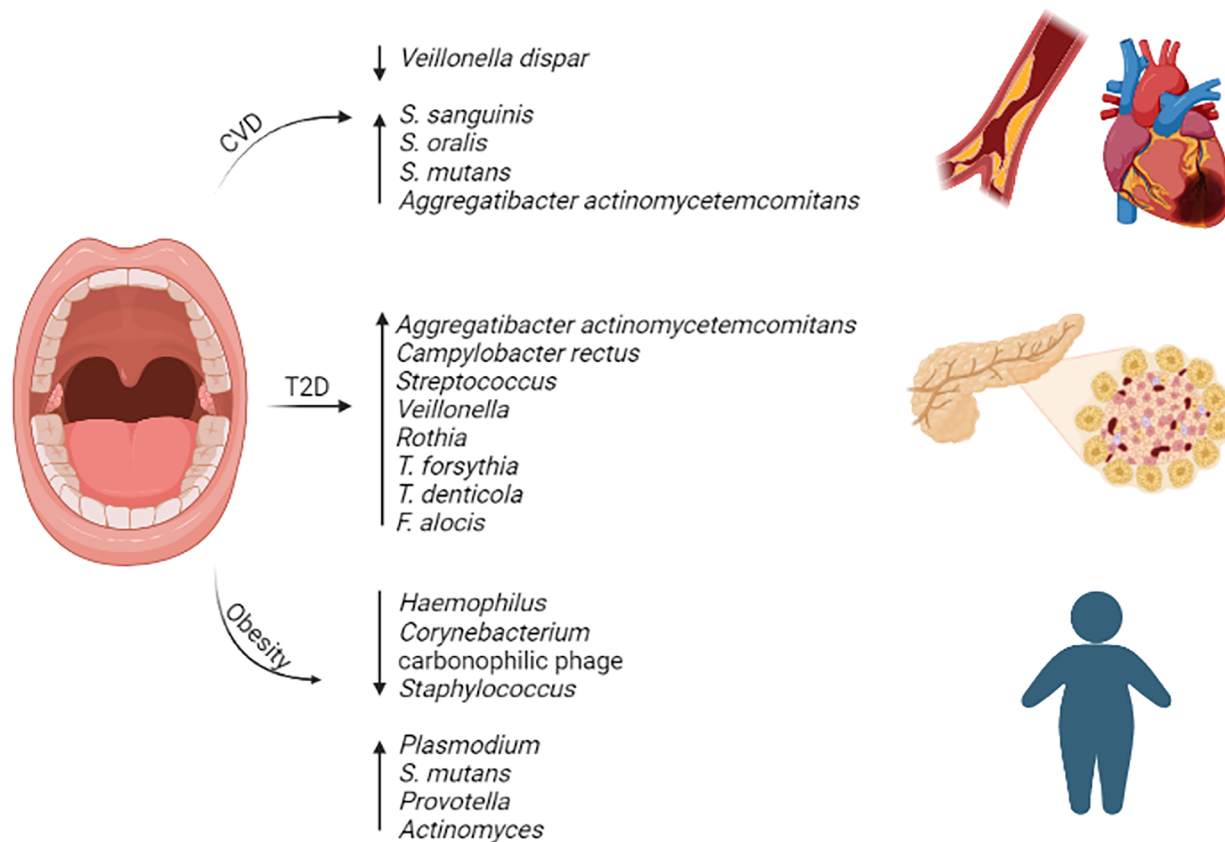
Figure 1. Oral microbiome bacterial enrichment or depletion in cardiometabolic syndrome (CMS). Cardiovascular disease (CVD), type 2 diabetes (T2D), and obesity.

periodontitis sites were the red-complex bacteria, such as *Aggregatibacter actinomycetemcomitans* and *Campylobacter rectus*.[67] Miranda et al. (2017) investigated the impact of glycemic control on the abundance of periodontal pathogens *(Treponema denticola, Porphyromonas. gingivalis, Tannerella forsythia, Eubacterium nodatum, Parvimonas micra, Fusobacterium nucleatum ssp., and Prevotella intermedia)* in T2D who presented generalized chronic periodontitis.[68] They collected subgingival biofilm samples from patients with poor and good glycemic control.[68] The analysis revealed individuals with poor glycemic control had significantly higher levels of *Fusobacterium. nucleatum* ssp. and detection frequencies of *Tannerella forsythia, Eubacterium nodatum, Parvimonas micra*, and *Fusobacterium nucleatum* ssp, indicating poor glycemic control is associated with elevated levels and frequencies of periodontal pathogens.[68] In another study, researchers found an increase in aciduric species, including *Streptococcus, Veillonella,* and *Rothia,* in T2D patients who adhere to a Mediterranean diet.[69]

Furthermore, specific oral microbiome profiles have been associated with the development of insulin resistance. Recent studies investigated the subgingival plaque of periodontitis patients with and without diabetes and found that periodontitis diabetic patients had significantly higher levels of *Tannerella forsythia*, *Treponema denticola,* and *Filifactor alocis*.[70] Notably, nondiabetic patients also showed a correlation between red complex species and *Filifactor alocis* and *Fretibacterium fastidiosum*.[70] These findings suggest that the oral microbiome may play a role in the development and progression of diabetes and periodontitis.

Hypertension

Hypertension is a major contributor to global morbidity and mortality.[71] It is associated with various metabolic and cardiovascular complications, posing a significant burden on global health and leading to years of life lost as a result of disability.[71,72] The current diagnostic parameter for hypertension is based on systolic pressure values

above 130 mmHg and diastolic pressure above 80 mmHg.[73]

The salivary microbiome has emerged as a potential factor associated with hypertension. A 2015 study revealed a reciprocal correlation between a chlorhexidine-based mouthwash and *Veillonella dispar,* a nitrate-reducing bacteria, along with an increase in systolic blood pressure.[74] However, our earlier study demonstrated an increase in nitrate-reducing microbes in hypertensive patients,[71] indicating possible variations in microbial profiles among different hypertensive populations.

A recent study conducted on 1190 Qatari individuals investigated the differences in the salivary microbiome of hypertensive and normotensive individuals.[75] The analysis of differential abundance revealed that *Bacteroides* and *Atopobium* were significant members associated with hypertensive groups.[75] Normotensive individuals exhibited higher alpha diversity compared to hypertensive individuals and with beta diversity the normotensive individuals were significantly different from hypertensive individuals.[75]

Studies exploring the subgingival microbiome in relation to hypertension have shown intriguing findings. In one study, in subgingival plaque of hypertensive individuals exhibited an increased abundance of *Actinobacillus actinomycetemcomitans,* while *Streptococcus* was significantly more abundant in normotensive individuals.[76] Additionally, *Treponema denticule* was more prevalent in supragingival region and prosthetic materials of hypertensive patients.[76] Furthermore, salivary nitric oxide levels were inversely associated with hypertension.[76]

Elevated relative abundance of *Fusobacterium* in subgingival samples of hypertensive individuals has been reported.[77] Moreover, a decrease in the relative abundance of *Actinomyces* and increase in *Selenomonas* in subgingival plaque specimens was correlated with elevated blood pressure.[77] Furthermore, the relative abundance of *Streptococcus* and several of its species were decreased in saliva and oral samples of hypertensive and ischemic stroke compared to individuals with no CVD.[77–79]

Dyslipidemia

Dyslipidemia, is defined as elevated concentrations of plasma triglycerides, reduced level of HDL concentration, and an increase in the levels of Low-Density Lipoprotein (LDL) concentrations. Dyslipidemia and pro-inflammatory cytokines play a crucial role in the development of atherosclerosis, a major underlying factor contributing to CVD.

Furthermore, the role of dyslipidemia in CVD is significant and is causing a health problem worldwide, with an increased mortality rate from 12.1 million in 1990 to 18.6 million in 2019. Research has identified that the microbiome may play a crucial role in CVD development. Oral microbiome dysbiosis can lead to gut dysbiosis by traveling through saliva, which can result in the release of endotoxins into the circulation, promoting CVD, heart failure, and left ventricular dysfunction.[80] Heart failure is also associated with lipopolysaccharide, a gram-negative cell wall product that activates dysregulated systematic inflammation.[81]

Elevated levels of *Aggregatibacter actinomycetemcomitans* were detected in saliva samples and subgingival plaque, and have been associated with ischemic stroke and various cardiovascular conditions, including coronary artery disease, acute coronary symptoms, and valvular heart disease.[82–85] Furthermore, it has been found in samples of coronary artery atherosclerotic tissue, suggesting its potential role in atherosclerosis development.[83,86] The effect of periodontitis has been associated with the development of CVD. Individuals with periodontitis experience endotoxins in their bloodstream, leading to low-level inflammation.[84,87] Chronic inflammation can accelerate atherosclerotic plaques, increase inflammation permeability of the blood vessels, and increase the risk of thrombosis.[84,87] Interestingly, a study found an inverse relationship between the levels of IgG antibodies to *Tannerella forsythia* and CVD mortality risk, specifically in men who had previously had heart attacks.[88] These findings suggest a complex relationship between the oral microbiome and CVD, which requires further investigation.

Multiple species of *Fusobacterium* were detected in both subgingival plaque and samples of coronary artery atherosclerotic plaques.[89] *Porphyromonas gingivalis* was identified in both subgingival and coronary artery atherosclerotic

plaque samples either independently or in conjunction with several other species, such as *Eikenella corrodens*, *Tannerella forsythia*, *Tannerella denticola,* and *Campylobacter rectus.*[89,90]

Studies have explored the association between the oral microbiome and heart valve defects. One study sampled the heart valve during heart valve replacement surgery and cut it into two halves; each part was cultivated in different conditions (aerobic and nonaerobic).[91] Seven gram-positive bacteria were identified; three were typical oral bacteria *(Streptococcus sanguinis, Streptococcus oralis,* and *Streptococcus sp.)*, while *Cutibacterium acnes,* which was the most abundant species and is also part of the skin normal flora.[91,92] A prior study also found that cardiac valve samples exhibiting high rates of gingivitis and/or periodontitis contained oral bacteria, specifically *Streptococcus mutans.*[93] This confirms a previous study where *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* were the abundant oral bacteria in cardiovascular specimens.[94] In addition, *Veillonella* was detected in whole mouth samples and in samples of carotid artery atherosclerotic plaque.[95]

Obesity

Obesity is a complex metabolic disorder characterized by excessive adipose tissue accumulation that increases the risk of chronic disease, including T2D, CVD, and certain types of cancer. Recent research has focused on the role of oral microbiome in the pathogenesis of obesity. Studies have reported alterations in the composition and diversity of obese oral microbiome compared to lean individuals. Tam et al. (2018) conducted a study to explore whether obesity influences the composition and diversity of the oral microbiome.[96] They collected subgingival and saliva samples from 18 patients with T2D, including 6 in which are obese (BMI $\geq$ 30 Kg/m^2) and 12 non-obese (BMI $<$ 30) most of them had periodontitis.[96] The study revealed in the subgingival of normal weight individuals there was a higher abundance of Bacteroidetes, Spirochetes, Firmicutes, *Treponema* spp., *Selenomonas* spp., and *Filifactor* spp.[96] On the other hand, obese individuals had a greater abundance of Proteobacteria, Firmicutes, *Chloroflexi* spp., and *Campylobacter* spp., with Bacteroidetes being nearly absent.[96] Similar patterns were observed in saliva with normal weight individuals; normal weight individuals had a higher abundance of Bacteroidetes and Firmicutes, while obese individuals had a higher abundance of Firmicutes.[96] The differences in microbial composition and diversity between obese and normal weight individuals were statistically significant indicating reduced species diversity in the obese group.[96]

Certain bacterial species, such as *Provotella* and *Actinomyces,* have been shown to increase the salivary microbiome of obese individuals without periodontitis.[97] However, recent studies have found that obese individuals have a significantly low abundance of specific bacteria, including *Haemophilus*, *Corynebacterium*, *Carbonophilic* phage, and *Staphylococcus*, but an increase in the abundance of *Plasmodium*, *Streptococcus genus*, and *Streptococcus mutans.*[98,99] In our previous study, we observed a higher abundance of proteobacteria and Firmicutes/Bacteroidetes ratio (a recognized obesogenic microbiome trait) in obese insulin-resistant and nondiabetic adults.[100]

In another study, 647 obese and 969 non-obese individuals' mouth rinse samples were analyzed.[101] Five taxa in Firmicutes and two each in Proteobacteria and Actinobacteria were significantly associated with increased obesity.[101] *Bifidobacterium* and *Lactobacillus* were associated with decreased obesity prevalence, lower weight gain and BMI.[101] A recent study on saliva samples on 3–4 years of age using shotgun metagenomics. Firmicutes, Actinobacteria, and Bacteroidetes phylum were linked to weight gain during the initial two years of life.[102] As BMI increased, the diversity of the oral microbiome decreased.[102] This suggests that alterations in body composition influenced the diversity of the oral microbiome, potentially contributing to an elevated risk of developing diseases in adulthood.[102,103]

Impact of smoking on cardiometabolic syndrome

The impact of smoking on the oral microbiome is significant, and it is also considered as an

underlying cause and a significant risk for CMS. Meta-analysis studies have shown that smokers have a 1.26 times higher chance of developing CMS than nonsmokers.[104] It has also been indicated that smokers and passive smokers are associated with increased susceptibility to metabolic disturbances among adolescents.[105]

Smoking has been associated in promoting insulin resistance leading to diabetes as well as hypertension and dyslipidemia.[106,107] Notably, smoking is associated with visceral obesity, which plays a role in CMS. Multiple studies have shown a positive correlation between smoking and visceral obesity due to its harmful effects on adipose tissue, altering its secretion pattern, lipolysis, and differentiation.

However, smoking cessation can reduce the risk of developing CMS and CVD and subsequently lower mortality rates.[108,109] Nevertheless, it should be emphasized that lifestyle modification should occur alongside smoking cessation, as it may lead to weight gain if not managed correctly.[110] A recent study conducted found smokers had increased levels of dyslipidemia, body mass index, and central obesity, in addition to higher von Willebrand factor (vWF) protein functional activity increased troponin I levels in smokers, indicating a higher susceptibility to cardiovascular mortality among smokers.[4]

Overall, there is a lack of studies on the impact of smoking on CMS and cardiovascular health is multifaceted, involving various physiological factors, and understanding its influence on the oral microbiome adds to the complexity of its effects on overall health.

The impact of smoking on the oral microbiome

Smoking has a profound impact on the oral microbiome in various populations. Cigarette smoke contains several toxic compounds that affect the gut and oral microbiota and induce dysbiosis.[111] The toxic compounds include nicotine, heavy metals, aldehydes, nitrosamines, polycyclic aromatic hydrocarbons, and more, which are inhaled into the lungs as aerosol particles or free in a gaseous state.[112–115] The toxic compounds reduce endogenous antioxidants, increase pro-inflammatory factors concentration in the blood, and increase lipid peroxidation and oxidative stress.[112,116–119]

When smoking, the oral cavity is the first that comes into direct contact with these toxic compounds making it the most affected part of the body. Toxic compounds found in cigarettes disrupt the oral microbiome's ecological balance through the formation of unstable bacterial growth in biofilms, increasing saliva acidity and reducing oxygen levels, altering bacterial attachment to mucosal surfaces, inducing antibiotic resistance, and affecting the host's immune cells.[120–122]

The salivary microbiome may be influenced by various factors, including host genetics, diet, and environmental factors.[123] In an American adult study using 16S rRNA oral wash samples, smokers had a lower abundance of the proteobacteria phyla and *Neisseria, Porphyromonas,* and *Capnocytophaga* compared to never smokers.[5] Meanwhile, *Atopobium, Veillonella,* and *Streptococcus* were enriched.[5] The functional analysis from inferred metagenomics revealed that the depleted bacterial genera were involved in carbohydrate, energy metabolism, and xenobiotic metabolism.[5] In contrast, the increased bacterial genera were anaerobes, thus supporting the oxygen depletion hypothesis.[5]

A Puerto Rican study using 16S rRNA, chemokines, and cytokine analysis revealed taxonomic differences between smokers and nonsmokers, which was correlated with enhanced inflammatory responses.[124] These factors have been linked with carcinogenesis and inflammation in the oral cavity.[124] Proteobacteria was highly enriched in smokers and has been associated with CVD and metabolic conditions.[125]

A Chinese 16S rRNA study found that alpha diversity differed between smokers and never smokers. *Actinomyces* and *Veillonella* were enriched in smokers, which are nitrite-producing bacteria that increase acidity.[7] *Moryella, Bulleidia*, and *Moraxella* were significantly enriched in the smoking status.[7] Acid production pathways were enriched in smokers.[7] In a Qatari salivary microbiome study, smoking increased the Bacteroidetes at the phylum level and *Prevotella* at the genus level. Proteobacteria and Synergistetes at the

phylum level, *Lactococcus, Corynebacterium, Gemella, Capnocytophaga,* and *Streptococcus* at the genus level were significantly higher in the non-smokers.[7] In another study of low-income African Americans participants, mouth rinse samples were collected and analyzed using 16S rRNA sequencing and showed higher levels of the probiotic genera *Bifidobacterium* and *Lactobacillus*, as well as the phylum Actinobacteria in smokers compared to never-smokers. In contrast, the phylum Proteobacteria was depleted in current smokers (Yang et al., 2019).[126] A Jordanian study aimed to investigate the salivary microbiome using high throughput next-generation sequencing found *Streptococcus, Prevotella*, and *Veillonella* showed significantly elevated levels among smokers at the expense of *Neisseria* in nonsmokers.[127]

Tongue samples have also been studied to learn the effects of smoking on the tongue microbiome. Tongue microbiomes of East Asian subjects who were current, former, or never smokers using 16S rRNA amplicon sequencing.[128] Their results showed significant differences in microbiome composition and metagenomic functions between current and never smokers but not between former and never smokers.[128] Several genera, such as *Streptococcus* and *Megasphaera*, showed increase abundance in current smokers, while others, such as *Neisseria* and *Capnocytophaga*, were less abundant.[128] The same group also conducted metagenomic sequencing on the tongue microbiome and single-nucleotide variant (SNV) profiles and gene content.[129] They found that beta diversity between never and current smokers was significantly different, and the SNV profiles of *Actinomyces graevenitzii, Megasphaera micronuciformis, Rothia mucilaginosa, Veillonella dispar*, and one *Veillonella sp.* were significantly different between never and current smokers. Furthermore, genes related to the lipopolysaccharide biosynthesis pathway in *Veillonella dispar* were more frequently present in current smokers.[129] Suzuki et al. (2022) used bar-coded pyrosequencing analysis to identify bacterial composition in resting saliva and tongue coating; the study demonstrated a significant difference in microbiome richness and diversity between saliva and tongue but not between smokers and nonsmokers.[130] Saliva samples of smokers were enriched with the genera *Treponema* and

Selenomonas; however, tongue samples from smokers were enriched with the genera *Dialister* and *Atopobium*. The study also found that the genera associated with periodontitis and oral malodor were more abundant in smokers' saliva and tongue and were positively associated with lifetime exposure to smoking.[130]

Al-Bataineh et al. (2020) aimed to investigate the oral microbiota of 105 adults using shotgun metagenomics and compared the functional capabilities of the oral microbiome in smokers and nonsmokers. There was an increase in the relative abundance *of Veillonella dispar, Leptotrichia spp.,* and *Prevotella pleuritidis* in smokers' buccal swap samples.[131] Functional profiling showed that smokers had an enrichment of tricarballylate utilization and lactate racemization, while smokers with high nicotine dependence had an enrichment of xanthosine utilization, p-Aminobenzoyl-Glutamate utilization, and multidrug efflux pump in *Campylobacter jejuni* biosynthesis modules.[131] A Hungarian study also used shotgun analysis and found an increase of *Prevotella* and *Megasphaera* genera in smokers, which has been associated with facilitating disease development.[6] In contrast, the overall diversity and composition did not differ significantly between smokers and nonsmokers.

It is worth mentioning studies that have categorized their sample size into three groups, current, former, and never smokers, revealed that there is no significant difference in the oral bacteria abundance between former and never smokers, indicating that significant bacteria depletion caused by smoking may be reversed following smoking cessation.[5,7] Furthermore, these studies have shown that the oral microbiome of smokers is enriched with anaerobic and facultative anaerobe bacteria while being depleted of aerobic bacteria, indicating smoking alters oxygen availability. A summary of the microbiome profile is presented in Table 1.

Variability in the oral microbiome among different populations

Research suggests there is a significant variation in the oral microbiome among different populations. Some studies have found no apparent geographical distribution,[132,133] while others have

Table 1. Studies of smoking effects on oral microbiome across diverse populations.

Authors	Study design	Sample size	Groups	Sampling site	Sequencing region	Findings General	Findings Nonsmokers	Findings Smoker
[128]	**Cross-sectional**	1616	Current smokers: 59 Former smokers: 477 Never smokers: 547	Mouth rinse samples	16S rRNA gene deep sequencing	——		↑Actinobacteria ↓Proteobacteria ↑Bifidobacterium ↓Lactobacillus
[133]	**Cross-sectional**	105	Current smokers: 55 Never smokers: 50	Buccal swab	Shotgun metagenomis	Firmicutes Proteobacteria Actinobacteria, Bacteroidetes Prevotella Veillonella		↑Veillonella dispar ↑Leptotrichia spp. ↑Prevotellapleuritidis ↑**in high nicotine dependent individuals:** ↑Streptobacillus hongkongensis ↑Fusobacterium massiliense ↑Prevotella bivia ↓Haemophilus_A ↓Gemella cuniculi ↓Neisseria subflava_B ↓Gemella haemolysans_B ↓Neisseria perflava ↓Streptococcus oralis_BA ↓Streptococcus mitis_AT
[129]	**Cross-sectional**	100	Current smokers: 51 Never smokers: 49	Saliva samples	V3-V4 16S rRNA gene sequencing	Firmicutes, Bacteriodetes, Proteobacteria, Actinobacteria, Fusobacteria, and Saccharibacteria Streptococcus, Prevotella, Vellionella, Rothia, Neisseria, and Haemophilus	↑Proteobacteria ↑Fusobacteria ↑Candidate_division_SR1 ↑Synergistetes	↑Firmicutes ↓Proteobacteria ↓Fusobacteria ↑Streptococcus, ↑Prevotella ↑Veillonella ↓Neisseria
[6]	**Cross-sectional**	22	Current smokers:11Never smokers: 11	Saliva samples	Shotgun metagenomics	Prevotella, Velillonella and Streptococcus, Neisseria, Oribacterium, Capnocytophaga, and Porphyromonas	↑Neisseria ↑Oribacterium ↑Capnocytophaga ↑Porphyromonas	↑Prevotella, ↑Megasphaera ↑Veillonella ↑Streptococcus ↓Neisseria ↓Oribacterium ↓Capnocytophaga ↓Porphyromonas

(Continued)

Table 1. Continued.

Authors	Study design	Sample size	Groups	Sampling site	Sequencing region	Findings General	Nonsmokers	Smoker
5	**Meta-analysis**	4 separate data sets. 1204 (1) PLCO-a: 261 (2) PLCO-b: 400 3) CPS-II-a: 203 4) CPS-II-b: 340	PLCO-a: 261 Current smokers: 35 Former smokers: 126 Never smokers: 100 PLCO-b: 400 Current smokers: 41 Former smokers: 165 Never smokers: 194 3) CPS-II-a: 203 Current smokers: 23 Former smokers: 103 Never smokers: 77 4) CPS-II-b: 340 Current smokers: 13 Former smokers: 177 Never smokers: 150 Former smokers: 177 Never smokers: 150	Oral wash samples	V3-V4 16S rRNA gene sequencing		↑Proteobacteria	↑Firmicutes ↑Atopobium, ↑Streptococcus ↑Veillonella ↑Bifidobacterium ↑Lactobacillus ↓Proteobacteria ↓Capnocytophaga, ↓Neisseria ↓Porphyromonas, ↓Haemophilus ↓Aggregatibacter ↓Peptostreptococcus ↓Leptotichia
126	**Cross-sectional**	34		Saliva samples	V3-V4 16S rRNA gene sequencing		Firmicutes (66%), Bacteroidetes (16%), Actinobacteria (5%), Fusobacteria (5%) Proteobacteria (4%) Streptococcus (35%), Veillonella (10%), Prevotella (8%), Porphyromonas (5%),	↑Proteobacteria (40%) Firmicutes (29%) Bacteroidetes (23%) Fusobacteria (5%) Actinobacteria(2%). Streptococcus (15%) Haemophilus (14%) Prevotella (13%) Neisseria(13%), Porphyromonas (9%), Veillonella (6%), Fusobacterium (5%) Aggregatibacter (2%) Staphylococcus (2%) Actinobacillus (1%)

(Continued)

Table 1. Continued.

Authors	Study design	Sample size	Groups	Sampling site	Sequencing region	Findings General	Findings Nonsmokers	Findings Smoker
7	**Meta-analysis**	316	(1) Current smokers:139 (2) Former smokers:7 (3) Never smokers:150	Saliva samples	V3-V4 16S rRNA gene sequencing		↑Proteobacteria↑ Neisseria	↑Rothia dentocariosa ↑Prevotella melaninogenica ↑Prevotella pallens ↑Bulleidia moorei ↑Rothia aeria ↑Actinobacillus parahaemolyticus ↑Haemophilus parainfluenzae ↓Proteobacteria ↓Peptococcus ↓Lautropia ↓Eikenella ↓Kingella ↓Neisseria ↓Cardiobacterium ↓Aggregatibacter ↓Haemophilus ↓Moraxella ↓Rothia aeria, ↓Neisseria oralis ↓Neisseria subflava ↓Actinobacillus parahaemolyticus ↓Haemophilus parainfluenzae
134	**Cross-sectional**	997	(1) Current smokers: 264 (2) Never smokers: 733	Saliva samples	V1-V3 16S rRNA	Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, and Saccharibacteria Prevotella, Porphyromonas, Streptococcus, Veillonella, Capnocytophaga, Haemophilus, Gemella, Alloprevotella, Granulicatella, Camphylobacter, Leptotrichia, Megasphaera and Neisseria	↑Proteobacteria ↑Synergistetes ↑Lactococcus, ↑Corynebacterium ↑Gemella, ↑Capnocytophaga ↑Streptococcus	↑Bacteroidetes ↑Prevotella
132	**Cross-sectional**	50	(1) Current smokers: 18 (2) Never smokers: 32	Saliva and tongue samples	16S rRNA	**Saliva** Streptococcus, Prevotella, Neisseria, and Actinomyces **Tongue:**Streptococcus, Prevotella,		**Saliva** ↑Treponema ↑Selenomonas. ↓Capnocytophaga ↓Cardiobacterium **Tongue** ↑Dialister ↑Atopobium ↓Haemophilus ↓Gemella ↓Peptostreptococcus ↓Granulicatella ↓Catonella ↓Peptostreptococcaceae

(Continued)

Table 1. Continued.

Authors	Study design	Sample size	Groups	Sampling site	Sequencing region	Findings General	Nonsmokers	Smoker
[130]	**Meta-analysis**	2 separate data sets. 844 1)2016: 657 2)2017: 187	(1) 2016: 657 Current smokers: 144 Former smokers: 129 Never smokers: 384 2) 2017:18 Current smokers:40 Former smokers:41 Never smokers:106	Tongue samples	V3-V4 16S rRNA gene sequencing			↑Actinobacteria ↑Firmicutes
[131]	**Cross-sectional**	286	(1) Current smokers: 52 (2) Never smokers:234	Tongue samples	Metagenomic sequencing			↑Porphyromonas endodontalis ↑Streptococcus oralis ↑Streptococcus parasanguinis ↑Veillonella dispar ↓Neisseria subflava ↓Lautropia mirabilis ↓Neisseria flavescens

reported a significant association between salivary microbiome and geographical location.[134,135] For instance, Nasidze et al. (2009) group sequenced 16s rRNA from 10 individuals from 12 different countries and found that the variation in microbiome might be influenced with the distance of each country from the equator.[135] However, despite the geographical diversity the study did not show any significant bacterial clustering depending on the geographical location, the bacterial composition of individuals differed from one individual to another, and it is not uniform across all individuals.[135] In another study, Li et al. (2014) conducted a study that explored oral microbiome variation from three different groups from different climates and regions (Alaska, Germany, and Africa). They found significant differences in alpha and beta diversity among the different groups, with Germans having the highest alpha diversity and the lowest beta diversity and Africans having the lowest alpha diversity and highest beta diversity. Using UniFrac, network, ANOSIM, and correlation analyses, they found similarities between the Germans' and Alaskans' salivary microbiome.[136]

Similarly, Clarke et al. (2022) found significant differences in oral microbial diversity from four different geographical areas: Thailand, Chile, South Africa, and Barbados. Bacteroidetes and Proteobacteria were the two most abundant phyla, but there were significant differences in their prevalence between countries.[137] A recent Qatari study compared the salivary microbiome of the Qatari population to that of various other populations, including Bangladesh, British, Brazilian, Japanese, South Korean, American, and German.[134] The data was retrieved from the National Center for Biotechnology Information/Sequence Read Archive (NCBI/SRA) bioprojects.[134] The study found differences in microbial composition at the phylum and genus level, with the Qatari population resembling the German population in the abundance of *Bacteroidetes*, while the other countries had a predominance of the *Firmicutes* genus.[134] A recent study compared the oral and skin microbiome from two regions in Italy and found no significant difference in the oral microbial beta diversity.[138] However, the study identified trends in the abundance of specific bacteria depending on age and smoking habits.[138] It has been reported the abundant phyla in the salivary microbiome, in general, are Actinobacteria, Bacteroides, Firmicutes, Fusobacteria, Proteobacteria, Spirochetes, and Saccharibacteria.[139] Additionally, a study conducted on supragingival plaque samples from children residing in the same geographical location and representing four ethnic groups (Caucasian, Hispanic, Burmese, and African American)[140] revealed significant differences in alpha and beta diversity among the ethnic groups, with Burmese children exhibiting the most complex microbial community.[140] Burmese and Caucasian children had a higher microbial similarity compared to other ethnic groups.[140] Therefore, these findings highlighted the significant variations of the microbiome in supragingival samples among children.[140] Similarly, a study conducted in the Kingdom of Saudi Arabia, in Jeddah city, on four different families from different ethnicities (Saudi, Sudanese, Yemeni, and Indian) showed a variation in the abundance of bacteria among the families.[141]

Furthermore, saliva samples were analyzed from eight different ethnic groups from southern Africa, suggested that ethnicity did not shape the oral microbial profiles of the population, but socioeconomic status could.[142] In contrast, a study in Gansu Province, China, where dental plaque samples were sequenced, showed geographic location had a significant influence on the composition of the oral microbiome in the same ethnic group.[143]

Understanding the interplay between smoking, ethnic-related differences in the oral microbiome, and the pathogenesis of CMS could hold crucial implications for tailored treatment strategies. The oral microbiome's role as a potential mediator in the association between smoking and CMS warrants further investigation, as it may offer new insights into preventive and therapeutic approaches for individuals at risk. Ultimately, elucidating the complex relationship between the oral microbiome, smoking, and CMS could pave the way for personalized interventions and improved management of cardiometabolic health.

Future direction

The predominant existing studies are correlational rather than causational relationships between oral

dysbiosis and CMS. Therefore, there is a need for causation studies to understand the direct impact of oral microbiome on CMS development and progression. Furthermore, to our knowledge, most studies focused on hyperglycemia, hypertension, dyslipidemia, and obesity rather than the syndrome itself. It is crucial to comprehensively study the mechanisms through which smoking induced changes in microbial composition impacts immune, metabolic, and physiological functions. Additionally, exploring variations in the oral microbiome across geographical locations and ethnicities while considering environmental factors such as diet, host genotype, socioeconomic status, lifestyle could lead to valuable insights. This could provide valuable insights into the association between ethnicity, regional location, and disease susceptibility, thus, establishing a foundation for personalized approaches to disease prevention and treatment strategies. Moreover, future research should investigate oral microbial dysbiosis's impact on the metagenomic content to identify specific microbial taxa and functional pathways influenced by smoking and contributing to CMS. Conducting larger and longitudinal studies can improve statistical power and enhance generalizability, providing crucial insights into the underlying pathogenesis of smoking-related diseases and help in the development of novel targeted therapies.

Conclusion

In conclusion, this narrative review explored the impact of oral microbiome dysbiosis in periodontitis and CMS. Smoking exerts a significant influence on the oral microbiome, leading to dysbiosis that disrupts the ecological balance of the oral microbiome and is linked to the development of oral and systematic diseases. However, the reviewed studies had certain limitations, including variation in sample size, methodologies employed, and inconsistent results from different populations, which made it challenging to establish a direct correlation between smoking-induced dysbiosis and CMS in this review. Nevertheless, it is crucial to acknowledge that smoking has detrimental effects on the oral microbiome and has the potential to contribute to the

development of CMS. To gain deeper understanding of the specific effects of smoking on the oral microbiome and its role in disease development, further studies with larger sample sizes with a specific age group is warranted. By addressing these knowledge gaps, we can enhance our comprehension of the complex relationship between smoking, oral microbiome dysbiosis, and the pathogenesis of CMS, paving the way for more effective preventive and therapeutic strategies in the future.

Authors' contributions

LIM: Manuscript draft writing. ZZZ, FMB: reviewing the manuscript. M.A: conception and reviewing, MA and FMB; Funding acquisition. All authors read and reviewed the final manuscript.

Disclosure statement

The authors declare that they have no competing interests

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