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Periodontal Microbiome Part I: A Literature Review

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PERIODONTAL MICROBIOME: NORM AND ALTERATIONS. A LITERATURE REVIEW. PART I.

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Abstract

Periodontal disease is one of the most chronic infectious diseases. It is initiated by a reversible inflammatory condition commonly called gingivitis. If untreated, it progresses to an irreversible condition called periodontitis, if not resolved, it leads to tooth loss. Periodontitis is a multifactorial disease; one of these factors is the periodontal microbiome. Moreover, the microbiome is altered based on the status of the host, the overall health, gingivitis, and periodontitis. Taxonomically, the microbiome is defined as being a variety of microorganisms called health-associated species. They consist of gram-positive cocci and rod with gram-negative species. In this state, the microbiome participates in various physiological functions. While in gingivitis, the microbiome shifts in its composition and can now initiate an inflammatory condition. While in periodontitis, the microbiome is changed to a state where new microorganisms emerge which initiate a periodontal tissue destruction phenomenon. These microorganisms are referred to disease-associated species. Environmental factors can alter the microbiome composition. Smoking and diabetes are two strong extrinsic factors that negatively shape the microbiome into a more aggressive form. Individuals with poor glycemic control or consume tobacco products are more prone to periodontal diseases because their microbiome is rich in bacterial complexes which negatively affects the periodontium. The effect of antibiotic consumption on the microbiome was more or less advantageous; it was observed that not all antibiotics affect the microbiome composition.

Keywords: Microbiome – periodontium – gingivitis – periodontitis – smoking.

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LE MICROBIOME PARODONTAL: NORMES ET ALTERATIONS. UNE REVUE DE LA LITTERATURE. PARTIE I

Résumé

La maladie parodontale est l'une des maladies infectieuses les plus chroniques. Elle est initiée par une affection inflammatoire réversible communément appelée gingivite. Si elle n'est pas traitée, elle évolue vers une affection irréversible, la parodontite. Mal ou non traitée, elle conduit à la perte des dents. La parodontite est une maladie multifactorielle, l'un des facteurs majeurs de morbidité étant le microbiome parodontal. Par ailleurs, le microbiome est altéré en fonction de l'état de l'hôte, c'est-à-dire la santé générale, la gingivite et la parodontite. Taxonomiquement parlant, le microbiome est composé d'une variété de micro-organismes appelés espèces. Ils sont constitués de Cocci à Gram positif et de bâtonnets avec des espèces à Gram négatif. Dans cet état, le microbiome participe à diverses fonctions physiologiques. En cas de gingivite, le microbiome change de composition et déclenche une maladie inflammatoire. Dans le cas de parodontite, de nouveaux micro-organismes émergent et initient un phénomène de destruction des tissus parodontaux. Ces micro-organismes sont alors appelés « espèces associées à des maladies ». Des facteurs environnementaux peuvent modifier la composition du microbiome. Le tabagisme et le diabète sont des principaux facteurs extrinsèques qui façonnent négativement le microbiome en une forme plus agressive. Les personnes ayant un mauvais contrôle glycémique ou consommant des produits du tabac sont plus sujettes aux maladies parodontales car leur microbiome est riche en complexes bactériens qui affectent négativement le parodonte. L'effet de la consommation d'antibiotiques sur le microbiome est plus ou moins avantageux ; il a été observé que les antibiotiques n'affectaient pas tous et systématiquement la composition du microbiome.

Mots clés : microbiome – parodonte – gingivite – parodontite – tabagisme.

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Introduction

Periodontal diseases are one of the most common chronic infectious diseases worldwide. It is composed by a set of inflammatory conditions affecting the supporting structures of the teeth, initiated at the gingival level, and going deeper into the supporting components of the periodontium, the connective tissue and alveolar bone [1]. In severe cases, untreated periodontitis can lead to tooth mortality [2].

Several etiological factors are present for the development of periodontitis. The primary etiological factor is the presence of subgingival bacteria and the spread of their toxins. Microbiological studies have identified around 150 to 800 different species present in the dental plaque [3]. Unfortunately, none of these 800 species is considered particularly virulent to initiate the onset of periodontal disease, but rather the entire microbiome as a whole [4].

Tissue destruction observed in periodontal diseases is not a consequence of dental plaque or poor oral hygiene, but rather the reaction to this stimulus, in other words, host response. It is now clear that periodontal tissue destruction is a consequence of an upregulated host response stimulated by the presence of dental plaque and microbial profiles. Therefore, periodontitis can be regarded as a "dysbiotic state", defined as a condition in which the balanced state of the ecosystem is perturbated [5]. Disruption of the finely tuned equilibrium microbial ecosystem can be caused by local, environmental, and genetic factors, which are mentioned later in this review.

Conversely, in periodontal health, a balance between "good" and "bad" bacteria is present in the biofilm, hence, the microbiome and the host coexist in a "symbiotic state" where colonization of pathogenic microorganisms is prevented, resident bacteria contribute to host physiology and homeostasis, and microbial attacks are well controlled by the host response, thus, maintaining a state of health. On the other hand, when the bacterial balance is disturbed, a dysbiotic state is initiated, and the attacks may be too strong for the host to control them, hence initiating a host response dedicated for tissue destruction [6]. In general, the dysbiotic state is considered either the cause of periodontal homeostatic disruption mechanism, which leads to an increased inflammatory reaction resulting in periodontal tissue breakdown, or, according to an alternative pathway, the inflammatory changes act as an environmental stress that induce a bacterial dysbiosis which lead to periodontal destruction [7]. Therefore, periodontal disease is either regarded as a polymicrobial perturbation of the host homeostasis, or inflammation-driven disruption of the periodontal microbial homeostasis. In both case scenarios, this leads to a subgingival dysbiosis and host-mediated periodontal tissue loss. Hence, the shift between symbiosis and dysbiosis is primary related to the interplay between the subgingival biofilm and the host immune response and secondary related to other local, environmental, and genetic factors [8].

The newer dysbiosis concept allows an ecological view on the microbial changes, suggesting that periodontal therapy could re-establish an eubiotic host-microbial area. The newer concept was retrieved from the information that the lack of host-compatible organisms, with the presence of pathogenic organisms in the dental biofilm could be an important triggering factor for disease progression. Nevertheless, to this day there is still no clear definition for the microbiome shift in terms of bacterial quantity needed to shift the environment from a symbiotic to a dysbiotic state.

Although bacterial species with different pathogenic effects in the biofilm were previously described [9], the role of aggregate microbial community in the biofilm is more important that the individual constituents [10]. Thus, to further increase bacterial determinant in periodontitis and the contribution of the microbiome, it is more important to study the bacterial communities in susceptible hosts that individual bacterial species. We aimed in this literature review to summarize the available evidence related to microbiome changes from health to gingivitis to periodontitis, and state the factors that negatively affects the equilibrium state of health.

Healthy microbiome: What is the norm?

The oral microbiome is diverse which facilitates various functions for the human body such as, development of mucosal immunity, food digestion, and tissue homeostasis. The nature of the microbiome and its composition is not static over time due to the multiple endogenous and exogenous perturbations [11]. Its composition represent a cocktail of bacteria, archea, fungi, protozoa, and viruses. Some microorganisms are strong enough to live independently, but others lack the genetic code for essential functions, this makes them dependent on other organisms for growth, either by growing close to other bacteria in an epibiotic state or demonstrating frank parasitism by invading the cells of other bacteria [12].

Profiling studies have identified over 300 different bacterial specie in a single human microbiome at any period, revealing that these species are stable over time, to the point that they can be used in forensic dentistry in identifying different individuals [13]. On the other hand, although different individuals present different microbiome composition, broad functional similarities were present between different bacterial communities [14].

In a healthy state, the oral microbiome presents itself in a well-balanced dynamic system [15]. Early microscopic studies of healthy subgingival communities revealed that gram-positive cocci and rods are numerically dominant [16]. A cultivation study performed by Moore and Moore showed that Actinomyces naeslundii were the most frequently reco-

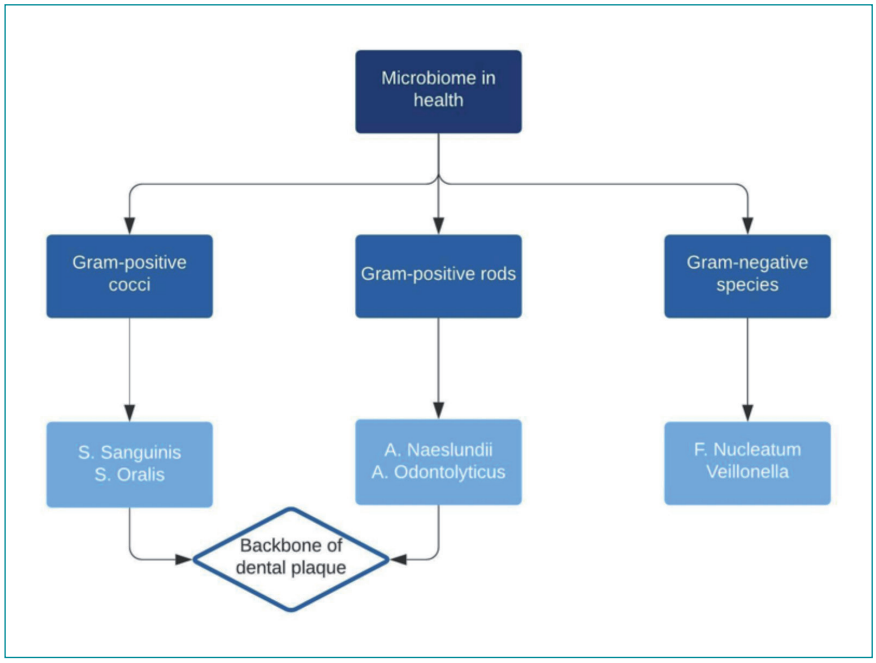

Fig.1: Microbiome in health.

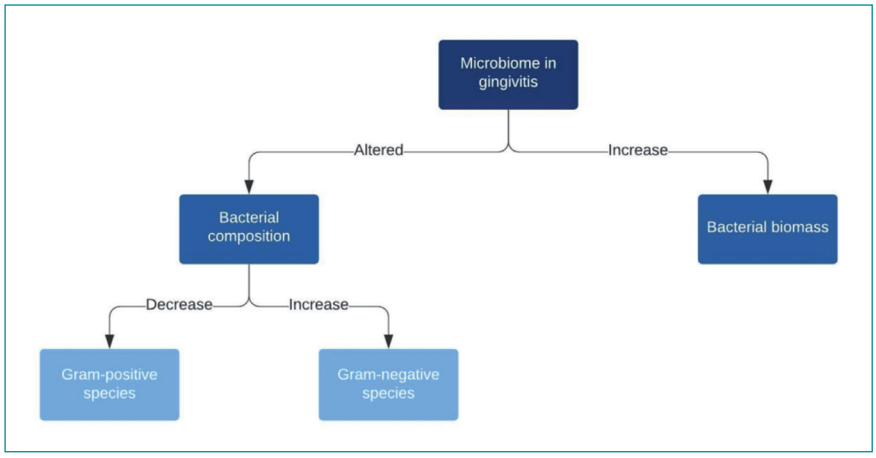

Fig.2: Microbiome in gingivitis.

vered subgingival species in health [17]. Other Actinomyces species such as A. meyeri and A. odontolyticus also appeared in large numbers in healthy communities [18]. In general, Actinomyces species are gram-positive rods, which colonize in early stages of tooth eruption. They also can coaggregate with other microorganisms such as Streptococcus, together they form the backbone of dental plaque [19]. Other cultivation studies of subgingival plaque in health identified gram-positive Streptococcus sanguinis, S. oralis, and S. intermedius [17]. On the other hand, gram-negative species are also present in health, such as F. nucleatum, Veillonella species, and Capnocytophaga species, which are important components of periodontal-free sites.

In summary, subgingival biofilms in health comprise gram-positives and a few but numerically abundant gram-negative species. These taxa have the potential to organize into spatially arranged consortia in which specific species physically and metabolically interact (Fig. 1).

Alteration in microbiome: Gingivitis

In the majority of individuals, when the microbial challenge interacts with the immune response of the host, a balanced, proportionate response characterized by reversible and minimal tissue inflammation with no tissue loss occurs. This interaction is orchestrated by endogenous and exogenous factors of the individual host. When these factors are not controlled, the outcome of the interaction between the microbial profiles and host response leads to a deregulated reaction characterized by an increased inflammatory response, elevated phagocytic cell recruitment, and increased gingival crevicular fluid flow. This results in an altered signaling response to the bone and connective tissue compartment, leading to the apical migration of junctional epithelium, epithelial ulceration, and bone loss [12].

Concerning the change of microbial profile form health to gingivitis, microbiologic cultivation studies have shown that a shift into the dominant species occurs in the subgingival community. An increased number of Gram-negative morphotypes, such as, rods, filaments, and spirochetes occur after 2-3 weeks of plaque accumulation [20]. Other species such as Prevotella, F. nucleatum, Tannerella, and Selenomonas were increased proportionately after experimental plaque accumulation [21]. These bacteria are able to over stimulate the levels of inflammatory mediators such as IL-1α, IL-1β, and lactoferrin in gingival crevicular fluid. These changes correlate to the clinical findings of gingival inflammation, redness, swelling and bleeding [20].

As discussed previously gingivitis-associated species increase in number, but on the other hand, gram- positive health-associated species decrease in abundance. R. dentocariosa was significantly decreased from 25% to 2% in the gingivitis communities. Other species such as Propionibacterium and Stenotrophomonas maltophila were decreased too [20].

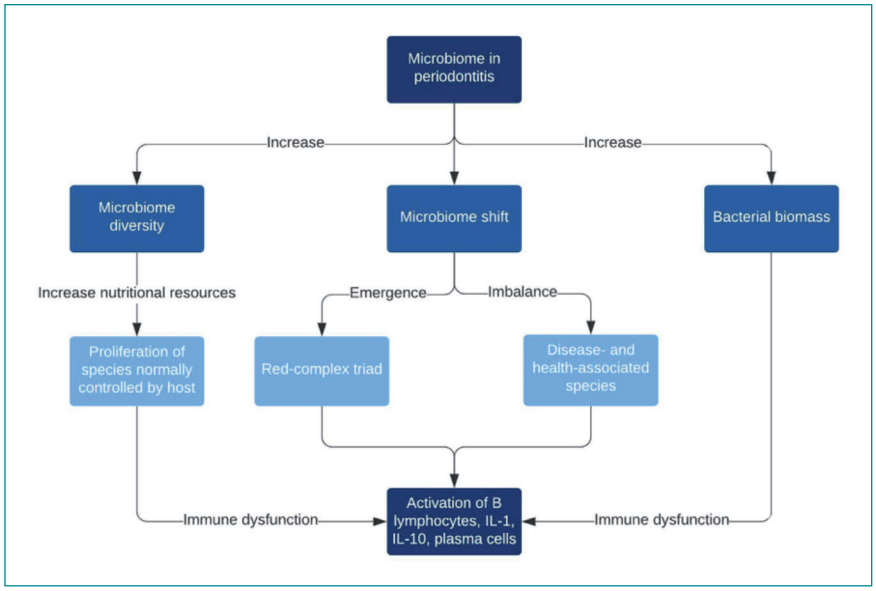

Fig. 3: Microbiome in periodontitis.

Finally, not only an increase in bacterial proportion occurs, but also bacterial biomass increases too. A 3-log increased in bacterial biomass was observed from health to gingivitis. It has been suggested that the influence of increased biomass is much greater that in the increased in bacterial proportions [20].

In summary, the development of gingivitis occurs concomitantly with an increase in bacterial biomass and a large shift in the composition of subgingival communities with depletion of gram-positive species and enrichment of gram-negatives (Fig. 2).

Alteration in microbiome: Periodontitis

During the development of periodontitis, a shift in the composition of bacterial communities occurs, with the development of different species to those enriched in health or gingivitis [22]. Several drivers could play a role in this microbial shift. Firstly, the ability of certain microorganisms to weaken the immune response and induce an increased inflammatory reaction could be responsible for this shift. Porphyromonas gingivalis act as a "keystone" in the development of periodontal disease. It initiates a dysbiosis state between the host and the microbiome by evading the immune response and triggering and maintaining an inflammatory reaction [23]. Secondly, the nature of the oral cavity could be considered inflammophilic [24]. Periodontal pathogens are always present in the oral cavity, whether in a state of health or disease. They could be responsible for triggering a persistent, but low, inflammatory reaction. This inflammatory response could act as protected site for inflammophilic organisms in which they can colonize [23]. Lastly, it has been shown that the subgingival ecosystem in periodontitis represents a site of immune dysfunction which facilitates the proliferation of species which were normally controlled by the host defense system [25].

Socransky grouped different species into different complexed by using DNA-DNA hybridization technique. Species in the red complex triad, which are composed of P. gingivalis, B. forsythus, and T. denticola, exhibited a very strong relationship with pocket depth, their prevalence increased with increasing pocket depth. Likewise, the orange complex triad such as F. nucleatum, P. intermedia and P. nigrescens, were present in increased pocket depth. It was observed that sites with the absence of the previously mentioned bacteria had the shallowest pocket depth and vice versa [22]. On the other hand, the red complex triad has been shown to play a role in the disruption of homeostasis through inhibition of IL-8 and toll-like receptor 4 signal regulation [26].

Concerning the health-associated species, two Actinomyces species such as, Rothia spp., and S. sanguinis appear to be the main taxa depleted [27]. Other studies have identified that in periodontitis, health-associated species are significantly increased compared to their number in periodontitis-free sites, but this increase is overwhelmed by the simultaneous increase in the number of periodontitis-associated species. The rise in the number of health-associated species in periodontitis is still not fully understood [20]. It is interesting to mention that health-associated species are still present in periodontitis, and diseased-associated species are present in a healthy state, which emphasize the principle that dysbiosis is due to the changes from the dominant species rather that new colonization periodontitis-associated species [28].

Similar to gingivitis, an increase in bacterial biomass occurs in periodontitis. In health, the disease-associated species comprise about 5% of the total biomass, while in periodontitis sites, the biomass of these species increase to 50%, showing a 4-log increase in their load [29]. The increase in bacterial biomass is relatively related to the increased proportions of archaea. They have the ability to remove the hydrogen from the environment, thus creating more thermodynamically favorable conditions for anaerobic bacterial growth [20].

In conclusion, a shift in microbiome quality and quantity occurs in periodontitis. The emergence of the red-complex triad initiates a dysbiotic state which stimulates an immune response that negatively affects the supporting structures. On the other hand,

health-associated species are depleted which can no longer go back to the symbiotic state of health. Lastly, the increased biomass of microbes plays another mechanistic pathway to further deteriorate the condition (Fig. 3).

What are the metabolic changes from health to disease?

Periodontitis sites possess an increased pathogenic activity compared the periodontal-free sites. Dabdoub et al. demonstrated that periodontitis communities have upregulated genes encoding for lipid-A biosynthesis, iron acquisition, and antibiotic resistance. The study also showed increased virulence factor in the genome to 33.1% compared to 8.9% in health [30]. A technology called metatranscriptomic RNA sequencing allows the evaluation of difference in metabolic activities in specific species in different communities. Comparing metatranscriptome between health and periodontitis, periodontally affected sites showed increased gene transcripts related to flagellar motility, peptide transport, and beta-lactam degradation [31].

Core species are organisms which do not change in proportions from health to disease. It has been shown that the core specie F. nucleatum upregulates lysin fermentation pathway which correlates to the increased anaerobiosis of periodontal pockets [32]. The presence of red-complex species further activates metalloproteases, peptidases, and proteins involved in iron metabolism, indicating that P. gingivalis, B. forsythus, and T. denticola present important virulence factors. Interestingly, transcriptional profiles of health-associated species, such as, streptococcal species, could undergo a more pathogenic status. Comparing baseline vs diseased sites, these species had upregulated virulence factors [33].

In conclusion, periodontally-affected sites possess increased metabolic activity compared to perdiodontal-free sies. Periodontal pockets occupy a different microbiome, qualitatively and quantitively, this change causes increase gene activity related to soft and hard tissue destruction.

Factors inducing dysbiosis

Several factors negatively affect the symbiotic balanced state of the microbiome, some are endogenous factors and others, which play a greater role, exogenous factors. The first line of defense against bacterial attacks is the gingival crevicular fluid (GCF) at the gingival crevice. Upon microbial accumulation, the GCF triggers a host response resulting in an increased flow from adjacent tissues into the sulcus [34]. It has been shown that GCF efflux is gradually increased from health to gingivitis to periodontitis. The increased flow of GCF results in increased proteinase-rich taxa, which are associated in periodontally-affected sites [35]. The second endogenous factor is the presence or absence of oxygen. Oxygen plays a crucial role in the composition of the microbiome. Healthy and gingivitis communities have a high tolerance to oxygen, while on the contrary, periodontitis communities cannot tolerate the presence of oxygen. Hence, oxygen plays a role in defining the qualitative nature of the periodontal microbiome [36].

The magnitude of many bacterial species is primary affected by the interactions with other bacteria, and secondary by environmental factors such as, tobacco smoking, diabetic control, antibiotics, and antimicrobials. These factors either upregulate bacterial activity or downregulate it. A dynamic balance state between the host and its microorganism determines a state of health [37].

What are tobacco effects?

50 years ago, Pindborg reported detrimental effect on the oral cavity in smoker individuals [38]. Ever since, numerous studies reported clinical, biochemical, and microbiologic findings linking tobacco products with the severity of periodontal disease. The Pathogenic bacteria in smokers possess a stronger bond towards epithelial cells, hence faster colonization, and aggregation, leading to a qualitative and quantitative shift the microbiome, increasing its pathogenicity, and decreasing the protective effect of the host. The nicotinic effect decreases local oxygen tension, leading to increased growth of anaerobic bacteria. Lastly, smokers show less pocket depth reduction than non-smokers after non-surgical periodontal therapy [37].

Smokers have increased susceptibility to be infected with red-complex bacteria [39]. A study showed that smokers had 2.3 times greater risk to be infected with T. forsythia and P. gingivalis compared to non-smokers [40]. Not only red-complex bacteria are increased but also other complexes. A retrospective study by Haffajee and Socransky proved that smokers had increased bacterial species from the orange complex in periodontal pockets >4mm compared to non-smokers [41]. This increased susceptibility revealed that smokers had different colonization over 7 days compared to non-smokers. Early biofilm-forming pathogens were observed in the supra- and sub-gingival microbiome of smokers [42].

In conclusion, these findings suggest that tobacco products decrease the ability of the periodontal microbiome to reset itself and go back to the symbiotic state, hence, decreasing the resistance to future diseases and increasing tissue loss due to the continuous host stimulus [43].

Diabetes and microbiome alteration

Diabetes have long been known to be a major risk factor for the initiation and progression of periodontal disease, described as a bidirectional adverse relationship, meaning that worse the diabetic control contributes to worse periodontal clinical findings, and vice versa [44]. Studies have shown that periodontally healthy diabetic subjects possess increased prevalence of periodontal pathogens from the red and orange complexes. Lower health-associated species and higher disease-associated species such as gram-positive and gram-negative

anaerobic organisms were observed in periodontal healthy diabetic subjects. This framework indicates that even if diabetic subjects were periodontally healthy, they possess an altered microbiome that would be ready to initiate an immune response to cause periodontal destruction [45].

Concerning the metabolic control in periodontally affected individuals, normoglycemic and diabetic individuals showed distinct periodontal microbiome [46]. The degree of metabolic control has been proven to shape the microbiome communities. This is due to the fact that fermenting species such as, Streptococcus anginosus and Filifactor alocis, have increased glucose availability essential for their growth [47].

This concludes that diabetic individuals, whether controlled or uncontrolled, possess an altered microbiome community in which the less traditional pathogenic bacteria could initiate a periodontal response devoted for tissue loss. This could explain the fact that diabetic individuals have increased risk to develop periodontal disease.

Antibiotics: Do they change the microbiome composition?

Most of the clinical and microbiological studies showed a significant benefit of using adjuvant systemic antibiotics with non-surgical periodontal therapy, even though their long-term significance is still debated [48]. The effectiveness and their use have been questioned due to the risk of developing a bacterial resistance. On the other hand, a proper guideline to instruct clinicians on when and how to use adjunct antibiotics is still absent [49]. A recent systematic review was conducted by Dilber et al. in 2020 to address this problematic. 30 clinical studies were allocated in this study with low level of bias. The results showed no noticeable differences between antibiotic and placebo for the antibiotics: clarithromycin, roxithromycin, and the combination of amoxicillin and clavulanic acid. After 6 months, there was also no difference in the number of reduced genera between azithromycin and placebo [50].

On the other hand, Hagenfeld et al. studied subgingival samples of 89 patients with chronic periodontitis before and two months after non-surgical periodontal therapy. One of the two groups received orally administered amoxicillin and metronidazole (500 and 400 mg respectively 3 times per day for 7 days). Adjunctive antibiotics were able to induce a microbiome shift by statistically reducing disease-associated species such as, P. gingivalis, T. forsythia, T. denticola, and A. actinomycetemcomitans, and noticeably increasing genera containing health-associated species. On the other hand, mechanical therapy alone did not statistically affect any disease-associated taxa [51].

Lastly, a study performed by Morales et al. was conducted to evaluate the effect of probiotic, antibiotic, and placebo effects on periodontal microbiome after non-surgical periodontal therapy. 47 systemically healthy volunteers with chronic periodontitis were recruited and monitored clinically and microbiologically using Polymerase chain reaction, at baseline, 3, 6 and 9 months after therapy. The results showed that adjunctive use of probiotic L. rhamnosus SP1 sachets and azithromycin during initial therapy resulted in similar clinical and microbiological improvements compared with the placebo group [52].

In summary, it was found that bacterial changes occur in both situations, i.e., after non-surgical periodontal therapy with and without systemic antibiotics. The results suggested that the microbial dynamics after therapy are similar with and without antibiotics and might be only emphasized in single bacterial genera by adjunctive antibiotics.

Conclusion

The symbiotic microbiota in health is dominated by health-associated species (green-complex) and low abundances of species associated with gingivitis (orange-complex) and periodontitis (red-complex). Gingivitis is characterized by an increased biomass comprising both green and particularly orange species and an associated increase in inflammation. In periodontitis, biomass is further increased, and the red-complex species become increasingly dominant in the dysbiotic microbiota. Furthermore, the gene expression profiles of the green and orange species are modified with increased expression of virulence determinants. This is accompanied by the development of a deregulated inflammatory response and tissue destruction. The microbiome shift is accompanied by several environmental factors. It has been proved that tobacco products and the metabolic level of a diabetic subject could further shape the microbiome into a highly active metabolic community. Taken together, the inflammatory response can contribute to microbiome changes and expression of bacterial virulence factors. Active control of excess inflammation can positively impact management of dysbiosis and periodontitis.

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