Microbiological Approach to Infections Derived from Periodontal Microbiome

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Abstract

In modern periodontics microbiological diagnostic approach and methods provide better understanding in periodontal microbiome including the impact of its pathogenetic properties to the development of the disease and it is still developing. The aim of this review is to discuss the recent advancement and knowledge in periodontal microbiome and clinical application of microbiological diagnostic methods.

Keywords: Periodontitis; Peri-Implantitis; Microbiology; Periodontal Microbiome

It is considered that different bacteria play a key role in the development of oral disease particularly periodontal disease and peri-implantitis as well as peri-implant mucositis. The bacterial etiology is rather complex with polymicrobial communities involved. Different authors in the field found out that there are hundreds of bacterial species which are not cultivated in everyday diagnostic routine procedures in the human oral cavity meaning that the role of oral microbiome in health and disease is not yet fully understood. Besides bacteria, there are many factors that may contribute to the pathogenic process of these diseases such as genetic, epigenetic, proteomic and metabolic factors. Smoking, stress, poor oral cavity hygiene, systemic diseases, HIV infection, immunosuppressant medications, anticoagulants or steroids and biofilm infections are well known epigenetic factors which may strongly influence changes in tissue behavior and consequently enhance development of periodontal disease, peri-implantitis and peri-implant mucositis. Furthermore, infection and inflammation of periodontal tissue could reach distant sites via bloodstream and contribute the development of several systemic diseases. It is well known that individuals suffering from periodontal disease have a higher risk of developing diabetes, blood vessel disease such as atherosclerosis, stroke or heart attack, chronic obstructive pulmonary disease or in pregnancy premature delivery of a low birth weight infant. Biofilm infection is caused by different microorganisms with their specific pathogenic properties which may influence to the inflammatory process like their metabolic and toxic products as well as necrotic cells. It is understood that host response, immune and inflammatory responses are playing the critical role to the pathogenesis of such oral diseases. The innate immune system is usually activated by initial local inflammatory reaction that is caused by bacteria which colonize tooth surface and gingival sulcus. The initial localized response is amplified by release of many different cytokines and mediators that propagate inflammation through gingival tissues. The cytokines have been recognized as cell products of different kinds of cells such as leukocytes, resident mesenchymal cells (fibroblasts and osteoblasts), dendritic cells and endothelial cells and they are produced by many cell types in periodontium. Herpes viruses, particularly Cytomegalovirus and Epstein-Barr virus are more frequently found in periodontal and gingival lesions than in periodontally healthy sites. It seems that infection with these two viruses due to the variety of their properties may impair immune defenses of periodontal tissue thus permitting the overgrowth of periodontopathogenic bacteria. Haraszthy and his colleagues reported that in 44% of atheroma at least
one of periodontopathogenic bacteria could be found [1]. Traditionally, these are non-spore forming, obligately anaerobic, Gram-negative, rod-shaped bacteria and only a few of them are considered to be involved in these infections. Experts in the field who gathered at the 1996 World Workshop in Periodontics concluded that bacteria involved in the etiology of periodontal disease are Aggregatibacter actinomycetemcomitans (previously Actinobacillus actinomycetemcomitans), Porphyromonas gingivalis, Tannerella forsythia (previously T. forsythenis) and Treponema denticola. According to them these bacteria and some other like Prevotella intermedia, Prevotella nigrescens and Fusobacterium nucleatum which may also be included into the pathogenic process are divided in three groups depending on their pathogenic potential named red (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia), orange (Prevotella intermedia, Treponema denticola) and yellow (Prevotella nigrescens, Fusobacterium nucleatum, Parvimonas micra, Eikenella corrodens) complex of subgingival and supragingival plaque. Each of these complexes demonstrates a certain level of bacterial pathogenic properties. Generally, the four periodontal pathogens have been identified in the peri-implant sulci of healthy implants of partially edentulous patients but not around implants in completely edentulous patients. But accumulated evidence shows that periodontal pathogens can survive at non-dental sites a long period of time, from which they further colonize the peri-implant sulcular [2]. Because implantation may alter the composition of the periodontal microbiome it is very important that periodontal health status of these patients should be carefully monitored before implantation. Several studies have showed that periodontopathogenic bacteria have developed resistance to usually administered antibiotics in therapy of these infections. The main mechanism of resistance is the production of β-lactamases by Prevotella, Porphyromonas and Fusobacterium strains to penicillin and amoxicillin. Also an increase in resistance to piperacillin, clindamycin, metronidazole and ciprofloxacin has been noticed. Moreover the rate of resistant strains is not equally distributed in all parts of the world. That is why susceptibility testing may be needed in individual patient therapy. CLSI (Clinical and Laboratory Standard Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines recommendations are that susceptibility testing should be done in case of long-term therapy, previously failed treatment, when choice of antibiotic is critical for the therapy or when the susceptibility model is unpredictable. In order to gain these goals isolation, identification and susceptibility testing should be done. All these laboratory procedures are time consuming, expensive and very complex. Usually, there are two microbiological approaches: cultivation on nutrition media or molecular methods, commonly PCR. For the bacterial culture a transport media for anaerobic bacteria should be used. Cultivation may take up to seven days, which is followed by identification and eventually susceptibility testing. Contrary by PCR testing of bacteria or viruses can be detected depending on the primer. Comparing detection of periodontopathogenic bacteria by bacterial culture versus PCR Urban and his colleagues have find out that the overall agreement between both methods was excellent for Eubacterium nodatum, Tannerella forsythia and Porphyromonas gingivalis (97 - 92%), fair for Capnocytophaga sp, Eikenella corrodens, Actinobacillus actinomycetemcomitans and Prevotella intermedia (91 - 89%) and poor for Fusobacterium nucleatum, Parvimonas micra (Micromonas micros) and Campylobacter rectus (86 - 78%). Discrepancies in the results may be explained by inability of culture method to distinguish between closely related taxa (e.g. P. intermedia/Prevotella nigrescens), and problems of keeping periodontopathogen bacteria viable, which is required for successful detection by standard culture method. They have concluded that cultivation may be substituted by molecular methods in microbiologic diagnostics of progressive periodontitis when there is no possibility of cultivation in routine microbiological laboratory [3]. Species that are phenotypically closely related are very hard to be distinguished even though some laboratories tried to differentiate them not only by biochemical tests but also with gas-liquid chromatography. Some other methods have also been used such as ribotyping, multiplex polymerase chain reaction or sodium dodecyl sulfate-poly-acrylamide gel electrophoresis. All of them are time consuming and some of them exhibit low level of reproducibility. However, there is a newly developed matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) analysis capable of distinguishing these phenotypically-indistinguishable species which are differently distributed in regions around the world [4]. The microbial composition of subgingival biofilm of implant patients or periodontal disease should be assessed in order to formulate adequate prevention measures and more specific treatments.

**Conclusion**

Besides genitic, epigenetic, proteomic and metabolic factors, periodontal microbiome plays an important role in periodontal tissue inflammation as well as in contribution the development of several systemic diseases. In this review the gradual improvement of microbiological diagnostic approach is showed. The better understanding of periodontal microbiome may contribute to the development of more adequate prevention measures and more specific treatments.

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