MICRO BIOLOGY OF PERIODONTAL DISEASE- A REVIEW

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Abstract:

Periodontal diseases are inflammatory and destructive diseases of the dentogingival complex associated with specific periodontal pathogens inhabiting periodontal pockets. Periodontal diseases lead to damage of the periodontal tissues supporting the teeth (bone and connective tissue) and affect the quality of life of the affected individuals: poor alimentation, tooth loss, social and financial problems. Although it is generally considered that the disease has multifactorial etiology, data show that some specific Gram-negative microorganisms in the subgingival plaque biofilm play a major role in the initiation and progression of periodontitis. *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* form a consortium in the subgingival biofilm and are regarded as the principal periodontopathogenic bacteria. Other microorganisms that have been implicated as predominant species in the disease process are: *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Campylobacter rectus*, *Peptostreptococcus migros*, *Eikenella corrodens*. In periodontitis, the initiation of the disease is the colonization of the tissues by these pathogenic species. The next step is bacterial invasion or invasion by pathogenic products into the periodontal tissues, interactions of bacteria or their substances with host cells, and this directly/indirectly causes degradation of the periodontium, resulting in tissue destruction.

Keywords: periodontal disease, periodontal pathogens, microbiology.

Introduction:

The research on the microbiology of periodontal health and diseases has been the focus of intense investigation for several decades. Microbial biofilm in the gingival sulcus around teeth is the most important cause of the periodontal diseases. It is a structured community of bacterial cells enclosed in a self produced polymeric matrix.[1] Although more than 700 different species are present in subgingival microbiota [2-4] only a few of these species are actually involved in the initiation and progression of the periodontal disease process. Moreover, research on the etiopathogenesis of periodontal diseases has suggested the role of environmental behavioral[6-

7], and [8] risk factors in periodontal disease progression but most, if not all forms of periodontitis, should be viewed primarily as infectious diseases. Many technological advances have occurred in molecular techniques in the last few decades which have provided us the capability of performing high- throughput analysis of a large number of samples, circumventing some of the limitation of culturing techniques.

Historical Aspect

Dental plaque the period from 1880 to 1930 is known as 'Golden age of microbiology'[9]. Wet mounts or stained smear. Scientists identified four different groups of potential etiologic agents

(amoebae, siprochetes, fusiforms and streptococci) for various periodontal diseases using the techniques available at that time (wet mounts or stained smear microscopy). Researchers suggested the specific plaque hypothesis based on these findings. However with the advancements in bacterial identification techniques, many other bacterial species were identified in dental plaque derived from periodontitis patients. Studies conducted between 1930 and 1970 failed to identify any specific microorganism as the etiologic agent of periodontal diseases which leads to the proposal of non-specific plaque hypothesis, according to which gross accumulation of dental plaque would be necessary and sufficient to cause periodontitis.

Later on, with the advancement in the field of microbiology, immunology and molecular biology, numerous studies concluded a putative pathogenic role of many bacteria, mainly gram negative species in the etiopathogenesis of the

diseases. These species include *A. actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Campylobacter Rectus*, *Fusobacterium nucleatum*, and *Treponema denticola*.

Virulence Factors produced by these micro organisms have been identified and their role in the periodontal destruction is well established.

BACTERIAL TAXONOMY

Systemic classification and categorization of organisms into ordered groups is called taxonomy. A working knowledge of bacterial taxonomy is essential for understanding various bacterial species involved in the periodontal disease progression. Although higher organisms are classified according to their evolutionary pathways (i.e. phylogenetically), bacteria cannot be similarly categorized because of insufficiency in their morphological features.

Bacterial classification according to phenotypic features-
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Morphology – (Cocci, bacilli, Siprochetes)

Staining properties – (Gram- positive, Gram- negative etc.)

Spore formers or non –spore formers.

Cultural requirements- (Aerobic, Facultative, Anaerobic, Anaerobic etc.

Biochemical reactions (saccharolytic and asaccharolytic according to sugar fermentation reaction)

Antigenic structure (serotypes)

Establishing an organism as periodontal pathogen.

- Periodontal diseases are caused by a variety of micro- organisms that reside at or below the gingival margin in the form of plaque biofilm. Because there is a complex microbiota involved in the formation of plaque biofilm, first we need to distinguish the pathogenic bacterial species from the host compatible species.

- Secondly, we need to devise treatment methods that can decrease the pathogens while maintaining host – compatible species.

- To establish an association of an organism with disease pathogenesis, three rules for experimental proof for the pathogenicity of an organism were presented in 1883 by the German bacteriologist, Robert Koch, and a fourth rule was appended by smith (1905)[10]. These rules of proof are often referred to as Koch's Postulates.

These are,

The suspected casual organism must be constantly associated with the disease.

The suspected casual organism must be isolated from an infected host and grown in pure culture.

When a healthy susceptible host is included with the pathogen from pure culture, symptoms of the original disease must develop.

The same pathogen must be re- isolated from host infected under experimental conditions.
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However, periodontal diseases are not caused by a single organism which is the cornerstone of Koch's postulates. To better identify periodontopathogenic bacterial candidates Koch's postulates were replaced with Socransky's postulates[11].

These include the following:

The organism must be found in relatively high numbers in proximity to the periodontal lesion.

The organism must be absent, or present in much smaller numbers in periodontally healthy subjects or in subjects with other forms of periodontal disease.

The organism must have high level of serum, salivary and gingival crevicular fluid antibody developed against it in periodontally diseased subjects.

The organism must be found to produce virulence factors in vitro, which can be correlated with clinical histopathology.

The organism must mimic similar pathogenic properties in an appropriate animal model.

Clinical improvement following treatment must eliminate the putative pathogen from the periodontal lesion.

SUBGINGIVAL MICROBIAL COMPLEXES

In a landmark study, Socransky et al. (1998) examined over 13000 subgingival plaque samples from 185 adult subjects and used DNA hybridization methodology and community coordination techniques to demonstrate the presence of specific microbial groups within the dental plaque.

1st complex (Red complex): *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola*.

2nd complex (Orange complex): *Eubacterium nodatum*, *Campylobacter rectus*, *Campylobacter showae*, *Streptococcus constellatus*, and *Campylobacter gracilis*.

3rd complex (Yellow complex): *Streptococcus sanguis*, *S. oralis*, *S. mitis*, *S. gordonii*, and *S. intermedius*.

4th complex (Green complex): *Campylobacter concisus*, *Eikenella corrodens*, and *Actinobacillus actinomycetemcomitans* serotype a.

5th complex (purple complex): *Veillonella parvula* and *Actinomyces odontolyticus*.

Figure 1: The subgingival microbial complexes as proposed by Socransky et al (1998)

PERIODONTAL CONDITION AND ASSOCIATED MICRO-ORGANISMS

Periodontal Health	Streptococcus oralis, Streptococcus sanguis, Streptococcus mitis, Actinomyces gerencseriae, Actinomyces naeslundii, Fusobacterium species, Prevotella nigrescens, Veillonella species.
Gingivitis	Lactobacillus species, Actinomyces naeslundii, Peptostreptococcus micros, Streptococcus anginosus, Fusobacterium nucleatum, P. intermedia, Winonalla parvula, Campylobacter species, Haemophilus species, Selenomonas Species, Treponema species.
Periodontitis with slow/moderate rate of progression (Grade A/B)	Eubacterium brachy, Eubacterium nodatum, Mogibacterium timidum, Parvimonas micra, Peptostreptococcus stomatis, Parvimonas micra, Tannerella forsythia, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Prevotella loescheii, Dialister pneumosintes, Campylobacter rectus, Treponema species.
Periodontitis with rapid rate of progression (Grade C)	Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Prevotella nigrescens, Eikenella corrodens, Selenomonas sputigena, F. nucleatum, Campylobacter rectus, Peptostreptococcus micros, Campylobacter concisus.
Refractory periodontitis	Aggregatibacter actinomycetemcomitans, Streptococcus constellatus/intermedius, Tannerella forsythia, P. gingivalis, T. denticola, Campylobacter rectus, Eikenella corrodens.
Necrotizing ulcerative gingivitis/periodontitis	Prevotella intermedius, Treponema species, Selenomona species, Fusobacterium species, Candida species.

Periodontal pathogen, and their virulence factors, their actions:

Microorganism	Adhesion/Colonization	Virulence factors	Actions
<i>Aggregatibacter actinomycetemcomitans</i>	Pilli Vesicle Capsular antigen	<ul style="list-style-type: none"> Leukotoxin Cytolethal distending toxin (CDT) Fc binding protein Collagenases LPS Proteases CagE-homologue OMP-1 	<ul style="list-style-type: none"> Kills neutrophils, lymphocytes and monocytes Apoptosis of lymphocytes. Deactivation of Ig's Disintegration of collagen Potent stimulator of IL1, PGE2 and TNFα Cleavage of Ig's. Host cell cytotoxicity Induce apoptosis of epithelia, endothelia, osteoblasts, and lymphocytes; activate CD4+ T-cell-mediated immune response associated with osteoclastogenic activity Induce IgG and CD4 + T-cell-mediated immunity

Microorganism	Adhesion/Colonization	Virulence factors	Actions
<i>Aggregatibacter actinomycetemcomitans</i>	Pilli Vesicle Capsular antigen	<ul style="list-style-type: none"> OMP-100 GroEL (Hsp60) 65-kDa protein Surface proteins (SAM: 14–79 kDa) 	<ul style="list-style-type: none"> Bacterial adhesin, invasin, and serum resistance factor; induce host cytokine production. Stimulate pro-inflammatory cytokine release; modulate antibacterial immunity; stimulate bone resorption in vitro (via osteoclast activity) Modulate immune response by binding to IL-10R (CSIF) Stimulate protective immunity (via IgG activity)

Micro-organism	Adhesion/Colonization	Virulence factors	Actions
<i>P.gingivalis</i>	Pilli Vesicle Capsular antigen	<ul style="list-style-type: none"> Proteases (gingipains) LPS Hemolysins Volatile sulfur compounds Ammonia Indole 	<ul style="list-style-type: none"> Degradation of Ig, complement factors. Potent stimulator of IL1, PGE2 and TNFα Causes agglutination and lysis of erythrocytes. Host cell cytotoxicity Host cell cytotoxicity Host cell cytotoxicity

Micro-organism	Adhesion/Colonization	Virulent factors	Actions
<i>T. denticola</i>	Major surface protein (MSP) Fibronectin binding adhesins	<ul style="list-style-type: none"> LPS Proteases Dentilisin Major surface protein (MSP) Phospholipase C (PLC) H₂S and methyl mercaptan 	<ul style="list-style-type: none"> Potent stimulator of IL1, PGE2 and TNFα Degradation of extracellular matrix Pleiotropic effects. Crucial for the virulence of T.denticola Cytotoxic for a wide variety of cells Directly or indirectly damages tissue by hydrolysis of membrane phospholipids Cytotoxic effects that is primarily due to inhibition of cytochrome oxidases.

Micro-organism	Adhesion/Colonization	Virulent factors	Actions
<i>T. denticola</i>	Major surface protein (MSP) Fibronectin binding adhesins	<ul style="list-style-type: none"> • LPS • Proteases • Dentilisin • Major surface protein (MSP) • Phospholipase C (PLC) • H₂S and methyl mercaptan 	<ul style="list-style-type: none"> • Potent stimulator of IL1, PGE₂ and TNFα • Degradation of extracellular matrix • Pleiotropic effects. Crucial for the virulence of <i>T.denticola</i> • Cytotoxic for a wide variety of cells • Directly or indirectly damages tissue by hydrolysis of membrane phospholipids • Cytotoxic effects that is primarily due to inhibition of cytochrome oxidases.

Microorganism	Adhesion/Colonization	Virulence factors	Actions
<i>P. intermedia</i>	Bacterial surface adhesins	<ul style="list-style-type: none"> • Proteases • Hemolysins • LPS 	<ul style="list-style-type: none"> • Degrade matrix components, host cell receptors and Ig's • Lysis of RBC's • Potent stimulator of IL1, IL6 and IL8 release. Causes periodontal tissue destruction and alveolar bone resorption. T

VIRUSES

More recently, viruses including Cytomegalo, Epstein Barr, Papilloma and Herpes simplex have been proposed to play a role in the etiology of periodontal diseases, possibly by changing the host response to the local subgingival microbiota (Contreras & Slots 2000)[11].

Human Cytomegalo, Epstein Barr and Herpes simplex viruses were found more frequently in deteriorating periodontal sites than in control, stable periodontitis sites in the same subject (Kamma et al 2001)[12].

Figure 2: Herpesviruses in destructive periodontal disease.

FUNGI

Hannula J, Dogan B, Slots (2001) showed geographical differences in the subgingival distribution of *C. albicans* serotypes and genotypes and suggested geographic clustering of *C. albicans* clones in Subgingival samples of Chronic Periodontitis patients[12].

Reynaud AH (2001) found a weak correlation between yeasts in periodontal pockets[13].

Rubio NA et al (2015) also showed fungal invasion of connective tissue in patients with gingival-periodontal disease[14].

NEWER PERIODONTAL PATHOGENS:

<p><i>Bdellovibrio bacteriovorus</i></p> <p>are small flagellate, motile, G-ve obligatory predators which invade and kill other Gram-negative bacteria.</p> <p>It attacks <i>Aa.comitans</i>.</p>	<p><i>Dialister pneumosintes</i></p> <p>G-ve, Obligately anaerobic, rod-shaped.</p> <p>Implicated in periodontitis (Ghayoumi et al, 2002).</p> <p>Detected in 83% of patients having severe periodontitis (Contreras A. et al. 2000, Slots 2002)</p>	<p><i>Treponema lecithinolyticum</i></p> <p>Saccharolytic spirochaete.</p> <p>More pronounced in aggressive periodontitis. (Park et al 2002)</p> <p><i>T. socranskii</i>, <i>T. lecithinolyticum</i> good indicators for periodontitis. (Annette Moter et al 2006)</p>	<p><i>Filifactor alocis</i></p> <p>G+ve, obligately anaerobic rod.</p> <p>GAP /CP harboured <i>F. alocis</i>, it was rarely detected in the control group. (Schlafer et al 2010).</p> <p>Virulence properties that may enhance its ability to persist in periodontal pocket. (Wilson Aruni et al. 2011)</p>

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MICROBIAL DIAGNOSTIC TESTING METHOD DESCRIPTION PROS CONS:

METHOD	DESCRIPTION	PROS	CONS
Bacterial Culture	<ul style="list-style-type: none"> - Currently the gold standard. - Determines the presence of the different species 	<ul style="list-style-type: none"> - Evaluates the antibiotic susceptibility - Estimates the number of isolated bacteria 	-No culturable bacteria (<i>T. denticola</i> and <i>T. Forsythensis</i>)
Immunological diagnostic methods	<ul style="list-style-type: none"> - Direct immunofluorescence - Indirect immunofluorescence - Flow cytometry - Latex agglutination - Enzyme-linked immune absorbent assay (ELISA) 	<ul style="list-style-type: none"> - Used to establish the nature of the bacteria in the biofilm - Calculates the percentage of bacteria - Greater sensitivity and specificity 	Cannot be used to assess bacterial susceptibilities

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METHOD	DESCRIPTION	PROS	CONS
Enzymatic detection methods	<ul style="list-style-type: none"> - Doesn't detect bacterias directly, determines the presence of enzymes that bacterias produce - BANA (benzoil arginine naftil amida) 	<ul style="list-style-type: none"> - Enables detection of certain bacterial species capable of producing tripsin enzymes as a virulence factor 	<ul style="list-style-type: none"> - Low specificity and sensitivity - High concentrations of microorganisms
Molecular biology techniques of DNA and RNA	<ul style="list-style-type: none"> - Polymerase chain reaction (PCR): amplifies DNA strands 	<ul style="list-style-type: none"> - High specificity - Diagnosis of major periodontal pathogenic bacteria - Does not need living microorganisms 	<ul style="list-style-type: none"> - Limited bacterial quantification - Ease of contamination in the process. - Inability to provide information on the sensitivity to antibiotics

Conclusion

The results of current genome study projects of several periodontopathogens will provide detailed information about the etiology of periodontal diseases, and will likely show new possibilities for the treatment and prevention of periodontal diseases. In the near future, it is expected that the correlation between biofilm maturation and activation of specific genes of the inner microorganisms will be clarified at the molecular level.

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