M ICROBIAL BIODIVERSITY AND ANTIBIOTICS IN PERIODONTAL DISEASES. A LITERATURE REVIEW

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ABSTRACT

The Oral cavity is a reservoir of bacteria with high biodiversity, however, not all species can cause invasive diseases. Identification of pathogenic bacteria in the oral lesions is challenging because cultivation of specimens from this non sterile site invariably yields a diversity of bacteria, making associations between cultivated bacteria and particular oral lesions difficult. Periodontitis is a multifactorial disease which might have a systemic or hereditary component in addition to its bacterial aetiology. It is highly prevalent worldwide, affects 30-50% of the population in developed countries, but only ~10% present with severe forms. The microbial aetiology of periodontal disease has been proved since long time ago, approximately 500 species or more have been detected in the gingival sulcus, some of them are widely regarded as major pathogens in periodontitis. Periodontal microbiota are more heterogeneous than earlier believed, in dentistry, gram-negative organisms were considered to be the predominant bacteria in periodontitis; however, gram-positive organisms found in deep, diseased sites are proposed to be the most important pathogens in periodontitis recently. Using antibiotics in controlling periodontal diseases is essential, which either suppress the growth of microorganism or destroy them. Drugs used to treat periodontal disease can be used systemically or locally, however, some periodontopathic bacteria can develop resistance to certain antimicrobials specially in refractory periodontitis and aggressive periodontitis cases.

KEY WORDS: Periodontal pathogens, Periodontal disease, Microbial biodiversity, Antibiotic resistance

INTRODUCTION

Periodontitis is a highly prevalent, chronic microbial inflammatory oral disease, initiated by a microbial dental plaque biofilm, which has negative and profound impacts on many aspects of daily life. Periodontal diseases, which include gingivitis (where the inflammation is confined to the gingiva and is reversible with dental care) and periodontitis (where the inflammation spreads, and results in tissue destruction and alveolar bone resorption), are the most common types of disease in humans worldwide.∗ The current concepts of
etio-pathogenesis suggest that gingivitis is initiated due to the enzymatic effects and toxins released by the pathogenic microorganisms of the dental plaque biofilm. It is known that retention of plaque close to the gingiva is hastened by formation of supragingival dental calculus. This leads to plaque induced gingivitis which eventually progress to periodontal pockets. Severe periodontitis that threatens tooth retention affects 10–15% of adults in the majority of populations investigated and ranged from 1%, among 20- to 29-year-old, to 39%, among individuals >65-year-old while moderate periodontitis affects 40–60% of adults in all populations.²,³

The human subgingival environment is a complex environmental niche where microorganisms meet to form diverse biofilm communities that exist in close proximity to the host. Bacteria constitute the most abundant, diverse and ultimately well-studied component of this biofilm with about 500 bacterial taxa reported to occur in this niche. Approximately 500 species have been detected in the gingival sulcus, among them are Porphyromonas gingivalis (P. gingivalis) and Tannerella forsythia, which are widely regarded as major pathogens in periodontitis.⁴ Subgingival microbiota were classified into several complexes indicated by various colors; the colors (varying from red to yellow) have different connotations, with red being the most pathogenic and yellow being less invasive. Periodontal microbiota are more heterogeneous than earlier believed. In dentistry, gram-negative organisms were considered to be the predominant bacteria in periodontitis; however, gram-positive organisms found in deep, diseased sites are proposed to be the most important pathogens in periodontitis.⁵ As these periodontopathic bacteria grow in a complex, polymicrobial dental plaque biofilm, often exhibit altered phenotypes, such as increased antibiotic resistance.

The stable structural properties and close proximity of the bacterial cells within the biofilm appears to be an excellent environment for horizontal gene transfer, which can lead to the spread of antibiotic resistance genes amongst the biofilm inhabitants.⁶ But it worth to mention that not all bacteria are harmful to the host we have probiotics which are live microorganisms, when administered in adequate amounts, may confer a health benefit on the host. Probiotics consist of individual or multiple live bacterial species (such as lactobacilli and bifidobacteria).⁶,⁷ In this review the authors tried to highlight some important gram negative periodontopathic bacteria and frequently used antibiotics to control them.

How to classify oral microbes as periodontopathogens (Koch’s postulate)

Koch’s postulates are four criteria designed to establish a causative relationship between a microbe and a disease. If we apply this in relation with periodontal diseases we can formulate the following:

1. Specific bacteria are found in high numbers in periodontal pockets, whereas their numbers are low at healthy sites. Different species are associated with the different forms of periodontal disease.
2. The organisms show periodonto-pathogenicity in animal models.
3. Periodonto-pathogens have numerous virulence factors that enhance their colonization, disable host defenses, and cause tissue destruction directly or by activations of an inflammatory response.
4. The presence of antibodies to periodontopathogens antigens in serum, saliva, and gingival crevicular fluid in patients with severe periodontal lesions.
5. Antibody levels are low in periodontally healthy persons and in patients treated for periodontitis.
6. Therapy directed at elimination of periodontopathogens from diseased sites is usually followed by improvement in the clinical signs of disease.
The studies on the relations between some specific bacterial strains and periodontal diseases began in 1960 with the isolation of certain bacterial morphotypes from healthy and diseased sites (14). The specificity of periodontal microflora is strongly supported by the identification of A. actinomycetemcomitans as a pathogen associated with localized aggressive periodontitis (13, 21). Subsequent crosssectional and prolonged studies aimed to associate bacterial species with health and disease. These investigations provide enough evidence to relate a small group of periodontopathogens with periodontitis and these bacteria have been defined as key periopathogens: A. actinomycetemcomitans, Tannerella forsythia and Porphyromonas gingivalis. They are strongly related with the initiation of periodontal disease, disease progression and unsuccessful periodontal therapy.

Possible etiologic agents of periodontal disease

- Actinobacillus actinomycetemcomitans
- Porphyromonas gingivalis
- Tannerella forsythia (Bacteroides forsythus)
- Treponema denticola
- Prevotella intermedia
- Fusobacterium nucleatum
- Eikenella corrodens
- Campylobacter rectus (Wolinella recta)
- Peptostreptococcus micros
- Streptococcus intermedius

*P. gingivalis*

*P. gingivalis* is a gram-negative oral anaerobe, which is a major cause of periodontitis. *P. gingivalis*: doesn’t survive at 10% O\(_2\) or 20% O\(_2\), but does at 3% and 6% O\(_2\). It participates in severe forms of periodontitis, and it is a prominent component of the oral microbiome and a successful colonizer of the oral epithelium. A series of reports over the years suggest that infection with *P. gingivalis* is associated with several systemic diseases, including CVDs, preterm births, low birth weight, rheumatoid arthritis, and DM.\(^{11,12}\)

*P. gingivalis* is released from the sulcus into the bloodstream. Human trials and animal experiments have confirmed the presence of *P. gingivalis* in liver tissues. Furthermore, the periapical granuloma, which served as a persistent and sustainable supply source of the *P. gingivalis* and its products, may lead to chronic liver injury.\(^ {10}\) In a study where the incidence rate of *P. gingivalis* was compared between NAFLD patients and non-NAFLD control subjects, it was found that the detection frequency of the *P. gingivalis* infection in NAFLD patients was significantly higher.\(^ {11}\) Notably, the detection frequency of *P. gingivalis* in the patients with NASH was also markedly higher than that of the non-NAFLD control subjects. In the same study, increased body and liver weights, accumulation of lipids in the liver, and increases in ALT and triglyceride (TG) levels were observed in high fat diet-induced steatosis mice that had received a direct injection of *P. gingivalis*. Animal experiments have also shown that dental infection of *P. gingivalis* may exacerbate the pathological progression of NASH from simple steatohepatitis to steatohepatitis with fibrosis. These results indicate that the presence of the *P. gingivalis* infection maybe an independent predictor for the development of NAFLD and may contribute to the progression of other LD.\(^ {12,13}\)

*Aggregatibacter actinomycetemcomitans* (A. actinomycetemcomitans)

First recognized as a possible periodontal pathogen in LJP (Newman et al.,1976), majority of Localized Juvenile Periodontitis (LJP) patients have high antibodies (Ab) titers against Aa. Successful therapy lead to elimination or significant decrease of the species, potential virulence factors; leukotoxin, cytolethal distending toxin, invasion, apoptosis, induce disease in experimental animals, elevated in “active lesions”, compared with non-progressing sites. Virulent clonal type of Aa in LJP
patients exhibit specific RFLP pattern, while healthy patients exhibit other patterns, increased leukotoxin production by Aa strains isolated from families of African Origin, associated with refractory periodontitis in adult patients.\textsuperscript{14-24}  

\textit{A. actinomycetemcomitans} is an exogenous bacterium, which is associated with periodontitis in young individuals and has the ability to produce virulence factors. Studies have shown that \textit{A. actinomycetemcomitans} generates certain products, which may inactivate and evade immune defense. The most investigated products of \textit{A. actinomycetemcomitans} are leukotoxin and repeats in toxin.\textsuperscript{16} A previous study showed that the injection of \textit{A. actinomycetemcomitans} into mice induced immunosuppression and suppressed the IgG response to red blood cells. The administration of \textit{A. actinomycetemcomitans} has also been reported to induce systemic inflammation in apolipoprotein E-deficient mice. In an animal study, \textit{A. actinomycetemcomitans} was present in liver tissue after intravenously inoculating mice with live \textit{A. actinomycetemcomitans}, and may have induced moderate hepatic inflammation. The \textit{A. actinomycetemcomitans} infection displayed more severe inflammatory changes in the liver, which positively correlated with serum markers of inflammation, such as interleukin (IL)-1β, IL-12, IL-10, IL-6, tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ).\textsuperscript{17-25}  

\textbf{Antibiotics}  

A single antibiotic cannot eliminate all the periodontopathic bacteria, since periodontal microbiota are more heterogeneous, therefore, it may be beneficial to use more than one antibiotic, serially or in combination.\textsuperscript{5} This combination formula should take into consideration the fact that bacteriostatic antibiotics can not be given in combination with bactericidal ones. If both types of drugs are required, they should be given serially i.e. one after the other.\textsuperscript{5,6}  

The most common antibiotics used are:  

\textbf{Amoxicillin}  

Amoxicillin is broad-spectrum penicillin and for periodontal therapy, it is often combined with clavulanate, which inhibits beta-lactamases produced by some bacteria. It is highly acid stable and over 90\% of administered dose is absorbed. It is a bactericidal drug that inhibits the synthesis of bacterial cell walls and results in cellular disruption due to high osmotic pressure. Since penicillins act during the synthesis of cell walls, it is most effective against multiplying bacteria.\textsuperscript{6,28} It is used in combination with metronidazole for treatment of chronic periodontitis and aggressive periodontitis.\textsuperscript{7} In addition, Augmentin in doses of 250 – 500 mg t.d.s may be of value in treating periodontitis refractory to treatment. Except for allergic reactions, penicillin toxicity is extremely low and it is one of the safest known drugs.\textsuperscript{28,29}  

\textbf{Precautions}  

In dental practice penicillins is contraindicated with patients under oral contraceptives as penicillin could decrease the efficiency of this drugs.  

In patients with systemic lupus erythromatosis (SLE) as penicillin will attack the nuclear material due to absence of nuclear membrane in those patients causing exaggeration of the condition.  

\textbf{Metronidazole}  

Metronidazole is a nitroimidazole compound, which is bactericidal to anaerobes and is believed to disrupt bacterial DNA synthesis. It is most effective against obligate anaerobic gram-negative bacilli (fusobacterium). It is also active against obligate anaerobic cocci (peptostreptococcus species).\textsuperscript{6} It is secreted in higher concentrations in the crevicular fluid; therefore, it has application in the field of periodontal medication. This drug administered systemically (750–1000 mg day for 14 days) reduces the growth of anaerobic flora and decreases
the clinical and histopathologic sings of periodontitis. The most common regimen is 250 mg t.d.s for 10–14 days. Clinically is used in the treatment of refractory periodontitis, especially in combination with amoxicillin. It is also used in treatment of acute necrotizing ulcerative gingivitis and severe odontogenic infections. The most common adverse effects are antabuse reaction when alcohol is ingested concomitantly. It also inhibits warfarin metabolism and prolongs prothrombin time. It should be avoided in patients taking lithium.

**Tetracyclines**

Tetracyclines are the most widely prescribed agents for periodontal therapy and are broad-spectrum antibiotics. Tetracyclines are bacteriostatic in action and retard the growth of organisms by inhibiting protein synthesis. The adult dose for tetracycline is 250–500 mg q.i.d, doxycycline 100 mg o.d and minocycline 100 mg b.d. These antibiotics are secreted in the crevicular fluid in higher concentrations and are effective against a number of oral gram-negative and gram-positive cocci and bacilli. These antibiotics are indicated in the treatment of generalized and localized aggressive periodontitis as well as periodontitis refractory to treatment. However, with the emergence of resistant species of bacteria, tetracyclines are currently replaced by more effective combination antibiotic therapy. Adverse effects of tetracycline include retardation of bone growth (transient), photosensitivity, permanent discoloration of developing teeth, teratogenesis, as well as hepatic and renal toxicity in susceptible individuals.

When prescribing tetracyclines we should advice the patients not to take it with dairy products which rich in calcium, this calcium due to its chelation reaction renders the tetracyclines ineffective, same also can be expected with antacids.

**Clindamycin**

This antibiotic inhibits bacterial protein synthesis and is usually bacteriostatic but bactericidal in high doses. This drug binds to a specific subunit of bacterial ribosome, thereby inhibiting protein synthesis. It is of particular efficacy in treating periodontal disease because of its ability to penetrate bone. Levels in the GCF are usually above the minimum inhibitory concentration for periodontal pathogens. Clindamycin is of value in the treatment of periodontitis refractory to therapy, either alone or in combination with Augmentin. The dose is 150 mg t.d.s or q.i.d for 7 – 10 days. Adverse effect of the drug is diarrhea and gastric upset if taken on empty stomach. Its use has been linked to the development of ulcerative colitis.

**Ciprofloxacin**

It is a quinolone, active against gram-negative rods including many periodontal pathogens. It does not suppress streptococcus species, which are associated with periodontal health, thus promoting a micro flora associated with periodontal health. Currently, this drug is the only one to which all strains of Actinobacillus actinomycetemcomitans are susceptible. The recommended dosage is 500–750mg b.d. for 7–10 days. This drug has been used in combination with metronidazole. The adverse effects include gastrointestinal upset, oral candidiasis, headache, restlessness, hypersensitivity, hyperpigmentation and photosensitivity.

**Host Modulation Therapy**

The destruction of periodontal tissues is due to the summation of two factors: A- The bacteria and bacterial products: Bacterial invasion, enzymes specially collagense enzyme and other noxious bacterial toxic products cause periodontal tissues destruction. B- The host defense mechanism:

The cells of the host as Neutrophils, during its response to the bacteria and bacterial products, it also release enzymes specially collagenase, and other products which also lead to periodontal tissues destruction.
**Drugs used in Modulation Therapy**

Host modulation is a mean of reducing/ stopping the host-mediated aspect of periodontal tissue destruction, as most of the tissue destruction in periodontal disease is host-mediated (pro-inflammatory cytokines: IL-1, PGE2, TNF-alpha). The concept is to combine host modulation with conventional therapy to achieve best results. Subantimicrobial Dose Doxycycline is the only FDA approved modality. 20 mg doxycycline is taken twice daily for 3-9 months. Its therapeutic effect is by collagenase, osteoclast and cytokine inhibition in alveolar bone, connective tissue and epithelium. At this low dose, there is no antimicrobial effect and hence no bacterial resistance and other side effects.

I. Doxycycline hyclate (Periostat)

Periostat: is available in 20mg capsule of doxycycline hyclate for use by patients twice daily. It does not produce antibacterial effects because the dose of 20 mg twice daily is too low to affect bacteria., so resistant to this medication has not been seen.

Mechanism: by suppression of the activity of collagenase, particulary that produced by poly morphnuclear leukocytes.

Advantages

1. 50% improvement in clinical attachment levels in pockets with probing depth 4 to 6mm.
2. 34% improvement in pockets with probing depths 7mm.
3. Attachment loss was prevented in sites with normal probing depths (0-3mm).

II. Non Steroidal Anti-inflammatory Drugs (NSAIDs)

It may be of therapeutic value in treating periodontal diseases because of their ability to interfere with arachidonic acid metabolism and so inhibit the inflammatory process.

Some NSAIDs affect the response neutrophils (PMNs) to inflammation not related to prostaglandin.

Example: ibuprofen

- It inhibits PMN migration, reduces vascular permeability and inhibits platelet aggregation by inhibiting cyclo-oxygenase pathway.
- It is significantly inhibited radiographic alveolar bone loss
- NSAIDs showed beneficial effects after topical application
- Other e.g : ibuprofen, mefenamic acid and naproxen

III- Bisphosphonates A group of drugs showed beneficial effects as NSAIDs in treatment of periodontal diseases

Bisphosphonates (BPs) are drugs for inhibiting bone resorption, widely used for the treatment of osteoporosis, multiple myeloma and skeletal complications of bone metastases. BPs were proven to inhibit decreases in bone density and prevent bone fracture by reducing activation of osteoclasts and inducing apoptosis of osteoclasts. The two main categories of BPs are the non-nitrogen and nitrogen-containing BPs. Alendronate is an oral nitrogen-containing BP and the most commonly used drug to treat osteoporosis and osteopenia.

Even though BPs have clinical efficacy, a number of cases of osteonecrosis of the jaw involving a patient treated with BPs for a long period of time have been reported worldwide since Marx [9] reported the first case, and this has been recognized as a serious adverse effect of these drugs. A reduction in osteoclast activity can therefore be expected to shift the balance between formation and resorption towards increased net bone formation. During bone repair, bisphosphonates have an anti-catabolic, or net anabolic, effect. Bisphosphonates bind to bone mineral and are taken up by osteoclasts when the latter resorb bone, which inactivates...
the cell. This mechanism makes bisphosphonates highly osteoclast-specific; free bisphosphonate is quickly excreted via the kidneys. However, some macrophages may be affected, and there is in vitro evidence\textsuperscript{34,35} that osteoblasts are stimulated by concentrations of bisphosphonates that are unlikely to occur in vivo. Systemic bisphosphonate treatment can increase screw removal resistance. Screws can also be better fixed in bone if they are coated with bisphosphonates.\textsuperscript{36-38} Examples of bisphosphonate drugs are: Alendronic acid (Fosavance), Disodium etidronate (Didronel), Disodium pamidronate (Aredia) which may be administered IV.\textsuperscript{39}

CONCLUSION

The periodontal therapies mainly used are pocket debridement, regenerative procedures along with the administration of antibiotics and other adjunctive aids. However, some other equally effective treatments are to be studied as using nanomaterial based antibiotics which could control resistant periodontopathic bacteria specially in refractory cases.

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