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Review Article

Microbial dynamics: Understanding the triad of periodontal pathogens

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ABSTRACT

Periodontal diseases are complex inflammatory conditions of the oral cavity, affecting a significant portion of the global population. Central to the pathogenesis of these diseases is the intricate interplay between microbial pathogens, host immune response, and environmental factors. Among the diverse array of microorganisms inhabiting the oral microbiome, a triad of pathogens—Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola—commonly referred to as the

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1. Introduction

Periodontitis is a chronic inflammatory condition, that is characterized by microbially associated, host-mediated inflammation that results in loss of periodontal attachment.[1] Periodontitis can arise due to a multitude of etiological reasons. Parallel research into the aetiology of periodontal diseases began during the "golden age of microbiology" (1880–1920), when the etiologic agents of many medically significant infections were identified.[2]

The presence of subgingival bacteria and the dissemination of their toxins is the main etiological component. According to microbiological analyses, dental plaque contains between 150 to 800 distinct species.[3] There is definitely evidence that periodontal disease has a microbial aetiology, but its precise cause is still unknown.[4]

Subgingival bacteria are divided into six separate color-coded microbial complexes by Sigmund Socransky and his colleagues based on the likelihood that they were associated with periodontal health or periodontal disease. The "red complex" which includes the gram-negative anaerobes Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola is the most widely recognised complex in the world. These organisms are found in greater volumes and ratios in pathologies from active periodontal diseases.[5]

2. The Microbial Triad

Based on their virulence traits and strong connections to diseased locations, a triadic group of oral anaerobic bacteria including P. gingivalis, Treponema denticola, and Tannerella forsythia have historically been thought to be the primary cause of periodontitis.[6] Periodontitis is a dysbiotic condition brought on by a switch from gram-positive to gram-negative subgingival bacteria. A prolonged time

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period is required for the onset of periodontal dysbiosis, which gradually shifts the symbiotic relationship between the host and the bacterium into a pathogenic form.[7]

The first microbial complex that has been linked to disease is the orange complex, which is made up of anaerobic gram-negative species like Prevotella intermedia, Prevotella nigrescens, Prevotella micros, and Fusobacterium nucleatum. As the disease progresses, the orange complex transforms into the red complex, which is made up of Tannerella forsythia, Treponema denticola, and Porphyromonas gingivalis.[8]

At sites exhibiting progressive periodontitis, the red complex represents a component of the climax community in the biofilms. In the absence of members of the orange complex, individuals of the red complex are only seldom found. The orange complex's growing invasion caused the red complex to colonise other areas. The red complex species showed a very significant correlation between pocket depth and bleeding on probing.[7]

2.1. *Porphyromonas gingivalis*

P. gingivalis is a small, gram negative, black pigmented anaerobe. It has historically been recognised as a significant element of the periodontopathic microbiota implicated in the development of periodontal disease and the loss of bone and tissue.[9] The interaction (adherence) of P. gingivalis in the oral cavity is the earliest event in its pathogenicity.P. gingivalis uses numerous bacterial components, including fimbriae, proteases, hemagglutinins, and lipopolysaccharide, to achieve this.[10]

2.2. *Morphological characteristics*

P. gingivalis species members are anaerobic coccobacilli that are gram-negative, non-motile, asacchrolytic, and range in diameter from 0.5-0.8 to 1.0-3.5 m.[11] Their colonies are rounded and smooth. The colonies are originally white to cream-colored when they are developed on a blood agar surface. These colonies gradually (4–8 days) darken from the periphery to the centre, changing from a deep red to a black colour, which corresponds to the proto heme concentration.[8]

2.3. *Virulence factors*

These include

1. Capsule
2. Outer membrane and its associated LPS
3. Fimbriae
4. Proteinases
5. Selected enzymes

The capsule assures higher serum resistance, reduced PMN chemotaxis, and increased resistance to phagocytosis.[8] The P. gingivalis fimbriae's chemotactic capacity is an essential feature. The development of an inflammatory lesion, the progression of periodontal tissue loss, and the deterioration of bone might all be significantly influenced by this capacity to recognise host stimuli.[12]

About 20 main proteins, ranging in size from 20 to 90 KDa, are found in P. gingivalis. The effects of "major outer sheath membrane proteins" on epithelial cells, fibroblasts, and other bone cells have been the focus of several in vitro investigations.[13] According to Mihara and Holt, a 24 kDa protein was recovered from the outer membrane vesicles of P. gingivalis strain W50, and it was found to have the ability to stimulate thymidine-incorporated human gingival fibroblasts.[14,15]

The enormous amount of hydrolytic, proteolytic, and lipolytic enzymes that are generated by nearly all of the known strains of P. gingivalis is one of the possibly important virulence traits of this organism. Trypsin, thiol, caseinolytic proteinases, and two peptidases are significant proteases (proteinases capable of hydrolyzing peptide bonds) linked with P. gingivalis. The common word "gingipains" refers to both the Arg and Lys proteinases, which are cysteine proteinases.

2.4. *Treponema denticola*

2.4.1. *Morphological characteristics*

Spirochetes are long, thin, corkscrew-shaped gram-negative anaerobic bacteria, and darkfield and phase contrast microscopy render it easy to identify their distinctive movement and appearance. The outer sheath of T. denticola's spiral-shaped cells is made up of a delicate structure resembling an envelope.[16]

One of the first researchers to establish a connection between clinical indicators of inflammation, increased dental plaque, increased gingival exudate, bleeding on probing, periodontal pocket depth, and the loss of connective tissue attachment was Armitage et al. over two decades ago.[17] Other oral bacterial species, including P. gingivalis and F. nucleatum, interact with T. denticola. This co-aggregation most likely contributes to the development of periodontal disease.

Table 1: Virulence factors

Hydrolytic enzymes	**Hyalourodinase, Collagenase**
	Protease
	Phosphates
	Phospholipase

2.4.2. *Others are*

1. Major outer sheath protein (Msp)
2. OppA (ortholog)
3. FH like binding protein
4. Dentilism

5. Leucine rich repeat A.[8]

2.4.3. Tannerella forsythia

It belongs to the gram-negative Cytophaga Bacteroides family and is anaerobic. T. forsythia is more commonly related with gingivitis, aggressive periodontitis, and other types of periodontal disease.

Only a few numbers of potential virulence factors for T. forsythia have been discovered until now, such as the trypsin-like and PrtH proteases, the sialidases SiaH and NanH, the leucine-rich repeat (Lrr) cell surface-associated and secreted protein BspA, the N-acetyl-b-glucosaminidase hemagglutinin, and others.[8]

In addition to F. nucleatum, T. forsythia also creates mixed synergistic biofilms. F. nucleatum is regarded as a bridge bacterium because it can co-aggregate with both early- and late-colonizing bacteria, enabling the production of dental plaque.[18]

3. Dysbiosis and Polymicrobial Synergy in Periodontitis

Subgingival microbial communities undergo significant changes as periodontitis progresses, with the introduction of gram-negative species that are largely distinct from those enriched during gingivitis and outgrow taxa that are associated with health. The traditionally characterised red-complex triad, which consists of Tanneralla forsythia, P. gingivalis, and T. denticola, is one of the superior genera.[19]

Keystone pathogens initially subvert host immune response with the help of accessory pathogens, and then pathobionts overactivate it, causing homeostasis breakdown and harmful inflammation in vulnerable individuals. Instead of being caused by a single pathogen, disease is caused by polymicrobial synergy and dysbiosis, which disturbs the ecologically balanced biofilm linked to the homeostasis of periodontal tissue.[20]

Communal pathobionts overreact to the host reaction and develop in the inflammatory condition. A homeostatic commensal, as opposed to an accessory pathogen, tends to stabilise a eubiotic community by either actively combating potentially pathogenic bacteria or by producing antimicrobial peptides that selectively target prospective pathogens.[21]

Through the suppression of IL-8 and toll-like receptor 4 signal control, the red complex triad has been demonstrated to contribute to the breakdown of homeostasis. Periodontitis causes a change in the number and quality of the microbiome. When the red-complex triad first appears, a dysbiotic condition is activated, which in turn induces an immunological reaction that harms the supporting structures. On the other hand, microorganism that are connected to health are declining and can no longer return to the symbiotic condition of health. At the absolute least, another mechanism that contributes to the condition's continued deterioration is the growing biomass of microorganisms.[22]

The eradication of these organisms from the infection site remains a difficult process that necessitates the use of antibiotics. Finding targeted therapies for these infections has gained interest in response to the rise of drug-resistant variants. Ibuprofen (IB) and acetaminophen (APAP) are two regularly used medications. The cellular function, metabolism, and pathogenicity of red complex pathogens were discovered to be targeted by these two medications.[23]

Another well-known powerful medication with antibacterial action against red-complex infections is reserpine. Reserpine specifically targets efflux pumps and ABC transporters, two protein transporters known to be essential for bacterial cell viability.[24]

4. Detection of Red Complex Species

Until DNA-based approaches replaced their detection by culture and identification by biochemical methods, anaerobic culture techniques were the gold standard for identifying periodontal infections. For specific target species, the DNA-DNA checkerboard and qualitative or quantitative PCR were verified and employed in oral microbiology service laboratories.

The few subgingival microflorae connected to periodontal disease are Porphyromonas gingivalis, Tannerella forsythensis (formerly known as Bacteroides forsythus), and Treponema denticola which may hydrolyse the synthetic peptide N-benzoyl-DL-arginine-B-naphthylamide, also known as BANA, thanks to an enzyme similar to trypsin.

The APIZYM Kit's trypsin-like enzyme was used to modify the BANA hydrolysis test to create the commercially available BANA test (Oral B, Perioscan TM). BANA is a quick and reliable diagnostic tool that has demonstrated good agreement with the clinical indices used to identify periodontal disease.[7]

Currently, PCR is a common diagnostic and investigative technique in periodontology. The detection of genetic polymorphisms, along with the identification of several immunological and inflammatory markers at the mRNA expression level, can provide a greater understanding of the processes causing periodontal disease.[25]

A single particular species is directly detected using single target PCR from plaque samples taken from healthy and diseased individuals. Numerous bacterial species, including more common pathogenic species like P. gingivalis, T. denticola, and T. forsythia a, have been identified as candidates as probable pathogens for periodontitis in studies using the sequencing analysis of 16S rRNA genes from the oral cavity.[26]

5. DNA-DNA Hybridization Methods

A molecular diagnostic technique called nucleic acid hybridization is used to identify and quantify various types of microbes in DNA samples.

6. Immunodiagnostic Method

To find the target microorganisms, immunologic techniques identify bacterial antigens. Many methods, including flow cytometry, latex agglutination, immunofluorescent assays (IFA), enzyme-linked immunosorbent assays (ELISA), and membrane assays, can be used to detect this response.

6.1. Fluorescence in situ hybridization (FISH)

Luminous in situ Fluorescence or confocal fluorescence microscopy is used to see the hybridization process in the hybridization-based method known as hybridization.

7. Checkerboard Hybridization

Checkerboard hybridization is a semi-quantitative approach that permits simultaneous testing of several probes on DNA, RNA, tissue, bacterial, and viral samples.

8. Microarray Technology

By simultaneously measuring the relative concentrations of numerous different DNA or RNA sequences in a sample via hybridization and subsequently detecting the hybridization events, microarrays are tools that are made up of thousands of DNA probes bound on a solid support and are used to identify microorganisms and determine gene expression.

9. Conclusion

The pathogenic consortium known as the red complex, which includes P. gingivalis, T. denticola, and T. forsythia, has been postulated. The new species complexes can be a sign of a disease-prone environment or they might offer a synergistic pathogenicity that starts an aggressive disease process.

10. Conflict of Interest

None.

11. Source of Funding

None.

References

1. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(51):159–72.
2. Tatakis DN, Kumar PS. Etiology and pathogenesis of periodontal diseases. *Dent Clin North Am*. 2005;49(3):491–516.
3. Lourenço TGB, Heller D, Silva-Boghossian CM, Cotton SL, Paster BJ, Colombo APV, et al. Microbial signature profiles of periodontally healthy and diseased patients. *J Clin Periodontol*. 2014;41(11):1027–36.
4. Arora N, Mishra A, Chugh S. Microbial role in periodontitis: Have we reached the top? Some unsung bacteria other than red complex. *J Indian Soc Periodontol*. 2014;18(1):9–13.
5. Belibasakis GN, Belstrøm D, Eick S, Gursoy UK, Johansson A, Könönen E, et al. Periodontal microbiology and microbial etiology of periodontal diseases: historical concepts and contemporary perspectives. *Periodontol 2000*. 2023;p. 1–17.
6. Socransky SS, Haffajee AD. Periodontal microbial ecology. *Periodontol*. 2000;38(1):135–87.
7. Mohanty R, Asopa S, Joseph M, Singh B, Rajguru J, Saidath K, et al. Red complex: Polymicrobial conglomerate in oral flora: A review. *J Family Med Prim Care*. 2019;8(11):3480–6.
8. Shaikh HM, Patil S, Pangam T, Rathod K. Polymicrobial synergy and dysbiosis: An overview. *J Indian Soc Periodontol*. 2018;22(2):101–6.
9. Joshi V, Bhat K, Kugaji M, Ingalgi P. Occurrence of Aggregatibacter actinomycetemcomitans in Indian chronic periodontitis patients and periodontally healthy adults. *J Indian Soc Periodontol*. 2016;20(2):141–4.
10. Holt SC, Kesavalu L, Walker S, Genco CA. Virulence factors of Porphyromonas gingivalis. *Periodontol*. 1999;20:168–238.
11. Slots J, Listgarten MA. Bacteroides gingivalis, Bacteroides intermedius and Actinobacillus actinomycetemcomitans in human periodontal diseases. *J Clin Periodontol*. 1988;15(2):85–93.
12. Potempa J, Sroka A, Imamura T, Travis J. Gingipains, the Major Cysteine Proteinases and Virulence Factors of Porphyromonas gingivalis: Structure, Function and Assembly of Multidomain Protein Complexes. *Curr Protein Pept Sci*. 2003;4(6):397–407.
13. Mayrand D, Grenier D. Biological activities of outer membrane vesicles. *Can J Microbiol*. 1989;35(6):607–13.
14. Mihara J, Holt SC. Purification and characterization of fibroblast-activating factor isolated from Porphyromonas gingivalis W50. *Infect Immun*. 1993;61(2):588–95.
15. Mihara J, Yoneda T, Holt SC. Role of Porphyromonas gingivalis-derived fibroblast-activating factor in bone resorption. *Infect Immun*. 1993;61(8):3562–4.
16. Onishi S, Honma K, Liang S, Stathopoulou P, Kinane D, Hajishengallis G, et al. Toll-Like Receptor 2-Mediated Interleukin-8 Expression in Gingival Epithelial Cells by the Tannerella forsythia Leucine-Rich Repeat Protein BspA. *Infect Immun*. 2008;76(1):198–205.
17. Armitage GC, Dickinson WR, Jenderseck RS, Levine SM, Chambers DW. Relationship Between the Percentage of Subgingival Spirochetes and the Severity of Periodontal Disease. *J Periodontol*. 1982;53(9):550–6.
18. Honma K, Inagaki S, Okuda K, Kuramitsu HK, Sharma A. Role of a Tannerella forsythia exopolysaccharide synthesis operon in biofilm development. *Microb Pathog*. 2007;42(4):156–66.
19. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25(2):134–44.
20. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol*. 2015;15(1):30–44.
21. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol*. 2009;16(12):745–59.
22. Chakar C, Menassa G, Khayat R. Periodontal Microbiome Part I: A Literature Review. *Int Arab J Dent*. 2021;12(1):41–7.
23. Priyadharsini JV. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol*. 2019;90(12):1441–8.
24. Ushanthika T, Girija ASS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. *Nat Prod Res*. 2021;35(11):1893–8.
25. Maheaswari R, Kshirsagar J, Lavanya N. Polymerase chain reaction: A molecular diagnostic tool in periodontology. *J Indian Soc*

Periodontol. 2016;20(2):128–55.
26. Paster BJ, Dewhirst FE. Molecular microbial diagnosis. *Periodontol 2000*. 2009;51:38–44.

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