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Porphyromonas Gingivalis A Multitasking Pathogen.

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ABSTRACT

Porphyromonas gingivalis is a ubiquitous organism primarily responsible for causing chronic generalized periodontitis. The inflammatory properties of Porphyromonas gingivalis are more nuanced, and the organism can exhibit both pro- and anti-inflammatory properties, depending on the context. The end toxin and gingipains released by the bacteria sets up an inflammatory stage which is responsible for various distant systemic pathologies in human body. Porphyromonasgingivalis has evolved several mechanisms to evade host immune system by invasion of host cells and disrupting signaling pathways by cytokine and receptor degradation. This paper overviews the various systemic diseases caused from the infection of Porphyromonas gingivalis in the periodontal apparatus in the oral cavity.

Keywords: Porphyromonas gingivalis, periodontal disease, systemic diseases.

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INTRODUCTION

Porphyromonas (formerly Bacteroides) gingivalis, a Gram-negative anaerobe, has a diverse range of virulence factors. The introduction of Porphyromonas gingivalis **(**P. gingivalis) to the medical community was by Nisengard and Newman [1]. Porphyromonas is derived from Greek word porphyries which means purple and monas which means unit. P. gingivalis is a major habitat of sub gingival sulks. It is a non-motile, asaccharolytic, Gram-negative bacteria which is an obligate anaerobe. On culture with blood agar it shows black-pigmented colonies. [2].

This opportunistic pathogen of oral mucosa known to be involved in periodontitis may also have a vital role in mediating the progression of several multifactorial and obviously unrelated chronic systemic diseases. The host and the micro biota of the oral cavity normally exist in a stable immunoinflammatory relationship. P. gingivalis however has the ability to disturb this balanced state, leading to a dysbiotic host–microbial interaction [3]. The periodontal blood vessels undergo proliferation and enlargement in inflammatory conditions such as gingivitis and chronic periodontitis, which provides a larger surface area that in turn, facilitates the entry of microorganisms into the circulation which may in turn lead to lodgment of these bacteria into different target organs, resulting in infections [4]. It has been recognized as a risk element for many systemic diseases such as coronary heart disease, pulmonary infections, diabetes mellitus and pre-term to low birth weight deliveries [5].Several studies have validated that there exists a considerable relationship between periodontitis and oral squamous cell carcinoma (OSCC) and or digestive cancer [6]. It was also recovered from several cases of appendicitis and peritonitis in adults and children. In the oral cavity other than periodontitis it was found in other oral infections, such as infected root canals, peritonsillar abscesses, and odontogenic abscesses [7]. A good understanding of the path physiological mechanism of p.gingivalis therefore becomes necessary. Hence, the purpose of this review is to emphasize the pit fall that are unconnected to the connectivity between P.gingivalis and other local and systemic morbidities.

VIRULENCE FACTORS

GINGIPAIN:

They are the known factors expressed in tissue damage in periodontitis. P.gingivalis secretes Arg-gingipain (Rgp) and lys-gingipain (Kgp). Tissue damage by gingipains was from the degradation of matrix metalloproteinase, collagen, and fibronectin. They have the propensity to cleave subclass 1 and 3 IgG antibodies. In higher concentration of P. gingivalis gingipains trigger proinflammatory cytokines response such as IL-1β, IL-2, IL-6, TNF-α and IL-8 [8].

CAPSULAR POLYSACCHARIDE

The capsular polysaccharide (CPS) when present down regulates pro-inflammatory cytokines production IL-1β, IL-6, IL-8, and TNF-α, indicating host evasion responses [9]. The other studies have found that the CPS elicits host immune responses like polymorph nuclear neutrophils migration and time and dose dependent expression of cell migration chemokines like MCP-1, KC, MIP-2 and RANTES [10].

FIMBRIAE

Virulence factor of P. gingivalis is strongly associated with its fimbriae. They have been involved to be the main factors in cell adhesion, invasion, colonization and invading membrane vesicles into host cells [11]. By binding to cellular α5β1 integrins, they mediate adherence and impairment of the homeostatic controls of host cells [12]. These fimbriae are incidental with altering β2 integrins adhesive activity which further were up taken by monocytes using the CD14/TLR2/PI3K signaling complex, and may promote to intracellular evasion by P. gingivalis [12].

P.GINGIVALIS AND CANCER:

Several epidemiological studies have shown that P. gingivalis is in association with cancers like oral squalors cell carcinoma (OSCC), as it has the ability to spread systemically, and also helps in cell survival and proliferation [13,14]. P. gingivalis is now known to be a possible independent risk factor that increases the

probability of or digestive cancer death. Many studies have correlated higher levels of antibodies to P. gingivalis, in periodontal diseases with an increased risk of pancreatic cancer [15].

The study by Nagy KN et al [16] showed that the level of P. gingivalis was higher on the surfaces of OSCCs than that of the adjacent normal mucosa. P. gingivalis antibodies detected by immunohistochemistry within gingival carcinomas showed greater intensity of staining. Studies have also shown that infection with both P. gingivalis and Fusobacterium nucleate (F.nucleatum) helps in advancement of the tumor in oral-specific chemical carcinogenesis mouse model [17]. The possible mechanisms of P. gingivalis that help in the progression of OSCC include evasion of immune system, inhibition of apoptosis, carcinogen conversion, MMP-9 induction and dysbiosis of the oral micro biome [18].

The possible molecular basis for carcinogenesis mediated by P. gingivalis can be explained in different aspects. Chronic inflammation has been shown to be associated with the development of cancer. Prolonged IL-6 signaling and STAT3 activity helps in tumor progression [19, 20]. P. gingivalis increases the production of IL-6 in epithelial cells activating JAK2 and GSK3β pathways [20, 21]. Secondly, P. gingivalis secretes nucleoside diphosphate kinase (NDK) which inhibits P2X7 receptor activation by ATP thereby reducing IL-1β production from epithelial cells. This mechanism also suppresses apoptosis by degradation of ATP by NDK thereby promoting tumor genesis [22]. Thirdly, P. gingivalis inhibits apoptosis of epithelial cells by different mechanisms which include activation of Jak1/Akt/Stat3, increasing the Bcl2: Bax ratio (Bcl2 being antiapoptotic and Bax being proapoptotic), inhibiting the release of cytochrome c which helps in apoptosis, and the activation of downstream caspases [23]. P. gingivalis also cause upregulation of micro-RNAs like miR-203, which inhibit apoptosis in gingival epithelial cells [24]. Lastly, P. gingivalis also enhances cellular migration in OSCC cells by activation of various pathways like ERK1/2-Ets1, p38/HSP27, and PAR2/NF-κB pathways which induce expression of pro-matrix metalloproteinase (MMP)-9. Apart from the above mentioned mechanisms another possible P. gingivalis mediated carcinogenesis mechanism is the metabolism of potential carcinogens [25]. For example, P. gingivalis causes conversion of ethanol to acetaldehyde, which is capable of inducing DNA damage, mutagenesis and hyper proliferation of the epithelium. This could also help in explaining the epidemiological evidence of association between heavy drinking and development of some cancers [26].

P.GINGIVALISAND CARDIOVASCULAR DISEASES:

Cardiovascular diseases (CVD) account for 29% of deaths worldwide [27]. Various studies have demonstrated the presence of oral micro biota in atherosclerotic plaques. In a study conducted by Ford PJ et al [28], P. gingivalis was found to be present in 100% of atherosclerotic plaques using real-time PCR [28]. By interacting with the endothelial surface, P. gingivalisinduces proliferation of smooth-cell and hence causes impairment of the vasomotor function of endothelial cells [29]. On investigation of carotid athermanous plaques, Cairo Fet al [30] detected DNA of P. gingival sin 37%, along with other oral microorganisms from carotid atheroma patients [30]. P. gingivalisactivates a phagocytic cell which is triggered by bacterial LPS as a result of which levels of PGE2, IL-1 β, and TNF-□ increase. The macrophages can then convert into foam cells and induce the formation of proinflammatory cytokines. These cytokines are responsible for endothelial dysfunction [29].

Other studies have also shown that oral micro biome like Streptococcus sanguisand P. gingivalis contribute to development of thrombus by inducing platelet aggregation [31].A study by Deshpande RG et al [32] explained that P. gingivalis has the ability to actively attach and entre the endothelial cells of fetal bovine heart, bovine aortic, and human umbilical vein [32].P.gingivalis also releases proteolytic enzymes like gingipains R in large amounts. These enzymes then promote thrombus formation by causing activation of factor X, prothrombin, and protein C and subsequently leading to intravascular clot formation. Periodontitis stimulates to produce C-reactive protein (CRP) by liver, which will be deposited on severed blood vessels. CRP then binds to the injured cells and fixes complement, which will activate phagocytes thereby contributing to atheroma formation, by the release of nitric oxide [33].

P.GINGIVALISAND DIABETES MELLITUS:

Diabetes mellitus and periodontitis share a unique interrelation. Studies have reported that diabetes mellitus may flare-up the periodontal disease condition. On the contrary, recent researches have also revealed that periodontal diseases can be a risk factor for development of insulin resistance and diabetes mellitus [34]. The studyby Padmalatha G et al[35] showed high glucose levels in type II DM patients which lead to the development of periodontogenic flora due to reduced oxygen production and defense cells. Hence, diabetic patients have an increased susceptibility for more severe periodontal disease due to increased prevalence of P. gingivalis [35].

Ishikawa M et al[36] demonstrated that P.gingivalis can translocate to liver from the oral cavity and incorporates into hepatocytes. Insulin stimulation in hepatocytes normally cause increase in IRS-1 and Akt/GSK-3β phosphorylation, preceded by GS activation. Activated GS further increases glycogen synthesis and lower level of blood glucose implying that the existence of P. gingivalis in hepatic cells prevents this insulin signaling pathway resulting in decreased glycogen synthesis [36]. Study was conducted by Ishikawa M et al by inducing periodontitisin 4 week female Balb/c mice by inoculating SNAP26b-tagged P.gingivalis (SNAP-P.g.) into the oral cavity. This SNAP-P.g. was found from the liver of these mice using nested PCR analysis. There was an increased blood glucose level in these mice which inclined towards SNAP-P.g. translocation to the liver from the oral cavity. In same study SNAP-P.g. was also internalized in the human hepatoma cell line HepG2. They found that SNAP-P.g. put down the insulin-induced glycogen synthesis in HepG2 cells suggesting that P.gingivalis translocated to the hepatic cells from the periodontal apparatus may contribute to the advancement of diabetes mellitus by affecting hepatic glycogenesis [36].

Study by Taylor et al[37] showed that severe periodontitis caused increase in HbA1c levels in type 2 diabetes mellitus. Studies have also shown that periodontitis raises the incidence of type 2 diabetes mellitus among non-diabetics and that periodontal health is also related with insulin resistance [37,38]. Study byMakiura Net al [39] showed that subjects with increased HbA1c values, the P. gingivalis was more frequently detected after periodontal therapy than in subjects with lower values of HbA1c. Moreover, in patients with increased HbA1c, P. gingivalis with type II fimbriae was found, while in patients with reduced HbA1c values did not show these type II clones. This result suggested that high glycemic value in diabetes is affected by presence of P. gingivalis, with type II fimbriae clones, in periodontal pockets [39]. In diabetic mice, P. gingivalis inoculation resulted in tissue injury with significantly higher fibroblast apoptosis as compared to non-diabetic mice. This showed that diabetes can also alter the repair capacity in inflamed periodontal tissues [40].

P. GINGIVALIS AND PRETERM LOW-BIRTH-WEIGHT:

P. gingivalis was found in amniotic fluid with premature labor; however, a definitive role of this bacterium in premature delivery is yet to be confirmed [41]. Recurrent colonization of oral bacteria including P. gingivalis is common following the dental procedures, and even been seen with periodontitis during simple mastication, thus allowing P. gingivalis to spread through hematogenous route and reach the placenta [42].

Studies have shown that certain bacteria like Bacteroidesforsythus(B. forsythias), P. gingivalis, Action bacillusactinomycetemcomitans (A. actinomycetemcomitans) and Treponema denticola(T. denticola),that are known to be integrated in mature plaque and progressive periodontitis. These bacteria are found in high titer in mothers of preterm low-birth-weight infants than in control mothers. This data suggested that oral microbial burden is associated with preterm birth and low birth weight (PLBW) [43]. Study by Katz J et al [44] also showed that P. gingivalis may enter the placental tissue and be partly responsible for preterm delivery [44]. The growth of anaerobic P.gingivalis takes place in the first 10-12 weeks of gestation as the placenta in this stage is in a state of physiological hypoxia [45]. Study by Hasegawa-Nakamura K [46] showed the presence of P. gingivalis in chorion of high-risk pregnant women, and bacterial lip polysaccharide induces interleukin-6 and interleukin-8 production via TLR-2 in chorion-derived cells [46]. A study showed that women with increased levels of serum IgG against P. gingivalis had more possibility of giving birth to a PLBW infant compared to women with lower levels of IgG against P.gingivalis [47].

P. GINGIVALIS AND RHEUMATOID ARTHRITIS

Many researchers have shown the link between periodontitis and rheumatoid arthritis (RA). Periodontitis and RA, both, occur due to a disbalance between pro- and anti-inflammatory cytokines. The early treatment was tooth eradication. TNF-⍰, a proinflammatory cytokine, is common in both diseases regulating a cascade of inflammatory events [48]. P. gingivalisis even detected in synovial fluid. P. gingivalis causes proteolytic cleavage at Arg-X peptide bonds by arginine gingipains resulting in citrullination of host peptides and inducing autoimmune responses in RA. Autoimmune responses in subjects with RA may also be triggered due to the heat shock proteins (HSPs) of P. gingivalis. Studies have shown that treatment of periodontal disease along with routine RA treatment can further improve overall health of RA patients [49].By the generation of citrullinated host peptides, P. gingivalis also causes citrullination of carboxy-terminal arginines by bacterial peptidylarginine deiminase [49]. P. gingivalis possessesa number of endogenous citrullinated proteins that are absent in other oral bacteria [50]. The importance of citrullination in RA is given by the expression of citrullinated autoantigens in synovial fluid [51]. Thus in case of P. gingivalis infection, due to its ability to citrullinate host peptides may be the cause in development of RA.

Increased antibody titres against P. gingivalis are seen in RA patients and even tend to be seen along with disease-specific autoimmunity. Association of its titers with RA autoantibody and CRP concentrations were found to be higher level in periodontitis. This shows that P. gingivalis infection has a role in progression of RA [52].

P. gingivalis particularly decomposes lysine and arginine; the IgG3 CH2 and CH3 domains processed by P. gingivalis proteinase become powerful targets for the rheumatoid factor produced by rheumatoid cells. The IgG levels of P. gingivalis, Prevotella intermedia, Prevotellamelaninogenica and B. forsythias were found to be noticeably at high level in RA patients that with those of the control patients [53]. Hence, careful screening of RA patients and appropriate treatment of their periodontal condition is necessary.

P.GINGIVALISAND RESPIRATORY DISEASES:

Several mechanisms explaining how the oral bacteria are play role in the pathogenesis of respiratory tract infection have been proposed. One of these mechanisms involves aspiration of oral bacteria like P. gingivalis, A. actinomycetemcomitans etc. into the lungs which are then responsible to cause the infections; the enzymes associated with periodontal disease that are present in the saliva can cause modification of mucosal surfaces thereby promoting adherance and colonization of respiratory pathogens following which they are carried into the lungs; these enzymes also cause destruction of salivary pellicle on the bacteria and interfere with their clearance from the mucosa; the cytokines released in the periodontal infection may promote infection by respiratory pathogens by alteration of the respiratory epithelium [54].

P. GINGIVALIS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD):

Over the last two decades the association between periodontitis and COPD has been increasingly identified. Due to the anatomical position of oral cavity the bacteria can be easily carried to the lungs and cause seeding of the infection there [55]. COPD and periodontitis, both, are associated with inflammation and the destruction of the local connective tissue [56]. Poor oral hygiene contributes to increased mass and complexity of dental plaque, which may enhance bacterial interactions between innate plaque bacteria (P. gingivalis, F. nucleate) [5]. In a study by Madalli R et al by comparison between P. gingivalis from the sputum of COPD patients before and after oral prophylaxis showed a decline in the bacterial load [57].Takahashi T et al[58] concluded from their study thatP. gingivalis-related antibody titre could act as an autonomous factor for exacerbation tendency in COPD. Measurement of these titers can be a useful indicator for susceptivity to persistent exacerbations so as to formulate a treatment strategy for prevention of such exacerbations. [58].

P. GINGIVALIS AND ASTHMA

Although many studies have been conducted regarding periodontal and allergic diseases still it is not clear about the association between both. J W Card et al under took a study regarding modification of allergic airway inflammation in an established murine model of asthma by infection with the P. gingivalis. Their results indicated that P. gingivalis exerted differential regulatory effects on allergic airway inflammation that were dependent on relative to allergic sensitization. [59]. Arbes SJ Jr et al[60] investigated the relationship between

serum IgG levels to A. actinomycetemcomitans and P. gingivalis and asthma, wheeze, and hay fever. An inverse association was seen by them between P. gingivalis and the outcomes asthma, wheeze, and hay fever [60]. Study was conducted by Roberto Rivera and et al to make out the association between periodontitis and asthma among overweight adults. They found the subjects with severe periodontitis with asthma medication were less likely to have asthma, which strongly indicated an inverse association. (61)

P.GINGIVALISAND CNS:

Recently, periodontal pathogens are being linked with the neurodegenerative condition, Alzheimer's disease (AD), which is characterized by loss of memory. Even various clinical, epidemiological and molecular studies have shown that periodontitis has role with increased risk of dementia, including AD. An in vivo study in mice inoculated with P. gingivalis showed the evidence of P. gingivalis bacterial genomic DNA in the brain which was predicted to activate the complement cascade [62]. The Hajishengallis G et al[63] hypothesis explained that P. gingivalis may cause even the early development of a neurodegenerative condition. In their view, highly virulent strains of P. gingivalis access the CNS during healthy stages and only those individuals who are susceptible to inflammatory traits are likely to develop progressive inflammatory component representing neurodegenerative disease processes. [63] Thus, P. gingivalis at present may be a missing link in the process of infection driven inflammation which represents the early stage in the development of AD pathology along with appearance of hallmark proteins.

The study by Shapira L et al[96] shows that P. gingivalis, may have a role to play in the pathogenesis of CNS inflammatory disorders such as multiple sclerosis. P. gingivalis LPS induces the secretion of certain proinflammatory mediators by CNS glial cells. Injection of P. gingivalis into subcutaneous tissue in mice, along with experimental induction of autoimmune encephalomyelitis led to aggravation of the disease due to lymphocyte proliferation in the presence of encephalitogenic protein myelin basic protein [64].

CONCLUSION

The possible local persistent infections could exert systemic manifestations in a number of above mentioned ways. A good knowledge about the interactions between P. gingivalisand host cells at the cellular and molecular level therefore becomes necessary to improve the overall well-being of the host.So this compiled information accessible at this moment seems to justify that maintaining a good oral hygiene is not only important to prohibit oral diseases but also to support overall health of the individual.

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Abbreviations:

1. Base pair: bp
2. Guanine-cytosine: G-C
3. Transfer RNA: tRNA
4. Arg-gingipain: Rgp
5. Lys-gingipain: Kgp
6. Capsular polysaccharide: CPS
7. Tumor necrosis factor alpha: TNF-
8. Monocyte chemoattractant protein-1 : MCP-1, Keratinocyte chemoattractant: KC, Macrophage inflammatory protein: MIP-2, regulated upon activation normal T cell expressed and secreted: RANTES
9. Fusobacterium nucleate: F.nucleatum
10. Matrix metalloproteinase-9: MMP-9
11. Signal transducer and activator of transcription 3: STAT-3
12. Janus kinase 2- JAK2, Glycogen synthase kinase 3 beta: GSK3β
13. Nucleoside diphosphate kinase: NDK
14. Adenosine triphosphate: ATP
15. B-cell lymphoma 2: Bcl 2

16. MicroRNA: miRNA
17. Cyclin-dependant kinase: CDK
18. Polymerase chain reaction: PCR
19. Lipopolysaccharides: LPS
20. Prostaglandin E2: PGE2
21. C-reactive protein: CRP
22. Insulin receptor substrate-1: IRS-1
23. Bacteroidesforsythus: B.forsythus
24. Actinobacillusactinomycetemcomitans: A. actinomycetemcomintans
25. Preterm birth and low birth weight: PLBW
26. Heat shock proteins: HSP
27. Chronic obstructive pulmonary disease: COPD
28. The Third National Health and Nutrition Examination Survey: **NHANES III**
29. Alzheimer's disease: AD

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