ROLE OF SALIVARY BIOMARKERS IN DIAGNOSIS OF PERIODONTITIS – A LITERATURE REVIEW

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ABSTRACT
Periodontitis, a chronic inflammatory disease of oral cavity, caused by the complex interactions between host immune system and sub-gingival microflora leading to chronic gingival inflammation, loss of periodontal ligament attachment, dental impairment, and finally tooth loss. Prevalence of periodontal disease varies and reported a higher prevalence in Asian countries. The conventional diagnostic techniques used for periodontitis are inherently limited. Periodontitis immunopathogenesis studies and mediator analyzes in saliva allowed the identification of a number of disease-specific molecular biomarkers. In this review article, diagnostic role of various salivary biomarkers in periodontitis has been discussed. Original articles, systematic and narrative reviews related to relevant study published since 2009-2019 were included in the literature search through PubMed, Google Scholar, Web of Science and Scopus. Consequent assessments of these salivary biomarkers with analytical chair facilities will yield more accurate diagnostic results which will further aid in early detection and better clinical management of periodontitis patients.

KEY WORDS: Saliva (MeSH); Biomarker (MeSH); Periodontitis (MeSH); Diagnosis (MeSH)

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INTRODUCTION
Broadly speaking, the term "periodontal disease" comprises mainly of three classes gingivitis, chronic periodontitis and aggressive periodontitis. Periodontitis is ranked as the sixth most prevalent inflammatory gum disease worldwide. It is a multifactorial disorder and involves cascade of sequential inflammatory processes taking place between bacterial pathogens and host immune system. Three main bacterial pathogens are mainly associated with chronic periodontitis namely Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia whereas in case of aggressive periodontitis Aggregatibacter actinomycetemcomitans is the main causative pathogen. Moreover, genetic, environmental and behavioral risk factors are also believed to play crucial role in the pathogenesis of periodontitis.1,2 Gingivitis is the mild and reversible form of periodontal disease and occurs due to the accumulation of bacterial biofilm (dental plaque) on teeth adjacent to the gingiva. Periodontitis is the more severe and irreversible form of periodontal disease leading to chronic gingival inflammation, loss of cementum attachment, formation of deepened periodontal pockets and ultimately alveolar bone loss. Persistent bad breath, recession and bleeding of gums, mobile teeth, impaired mastication and painful jaws are frequent problems encountered in periodontitis patients. Moreover, periodontitis patients have detrimental effects on physical, psychological, and social aspects of life as compared to periodontal healthy individuals.3,4 Many etiological factors are associated with increased risk of periodontal disease. Some of them are modifiable (subject to change on intervention) and some are non-modifiable. Cigarette consumption is considered to be a major attributable risk factor for chronic periodontitis. The average estimated risk lies between 2 and 7. Hence, more detrimental tooth affects and poor periodontal status is related to smokers than non-smokers.5 Tobacco is another important modifiable contributable factor in the development of periodontitis and is responsible for more than half of adolescent periodontitis in the USA. Dentists examined participants for the use of tobacco and educated them to use standardized clinical requirements. On-modifiable risk factors include increasing age, male predilection, minor ethnicity, low socioeconomic background, genetic predisposition, dermatological, hematological, granulomatous, immunosuppressive and neoplastic conditions.6 Several systemic diseases such as adverse pregnancy, cardiovascular disease, stroke, pulmonary disease and diabetes mellitus are also associated with different forms of periodontal disease, but no causal relationships were identified.7

The prevalence and disease severity of periodontitis varies for different countries.8 The worldwide prevalence of periodontitis is nearly 10–15% of adult population and it is gradually increasing with age.9 However, in Asia the prevalence accounts for 15–20% of adult population.10 According to the

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Bacterial pathogens present in the responses also play a vital role in of periodontal disease. Host immune is not responsible for the pathogenesis However, pathogenic microflora alone identified from the dental plaque. species of microbial colonies have been inflammation and propagation of defensive mechanisms, leading to species (dental plaque) and the host the commensal residing microbial microbial imbalance that disrupt the The pathogenesis of chronic periodontitis is exaggerated through a microbial imbalance that disrupt the normal symbiotic relationship between the commensal residing microbial species (dental plaque) and the host defensive mechanisms, leading to inflammation and propagation of disease. Approximately, 800 different species of microbial colonies have been identified from the dental plaque. However, pathogenic microflora alone is not responsible for the pathogenesis of periodontal disease. Host immune responses also play a vital role in progression of periodontitis (figure 1). Bacterial pathogens present in the dental plaque triggers activation of intrinsic host cells which secrete pathogen-associated molecules (PAMPs) such as lipopolysaccharide (LPS) and glycoconjugates. These PAMPs in turn recruit and activate certain pro-inflammatory mediators including neutrophils, cytokines and osteoclasts (MMP-8, MMP-9, β-glucuronidase, Interleukin-1β and Interleukin-8). Macrophages and lymphocytes (T-and B-lymphocytes) are also activated and mediate release of other secondary inflammatory mediators (tumor necrosis factor alpha, interleukin-12, interleukin-17 and interleukin-1β). The ecological imbalance between microflora and neutrophil concentration is reflected by raised levels of Interleukin (IL)-β and IL-8. Consistent inflammatory response, ultimately leads to loss of soft and hard connective tissues of periodontium.

**METHODS**

The electronic databases were searched including PubMed, SCOPUS, Web of Science, PubMed Central (PMC), and Google scholar to conduct review literature. We used keywords like periodontitis, saliva, diagnosis and biomarkers as medical subject headings (MeSH) keywords to search for the relevant published articles. All original articles, systematic and narrative reviews published in English language since 2009-2019 were included in this study except case report or case series. Abstracts, duplicate papers as well research papers published in other languages were also excluded.

**DISCUSSION**

Assessment of breastfeeding facilities Conventional diagnostic methods for periodontal diseases relies on clinical and radiological examination of periodontal tissues but these techniques are inherently limited since they lack knowledge regarding current disease activity, severity and its episodic progression. Hence there is an utmost need to find innovative methods to overcome this limitation. In recent years, studies on various inflammatory and disease-specific biomarkers have paved way for saliva to hold potential role in field of salivary diagnostics. Salivary composition initially originates from blood, but through active transport and secretion processes taking place in the salivary glands, the actual saliva composition may change. Based on this biological mechanism, saliva has been widely accepted as putative tool for diagnosis, prognosis and evaluation of therapeutic intervention. The main advantage of opting saliva as a diagnostic medium is its accessibility through non-invasive and painless procedures which make clinical studies more patient-friendly.

**Salivary Biomarkers in Diagnosis of Periodontitis**

**Role of Cytokines in Periodontitis**

In a number of studies many cytokines such as IL-6, IL-8, IL-1β, macrophage inflammatory protein (MIP-1α), prostaglandin E2 and TNF-α have been reported to be significantly associated with periodontitis and gingivitis. IL-1β, is an established biomarker for periodontitis because its levels are observed to be much higher in patients of periodontitis than healthy controls. On the other hand, role of biomarker IL-6 in saliva is controversial. In majority of studies no significant association of salivary IL-6 is seen with periodontitis whereas, few studies demonstrated significant elevated levels of salivary IL-6 in periodontitis patients. However, salivary levels of granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-2, interleukin-3, interleukin-4, interleukin-
5, interleukin-10, interleukin-12 and interferon-γ had no significant association with periodontitis. In addition to this, Toll-like receptor-4 and 2, uric acid, IL-1β, aspartate transaminase and CCL3 (MIP-1α) are significantly associated with periodontitis.11-12 Moreover, these biomarkers also depicted positive correlations with clinical periodontal parameters including PPD, CAL and GI. Therefore, evaluation of these cytokines could be used as putative tools in diagnosis and prognosis of periodontal diseases.

**Role of Bone Biomarkers in Periodontitis**

Receptor activator of nuclear factor kappa beta ligand (RANKL), Osteoprotegerin (OPG), Osteocalcin and Osteonectin are chemokines that regulate bone metabolism and mediate bone loss characteristics of periodontitis. Alveolar bone loss is an important aspect of chronic periodontitis and molecular interactions takes place between bone metabolism and inflammation during periodontitis.13 RANKL upregulates bone resorption and its activation is stimulated by IL-1β and IL-6 cytokines. The ratio of RANKL to OPG determines bone cell resorption and turnover. Significantly elevated levels of sRANKL and lower levels of OPG were observed in chronic periodontitis patients as compared to healthy controls.14-16

Osteocalcin, also known as bone Gla-protein (BGLP) is a small (5.4kDa) calcium binding protein of bone. It is the most abundantly present non-collagenous protein in mineralized tissues. Serum osteocalcin is considered as a valid biomarker of bone turnover when resorption and formation are coupled and a specific marker of bone formation when both processes are uncoupled.17 The salivary levels of osteocalcin were significantly correlated with clinical attachment loss and hence it was concluded that osteocalcin can be used as vital diagnostic marker for periodontitis.18 Several other studies also supported the theory that osteocalcin is a biomarker related to bone turnover in periodontitis. It not only holds significant diagnostic potential but also can be used as prognostic marker to predict the likely outcome of the disease by determining its concentrations in periodontitis patients.19-22

Salivary concentrations of osteonectin and osteopontin bone biomarkers was also positively correlated with alveolar bone loss and other clinical periodontal parameters of chronic periodontitis

**Role of Systemic Inflammatory Markers in Periodontitis**

Calprotectin (neutrophil protein) is known as marker of systemic inflammation similar to procalcitonin. Levels of calprotectin are increased in the saliva of periodontitis patients. In a similar manner decreased salivary levels of the C-reactive protein (CRP); a protein involved in acute inflammation are seen in cases of periodontitis.23 Studies also suggested that salivary levels of the complement components C3 and C4 have a significant association with periodontitis. Saliva of periodontitis patients had low levels of C3 as compared to healthy individuals. Specific markers of cell injury and inflammation like alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are also significantly associated with periodontitis and their concentrations are enhanced in periodontitis patients.24 β-Glucuronidase is an enzyme found in neutrophil lysosomes and has role in the digestion of proteoglycans. Salivary level of this enzyme is directly proportional to the severity of periodontitis. Also, levels of glutathione peroxidase, which is a marker of neutrophil antioxidant capacity, are significantly raised in saliva of periodontitis patients.25

**Role of Growth Factors in Periodontitis**

Growth factors have diverse functions in immune responses and many of them are well correlated with periodontitis. Strong evidence in research suggests that levels of transforming growth factor (TGF-β), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) are increased in the GCF of periodontitis patients. However, fewer studies also demonstrate positive correlation of salivary hepatocyte growth factor (HGF) with periodontitis. HGF is released by gingival fibroblasts and control tissue regeneration and wound healing processes in periodontitis.26,27

**Matrix Metalloproteinases (MMP’s)**

MMPs are synthesized by different periodontal cell types such as fibroblasts, basal cells, endothelial cells and white blood cells. MMPs play a central role in major physiological functions like tissue remodeling, cell repair, homeostatic balance and immune responses. MMPs levels are upregulated during gene transcription, gene processing and cell activation processes.28 During periodontal disease this balance is disrupted and stimulates increased activation and release of MMP-8 and MMP-9. Matrix metalloproteinase 8 or neutrophil collagenase has been analyzed in several studies as a possible diagnostic biomarker of periodontal disease. Concentrations of MMP-2 and MMP-9 (salivary gelatinase A and gelatinase B respectively) are also elevated in periodontitis patients. On the contrary, levels of TIMP-1 are found to be much elevated in saliva of healthy individuals than periodontitis patients.29-32

**CONCLUSION**

This review article highlights the diagnostic role played by different salivary biomarkers in periodontitis. Based on the accuracy and efficacy of these biomarkers, dental practitioners can not only diagnose periodontitis at an early stage and reduce disease severity but also set up more efficacious and immediate treatment strategies. Quantitative analysis of these biomarkers in human saliva will help to improve healthcare outcomes in field of rapid point of care (POC) diagnostics. The identification and isolation of these salivary biomarkers underline the fundamental principles of salivary diagnostics by introducing quick, non-invasive and reliable diagnostic/screening procedures. Last but not least, implementation of further longitudinal and clinical studies will clarify role of these disease-specific biomarkers in pathogenesis of periodontitis and yield highly validated diagnostic results that would aid in better clinical management of periodontitis patients.

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Following authors have made substantial contributions to the manuscript as under:

HS & FS: Conceiving and designing the review, designed the literature search, screened results of searches, abstracted data from included studies and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

MMM, Sab K & Sad K: Conducted the literature search, screened results of searches, drafting the manuscript, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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