The Role and Significance of Biomarkers in the Diagnosis of Dental Disease: A Depth Review Study, Challenges, Limitations and Future Directions

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ABSTRACT

This research on the biomarkers in periodontal diseases emphasizes the use of salivary and gingival crevicular fluid biomarkers, like MMP-8, IL-1β, and RANKL, as basic tools for the early diagnosis, follow-up assessment of the progression of disease, and response to treatment. The application of non-invasive diagnostic approaches, like salivary diagnostics, was also recommended for enhanced accuracy and access. The biological mechanisms of the biomarkers were seen as the backbone of knowledge through which an individualized approach toward the management of diseases like periodontal diseases can be created.

Keywords

Periodontal disease; Biomarkers; Saliva-based diagnostics; Gingival crevicular fluid; Inflammatory markers; Non-invasive diagnostic approaches.

1. Introduction

It is well recognized that periodontal disease begins with inflammation and the breakdown of the tooth's supporting tissues, starts with the formation of dental plaque. It may gradually progress to periodontitis through such clinical features as the presence of periodontal pocket or loss of attachment unless such progresses to the loss of the tooth itself (Ginting et al., 2021; Al- Hajri et al., 2017). Often, it is asymptomatic in the initial stages, leading patients to dental care when the disease has progressed significantly (Baek et al., 2023). The criticality of the requirement for early diagnosis is established due to the fact that proper and timely intervention may prevent progression of periodontal disease and its complications.

There are numerous reasons why early diagnosis of periodontal disease is important. This allows for the possibility of the application of non-surgical treatments such as scaling and root planning that can be quite effective in the management of the disease

in its early stages (Baek et al., 2023; Georgieva, 2020). Through various researches, it has been proved that if early diagnosed, the disease can minimize the risks of having severe periodontal conditions and its subsequent effects on the health status, for example, cardiovascular diseases and stroke (Patel et al., 2020; Linden et al., 2013). Furthermore, with early diagnosis, educating a patient about the disease process motivating them to adhere to preventions helps in the optimal final outcomes of oral health (Georgieva, 2020; Mutamuliza et al., 2015).

Furthermore, systemic manifestations of periodontal disease require its earlier diagnosis. It is known that chronic periodontitis is associated with other systemic diseases, such as diabetes, cardiovascular disease, and even renal failure (Patel et al., 2020; Demmer et al., 2010; Ioannidou et al., 2010). The periodontal disease can also contribute to a rise in systemic inflammatory markers and thus to a worsening of such conditions (Nakib et al., 2013; Nijakowski et al., 2022). Detection of periodontal disease at an early stage helps in local treatment, and also prevents the diseases from aggravating systemic health.

Periodontal disease continues to be a matter of concern for oral health because the symptomless nature of the disease requires vigilant and periodical visits that could allow its detection early enough. Therefore, aggressively managing periodontal disease is a crucial strategy for preserving systemic as well as oral health.

Table 1: Categories of Periodontal Biomarkers.

Category	Examples	Role	Key References
Diagnostic	MMP-8, IL-1β, TNF-α	Identify disease presence and severity by assessing inflammation markers.	Kinney et al., 2013; Gomes et al., 2023
Prognostic	IL-17A, OPG, MMP-9	Indicate likelihood of disease progression and severity.	Relvas et al., 2023; Sexton et al., 2011
Predictive	miRNAs (miR-146a), RANKL	Forecast responses to treatment and risk of recurrence.	Han et al., 2020; Bostancı et al., 2021

The biomarkers are the measurable indicators of normal biological processes or other pathological processes or indicate the pharmacologic responses to a therapeutic intervention. For periodontal disease, these biomarkers are important because they highlight the host's response to bacterial invasion, inflammation, and tissue destruction as part of the disease process. Some of the specific biomarkers for periodontal disease were identified in saliva and GCF by several studies. Very significant in indicating periodontal inflammation and degradation of tissues is the presence of salivary biomarkers, including interleukin (IL)-1β, C-reactive protein (CRP), and matrix metalloproteinase-8 (MMP-8). These biomarkers have been related to an increased severity of periodontal diseases, and high salivary levels have been associated with such, considered useful for diagnosis purposes as well as monitoring the progression of the disease (Hendek et al., 2015; Gomes et al., 2023; Kinney et al., 2013). Additionally, the salivary proteins and peptides present might even provide a clue on

the processes of inflammatory host response as well as those in respect to tissue remodeling during periodontal disease (Satoh, 2014).

Another source of rich biomarkers that will give information on the status of the periodontium is GCF. Biomarkers including osteoprotegerin and MMP-8 in GCF have been related to gum disease activity and progression Kinney et al., 2013; Al‐Sabbagh et al., 2011). The collection of GCF offers the benefits of being a non-invasive procedure with direct reflections of pathological process occurring within the periodontal tissues (Amr et al., 2019). In the study, microbial enzymes, such as β-glucuronidases, were also considered as potential markers of the severity of periodontal disease. Elements of both microbes and hosts were noted in the pathogenesis of the disease (Lietzan et al., 2023). MicroRNAs have also very recently been used as potential biomarkers. Some miRNAs in saliva have been linked with periodontal disease and have been identified as non-invasive markers (Han et al., 2020; Al‐Rawi et al., 2020). It has been suggested to enhance the accuracy of the diagnosis that a panel of biomarkers rather than one be used as the different biomarkers could signify the different aspects of the disease process (Kim et al., 2021; KC et al., 2019).

2. Understanding Biomarkers in Periodontal Disease

There are three sorts of biomarkers in general for periodontal diseases: diagnostic, prognostic, and predictive. This forms a unique classification, which represents a vital role in both the treatment and understanding of periodontal diseases. Diagnostic Biomarkers are employed for the detection of periodontal disease. Salivary biomarkers are used in diagnosing periodontal conditions as several research studies have shown. MMP-8 was detected as a consistent, reproducible diagnostic marker proportional to the severity of connective tissue degradation and the degree of periodontal disease (Sexton et al., 2011; Nagarajan et al., 2019). In GCF, alkaline phosphatase and beta-glucuronidase have been reported with more than 77% diagnostic accuracy regarding the predictability of gum disease (Baliban et al., 2012). In addition to that, salivary biomarkers, like interleukin-1 beta (IL-1β), have also been emphasized; in fact, it is one of the most potent predictors of periodontal disease (Zhang et al., 2021).

Prognostic biomarkers provide an estimate of the most probable future of periodontal disease. They are indicators of the further course of the disease considering biological indicators at present. For example, IL-1β, IL-17A, and osteoprotegerin (OPG) in saliva have been linked with the severity and complexity of periodontal disease, thereby acting as a potential prognostic indicator (Relvas et al., 2023). Other biomarkers that exist in GCF include MMP-8 and neutrophil elastase. These biomarkers have been linked with disease activity and progression and, therefore, useful for prognosis (Kinney et al., 2013). Predictive capability for disease progression is of significance to the implementation of timely intervention and management strategies.

Table 2: Key Salivary Biomarkers in gum Disease.

Biomarker	Source	Role in Periodontal Disease	Key References
MMP-8	Saliva	Indicates connective tissue breakdown and inflammation.	Hendek et al., 2015; Kinney et al., 2013
IL-1β	Saliva	Signals inflammatory activity and tissue resorption.	Gomes et al., 2023; Zhang et al., 2021

TNF-α	Saliva	Amplifies inflammation and promotes alveolar bone loss.	Teles et al., 2010; Hussien, 2023
miR-146a	Saliva	Reflects microbial and host response regulation.	Han et al., 2020; Al-Rawi et al., 2020
Calcium	Saliva	Related to dental plaque mineralization.	Kumar et al., 2018

Predictive Biomarkers are utilized in the prediction of the reaction to specific treatments or even recurrences of disease. This research interest focuses on identification of biomarker signatures able to predict periodontal health/disease effectively. For example, employment of salivary MMP-8 alongside other biomarkers in predictive diagnostics related to treatment outcomes and recurrent incidences of the disease (Sexton et al., 2011; Bostancı et al., 2021). Another study found that miRNAs might be included as potential biomarkers because they can be considered predictive markers for the progression of periodontal disease and response to treatment (Han et al., 2020; Al‐Rawi et al., 2020). Such biomarkers are likely to make a difference in the personalization of treatment in the periodontal therapy field. The three types of categories of biomarkers in periodontal diseases, that are diagnostic, prognostic, and predictive aid in providing a broad general understanding of the existence and progression of the disease while also describing its response to specific treatments. Continued studies would further develop these classifications towards developing better outcomes in clinic, through enhanced biomarker application.

Periodontal disease is characterized by the involvement of intricate biological processes: most importantly, inflammation, tissue loss, and bone loss. Different biomarkers in saliva and gingival crevicular fluid are seen as indicative of disease progress and severity. Inflammation is the core of periodontal disease; it is what causes tissue loss and bone loss. Pro-inflammatory cytokines, such as IL-1β and TNF-α, are biomarkers of periodontal disease because they have been consistently found in cases of periodontal diseases. The degree of inflammation and tissue destruction associated with periodontal diseases is related to high levels of such cytokines (Gomes et al., 2016; Teles et al., 2010). For example, IL-1β was demonstrated to have a high correlation with clinical manifestations of periodontitis, therefore, making it a key inflammation mediator (Teles et al., 2010). Levels of TNF-α were found to be associated with worsening of periodontal disease when there are risk factors such as smoking and diabetes. The value of TNF-α has been postulated to be a salivary biomarker for disease progression (Gomes et al., 2016).

The activity of matrix metalloproteinases specifically secreted during inflammation, MMP-8, causes the destruction of connective tissue, which is the main cause of tissue destruction in periodontal disease (Sexton et al., 2011). Salivary MMP-8 has emerged as a valid indicator of periodontal disease representing the level of connective tissue degradation due to the effects of inflammation. Furthermore, some bacteria, like Porphyromonas gingivalis, can activate MMPs, thus promoting tissue destruction (Gürsoy et al., 2011). This bacterium-host interaction highlights the contribution of

microbial factors to the disease mechanism of periodontal disease because they can promote the inflammatory reaction and tissue degradation.

The bone resorption process is also another major part of periodontal disease often due to the inflammatory mediators and osteoclasts activity. OPG and RANKL are key controllers of osteoclastogenesis that have been studied as a potential marker for periodontal disease (Sexton et al., 2011; Kuboniwa et al., 2016). The levels of OPG and RANKL would represent the activity of resorption processes of bones in the periodontal tissues. There is a raised RANKL level with down-regulated OPG levels which suggest increased osteoclast activity. This would cause additional loss of bones in periodontal disease (Sexton et al., 2011; Kuboniwa et al., 2016).

3. Types of Biomarkers in Periodontal Disease

Inflammatory Biomarkers:

These major cytokines in periodontal disease development include TNF-α, IL-1β, and IL-6. Because periodontal infections induce the synthesis of these pro-inflammatory cytokines, they are thought to play an important role in orchestrating the inflammation that characterizes periodontal disease. IL-1β especially is produced during the natural course of periodontal disease progression. Since IL-1β levels have been shown to be elevated and connected with the severity of gingivitis, patients with periodontal disease had much higher salivary IL-1β levels than healthy people (Gomes et al., 2016; Kim et al.,2021). This cytokine has been documented to cause bone resorption, an extremely critical step in advanced stages of gum disease (Kumar et al., 2018). In addition, its correlation with clinical parameters indicative of severity of periodontal disease suggests that it may be used as a biomarker for screening purposes (Gomes et al., 2016; KC et al., 2019). IL-1β action, therefore, results in the production of MMPs, which continues to enhance tissue destruction as they degrade components of the extracellular matrix and degrade parts of the periodontal structures (Jir et al., 2018).

TNF-α is another important cytokine related to periodontal diseases. It is secreted by various immune cells. It is involved in inflammatory processes. It has been found to be at very high levels during periodontal diseases, and in proportion to the severity. It has been found proportionate to the severity of diseases (Swetaa et al., 2021; Alamelu et al., 2020). TNF-α not only causes inflammation but also stimulates osteoclasts, which are responsible for the loss of alveolar bone; it is one of the key features of severe gum disease (Hussien, 2023; Gür et al., 2023). The cytokine has the potential to stimulate other inflammatory mediators' production, thus amplifying the inflammatory response and contributing to periodontal tissue destruction (Thahir et al., 2022; Jain et al., 2020).

IL-6 also contributes to the inflammatory environment in periodontal disease. It is implicated in the recruitment of immune cells to the site of infection; besides, it plays a role in the progression of periodontal tissue destruction (Jain et al., 2020; KC et al., 2019). IL-6 was reported in high concentrations in the gingival crevicular fluid of patients with periodontitis and appears to play a role in localized inflammation (Toyman et al., 2014). IL-6 may be associated with other systemic diseases such as cardiovascular disease because of its systemic impact on periodontal disease (WANG et al., 2022).

In pathological conditions such as periodontal disease, zinc-dependent endopeptidases known as (MMPs) play a crucial role in the degradation of ECM components. In the case of periodontitis, MMP-8 and MMP-9 have been identified as significant markers of tissue damage. The levels of such MMPs in gingival tissues and saliva are directly related to the severity of inflammation and tissue devastation caused by periodontal disease; consequently, they become markers of diagnostic importance.

MMP-8 is generally referred to as neutrophil collagenase and is more or less of polymorphonuclear leukocytes' origin, and has been described as the primary enzyme for the degradation of collagen-the primary constituent of the periodontal extracellular matrix by Gupta et al., 2014; Sioustis et al., 2021. The MMP-8 levels have been seen to be significantly higher in inflamed gingival tissues than in healthy controls. Hence, it is found to play a role in connective tissue destruction (Lin et al., 2020; Sioustis et al., 2021). For instance, Lin et al. observed an upregulation of MMP-8 that was 1.8-fold in inflamed tissue, while MMP-9 expressed a very high fold-increase at 13.6 thus indicating participation of enzymes in pathologically related processes of periodontitis (Lin et al., 2020). On top of that, MMP-8 concentration correlates well with collagen degradation products indicating that it can be considered an effective biomarker for monitoring the severity of tissue loss. Zhang et al., (2018) and Al-Majid et al., (2018)

The role of MMP-9 in periodontal diseases, however, is more as that of an enzyme degrading some of the ECM components thus promoting inflammatory responses. As indicated by Grant et al., (2022), Mohammed et al., 2022. Saliva and gingival crevicular fluid (GCF) of periodontitis patients have been shown to contain elevated levels of MMP-9, which may help in the diagnosis of this disease (Grant et al., 2022; Mohammed et al., 2022). These MMPs in oral fluids not only help in diagnosing periodontal disease but also provide information about the continuous tissue remodeling process that is going on with the advancement of the disease. Mohammed et al., 2022; Özcan et al., 2016.

In addition, the balance of MMPs and TIMPs determines the health of the periodontium. An imbalance between increased MMP activity and decreased TIMP is found in most cases of periodontitis, resulting in a higher rate of tissue breakdown (Fenol et al., 2014; Popat et al., 2014). For example, MMP-8/TIMP-1 has been reported to be elevated and considered a potential severity marker of periodontal disease through increased tissue destruction (Al-Majid et al., 2018). This indicates that besides acting as biomarkers, MMPs also act as targets in the treatment of periodontal disease. In a word, MMP-8 and MMP-9 as biomarkers of periodontal diseases, indicate crucial tissues destruction which in high contents in the gingival tissue, saliva will have some proportion to be proportional with higher disease status and break tissue. A knowledge about their importance in this context is, therefore very crucial to upgrade diagnosis and the way for treatments through clinical research.

Bone Resorption Biomarkers:

Biomarkers such as RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand), osteoprotegerin (OPG), and CTX (C-terminal telopeptide of type I collagen) are

crucially related to the regulation of alveolar bone loss, especially periodontal disease. The key cytokine involved in osteoclastogenesis is RANKL, and OPG acts as a decoy receptor that would inhibit RANKL's activity and therefore regulate the bone resorption process. The balance between RANKL and OPG is essential; increased RANKL/OPG is related to elevated osteoclast activity, followed by loss of alveolar bone (Li et al., 2023; Sojod et al., 2017).

There are some works which indicate that RANKL is a potent driver for differentiation and also an activator of the osteoclast. An illustration is the experimental periodontitis models, where augmented production of RANKL has been found in the tissues with the periodontia leading to enhanced bone cells formation and bone lysis (Settem et al., 2021; Malcolm et al., 2015; Zhang et al., 2023). Inflammatory cytokines, such as those produced by bacterial infections, can enhance alveolar bone loss by increasing the expression of RANKL (Zhang et al., 2023; Melgar-Rodríguez et al., 2015). OPG counteracts the activities listed above because it interferes with the interaction between RANKL and the RANK present on precursors of osteoclasts (Sağlam et al., 2013; Sağlam et al., 2015). Recent therapeutic modulation studies on the RANKL/OPG axis have indicated potential use as an interventional target for diseases afflicting periodontiums and can modulate alveolar bone loss (Gao et al., 2021; Kızıldağ et al., 2019; Cafferata et al., 2020).

Table 3: Challenges in Biomarker Implementation

Challenge	Explanation	Key References
Validation complexities	Difficulty in establishing sensitivity and specificity across diverse populations.	Gogate et al., 2021; Disanto et al., 2017
Variability in methodologies	Differences in sampling, analysis techniques, and study cohorts lead to inconsistent results.	Posti & Tenovuo, 2022
Regulatory hurdles	Lengthy processes and unfamiliarity with diagnostic requirements hinder clinical translation.	Zhang & Chan, 2010; Füzéry et al., 2013
Integration of digital biomarkers	Successful integration requires robust collaboration and consistency in defining and utilizing digital markers.	Vasudevan et al., 2022

Though it is not in the RANKL/OPG signaling pathway itself, CTX acts as a biomarker of bone resorption. High serum and urine levels of CTX have been shown to be indicative of high osteoclastic activity, and thus the turnover rate of bones can be termed elevated, suggesting an ongoing loss of alveolar bone in periodontal diseases (Jin et al., 2013; Alpan & Çalışır, 2022). In several studies, it has been shown that decreased expression of RANKL and increased expression of OPG result in decreased CTX levels and consequently reduced osteoclastic activity and subsequent bone resorption (Arabacı et al., 2015; Jin et al., 2013). RANKL and OPG play a key role in the pathogenesis of alveolar bone loss, especially within the scope of periodontal disease. The interrelation of these biomarkers and the level of CTX could therefore be helpful for determining bone remodeling within periodontal tissues.

Understanding such relations will provide a clue toward the design of targeted therapy for alleviating alveolar bone loss caused by periodontitis.

Microbial Biomarkers:

The components involved are critical biomarkers assessing pathogenic bacterial activity especially with lipopolysaccharides, proteins and metabolites. Lipopolysaccharides, a part of the outer wall of Gram-negative bacteria escape into the blood system because of infections or dysbiosis gut leading to an enhancement of intestinal permeability. Numerous illnesses have been linked to elevated serum levels of LPS, such as metabolic-associated fatty liver disease, where it is used as a marker for gut barrier dysfunction and systemic inflammation (Zhang et al., 2022). Blood LPS may also be an indicator of other pathological conditions, such as venous thromboembolism, in which the levels of lipopolysaccharide-binding protein are correlated with LPS load and gut microbiota health (Jensen et al., 2022).

Other than LPS, proteins of pathogenic bacteria are the most important biomarkers. Elastase and LasA produced by Pseudomonas aeruginosa are secreted proteases whose expression is enhanced during infection. Their levels can be measured to identify bacterial infections and track the effects of antibiotics (Buss et al., 2018). Such presence may also allow the differentiation between bacterial and viral infection, for example, calprotectin in throat swabs (Lown et al., 2022). Such proteins do not only indicate the presence of pathogens but also the physiological state of the host's immune response. Metabolites, specifically phospholipids, have become sensitive biomarkers for bacterial infections. The levels of PC in CSF have been used to distinguish between bacterial and viral CNS infections. Specific species of phosphatidylcholine have been correlated with the severity of bacterial meningitis (Al-Mekhlafi et al., 2021, Araujo et al., 2020).

The identification of these metabolites using advanced techniques like mass spectrometry increases the accuracy of diagnosis and illuminates the pathophysiological processes associated with bacterial infections. Moreover, the metagenomic sequencing has been proven to be an effective tool for the detection of pathogenic bacteria, especially in central nervous system infections, through the detection of microbial DNA (Wang, 2024; Xing et al., 2020). This further supports the fact that microbial components are biomarkers because it gives a complete characterization of microbial populations and distinguishes specific infections based on their genetic fingerprints.

Oxidative Stress Biomarkers:

Therefore, is an emerging etiology in the pathogenesis of periodontal disease, particularly chronic periodontitis, wherein the markers for the said stress, malondialdehyde, and ROS correlate with tissue damage and the severity of gum disease. MD A by-product of lipid peroxidation, is a good biomarker for oxidative stress which has been found at consistently higher levels in patients suffering from chronic periodontitis than in healthy controls. This elevation suggests that oxidative

stress does have a role in the inflammation characteristic of periodontal disease (Mannan et al., 2020; Shetty et al., 2017; Aziz et al., 2013).

The relationship between OS and periodontal destruction may be explained by how ROS contributes to tissue inflammation and destruction. ROS produced by activated neutrophils and other immune cells in response to the periodontal pathogens cause oxidative degradation of cellular components, which include lipids, proteins, and DNA (Jha et al., 2022; D'Aiuto et al., 2010). High levels of oxidative markers, such as 8-hydroxydeoxyguanosine (8-OHdG), have been linked to DNA damage in periodontal tissues and further implicates oxidative stress as a component in the disease progression (Jha et al., 2022; Boşca et al., 2016). The oxidative damage does not only worsen inflammation but also the healing processes that result in the loss of periodontal attachment and alveolar bone (Baltacıoğlu et al., 2014).

Studies that describe associations between oxidative markers with inflammatory cytokines, such as interleukin-6, and C-reactive protein (D'Aiuto et al., 2010; Zhang et al., 2022) have made known the interplay between oxidative stress and systemic inflammation. Hence, this interaction demonstrates the sophistication of periodontal diseases wherein oxidative stress is also reflective of systemic inflammatory response mechanisms that may have resultant implications for general health and concurrently reflective of local tissue destruction. The persistent oxidative stress caused by chronic periodontal inflammation has been linked to more severe types of periodontitis, including aggressive periodontitis (Baltacıoğlu et al., 2014; Zhang et al., 2022).

Genetic and Epigenetic Biomarkers:

Numerous genetic variations implicated in the host immune response and inflammatory processes have been linked to susceptibility to periodontal disease. The intricate interaction between genetics and environmental variables is shown by the hundreds of studies that have found certain genetic variations linked to an elevated risk for periodontal diseases. One of the main genes that was found to be linked to susceptibility for periodontal disease is the NLRP3 gene. Mahmood & Abbas Mahmood & Abbas (2023) demonstrated that polymorphisms in the NLRP3 gene lead to increased production of cytokines once activated, hence the patients with such genes have increased susceptibility to develop periodontitisThis understanding supports the general awareness that the genetic variation causes variations in inflammatory responses and that this forms a crucial link in the pathogenesis of periodontal diseases.

Another important genetic determinant is the MMP-1 gene. As Fadhil et al.The MMP-1 -1607 2G allele has been related to the overexpression of MMP-1, a key contributor to tissue remodeling and inflammation associated with periodontal disease. Thus, it would mean that those who carry this allele are more susceptible to periodontitis because of increased processes of tissue breakdown (Fadhil et al., 2022). Moreover, the HLA system has also been under focus regarding periodontal disease. Li et al. Li et al. (2017) concluded that specific HLA alleles have association with rheumatoid arthritis as well as aggressive periodontitis. The common genetic predisposition towards it has been indicated by the implication of such an association, as the result is more determined by the variation in the genes related to immune response.

More extensive genetic research has identified a few more variants associated with periodontal disease in addition to these specified genes. Nibali et al. (2017) showed that variants in ACE, CD14, and MMP2 were involved in the pathogenesis of periodontal disease since they play a role in inflammation and tissue remodeling. In addition, the genetic dysbiosis concept of Nibali et al. (Nibali et al., 2016) suggests that genetic variations can influence the composition of subgingival microbiome, thereby adding to the susceptibility of the disease. The heritability of periodontal disease has also been studied. Nibali et al. As revealed by a systematic review, according to Nibali et al. (Nibali et al., 2019), a substantial genetic component is involved with periodontal disease: heritability estimates pointed out that genetic factors form most of the risk. This was also reflected in findings from Silva et al. (Silva et al., 2023). A considerable amount of the risk in periodontitis is due to genetic factors and, therefore, a considerable variability in susceptibility to periodontitis, especially in those who carry a monogenic syndrome.

In summary, genetic polymorphisms are a critical determinant of susceptibility to periodontal disease. Important genes like NLRP3, MMP-1, and many alleles of the HLA group have been associated with an increased risk, suggesting that genetic predisposition plays a fundamental role in the pathogenesis of periodontal diseases. Thus, the complexity of the etiology of periodontal disease relates to its potential for individualized prevention and treatment strategies. Most medical specialties, including neurology, cancer, and metabolic disorders, have long recognized that epigenetic changes—specifically, Gene methylation are a crucial indicator of the onset of numerous illnesses. Among the commonest types of epigenetic changes, DNA methylation, involves attaching a methyl group to the cytosine residues inside DNA to regulate gene expression in such a manner that the actual DNA sequence stays unchanged. It is a promising target for the development of biomarkers since it plays a significant role in the pathophysiology of many diseases.

In oncology, DNA methylation patterns have been quite widely explored biomarkers for cancer findings and prognosis. For example, the hypermethylation of specific genes, like GPX3, has been associated with worse outcomes in myelodysplastic syndromes and therefore useful in predicting transformation and disease progression (Zhou et al., 2016). Likewise, variations in the SHOX2 methylation level associated with the diagnosis of lung cancer; therefore, such epigenetic changes are regarded as excellent biomarkers for the early diagnosis and control of lung cancer (Kneip et al., 2011; Schmidt et al., 2010; Dietrich et al., 2012). A study showed that methylation profiles of TBX2 and TBX3 could classify bladder cancer patients into distinct molecular grades, thereby predicting the disease's progression (Beukers et al., 2015). Such results emphasize the potential of DNA methylation as a common biomarker for all cancers, thereby enabling personalized treatment plans.

Besides cancer, DNA methylation plays an important role in chronic diseases, such as non-alcoholic fatty liver disease and neurodegenerative disorders. It has been demonstrated that the levels of global gene methylation correlate with the histological severity of NAFLD, and their monitoring may provide insight into the disease's

progression and therapeutic interventions (Lai et al., 2019). Abnormal gene methylation patterns have been linked to disease pathology and progression in neurodegenerative diseases such as Alzheimer's and Huntington's disease. For example, in Alzheimer's disease patients, the levels of DNA methylation were elevated in the globe and correlate with increased plasma homocysteine levels that are known to increase risk of the disease (Zadel et al., 2018). This suggests that DNA methylation reports on not just the disease status but may also contribute to mechanisms of disease.

The analysis of DNA methylation is also sensitive and accessible, which provides a strong rationale to position this technology for use within the clinical realm. DNA methylation can be assessed quantitatively from highly minute amounts of DNA, opening the possibility of developing non-invasive testing methods. For example, stool samples could be used for colorectal cancer screening Chen et al. (2019), whereas plasma analyses may be a means of diagnosing lung cancer Kneip et al., 2011; Pineau et al., 2021). This non-invasive nature makes the application of DNA methylation as a biomarker even more viable for routine clinical practice, allowing for early detection and monitoring of disease progression.

This evidence is pretty compelling about the role of gene methylation as a biomarker for disease progression. The approach could be very beneficial to several diseases such as cancer and neurodegenerative diseases. Such an application could provide diagnostic accuracy, better prognosis assessments, and improve therapeutic strategy. As this continues, understanding the DNA methylation patterns may offer much towards including such biomarkers in the management of clinical patients and possibly improving the outcomes for these patients.

4. Diagnostic Potential of Biomarkers

Biomarker-based diagnostic devices have improved significantly the sensitivity and specificity of periodontal disease detection, primarily because of the identification of specific salivary and gingival crevicular fluid (GCF) biomarkers that have been correlated with the presence and severity of the disease. The Biomarkers could provide more detailed insights into periodontal status than conventional approaches that basically depend on clinical judgment. In addition, it has been found that salivary biomarkers such as matrix metalloproteinase-8 increase the sensitivity and specificity of the diagnostic accuracy. MMP-8 has a highly elevated level in periodontal disease, and it is considered as an excellent marker in disease severity and progression monitoring. Clinical parameters like probing depth and attachment loss are correlated with the levels of MMP-8 and insights have been given regarding inflammation mechanisms that prevail in periodontal diseases (Morais et al., 2017).

The development of point-of-care tests for MMP-8 enables rapid and non-invasive screening and could be very useful in large population studies (Lähteenmäki et al., 2020). Moreover, the study of gingival crevicular fluid has indicated the presence of several host-derived biomarkers, like interleukins (IL-1β and IL-6), other inflammatory mediators that are assumed to be a strong marker for gum disease (KC et al., 2019; Kinney et al., 2013). These biomarkers will enhance the sensitivity of diagnosis as disease may be detected at earlier stages. In this way, intervention is likely to occur at the right time (Arias-Bujanda et al., 2019). This systematic review conducted by Lima et al. is on the additive use of both bacterial and host-derived

biomarkers, whose combination increases diagnostic success (Lima et al., 2016). In addition, several biomarkers have been suggested to be added into diagnostic panels for specificity increases. For example, there is a suggestion that salivary markers combined with clinical examination are going to classify periodontal disease states correctly and differentiate between gingivitis and periodontitis (Zhang et al., 2021). This multi-faceted approach increases the precision of the diagnosis and provides information related to the progression of the disease and response to therapy (Ramenzoni et al., 2021).

Perhaps one of the most important tools used in biomarkers within the assessment of periodontal disease has been salivary diagnostics due to the convenience in availability of saliva and possibility for disease course following by non-invasive methods. Saliva, within its vast range of possible biomarkers, may thus be used to characterize the present status of the periodontium by different components which originate from microbial origin, cells of the host, as well as products of inflammation. Saliva is the source of multimodal information about periodontal disease.

According to research, it can be pointed out specific bacteria that are associated with periodontal diseases such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans through salivary analysis. Since these bacteria are present in saliva, it makes the method much easier compared to the traditional methods of using subgingival plaque samples (Haririan et al., 2014). Additionally, salivary diagnostics also enables finding host-derived biomarkers, such as matrix metalloproteinases and cytokines, which indicate an inflammatory response in the periodontal tissues (KC et al., 2019; Ebersole et al., 2012). For example, high concentrations of interleukin-1β (IL-1β) and MMP-8 are reportedly indicative of chronic periodontitis compared with a healthy state, thereby proving useful as reliable biomarkers (Ebersole et al., 2012).

Saliva not only plays a role in the microbial analysis but also as a protective agent for oral tissues. Saliva helps in mechanical removal of plaque, buffers acids, and has antibacterial action to contribute to periodontal health (Hussien & Maged, 2019). The role of saliva in protective function thus brings in a point for the relevance of salivary composition in the advancement of gum disease. Hence, they could be utilized to monitor therapeutic responses in patients undergoing periodontal therapy (Jeyasree et al., 2018; Sexton et al., 2011). Moreover, systematic reviews have been done on salivary biomarkers in support of their diagnostic sensitivity and specificity. For example, systematic review highlighted the possibility of some salivary biomarkers in the diagnostic use of gum disease and suggested that more research be done in the clinic to validate such biomarkers (KC et al., 2019). Another approach presented was the detection of nitrosative stress biomarkers in saliva as a new approach in the pathogenesis of periodontal disease, with the indication of a shift in salivary diagnostics (Toczewska et al., 2020).

Salivary diagnostics is a tool in the assessment of biomarkers for periodontal disease since it is non-invasive and includes an overall approach in the examination of microbial as well as host responses. It provides a diagnosis for periodontal conditions

by showing the nature of disease progression and the response to the treatment. Therefore, these research diagnostics will confirm and modify them; therefore, salivary diagnostics could be part of managing periodontal diseases.

5. Biomarkers in Monitoring Disease Progression and Treatment Response

Biomarkers are important for observing disease activity and progression in periodontal disease. These allow observation of both the immune host response and the microbial factors in disease etiology. Some of the latest breakthroughs in biomarker research identify various substances that can be measured in saliva and GCF as means of assessing the periodontal health status and predicting future disease progression. One of the identified significant indicators is a biomarker such as the microbial β-glucuronidase (GUS) activity. This is a marker associated with periodontal disease and reflects the presence of pathogenic microbes that have been captured by the host's immune response. It will thus be a useful indicator for predicting the onset and progression of periodontitis (Lietzan et al., 2023). The ability to track GUS activity could bring in early interventions and better treatment measures, especially as the global burden of periodontal disease remains sky-high.

There have been biomarkers studied over the predictive capability of MMP-8 in salivary diagnostics in the periodontal disease. The biomarker has been associated with the destruction of soft tissue. It has continually achieved distinction between healthy and diseased individuals, differentiating between periodontitis (Nagarajan et al., 2019). The combination of salivary biomarkers with pathogen detection, particularly the "red complex" pathogens, enhances the predictive accuracy for the subsequent progression of disease (Kinney et al., 2011). This multi-component evaluation offers a more holistic view for status determination of periodontal health. Moreover, GCF analysis offers the additional benefit of site-specific assessment of disease activity, whereas the analyses from saliva are often more generalized. Proteomic studies have identified a number of proteins in GCF that correlate with periodontal tissue turnover and disease progression (Satoh, 2014). For instance, the levels of inflammation cytokines, such as (IL-1β) and osteoprotegerin, whose levels have been associated with periodontal disease activity, can be used possibly as biomarkers for follow-up of the status of the disease (Kinney et al., 2013).

Apart from these, it has been demonstrated that saliva contains a level of calcium in correlation with periodontal health due to its association with the mineralization process of dental plaque (Kumar et al., 2018). The correlation between the saliva biomarkers with the clinical parameters like the probing depth and clinical attachment loss suggests the significance of saliva biomarkers to evaluate the disease progression and severity (Kader et al., 2021). Besides, several recent studies demonstrated that IL-1, IL-6, and TNF-α can be quantified in saliva as potential markers for inflammatory responses related to periodontal disease (Kim et al., 2021). Elevated levels of such cytokines in the saliva of patients with periodontal diseases compared to healthy controls establish them as potential clinical diagnostic biomarkers.

6. Challenges and Limitations

Translation of research-based biomarkers into routine clinical use at the scientific, regulatory, and logistical domains poses important challenges: among them is the need

for significant biomarker validation for clinical utility. For instance, it has been commented that, in the case of serum neurofilament light (sNfL) as a biomarker for neuronal damage in multiple sclerosis, all data needed to establish reference ranges, sensitivity, and specificity have to be acquired from diverse populations and conditions before it can be utilized in the clinic (Disanto et al., 2017). Other studies repeat this call for rigorous validation, additionally reporting that promising biomarkers often lack the crucial specification and sensitivity to translate them clinically (Gogate et al., 2021;, Parikh & Mansour, 2017).

Another major challenge is the variability of research methodologies between studies. Variability in sampling times, study cohorts, and analytical techniques can make the results inconclusive and, therefore, not translatable to clinical practice. For instance, in traumatic brain injury research, inconsistent methods have significantly limited the clinical applicability of blood-based biomarkers (Posti & Tenovuo, 2022). Also, due to their relative rarity and diversity, conditions like sarcomas can cause biomarker studies statistically unreliably (Orth et al., 2018). To overcome the hindrances and come up with ways to include publicly accessible genomic data in functional assays and translate, there remain still many troubles on this track.

Another significant barrier in this translation is related to regulation. The chain of biomarker discovery to clinical diagnostics usually long and complicated, calling for adequate evidence from front-line validation studies to merit significant investment in large trials (Zhang & Chan, 2010). It further complicates the landscape of regulation due to the fact that most researchers are not aware of the requirements for analytical and diagnostic procedures needed for clinical assays, which further disconnects the discovery of biomarkers with their clinical application (Füzéry et al., 2013). To ensure that biomarkers may be reliable in a variety of clinical settings, consistent test procedures and validation across several sites are also required (Disanto et al., 2017).

Additionally, the integration of digital biomarkers into clinical practice poses other challenges. As much as digital health technologies are expected to make the application of biomarkers more convenient, integration is only successful if the whole healthcare ecosystem collaborates (Vasudevan et al., 2022). This convergence requires a universal definition of digital biomarkers and the consciousness of exogenous factors on health that may further complicate the integration into the routine clinical workflow.

7. Emerging Research and Future Directions

The most recent periodontal disease biomarkers have enhanced the diagnostic ability and therefore increased the accuracy of diagnosis and treatment of the disease. Salivary and (GCF) biomarkers have been proposed as non-invasive, inexpensive substitutes for diagnosis. (aMMP-8) is now one of the most important biomarkers and is included in the latest classification system devised for periodontitis. Studies have established its usability for grading and staging periodontal disease, and point-of-care tests of aMMP-8 have yielded promising results in a clinical context (Sorsa et al., 2020; Lähteenmäki et al., 2020).

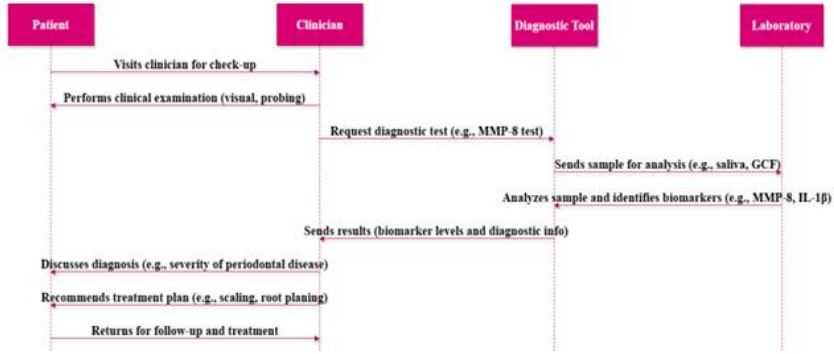

Figure 1. This sequence diagram shows the interaction between the patient, clinician, diagnostic tool, and laboratory during the diagnostic process of periodontal disease using biomarkers.

Furthermore, systematic reviews recently emphasized the accuracy of various molecular biomarkers in oral fluids for the diagnosis of periodontitis. Salivary biomarkers, especially those from GCF and saliva, are considered good indicators because they are easily collected and analyzed. Advances in the detection technology have opened up an avenue to point-of-care diagnostics, making it possible that automatic screening tools, including smart toothbrushes, would be established to facilitate early detection and referral to dental professionals (Blanco-Pintos, 2023; Grant et al., 2022).

Besides aMMP-8, various salivary biomarkers have also been studied. Recently, annexin A1 has been suggested as an indicator candidate for periodontal status; it has recently been found to be in smaller quantities in patients who suffered from periodontal diseases rather than in healthy cases (Casarin et al., 2023). Additionally, leucine-rich alpha-2 glycoprotein has been described with superior diagnostic efficiency from the level of C-reactive protein in distinguishing health or periodontal disease statuses. Hence, it also proves itself as a highly promising candidate biomarker with enhanced reliability. Another area that has been under focus is the role of (miRNAs) in periodontal disease, with promising non-invasive biomarkers found to be miR-146a, miR-155, and miR-203. The miRNAs are assumed to be regulated by microbial infections and can serve as a marker for periodontal disease progression (Al-Rawi et al., 2020; Han et al., 2020). Other studies have suggested inflammatory mediators like (IL-6) and (IL-8) as potential salivary biomarkers that have levels that are elevated with higher levels correlating to higher severity of periodontal disease (Rocha, 2024).

This would significantly enhance diagnosis and monitoring of gum disease with the potential integration of biomarkers into the clinical setting. It is by validation in well-controlled clinical studies that this bridge between discovery and practice is going to be formed to ensure that biomarkers will have reliable input on treatment decisions and management strategies for the patient (Bostancı et al., 2021; Luchian et al., 2022). In conclusion, the most recent biomarker discoveries related to periodontal disease indicate a future for non-invasive diagnostic tools that may help in early detection and personalized treatment approaches. The current research on salivary and GCF

biomarkers, including aMMP-8, ANXA1, LRG, and miRNAs, is reflective of the increased understanding of the biological basis of periodontal disease and its systemic implications.

8. Conclusion

The study brought into limelight the significant role that biomarkers will play in furthering diagnosis and treatment of periodontal diseases. Salivary and GCF biomarkers are quite promising in non-invasive diagnostic, monitoring of progression, and even treatment outcomes prediction. With problems like inconsistencies in methodologies and regulatory restrictions, ongoing research and clinical validation would help bring the benefits of these biomarkers to more common practices in periodontal care. These developments open up the avenue to precision medicine in dentistry, enabling personalized therapeutic approaches and improved results for patients.

Conflict of Interest

The authors declare they don't have any conflict of interest.

Author contributions

Each author has contributed to the study for data collection, manuscript review, editing and given their final permission for the publication.

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