Kurdistan Journal of Applied Research (KJAR)
Print-ISSN: 2411-7684 | Electronic-ISSN: 2411-7706

Review Article

Microbial and Inflammatory Salivary Biomarkers of Periodontal Diseases

Aram Mohammed Sha [a*], Hayder Raad Abdulbaqi [b], Syed Saad Bin Qasim [c]

[a] Department of Periodontics, College of Dentistry, University of Sulaimani, Sulaymaniyah, Iraq
[b] Department of Periodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq
[c] Department of Bioclinical Sciences, Faculty of Dentistry, Kuwait University, Safat, Kuwait

Submitted: 28 May 2024
Revised: 15 June 2024
Accepted: 18 June 2024

*** Corresponding Author**:
aram.hamad@univsul.edu.iq

Keywords: Saliva, Biomarkers, Microbial, Inflammatory, Periodontal diseases, Diagnosis, Prognosis.

How to cite this paper: A. M. Sha, H. R. Abdulbaqi, and S. S. Bin Qasim, "Microbial and Inflammatory Salivary Biomarkers of Periodontal Diseases", KJAR, vol. 9, no. 1, Jun. 2024, doi: 10.24017/science.2024.1.9

Copyright: © 2024 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0)

Abstract: Recent data reveals that severe periodontal diseases affect approximately 19% of global adult populations, impacting more than 1 billion individuals universally, ranking it as the sixth most predominant human disease. Diagnosis and treatment plans for periodontal disease entirely depend solely on the assessment of traditional clinical parameters. However, these parameters are not sensitive and are subject to error. Hence, microbial and inflammatory biomarkers of periodontal diseases in saliva have attracted of interest. This review aims to evaluate the salivary components as potential diagnostic tools for periodontal diseases. In addition to periodontal pathogens, interest in salivary biomarkers of periodontal disease is increasing, and a number of biomarkers have the potential to be accurate indicators of disease and the effectiveness of therapy. The most up to date studies examining the microbial and inflammatory biomarkers with both diagnostic and prognostic values in saliva were reviewed for the purpose of this study. It is apparent that saliva has many advantages over other oral fluids such gingival crevicular fluid and mouth rinse as a tool for diagnosis of periodontal disease. Biomarkers can identify and quantify periodontal risk through objective measures. Further, the combination of biomarkers seems to enhance the diagnostic and prognostic value of the biomarkers. Amongst the microbial biomarkers of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia* are widely used with some promising results. Whereas, the inflammatory salivary biomarkers of IL-1β and MMP-8 are widely used for diagnosing periodontitis and predicting the future treatment outcome.

1. Introduction

Periodontal diseases are generally classified to either gingivitis or periodontitis, they both considered as a chronic inflammatory disease of the periodontium that are triggered by the interaction between the oral microorganisms (periodontal pathogens) and the body's immune inflammatory response[1-4]. In predisposed patients, antigen and antibody response by the host stimulates variety of inflammatory responses, ultimately resulting in the destroying both soft and hard tissues around the teeth which finally lead to tooth loss [5]. Periodontitis which is the most destructive form of the disease, and it is among the most common bacterial diseases in humans which lead to irreversible damage to the periodontal tissues. Existing data suggests that 19% of adults globally suffer from periodontitis impacting more than 1 billion individuals universally [6]. In the United States, periodontitis has been reported to affect over 47% of the adult population [7], and approximately 70.6% of individuals in Germany are affected by periodontitis[8].

Periodontal diagnosis mainly relies on measuring clinical periodontal parameters, including clinical attachment loss (CAL), bleeding on probing (BOP), probing pocket depth (PPD), and alveolar bone level in radiograph [9]. The traditional comprehensive periodontal examination needs skilled dental experts and is somewhat difficult, particularly when doing assessments for extensive health surveys [10]. Thus, over the past twenty years, numerous studies have investigated the application of saliva, as a reliable and readily accessible source for the diagnosis of periodontal diseases [11-13].

Human saliva, a readily accessible biological fluid, contains various biomarkers related to diseases, making it a potential source for diagnosis. It is produced by major and minor salivary glands. Saliva can be gathered easily and painlessly, requiring no specialized equipment or skills [14]. It is crucial for diagnosing and predicting periodontal diseases, as it contains markers derived from both local and systemic sources [15]. This makes it a promising candidate for developing alternative diagnostic techniques[14]. Additionally, salivary analysis could be an economical way to perform high-sensitivity screening tests for periodontal disease [12].

Studies have investigated into the possibilities of employing salivary biomarkers linked to microbes or to the major elements of periodontal disease, such as inflammation, tissue loss, and bone remodeling. Numerous studies have displayed valuable markers for differentiating individuals with periodontal diseases from those who are periodontally healthy [16]. The advancement and integration of modern technologies that apply definite biomarkers in chairside settings are expected to raise clinical diagnostic and prognostic capabilities. Applying these technologies in different healthcare contexts and among communities with limited access to basic medical and dental services could significantly amend public health outcomes. Particularly, the use of biomarkers in diagnosing periodontitis periodontal diseases should enable early identification, intervention, and prevention, eventually improving overall oral and systemic health [17, 18]. Salivary biomarker investigation is increasingly critical for both diagnosis and prognosis purposes of periodontal diseases, while additional study is required to expand our understanding in this area. Therefore, this review aims to present salivary biomarkers as potential diagnostic tools for periodontal diseases.

2. Biomarkers

Biomarkers are described as measurable characteristics that can denote normal biological functions, disease processes, or response to treatment [19]. They are molecules that serve as a monitor for health conditions, the onset of diseases, treatment efficacy, and outcome. Biomarkers are delivered in both healthy people and those affected by particular systemic diseases. They are crucial in the life sciences and are increasingly important in diagnosing, monitoring, predicting treatment outcomes, and in drug invention [20]. Studies in oral and periodontal disease diagnostics are shifting toward techniques that can identify and quantify periodontal risk by using objective measures such as biomarkers.

Biomarkers are typically divided into the following groups:

- Biomarkers indicating the current status of the disease (active or inactive).
- Biomarkers indicating the later disease progression or outcome of treatment (prognostic biomarkers).
- Biomarkers indicating future disease onset in currently healthy sites.
- Necessity to use biomarkers for diagnosing periodontal diseases.

The conventional clinical diagnostic periodontal parameters, established over five decades ago, persist to serve as the fundamental paradigm for diagnosing periodontal diseases in clinical training nowadays. The parameters comprise PPD, BOP, CAL, plaque index, and radiograph to assess alveolar bone loss. These conventional clinical parameters were recognized for their simplicity, affordability, and minimally invasive nature [21]. Amongst them, BOP is believed to be the main conventional biomarker for periodontal disease and is regarded as the best obtainable predictor of disease. However, researchers observe that BOP can produce many false positives, while the lack of BOP is an extremely accurate undesirable indicator of disease activity [21]. Furthermore, these parameters are subjective clinical measures that can be time-consuming and might be ineffectively applied by the dentist. For

instance, relying solely on PPD measurements for diagnosis because of time restrictions can result in improper diagnosis, unsuitable therapy, and low rates of appropriate treatment interference.

Diagnosis and monitoring typically include painful invasive techniques, adding unnecessary pressure to a previously bad experience. The finding of microbial, immunologic, and molecular biomarkers in saliva offers a novel approach to evade these procedures, permitting the assessment of both healthy and diseased individuals using oral fluids. The use of salivary biomarkers is important as they assist as indicators for local, systemic, and infectious diseases. Initial diagnosis and management that rely on these biomarkers can aid in decreasing the severity and potential problems of the disease process [18, 22]. Because of its multifactorial nature and indistinct etiology, early diagnosis of periodontitis is still challenging. Recognizing the active phase of the disease and at-risk patients is an important challenge for dentists and practitioners. There is a persistent need for advanced diagnostic tests that can discover microbial challenges to the host. Innovative methods could precisely detect present disease activity, anticipate future vulnerable sites, and evaluate the efficacy of periodontal therapies. A new diagnostic paradigm could considerably improve the clinical management of periodontal diseases [23].

For effective prevention and treatment, early risk prediction and precise diagnosis of existing disease activity are essential. Therefore, periodontists would greatly benefit from an accurate diagnostic tool that can provide trustworthy data to assess the existence, severity, and effect of the disease [24, 25].Additionally, there is a lack of methods to recognize highly liable individuals who are in danger of disease progression [26].

Risk factors are acknowledged to affect the onset and development of periodontitis, along with host liability and numerous local and systemic factors. They can change biomarkers linked to the disease. To address these challenges, there has been a substantial attempt to progress further diagnostic and prognostic tests. Study of oral and periodontal diseases has concentrated on techniques to accurately recognize and quantify periodontal risk by using biomarkers [27].

The primary objective of biomarkers is to demonstrate the presence or absence of periodontal bacteria, inflammation of the periodontal tissues, the host's inflammatory immune response to a specific microbe, and damage to the host's tissue. Different agents, involving serum, saliva, subgingival biofilm, biopsies, and GCF, have been used for biomarker investigation. Despite the development of various biomarkers and diagnostic tests, only a limited diagnostic kit has been presented. These kits primarily involved biomarkers from GCF, but they failed to obtain traction in practice due to a lack of market research [27]. The problems described with these kits involved high cost, time-consuming procedures, complexity, and difficulty in explanation. Subsequently, saliva was selected as an alternative diagnostic means.

There are many obstacles for predicting periodontal diseases because it is still unclear what precise phenomena encourage the sequence of events that cause tissue damage and what biomarkers are needed to forecast disease initiation and progression. Biomarkers that are accountable for the initiation of the disease process are not the ones that are linked with inflammation. It is possible that the microbial biomarkers involved in gingivitis such as *Streptococcus sanguis* and *Actinomyces naeslundii* may vary from the biomarkers involved in periodontitis such as *porphyromonas gingivalis* [27].

A variety of oral fluid biomarkers, including host-derived proteins (such immunoglobulins), host cells (like PMNs), phenotypic markers, bacteria and their metabolites, hormones, ions, and volatile substances, have all been studied for periodontal diagnosis. As bacterial biofilm and the host interact intricately to cause periodontitis, it is unlikely that a single biomarker can reliably forecast the course of the disease. Thus, combining biomarkers may propose a more effective approach for predicting periodontal diseases [20].

Biomarkers used to determine the activity of disease, which are released into GCF and saliva, can be approximately classified into four categories based on their resources [9, 27]:

1) **Plaque microorganisms**: This includes lipopolysaccharides, metabolic byproducts, enzymes, DNA probes, and cultures of the leading bacteria of periodontal disease.

2) **Host originated**: such as, aspartate aminotransferase, lysozyme and lactoferrin.

3) **Breakdown product of connective tissue**: These encompass fibronectin, collagen-telopeptides, osteocalcin and proteoglycans and matrix metalloproteinases.

4) **Inflammatory markers:** This category includes CRP, PGE2, cytokines (interleukin 1β), TNF-α, antibacterial antibodies, interleukins, substance P, interferon-α and complement.

Table 1: Potential salivary indicators for the diagnosis of periodontal diseases.

Enzymes	Immuno-globulins	Proteins	Phenotypic Marker	Host cells	Ions	Bacteria	Volatile Com-pounds
a-glucosidase	IgA	Cystatins	Epithelial keratins	leuco-cytes (PMNs)	Cal-cium	Aa	Hydrogen sulfide
Alkaline phospha-tase	IgG	Epidermal growth factor				*T. forsythia*	Methylmer-captan
Aminopeptidases *b*galactosidas *b*glucosidase	IgM	Fibronectin Lactoferrin platelet acti-vating factor				Mycoplasmas *P. gingivalis* *P. intermedia*	Picolines
b-glucuronidase	IgA	Vascular en-dothelial growth fac-tor				*P. micros*	Pyridine
Caprylate, esteraseli-pae collagenase, elastase esterase, kininase gelatinase lysozyme kallikrein, myeloperoxidase trypsin						*P. nigrescens* *C. rectus* *T denticola*	

3. Saliva as a Resource of Biomarkers for Periodontal Diseases

Saliva is a unique fluid of the oral cavity that is a mixture of the major and minor salivary glands and serves as an indicator of the general health of the body and its systems [28]. Under normal circumstances, the daily production of saliva reaches to 0.5 to 1.5 liters, with its composition being 98% water and the remaining 2% containing electrolytes, mucus, antibacterial compounds, and different enzymes. It has many functions, including washing, solubilizing food substances, clearing food and bacteria, lubricating soft tissues, forming a bolus, diluting detritus, aiding in swallowing, easing speech, and helping in mastication [29].

GCF proposes several diagnostic advantages, such as its site-specific characteristic. However, diagnostic tools for periodontitis that rely on samples from the gingival sulcus have not been extensively accepted by operator. There are multiple reasons for this, including the lengthy duration of the test, the requirement for multiple sampling areas, blood contamination, and unlikely expectations of the test's accuracy. Last but not least, these tests are not cheap and cannot be carried out as a chairside test [12]. In contrast, saliva is obtainable, profuse, and can be sampled in larger volumes without requiring clinical facilities. Additionally, saliva comprises elements that exhibit the activity of all periodontal sites, offering a comprehensive view of the inflammatory position of the whole mouth, unlike GCF investigation, which concentrates on active disease sites [12].

Saliva comprises microorganisms that inhabit the oral cavity, as well as exogenous substances, providing insight into the host's association with the environment. Saliva can also be used to detect agents of tissue injury, such as cytokines, chemokines, and damaging cellular enzymes, which are produced during the pathogenesis of periodontal disease [27]. Saliva has been found to contain inflammatory markers as TNF-α, IL-1, -6, and -8, MMP 8 and 9, and TIMP-1, which have been associated with

oral and systemic disorders [30]. Moreover, increased levels of many biomarkers, including soluble, chemerin, visfatin, ALP, and AST, have been linked to periodontal disease [31-36].

4. Single versus Combination of Biomarkers for Periodontal Diseases

The technological advancements is highly acknowledged, researchers have been able to identify potential panels of periodontal microorganisms and salivary biomarkers that can better predict the stability and progression of periodontal diseases and differentiate patients from healthy person [37]. A study found that the combination of MMP-9, −8, and calprotectin biomarkers, together with the measurement of red complex pathogens in dental biofilm, effectively differentiate the severity of periodontal diseases [25]. Another investigation of salivary biomarkers examined the degradation of periodontal tissue revealed that MMP-8, OPG, TNF-α, IL-1β, TNF-α, and macrophage inflammatory protein-1α can predict the severity of the disease.

MMP-8 was found to be the most accurate biomarker for predicting therapeutic response. In the meantime, studies indicated that low levels of MMP-8, MMP-9, OPG, and IL-1β positively predict the stability of periodontal disorders [38] . A further novel diagnostic technique found that *P. gingivalis*, IL-1β, and MMP-8 together had a more accurate diagnostic ability to detect periodontitis than any one of the markers alone [10]. Amongst the biomarkers examined, hepatocyte growth factor and IL-1β are the most actively researched salivary biomarkers for periodontal disease [39] . Furthermore, studies have shown that salivary biomarkers of oxidative stress are associated with disease activity [40-42], and non-surgical periodontal therapy has also been shown to raise total antioxidant levels. Nevertheless, research on different salivary cytokines has shown conflicting results in terms of a "biomarker signature." Therefore, it is essential to carry out a top-notch study that gives sensitivity and specificity first priority. Determining whether salivary biomarkers can be effectively used as a diagnostic tool for the early identification of periodontitis is crucial.

5. Biomarkers of Periodontal Diseases

Numerous studies have shown that potential salivary markers can provide a substantial amount of extra diagnostic information and can be used as screening tools for prognosis, diagnosis, and monitoring the course of periodontal disease [32, 39]. Periodontal disease biomarkers can be categorized as follows:

5.1. Microbial Biomarkers of Periodontal Diseases

Periodontal diseases include different biological processes happening inside the gingival sulcus. In a healthy individual with an intact periodontium, the sulcus is inhabited by non-periodontopathic microorganisms. Bacterial load causes the development of periodontal disease due to a qualitative shift in the bacterial composition. Harmful bacteria will increase in thickness and mass, and saliva is measured as a reservoir for microorganisms and a latent medium for bacterial transmission [43, 44].

Certain microbial species that are present in saliva may also be present in dental biofilm, inside periodontal pockets, and on the surface of the tongue [45-48]. Saliva is an important means for observing periodontal health as it includes biomarkers that transit from the GCF to saliva, reflecting the periodontal status.

Many species, such as *P. gingivalis*, *Aggregatibacter actinomycetemcomitans* (Aa), *T. forsythia*, *Fusobacterium nucleatum*, *P. intermedia, P. nigrescens, T. denticola,* and *Mycoplasma*, have been identified as the leading periodontal pathogens [49-53]. Aa species is associated with periodontitis. Carriers may be at higher risk of developing periodontal diseases [54, 55]. *P gingivalis* has a high prevalence in periodontal diseases, and it is an important predictor of disease progression [10, 56-58]. *P. intermedia* is a considerable predictor of disease progression [32]. *T. forsythia* with inflammatory biomarkers such as IL-1β, IL-8, and MMP-8 demonstrates an increased predictability [10, 57, 58]. Similarly, *T. denticola* is highly prevalent in periodontal diseases and strongly associated with the disease progression and when combined with the inflammatory biomarkers it does increase the predictability [56].

5.2. Inflammatory Biomarkers of Periodontal Diseases

Numerous inflammatory biomarkers studied in saliva have been linked with oral diseases, including IL-1β, -6,-8, TNF-α, MMP-8, -9, and TIMP-1, and myeloperoxidase (MPO), [30, 59]. An important part of the immune system's defense against infection is IL-1β and it is well known that numerous cell types, including fibroblasts, macrophages, monocytes, and dendritic cells, release IL-1β. Pro-inflammatory IL-6 inhibits the effects of TNF-α and IL-1 and causes acute-phase reactions throughout inflammation. TNF-α released by macrophage, fibroblasts, mast cells, lymphoid cells, and endothelial cells and function is to stabilize immune cells. TNF-α with other cytokines encourage the acute-phase response and greatly contributes to local and systemic inflammation [60, 61].

MMPs are enzymes with the capability to disintegrate nearly all extracellular matrix (ECM). They are typically released by various types of cells, including macrophages, neutrophils, lymphocytes, osteoblasts, endothelial cells, and fibroblasts [62]. The role of MMPs differs depending on the physiological state. MMPs participate in wound healing, tissue development, and remodeling during normal physiological processes [63]. Additionally, by breaking down several non-matrix bioactive substrates, MMPs have anti-inflammatory properties [64]. Collagenases are the most efficient of the six subfamilies of MMPs, as they are capable of breaking various proteins, ECM components, and collagens I, II, and III. With gelatin as its primary substrate, the second group consists of gelatinases. Proteins included in basement membranes and extracellular matrix components can be broken down by gelatinases [65, 66].

MMPs can be activated through various mechanisms, and their activity can be controlled through several factors: growth factors, hormones, enzyme inhibition, and inflammatory cytokines. Endogenous MMP inhibitors can be broadly classified into two categories: α-macroglobulins and TIMPs. TIMPs exist in four different types: TIMP-1, -2, -3, and -4. MMP-8, also recognized as neutrophil collagenase or collagenase-2, and it is specifically associated with inflammatory diseases. It is released mostly by neutrophils and by other cell types, including macrophages, fibroblasts, epithelial cells, plasma cells, T-cells, and chondrocytes [67]. The concentration of MMP-8 is increased in inflammatory diseases such dental caries, periodontal disease, atherosclerosis, and cancer [68, 69]. MPO, an enzyme secreted from PMN, is intensely linked to inflammation [70]. It is commonly used as an inflammatory biomarker for both chronic and acute diseases [71, 72].

5.3. Salivary Enzymes as Biomarkers of Periodontal Diseases

Enzymes are proteins that hasten reactions by particular mechanisms. Approximately all biological cell processes require enzymes to emerge at significant rates. Human saliva contains an abundant supply of enzymes originating from epithelial cells, salivary glands, oral bacteria, GCF, and external resources such as microorganisms and food. The prominent salivary enzymes include peroxidase, alkaline phosphatase, lactate dehydrogenase, and amylase [73].

6. Biomarkers of Periodontitis: Salivary Matrix Mettaloproteinase-8 as an Example

Active MMP-8 that produced by neutrophils is the primary collagenase that causes periodontal tissue degradation. Healthy sites from those involving gingivitis and periodontitis can be distinguished by MMP-8 that was sampled from GCF [68, 74]. In patients, increased MMP-8 values in saliva correlate with clinical signs and symptoms of periodontitis [1, 16, 75]. Increased MMP-8 values are also discovered in the plasma of periodontitis patients [76]. Saliva levels of MMP-8 has shown to be directly associated with the severity of the disease, i.e higher level detected in the advance stage of the periodontal disease than in subjects with early stage of the disease [77].

Successful periodontal treatments, such as scaling and root surface debridement, decrease periodontal clinical parameters and the mean value of MMP-8 in saliva and GCF [68, 78, 79]. Higher levels of MMP-8 usually can be detected in subjects or sites of disease that would progress in future or do not respond to the periodontal therapy [77]. MMP-8 is considered a assuring candidate for diagnosing and anticipating the development of periodontal diseases in saliva [21]. Furthermore, after non-surgical periodontal therapy, substantial declines in salivary MMP-8 values have been observed, thus, indicating their probable capability to indicate periodontal disease activity [25]. Additionally, a transferable

diagnostic hand-held point of care tool calculated significantly elevated MMP-8 values in periodontitis patients, which were reduced after scaling and root surface debridement [80].

Specifically, increased values of MMP-8 were noticed in areas that did not heal after scaling and root surface debridement in smokers. At those sites, MMP-8 levels stayed constantly high during the following visits [78].In areas that remained active, MMP-8 values did not reduce significantly after therapy, in contrast to inactive sites[81]. Individuals with progressive periodontal disease also had greater baseline MMP-8 concentrations, with MMP-8 activity rising over time in comparison to those with non-progressive status [38].

It is important to mention that the application of salivary MMP-8 as a diagnostic indicator has restrictions. Smoking, a main cause of periodontal diseases, is linked with a lower concentration of salivary MMP-8 [82]. Smokers have lower MMP-8 concentrations in comparison to nonsmokers [83-85]. Because smokers' salivary MMP-8 levels are lower, it is not possible to use salivary MMP-8 as a reliable indicator of periodontitis in smokers. Cigarettes has been demonstrated to up-regulate MMP genes and corresponding proteins in human vascular endothelial cells[86]. In periodontal practice, the impact of cigarette smoking on the diagnostic usefulness of MMP-8 in saliva can be managed through the use of different antibodies and the specific ratio-mixing of enzymes and inhibitors. Salivary MMP-8 concentrations and the ratio of MMP-8 to TIMP-1 have been shown to be useful in differentiating periodontitis patients from controls [55].

7. Biomarkers of Periodontal Diseases: Cytokines as Example

The bacterial components of dental biofilm are now widely recognized as the key factor in the initiation and progression of periodontal disease. The pathophysiology and subsequent tissue loss are happened by the establishment of a persistent host immunological inflammatory response. Cytokines demonstrate a significant role in this process, mediating the interactions between periodontal tissue cells and immune cells [61, 87]. The study of cytokines in periodontal research dates back to the 1980s [88, 89]when IL-1, IL-2, and TNF-α were considered [90].

These experiments demonstrated elevated thymocyte proliferation induced by GCF in inflamed areas compared to non-inflamed areas, suggesting cytokine activity. However, the exact character of the mediators continued to be determined [88, 89]. With the introduction of ELISAs, IL-1β was the first cytokine to be specifically assessed in the gingival tissue of patients with periodontal diseases [91].

7.1. Interleukin-1β as Biomarker of Periodontal Diseases

Inflammatory and infectious triggers result in the production of IL-1β, which is crucial for the development of inflammatory diseases. When exposed to inflammatory triggers such as bacterial lipopolysaccharide, its active form induces immunological responses that regulate inflammation, bone resorption, and several cellular processes such as cell proliferation, differentiation, and death. Furthermore, at the region of infection or trauma, IL-1β promotes the recruitment of angiogenesis, phagocytes, epithelial cell repair, and control of chemokine and cytokine production by other immune cells [92]. Several clinical studies showed the utilization of IL-1β existing in GCF or saliva as a biomarker of periodontal diseases [93-96].

Subjects with periodontitis had considerably higher mean salivary IL-1β values than controls, and these differences were associated with the disease's clinical parameters related to periodontal diseases such as PPD, CAL and bone loss. Furthermore, higher risk of periodontal disease (by 45 times) has been reported in patients with elevated salivary level of MMP-8 and IL-1β [97]. Salivary IL-1β values were more abundant in diseased untreated individuals as compared to IL-6 and prostaglandin E2 [98]. These findings demonstrated a stronger correlation between periodontal diseases and salivary IL-1β than any of the other investigated mediators.

In terms of IL-1β's capacity to forecast bone loss, salivary levels of the biomarker showed a stronger positive connection with alveolar bone loss than with any other biomarker examined in saliva [99, 100]. These data support the hypothesis that increased GCF biomarkers of inflammation can help in identifying patients susceptible to progressive periodontitis. To conclude the disease condition and response to periodontal treatment, a longitudinal study by Sexton *et al.* [25] found that the degree of IL-

1β in saliva was strongly correlated with the severity of the disease and the response to therapy, suggesting that it might be used to monitor the stages of periodontal disease. Another study demonstrated that IL-1β GCF values in periodontitis individuals reflect disease severity and response to treatment [92]. Therefore, IL-1β might assist as a powerful biomarker of periodontitis, particularly when used with other biomarkers.

8. Total Salivary Protein

Total protein is a general measure of all proteins that exist in a solution. It is used to evaluate the alterations in whole protein secretion in blood or saliva that are linked with disease conditions or to assess the differences in the ratio of certain proteins to the total protein that are present in various oral fluids Such as saliva, GCF, and serum exudate or that happen in response to physiological alterations or disease conditions [101, 102]. Total protein is also occasionally adjusted to standardize concentrations of different proteins in saliva in various samples, as protein concentrations can differ significantly in response to triggers or changes in saliva flow [29].

In medically compromised individuals whose overall status deteriorates, salivary proteins have been demonstrated to be elevated. Elderly patients usually have a less-effective immune response compared to younger subjects. In chronic inflammatory diseases, like gingivitis or periodontitis plasma protein leakage, it can be determined using salivary protein and albumin levels [103].

9. Biomarkers' Limitations in Diagnosing Periodontal Diseases

The complicated nature of periodontal diseases makes it seem difficult to identify a single, highly sensitive and specific diagnostic biomarker for the purpose of predicting and detecting the disease [21]. Consequently, examining the combination of markers may be beneficial in identifying biomarkers that predict the disease progression and provide a more accurate assessment of the periodontal status. Notwithstanding, there is no particular laboratory or clinical test for monitoring patients with periodontal disease, and only a small number of salivary biomarkers have been included in clinical practice [104]. There was no appropriate marker that led to the main conceptual alterations in the periodontal diagnostics field, despite the progression of highly complex tools and approximately 30 years of research [105].

Despite widespread study, much of it has failed to offer clinicians dependable and practical information for progressing diagnosis and treatment plans for periodontal diseases [28]. Furthermore, the relationship between salivary biomarkers and periodontal disease has been investigated in a number of cross-sectional investigations. These studies mostly show correlations between biomarkers and the presence of periodontal disease or the ability of an individual biomarker to distinguish different status of the disease. This attempt mostly reveals past disease activity rather than the current process [39]. Besides, the review literature covers a little longitudinal study on biomarker levels during disease progression, while in clinically detecting periodontal disease progression, periodontal clinicians face dilemmas. It is clear from the presently obtainable evidence that a dependable clinical method to evaluate disease progression is not available, which impedes the utilization of biomarkers for prognosis and assessment of periodontal diseases. Moreover, procedural shortcomings and small sample sizes in many research studies render it challenging to achieve ultimate conclusions.

As a result, the statistical strength of these studies and their capability to establish any causal link between the examined markers and periodontal diseases are questioned, influencing both their external and internal validity [106].

10. Conclusions

Saliva represents the most valuable fluid in the oral cavity that serves as a reflection of the body's overall health. Salivary indicators can be used to predict systemic or oral diseases. Periodontitis is one of the destructive diseases that need to be diagnosed and detected at an early stage to prevent further destruction. For diagnosing periodontal disease in a dental clinic different periodontal parameter, including PPD, BOP, CAL, plaque index, and radiographs measuring alveolar bone levels, have been

used. Since biomarkers are signs of recent disease activity and predictors of upcoming disease initiation and progression in currently healthy areas, using biomarkers for diagnosing periodontal disease is less time- consuming and they can be used in clinical practice for detecting oral and periodontal disease. The microbial biomarkers of *P. gingivalis*, *Aa* and *T. forsythia* are widely used with some promising results. Biomarkers can identify and quantify periodontal risk through objective measures. There are many biomarkers in saliva, however, salivary cytokines, enzymes, and inflammatory biomarkers are the most helpful in periodontal diseases. Amongst the biomarkers examined so far, IL-1β and MP-8 are the most promising ones to be used for diagnosis and prognosis purposes of periodontal diseases.

Authors contributions: Aram Mohammed Sha: Supervision, Writing – original draft. **Hayder Raad Abdulbaqi:** Writing – original draft. **Syed Saad B. Qasim:** Methodology, data Curation, Formal Analysis.

Data availability: Data will be available upon reasonable request.

Conflicts of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: The authors did not receive support from any organization for the submitted work.

References

[1] B. Rai, S. Kharb, R. Jain, and S. C. Anand, "Biomarkers of periodontitis in oral fluids," *J. Oral Sci.,* vol. 50, no. 1, pp. 53-56, 2008. https://doi.org/10.2334/josnusd.50.53

[2] M. Yakob *et al.*, "Associations of periodontal microorganisms with salivary proteins and MMP-8 in gingival crevicular fluid," *J. Clin. Periodontol,* vol. 39, no. 3, pp. 256-263, 2012. https://doi.org/10.1111/j.1600-051X.2011.01813.x

[3] R. P. Darveau, "Periodontitis: a polymicrobial disruption of host homeostasis," *Nat. Rev. Microbiol*, vol. 8, no. 7, pp. 481-490, 2010.https://doi.org/10.1038/nrmicro2337

[4] A. A. Abdulkareem, F. B. Al-Taweel, A. J. Al-Sharqi, S. S. Gul, A. Sha, and I. L. Chapple, "Current concepts in the pathogenesis of periodontitis: from symbiosis to dysbiosis," *J. Oral Microbiol.,* vol. 15, no. 1, p. 2197779, 2023. https://doi.org/10.1080/20002297.2023.2197779

[5] B. L. Pihlstrom, B. S. Michalowicz, and N. W. Johnson, "Periodontal diseases," *Lancet,* vol. 366, no. 9499, pp. 1809-1820, 2005. https://doi.org/10.1016/S0140-6736(05)67728-8

[6] M. Nazir, A. Al-Ansari, K. Al-Khalifa, M. Alhareky, B. Gaffar, and K. Almas, "Global prevalence of periodontal disease and lack of its surveillance," *Sci. World J.,* vol. 2020, no. 1, p. 2146160, 2020. https://doi.org/10.1155/2020/2146160

[7] E. D. Beltrán-Aguilar, P. I. Eke, G. Thornton-Evans, and P. E. Petersen, "Recording and surveillance systems for periodontal diseases," *Periodontol. 2000,* vol. 60, no. 1, pp. 40-53, 2012. https://doi.org/10.1111/j.1600-0757.2012.00446.x

[8] B. Holtfreter, T. Kocher, T. Hoffmann, M. Desvarieux, and W. Micheelis, "Prevalence of periodontal disease and treatment demands based on a German dental survey (DMS IV)," *J. Clin. Periodontol,* vol. 37, no. 3, pp. 211-219, 2010. https://doi.org/10.1111/j.1600-051X.2009.01517.x

[9] G. C. Armitage, "The complete periodontal examination," *Periodontol. 2000,* vol. 34, no. 1, pp. 22-33, 2004. https://doi.org/10.1046/j.0906-6713.2002.003422.x

[10] U. K. Gursoy *et al.*, "Use of host-and bacteria-derived salivary markers in detection of periodontitis: a cumulative approach," *Dis. Markers,* vol. 30, no. 6, pp. 299-305, 2011. https://doi.org/10.3233/DMA-2011-0788

[11] W. V. Giannobile, T. Beikler, J. S. Kinney, C. A. Ramseier, T. Morelli, and D. T. Wong, "Saliva as a diagnostic tool for periodontal disease: current state and future directions," *Periodontol. 2000,* vol. 50, p. 52, 2009. https://doi.org/10.1111/j.1600-0757.2008.00288.x

[12] E. Kaufman and I. B. Lamster, "Analysis of saliva for periodontal diagnosis: a review," *J. Clin. Periodontol,* vol. 27, no. 7, pp. 453-465, 2000. https://doi.org/10.1034/j.1600-051x.2000.027007453.x

[13] H. Haririan, O. Andrukhov, M. Laky, and X. Rausch-Fan, "Saliva as a Source of Biomarkers for Periodontitis and Periimplantitis," *Front. Dent. Med.,* vol. 2, p. 687638, 2021. https://doi.org/10.3389/fdmed.2021.687638

[14] C. S. Miller *et al.*, "Current developments in salivary diagnostics," *Biomarkers Med.,* vol. 4, no. 1, pp. 171-189, 2010. https://doi.org/10.2217/bmm.09.68

[15] S. Trivedi and N. Lal, "Antioxidant enzymes in periodontitis," *J. Oral Biol Craniofac. Res.,* vol. 7, no. 1, pp. 54-57, 2017. https://doi.org/10.1016/j.jobcr.2016.08.001

[16] U. K. Gursoy *et al.*, "Salivary type I collagen degradation end-products and related matrix metalloproteinases in periodontitis," *J. Clin. Periodontol,* vol. 40, no. 1, pp. 18-25, 2013. https://doi.org/10.1111/jcpe.12020

[17] N. Christodoulides *et al.*, "Lab-on-a-chip methods for point-of-care measurements of salivary biomarkers of periodontitis," *Ann. N. Y. Acad. Sci.,* vol. 1098, no. 1, pp. 411-428, 2007. https://doi.org/10.1196/annals.1384.035

[18] S. S. Gul, A. A. Abdulkareem, A. M. Sha, and A. Rawlinson, "Diagnostic accuracy of oral fluids biomarker profile to determine the current and future status of periodontal and peri-implant diseases," *Diagnostics,* vol. 10, no. 10, p. 838, 2020. https://doi.org/10.3390/diagnostics10100838

[19] G. Downing, "Biomarkers Definitions Working Group. Biomarkers and Surrogate Endpoints," *Clin. Pharmacol. Ther.,* vol. 69, pp. 89-95, 2001. https://doi.org/10.1067/mcp.2001.113989

[20] W. A. Colburn, "Biomarkers in drug discovery and development: from target identification through drug marketing," *J. Clin. Pharmacol.,* vol. 43, no. 4, pp. 329-341, 2003. https://doi.org/10.1177/0091270003252480

[21] L. Zhang, B. S. Henson, P. M. Camargo, and D. T. Wong, "The clinical value of salivary biomarkers for periodontal disease," *Periodontol. 2000,* vol. 51, no. 1, pp. 25-37, 2009. https://doi.org/10.1111/j.1600-0757.2009.00315.x

[22] J. M. Yoshizawa, C. A. Schafer, J. J. Schafer, J. J. Farrell, B. J. Paster, and D. T. Wong, "Salivary biomarkers: toward future clinical and diagnostic utilities," *Clin. Microbiol. Rev.,* vol. 26, no. 4, pp. 781-791, 2013. https://doi.org/10.1128/cmr.00021-13

[23] V. V. Gupta, N. Chitkara, H. V. Gupta, A. Singh, R. S. Gambhir, and H. Kaur, "Comparison of salivary calcium level and ph in patients with aggressive periodontitis and healthy individuals: a clinico-biochemical study," *Oral Health Dent. Manag.,* vol. 15, no. 4, pp. 122-26, 2016.

[24] N. Buduneli and D. F. Kinane, "Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis," *J. Clin Periodontol,* vol. 38, no. s11, pp. 85-105, 2011. https://doi.org/10.1111/j.1600-051X.2010.01670.x

[25] W. M. Sexton, Y. Lin, R. J. Kryscio, D. R. Dawson III, J. L. Ebersole, and C. S. Miller, "Salivary biomarkers of periodontal disease in response to treatment," *J. Clin. Periodontol.,* vol. 38, no. 5, pp. 434-441, 2011. https://doi.org/10.1111/j.1600-051X.2011.01706.x

[26] J. M. Goodson, "Diagnosis of periodontitis by physical measurement: interpretation from episodic disease hypothesis," *J. Periodontol.,* vol. 63, no. 4s, pp. 373-382, 1992. https://doi.org/10.1902/jop.1992.63.4s.373

[27] M. Taba, J. Kinney, A. S. Kim, and W. V. Giannobile, "Diagnostic biomarkers for oral and periodontal diseases," *Dent. Clin. North Am.,* vol. 49, no. 3, pp. 551-571, 2005. https://doi.org/10.1016/j.cden.2005.03.009

[28] J. J. Kim, C. J. Kim, and P. M. Camargo, "Salivary biomarkers in the diagnosis of periodontal diseases," *J. Calif. Dent. Assoc.,* vol. 41, no. 2, p. 119, 2013.

[29] J. Y. Lee, J. W. Chung, Y. K. Kim, S. C. Chung, and H. S. Kho, "Comparison of the composition of oral mucosal residual saliva with whole saliva," *Oral Dis.,* vol. 13, no. 6, pp. 550-554, 2007. https://doi.org/10.1111/j.1601-0825.2006.01332.x

[30] N. Rathnayake *et al.*, "Salivary biomarkers for detection of systemic diseases," *PloS one,* vol. 8, no. 4, p. e61356, 2013. https://doi.org/10.1371/journal.pone.0061356

[31] N. Ghallab and O. Shaker, "Salivary-soluble CD44 levels in smokers and non-smokers with chronic periodontitis: A pilot study," *J. Periodontol.,* vol. 81, no. 5, pp. 710-717, 2010. https://doi.org/10.1902/jop.2010.090630

[32] Y. Nomura *et al.*, "Salivary biomarkers for predicting the progression of chronic periodontitis," *Arch. Oral Biol.,* vol. 57, no. 4, pp. 413-420, 2012. https://doi.org/10.1016/j.archoralbio.2011.09.011

[33] S. I. Tobón-Arroyave, D. M. Isaza-Guzmán, E. M. Restrepo-Cadavid, S. M. Zapata-Molina, and M. C. Martínez-Pabón, "Association of salivary levels of the bone remodelling regulators sRANKL and OPG with periodontal clinical status," *J. Clin. Periodontol.,* vol. 39, no. 12, pp. 1132-1140, 2012. https://doi.org/10.1111/jcpe.12012

[34] Z. A. Tabari, A. Azadmehr, A. Nohekhan, N. Naddafpour, and F. B. Ghaedi, "Salivary visfatin concentrations in patients with chronic periodontitis," *J. Periodontol.,* vol. 85, no. 8, pp. 1081-1085, 2014. https://doi.org/10.1902/jop.2013.130388

[35] S. H. Hassan, M. I. El-Refai, N. A. Ghallab, R. F. Kasem, and O. G. Shaker, "Effect of periodontal surgery on osteoprotegerin levels in gingival crevicular fluid, saliva, and gingival tissues of chronic periodontitis patients," *Dis. Markers,* vol. 2015, 2015. https://doi.org/10.1155/2015/341259

[36] E. Özcan, N. I. Saygun, M. A. Serdar, and N. Kurt, "Evaluation of the salivary levels of visfatin, chemerin, and progranulin in periodontal inflammation," *Clin, Oral Investig.,* vol. 19, pp. 921-928, 2015. https://doi.org/10.1007/s00784-014-1308-0

[37] C. A. Ramseier *et al.*, "Identification of pathogen and host-response markers correlated with periodontal disease," *J. Periodontol.,* vol. 80, no. 3, pp. 436-446, 2009. https://doi.org/10.1902/jop.2009.080480

[38] J. S. Kinney *et al.*, "Crevicular fluid biomarkers and periodontal disease progression," *J. Clin. Periodontol.,* vol. 41, no. 2, pp. 113-120, 2014. https://doi.org/10.1111/jcpe.12194

[39] K. M. Jaedicke, P. M. Preshaw, and J. J. Taylor, "Salivary cytokines as biomarkers of periodontal diseases," *Periodontol. 2000,* vol. 70, no. 1, pp. 164-183, 2016. https://doi.org/10.1111/prd.12117

[40] E. Baltacıoğlu *et al.*, "Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease?," *J. Periodontol.,* vol. 85, no. 10, pp. 1432-1441, 2014. https://doi.org/10.1902/jop.2014.130654

[41] N. Novakovic *et al.*, "Salivary antioxidants as periodontal biomarkers in evaluation of tissue status and treatment outcome," *J. Periodontal Res.,* vol. 49, no. 1, pp. 129-136, 2014. https://doi.org/10.1111/jre.12088

[42] Y. A. Villa-Correa, D. M. Isaza-Guzmán, and S. I. Tobón-Arroyave, "Prognostic value of 8-hydroxy-2′-deoxyguanosine and human neutrophil elastase/α1-proteinase inhibitor complex as salivary biomarkers of oxidative stress in chronic periodontitis," *J. Periodontol,* vol. 86, no. 11, pp. 1260-1267, 2015. https://doi.org/10.1902/jop.2015.150293

[43] G. Greenstein and I. Lamster, "Bacterial transmission in periodontal diseases: a critical review," *J. Periodontol,* vol. 68, no. 5, pp. 421-431, 1997. https://doi.org/10.1902/jop.1997.68.5.421

[44] M. Quirynen, M. De Soete, K. Dierickx, and D. Van Steenberghe, "The intra-oral translocation of periodontopathogens jeopardises the outcome of periodontal therapy," *J. Clin. Periodontol.,* vol. 28, no. 6, pp. 499-507, 2001. https://doi.org/10.1034/j.1600-051x.2001.028006499.x

[45] S. Asikainen, S. Alaluusua, and L. Saxen, "Recovery of *A. actinomycetemcomitans* from teeth, tongue, and saliva," *J. Periodontol.,* vol. 62, no. 3, pp. 203-206, 1991. https://doi.org/10.1902/jop.1991.62.3.203

[46] M. Umeda, A. Contreras, C. Chen, I. Bakker, and J. Slots, "The utility of whole saliva to detect the oral presence of periodontopathic bacteria," *J. Periodontol.,* vol. 69, no. 7, pp. 828-833, 1998. https://doi.org/10.1902/jop.1998.69.7.828

[47] D. L. Mager, A. D. Haffajee, and S. S. Socransky, "Effects of periodontitis and smoking on the microbiota of oral mucous membranes and saliva in systemically healthy subjects," *J. Clin. Periodontol.,* vol. 30, no. 12, pp. 1031-1037, 2003. https://doi.org/10.1046/j.0303-6979.2003.00418.x

[48] B. J. Paster, I. Olsen, J. A. Aas, and F. E. Dewhirst, "The breadth of bacterial diversity in the human periodontal pocket and other oral sites," *Periodontol. 2000,* vol. 42, no. 1, pp. 80-87, 2006. https://doi.org/10.1111/j.1600-0757.2006.00174.x

[49] B. von Troil-Lindén, H. Torkko, S. Alaluusua, H. Jousimies-Somer, and S. Asikainen, "Salivary levels of suspected periodontal pathogens in relation to periodontal status and treatment," *J. Dent. Res.,* vol. 74, no. 11, pp. 1789-1795, 1995. https://doi.org/10.1177/00220345950740111201

[50] A. Bolstad, H. Jensen, and V. Bakken, "Taxonomy, biology, and periodontal aspects of Fusobacterium nucleatum," *Clin. Microbiol. Rev.,* vol. 9, no. 1, pp. 55-71, 1996. https://doi.org/10.1128/cmr.9.1.55

[51] A. Yoshida *et al.*, "TaqMan real-time polymerase chain reaction assay for the correlation of Treponema denticola numbers with the severity of periodontal disease," *Mol. Oral Microbiol.,* vol. 19, no. 3, pp. 196-200, 2004. https://doi.org/10.1111/j.0902-0055.2004.00142.x

[52] Y. Sawamoto, N. Sugano, H. Tanaka, and K. Ito, "Detection of periodontopathic bacteria and an oxidative stress marker in saliva from periodontitis patients," *Oral Microbiol Immunol..,* vol. 20, no. 4, pp. 216-220, 2005. https://doi.org/10.1111/j.1399-302X.2005.00215.x

[53] H. Kurata, S. Awano, A. Yoshida, T. Ansai, and T. Takehara, "The prevalence of periodontopathogenic bacteria in saliva is linked to periodontal health status and oral malodour," *J. Med. Microbiol.,* vol. 57, no. 5, pp. 636-642, 2008. https://doi.org/10.1099/jmm.0.47706-0

[54] D. H. Fine *et al.*, "Macrophage inflammatory protein-1α: a salivary biomarker of bone loss in a longitudinal cohort study of children at risk for aggressive periodontal disease?," *J. Periodontol.,* vol. 80, no. 1, pp. 106-113, 2009. https://doi.org/10.1902/jop.2009.080296

[55] U. K. Gursoy *et al.*, "Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis," *J. Clin. Periodontol.,* vol. 37, no. 6, pp. 487-493, 2010. https://doi.org/10.1111/j.1600-051X.2010.01563.x

[56] S. S. Socransky and A. D. Haffajee, "The bacterial etiology of destructive periodontal disease: current concepts," *J. Periodontol,* vol. 63, no. 4s, pp. 322-331, 1992. https://doi.org/10.1902/jop.1992.63.4s.322

[57] J. Kinney, T. Morelli, T. Braun, C. Ramseier, A. Herr, and J. Sugai, "Saliva/pathogen biomarker signatures and periodontal disease progression," *J. Dent. Res.,* vol. 90, no. 6, p. 752-758, 2011. https://doi.org/10.1177/0022034511399908

[58] V. Baelum and R. López, "Defining a periodontitis case: analysis of a never-treated adult population," *J. Clin. Periodontol.,* vol. 39, no. 1, pp. 10-19, 2012. https://doi.org/10.1111/j.1600-051X.2011.01812.x

[59] E. Kaufman and I. B. Lamster, "The diagnostic applications of saliva—a review," *Crit. Rev. Oral Biol. Med.,* vol. 13, no. 2, pp. 197-212, 2002. https://doi.org/10.1177/154411130201300209

[60] C. A. Dinarello and B. J. Pomerantz, "Proinflammatory cytokines in heart disease," Blood Purif., vol. 19, no. 3, pp. 314-321, 2001. https://doi.org/10.1159/000046960

[61] P. M. Preshaw and J. J. Taylor, "How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis?," *J. Clin Periodontol.,* vol. 38, no. s11, pp. 60-84, 2011. https://doi.org/10.1111/j.1600-051X.2010.01671.x

[62] V. J. Uitto, C. M. Overall, and C. McCulloch, "Proteolytic host cell enzymes in gingival crevice fluid," *Periodontol. 2000,* vol. 31, no. 1, pp. 77-104, 2003. https://doi.org/10.1034/j.1600-0757.2003.03106.x

[63] C. Amălinei, I.-D. Căruntu, S. E. Giuşcă, and R. A. Bălan, "Matrix metalloproteinases involvement in pathologic conditions," *Rom. J. Morphol. Embryol.*, vol. 51, no. 2, pp. 215-228, 2010.

[64] J. Reynolds, "Collagenases and tissue inhibitors of metalloproteinases: a functional balance in tissue degradation," *Oral Dis..*, vol. 2, no. 1, pp. 70-76, 1996. https://doi.org/10.1111/j.1601-0825.1996.tb00206.x

[65] D. G. Armstrong and E. B. Jude, "The role of matrix metalloproteinases in wound healing," *J. Am. Podiatr. Med. Assoc.*, vol. 92, no. 1, pp. 12-18, 2002. https://doi.org/10.7547/87507315-92-1-12

[66] T. Tsuruda, L. C. Costello-Boerrigter, and J. C. Burnett, "Matrix metalloproteinases: pathways of induction by bioactive molecules," *Heart Fail. Rev.*, vol. 9, no. 1, pp. 53-61, 2004.https://doi.org/10.1023/B:HREV.0000011394.34355.bb

[67] T. Sorsa *et al.*, "Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation," *Ann. Med.*, vol. 38, no. 5, pp. 306-321, 2006. https://doi.org/10.1080/07853890600800103

[68] P. Mäntylä *et al.*, "Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis," *J. Periodontal Res.*, vol. 38, no. 4, pp. 436-439, 2003. https://doi.org/10.1034/j.1600-0765.2003.00677.x

[69] H. Saari, K. Suomalainen, O. Lindy, Y. Konttinen, and T. Sorsa, "Activation of latent human neutrophil collagenase by reactive oxygen species and serine proteases," B*iochem. Biophys. Res. Commun.*, vol. 171, no. 3, pp. 979-987, 1990. https://doi.org/10.1016/0006-291X(90)90780-Q

[70] J. M. Leppilahti, M. A. Kallio, T. Tervahartiala, T. Sorsa, and P. Mäntylä, "Gingival crevicular fluid matrix metalloproteinase-8 levels predict treatment outcome among smokers with chronic periodontitis," *J. Periodontol.*, vol. 85, no. 2, pp. 250-260, 2014. https://doi.org/10.1902/jop.2013.130156

[71] V. Loria, I. Dato, F. Graziani, and L. M. Biasucci, "Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes," *Mediators Inflamm.*, vol. 2008, 2008. https://doi.org/10.1155/2008/135625

[72] B. Ikitimur and B. Karadag, "Role of myeloperoxidase in cardiology," *Future Cardiol.*, vol. 6, no. 5, pp. 693-702, 2010. https://doi.org/10.2217/fca.10.75

[73] B. Mahalingam, P. Harikrishnan, R. Muthusamy, S. Kenniyan Kumar, M. Loganathan, and S. Sivanandham, "Salivary Enzymes in Health and Disease," *Int. J. Med. Rev.*, vol. 9, no. 4, pp. 389-396, 2022. doi: 10.30491/IJMR.2021.287845.1203

[74] N. Prescher *et al.*, "Rapid Quantitative Chairside Test for Active MMP-8 in Gingival Crevicular Fluid," *Ann. N. Y. Acad. Sci.*, vol. 1098, no. 1, pp. 493-495, 2007. https://doi.org/10.1196/annals.1384.01

[75] A. Salminen *et al.*, "Salivary biomarkers of bacterial burden, inflammatory response, and tissue destruction in periodontitis," *J. Clin. Periodontol.*, vol. 41, no. 5, pp. 442-450, 2014. https://doi.org/10.1111/jcpe.12234

[76] A. M. Marcaccini *et al.*, "Circulating matrix metalloproteinase-8 (MMP-8) and MMP-9 are increased in chronic periodontal disease and decrease after non-surgical periodontal therapy," *Clin. Chim. Acta*, vol. 409, no. 1-2, pp. 117-122, 2009. https://doi.org/10.1016/j.cca.2009.09.012

[77] J. M. Leppilahti *et al.*, "The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site-level clinical treatment outcome," *J. Periodontol.*, vol. 86, no. 6, pp. 777-787, 2015. https://doi.org/10.1902/jop.2015.140421

[78] P. Mäntylä *et al.*, "Monitoring periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test," *J. Periodontal Res.*, vol. 41, no. 6, pp. 503-512, 2006. https://doi.org/10.1111/j.1600-0765.2006.00897.x

[79] A. M. Marcaccini *et al.*, "Gingival crevicular fluid levels of MMP-8, MMP-9, TIMP-2, and MPO decrease after periodontal therapy," *J. Clin. Periodontol.*, vol. 37, no. 2, pp. 180-190, 2010. https://doi.org/10.1111/j.1600-051X.2009.01512.x

[80] A. E. Herr *et al.*, "Integrated microfluidic platform for oral diagnostics," *Ann. N. Y. Acad. Sci.*, vol. 1098, no. 1, pp. 362-374, 2007. https://doi.org/10.1196/annals.1384.004

[81] M. Hernández *et al.*, "Associations between matrix metalloproteinase-8 and-14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: a longitudinal study," *J. Periodontol.*, vol. 81, no. 11, pp. 1644-1652, 2010. https://doi.org/10.1902/jop.2010.100196

[82] R. J. Genco and W. S. Borgnakke, "Risk factors for periodontal disease," *Periodontol. 2000*, vol. 62, no. 1, pp. 59-94, 2013. https://doi.org/10.1111/j.1600-0757.2012.00457.x

[83] Y. Ding e*t al.*, "Gingival Crevicular Fluid and Salivary Matrix Metalloproteinases of Heavy Smokers as Indicators of Periodontal Health a," Ann. N. Y. Acad. Sci., vol. 732, no. 1, pp. 453-455, 1994.

[84] K. E. Liede, J. K. Haukka, J. H. Hietanen, M. H. Mattila, H. Rönkö, and T. Sorsa, "The association between smoking cessation and periodontal status and salivary proteinase levels," *J. Periodontol.*, vol. 70, no. 11, pp. 1361-1368, 1999. https://doi.org/10.1902/jop.1999.70.11.1361

[85] A. M. Heikkinen *et al.*, "Smoking affects diagnostic salivary periodontal disease biomarker levels in adolescents," *J. Periodontol*, vol. 81, no. 9, pp. 1299-1307, 2010. https://doi.org/10.1902/jop.2010.090608

[86] B. K. Nordskog, A. D. Blixt, W. T. Morgan, W. R. Fields, and G. M. Hellmann, "Matrix-degrading and pro-inflammatory changes in human vascular endothelial cells exposed to cigarette smoke condensate," *Cardiovasc. Toxicol.*, vol. 3, no. 2, pp. 101-117, 2003. https://doi.org/10.1385/CT:3:2:101

[87] K. S. Kornman *et al.*, "The interleukin-1 genotype as a severity factor in adult periodontal disease," *J. Clin. Periodontol.*, vol. 24, no. 1, pp. 72-77, 1997. https://doi.org/10.1111/j.1600-051X.1997.tb01187.x

[88] J. A. Charon, T. A. Luger, S. E. Mergenhagen, and J. J. Oppenheim, "Increased thymocyte-activating factor in human gingival fluid during gingival inflammation," *Infect. Immun.*, vol. 38, no. 3, pp. 1190-1195, 1982. https://doi.org/10.1128/iai.38.3.1190-1195.1982

[89] S. Mergenhagen, "Thymocyte activating factor (s) in human gingival fluids," *J. Dent. Res.*, vol. 63, no. 3, pp. 461-464, 1984. https://doi.org/10.1177/00220345840630031901

[90] C. A. Dinarello, "Historical insights into cytokines," *Eur. J. Immunol.*, vol. 37, no. S1, pp. S34-S45, 2007. https://doi.org/10.1002/eji.200737772

[91] J. Hönig, C. Rordorf-Adam, C. Siegmund, W. Wiedemann, and F. Erard, "Increased interleukin-1 beta (IL-1β) concentration in gingival tissue from periodontitis patients," *J. Periodontal Res.*, vol. 24, no. 6, pp. 362-367, 1989. https://doi.org/10.1111/j.1600-0765.1989.tb00883.x

[92] J. Hyun, H. Hirano, H. Takai, and Y. Ogata, "Effects of initial periodontal therapy on interleukin-1β level in gingival crevicular fluid and clinical periodontal parameters," *J. Oral Sci.*, vol. 57, no. 2, pp. 67-71, 2015. https://doi.org/10.2334/josnusd.57.67

[93] M. Relvas *et al.*, "Analysis of Salivary Levels of IL-1β, IL17A, OPG and RANK-L in Periodontitis Using the 2017 Classification of Periodontal Diseases—An Exploratory Observational Study," *J. Clin. Med.*, vol. 12, no. 3, p. 1003, 2023. https://doi.org/10.3390/jcm12031003

[94] S. Tobón-Arroyave, P. Jaramillo-González, and D. Isaza-Guzman, "Correlation between salivary IL-1β levels and periodontal clinical status," *Arch. Oral Biol.*, vol. 53, no. 4, pp. 346-352, 2008. https://doi.org/10.1016/j.archoralbio.2007.11.005

[95] J.-Y. Kim, K.-R. Kim, and H.-N. Kim, "The potential impact of salivary IL-1 on the diagnosis of periodontal disease: a pilot study," *Healthcare*, vol. 9, no. 6, p. 729, 2021. https://doi.org/10.3390/healthcare9060729

[96] T. Bolyarova-Konova *et al.*, "Concentrations of Interleukin-1ß in Gingival Crevicular Fluid and Saliva–a Potential Diagnostic Biomarker of Periodontal Diseases," *Folia. Medica.*, vol. 62, no. 4, pp. 825-830, 2020. http://doi.org/10.3897/folmed.62.e49872

[97] C. Chaussain-Miller, F. Fioretti, M. Goldberg, and S. Menashi, "The role of matrix metalloproteinases (MMPs) in human caries," *J. Dent. Res.*, vol. 85, no. 1, pp. 22-32, 2006. https://doi.org/10.1177/154405910608500104

[98] N. Y. B. Patricia, M. Donley, E. Hausmann, A. D. Hutson, E. F. Rossomando, and F. A. Scannapieco, "Candidate salivary biomarkers associated with alveolar bone loss: cross-sectional and in vitro studies," FEMS Immunol. *Med. Microbiol.*, vol. 49, no. 2, pp. 252-260, 2007. https://doi.org/10.1111/j.1574-695X.2006.00187.x

[99] F. Scannapieco, P. Ng, K. Hovey, E. Hausmann, A. Hutson, and J. Wactawski-Wende, "Salivary biomarkers associated with alveolar bone loss," *Ann. N. Y. Acad. Sci.*, vol. 1098, no. 1, pp. 496-497, 2007. https://doi.org/10.1196/annals.1384.034

[100] R. A. Reinhardt *et al.,* "Association of gingival crevicular fluid biomarkers during periodontal maintenance with subsequent progressive periodontitis," *J. Periodontol.*, vol. 81, no. 2, pp. 251-259, 2010. https://doi.org/10.1902/jop.2009.090374

[101] J. Furuholm *et al.*, "Salivary matrix metalloproteinase-8 in patients with and without coronary heart disease may indicate an increased susceptibility to periodontal disease," *J. Periodontal Res.*, vol. 41, no. 5, pp. 486-489, 2006. https://doi.org/10.1111/j.1600-0765.2006.00900.

[102] V. De Oliveira, A. Bessa, R. Lamounier, M. De Santana, M. De Mello, and F. Espindola, "Changes in the salivary biomarkers induced by an effort test," *Int. J. Sports Med*., vol. 31, no. 06, pp. 377-381, 2010. http://doi.org/0.1055/s-0030-1248332

[103] F. Oppenheim, "Preliminary observations on the presence and origin of serum albumin in human saliva," *Hel. Odontol. Acta*, vol. 14, no. 1, pp. 10-17, 1970.

[104] N. Buduneli and D. F. Kinane, "Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis," *J. Clin. Periodontol*., vol. 38, pp. 85-105, 2011. https://doi.org/10.1111/j.1600-051X.2010.01670.x

[105] G. C. Armitage, "Learned and unlearned concepts in periodontal diagnostics: a 50-year perspective," *Periodontol. 2000,* vol. 62, no. 1, pp. 20-36, 2013. https://doi.org/10.1111/prd.12006

[106] N. Rathnayake, D.-R. Gieselmann, A. M. Heikkinen, T. Tervahartiala, and T. Sorsa, "Salivary Diagnostics—Point-of-Care diagnostics of MMP-8 in dentistry and medicine," *Diagnostics*, vol. 7, no. 1, p. 7, 2017. https://doi.org/10.3390/diagnostics7010007