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Salivary Biomarkers for Gingivitis and Periodontitis

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Abstract

Saliva has been studied extensively for the identification of potential biomarkers for various diseases, including those of the oral cavity and also other systemic diseases. Especially, saliva has been shown to be extremely valuable for the potential diagnosis, prognosis, and monitorization of periodontal disease. While there is a considerable number of salivary biomarkers currently associated with periodontitis, salivary biomarkers concerning gingivitis are not as abundant. This review aims to provide a comprehensive overview of the existing salivary biomarkers for both gingivitis and periodontitis, demonstrating the need, use, and potential for additional biomarkers for gingivitis.

Keywords: Saliva; Biomarkers; Gingivitis; Periodontitis; Proteomics; Genomics; Epigenomics

Introduction

According to the Center for Disease Control, approximately 47% of adults above age 30 suffer from periodontal disease [1]. This finding is significant because periodontal disease has a considerable impact on one's systemic health, which can put individuals at increased risk of ischemic coronary events, coronary heart disease [2], myocardial infarction[2], cerebrovascular accident [3], diabetes [4], cancer [5], Alzheimer's disease [5], and more. Periodontal health is a state free of inflammation and disease in the gingiva and bone that support the teeth, allowing an individual to function without suffering any consequences. Periodontal health can exist prior to disease, and in some cases, it can be re-established once disease commences as long as it hasn't progressed beyond certain parameters [6]. When plaque builds up on teeth, it can lead to a periodontal disease termed gingivitis, which is inflammation confined to the gingiva. Gingivitis is reversible with the implementation of adequate oral hygiene. When inflammation results in tissue destruction and attachment loss, the gingivitis has progressed to periodontitis and the damage is irreversible [4]. In the early stages of periodontitis, patients are typically asymptomatic and therefore unaware of the condition until symptoms develop and the disease is more severe [4] (Figure 1). In periodontitis, collagen fibers in the periodontal ligament break down, forming a pocket with a probing depth greater than 3 mm between the gingiva and teeth [7]. These pockets make it difficult to access the plaque below the gingiva and usually leads to further attachment loss and alveolar bone resorption [4]. Once this condition has progressed into late stages, it is termed "advanced periodontitis which is characterized by gingival erythema and edema, gingival bleeding, gingival recession, tooth mobility, drifting of teeth, suppuration from periodontal pockets, and tooth loss" [4]. Periodontal disease is irreversible and the development of novel testing techniques for early detection can potentially decrease its progression.

Methods

We searched Google Scholar and PubMed databases for original research and review articles related to our topic. There were no date limits for this review and keywords used include, "saliva", "diagnostics", "periodontitis", "gingivitis", "biomarker", "proteomics", "genomics", "epigenomics", and "detection". All articles were screened and further analyzed for additional references. A total of 52 articles were identified and included in our article.

Diagnosis of Periodontal Disease

Currently, multiple modalities can be utilized to diagnose periodontal disease, some of which include clinical examination,

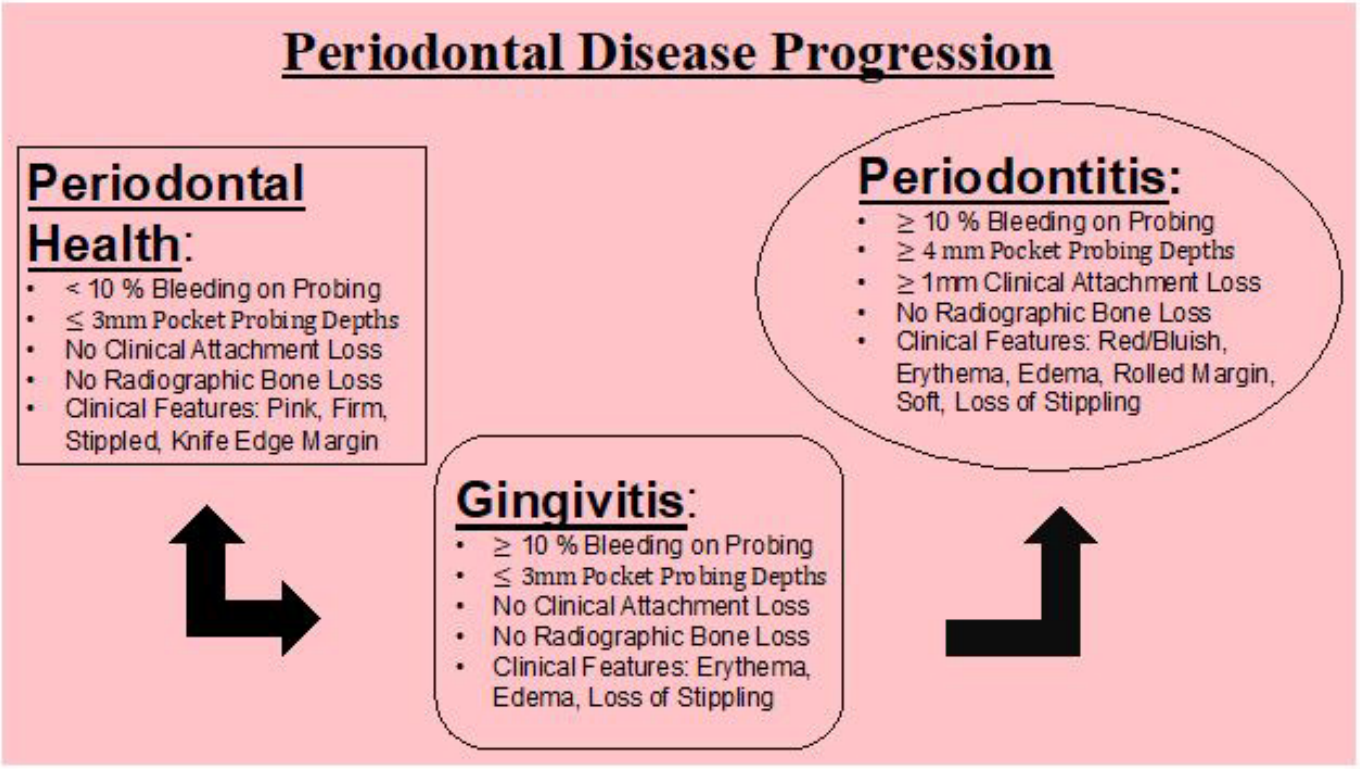

Figure 1: Progression of Periodontal Disease

histology via gingival biopsy [6], and analysis of biomarkers in body fluids. Current practice in dentistry often involves only clinical and radiographic information for the diagnosis and treatment of disease [8]. The assessment of periodontal destruction is the primary diagnostic method for evaluating periodontal disease. Clinical parameters used for assessment and diagnosis such as plaque index, bleeding upon probing, inflammation, edema, erythema, changes in color of gingiva [9], and probing depth are indeterminate when distinguishing health from disease in its early stages [10,11]. Particularly, the absence of pain in gingivitis provides individuals with no signs of their pathologic condition [12]. In addition, a discordance between biological and clinical health measures exists. In a study conducted by Nagarajan et al., 40% of gingivitis patients did not return to biological health after periodontal therapy as assessed with salivary biomarkers even though clinical measures indicated a return to health [8]. While clinical parameters do provide useful information, they do not predict non-responders to treatment, provide comprehensive data about current disease activity, nor inform health care providers of individuals who are at risk of periodontal disease [12].

Biomarkers show great potential in diagnosing periodontal disease in its early stages, ideally in the gingivitis stage when periodontal damage is still reversible. At present, there has been some progress in the development of reliable markers for periodontal disease. However, challenges remain in finding ideal markers that meet all of the following criteria [13]: identify and predict disease activity, distinguish active disease sites from inactive sites, and monitor response to treatment therapy. An ideal biomarker must be safe, reliable, altered with treatment, consistent across different ethnic and gender groups, cost efficient, easily measured, and can be used to diagnose, stage, and determine the prognosis of a disease [13].

Biomarkers for detection of periodontal disease can be found in gingival tissue, gingival crevicular fluid, saliva, serum, and various other sources. While biomarkers found in many of these sources are effective in the diagnosis of active disease, the use of saliva may be superior due to its high sensitivity [14], repeatability, and ease of collection, storage and shipment. Saliva collection does not require special equipment or a trained technician [14], is cost effective, and non-invasive [15], eliminating the potential for the patient and provider to contract an infectious disease [14]. The aforementioned characteristics of saliva make it applicable to a variety of circumstances including self-administered point-of-care (POC) tests for periodontal disease screenings. POC tests can inform individuals of their periodontal status prior to disease progression to a symptomatic state, leading to less substantial and more economical treatment [12]. The use of salivary-based POC tests could serve as an asset to low-income communities by providing accessible testing and monitoring of oral health for individuals at high risk [12].

V. Saliva as a Credible Bio fluid for Disease Detection

Saliva is a slightly acidic (pH 6 -7) [14] fluid produced by three major salivary glands and numerous minor glands [15]. Its production is regulated by the autonomic nervous system such that during periods of sympathetic stimulation, saliva is produced in smaller, more protein rich quantities. While under parasympathetic control, saliva is produced in larger quantities with lower viscosity. The two most effective methods of collecting whole saliva are the draining method and the spitting method, both of which can be collected with or without stimulation [16] and in large quantities. Stimulation of the salivary glands can be achieved via mechanical (*i.e.* chewing), electrical, taste, and pharmacologic stimulation [17].

Due to its convenience of use and ability to reflect the health status of the body, saliva is currently used as a diagnostic and monitoring tool in other scientific fields [15]. The potential use of saliva has been demonstrated in detecting local and systemic diseases such as Sjogren's syndrome, type-2 diabetes, as well as cancers in the head and neck [18]. Salivary constituents include proteome, transcriptome, metabolome, and microbiome, making salivary analysis a promising non-invasive diagnostic tool [15]. Saliva contains metabolites involved in disease pathways such as periodontal disease and oral cancer [15]. The scientific community's knowledge of salivary metabolites is progressing due to advanced testing. High-performance liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy accompanied by pattern recognition enable the identification of metabolites associated with disease onset, progression, and regression [15]. Salivary transcriptomic biomarkers are primarily identified via microarray and validated via quantitative real-time PCR (qPCR) [15].

With newly emerging data on salivary biomarkers, there are a multitude of developing technologies that can measure proteins, DNA, mRNA, electrolytes and small molecules found in saliva [15]. Currently, several modes of analysis are applicable to the detection and quantification of biomarkers in saliva. These methods include immunoassay, magnetic resonance spectroscopy, western blot, mass spectrometry, reverse transcription-polymerase chain reaction, microarrays,

enzymatic assays, and nanoscale sensors [17]. Technologies such as liquid biopsies and POC systems have facilitated a novel approach to diagnosing both periodontal as well as systemic diseases with high accuracy (Table 1). The use of these products has the potential to assist in the prevention of periodontal disease and improve treatment outcomes [15].

Salivary Molecular Constituents for Periodontal Diseases

Proteomic Biomarkers

Proteins and peptides are abundant in human saliva. Thus, the advent of recent proteomics techniques has led to the identification of a large number of salivary protein biomarkers, including many for periodontal diseases (Table 2). The most specific biomarkers for periodontitis in saliva are immunoglobulins (Ig), particularly the IgA, IgG and IgM classes. These immunoglobulins deter bacterial adherence and inhibit bacterial metabolism which alters the oral microbiota [17,19]. Several studies have found an increase in these immunoglobulins in patients with either chronic or aggressive periodontitis versus healthy patients. Studies have also shown that the level of these immunoglobulins decreases after periodontal therapy [17]. Lactoferrin, produced by the salivary glands, exhibits a protective mechanism against microbes by inhibiting microbial growth. This protein is elevated in patients with periodontal disease compared to healthy patients [19]. Peroxidase, an enzyme produced in saliva, also acts to prevent the establishment of periodontal disease. Patients with periodontal disease have been shown to have high levels of salivary peroxidase [19] compared to healthy controls. C-reactive protein (CRP), which is released during periodontal inflammation, is present at higher levels in patients with chronic and aggressive periodontal disease [19] versus healthy control patients. In a study comparing the salivary profiles of chronic periodontitis patients versus healthy controls, soluble CD14, IL-6 and, IL-4 were significantly higher while soluble toll-like receptor-2 (sTLRR-2), interleukin (IL)-17 and IL-10 were significantly lower in patients with chronic periodontitis [20]. Additionally, after a six-week evaluation of the salivary profiles post scaling and root planning (SRP) treatment, levels of sTLR-2 and IL-4 were comparable to patients with healthy gingiva [20]. Soluble CD44 [21], a transmembrane glycoprotein involved in cell adhesion and migration, aspartate aminotransferase (AST) [22], alanine transaminase (ALT) [22], and lactate dehydrogenase (LDH) [22] were also shown

Reference	Test Kits	Functions	Technique
Buduneli[13], Srivastava[40], Kaczor-Urbanowicz[15]	MyPerioPath®	Detects type and concentration of bacteria	DNA PCR
Buduneli[13]	OMNIgene®	P. intermedia, A.a, F. nucleatum, E. corrodensm C. rectus, T. forsythia, T. denticola	DNA probe
Buduneli[13]	Evalusite®	Identifies antigen of *A. actinomycetemcomitans, P. intermedia, P. gingivalis*	Membrane-based enzyme immunoassay
Buduneli[13], Srivastava[40], Rathnayake[41]	MyperiodID®	Determines genetic susceptibility to periodontal disease via Interleukin-1A and Interleukin-1β	Molecular testing
Buduneli[13]	Integrated microfluidic platform for oral diagnostics (IMPOD)®	Quantifies matrix metalloproteinase-8, tumor necrosis factor-α, interleukin-6 and C-reactive protein in 3-10 minutes	Electrophoretic immunoassays
Buduneli[13], Rathnayake[41]	PerioSafe®	Detects matrix metalloproteinase-8	Lateral flow technology with ELISA
Lee[42]	Lab-on-a-chip (LOC)	Measures interleukin-1β, C-reactive protein, and matrix metalloproteinase-8	Microfluidic immunoassay

Table 1: Commercially available POC tests for periodontal disease

Biomarker	Periodontal Disease	Elevated/Decreased	Statistical Significance	Reference
MMP-8	1. Gingivitis and Periodontitis 2. Periodontitis	1. Elevated 2. Elevated	1. P < 0.001, 2. P < 0.001	Rai[43], Lahdentausta[23]
sTLR-2	Chronic Periodontitis	Decreased	*P* = 0.01	Prakasam[20]
IL-17	Chronic Periodontitis	Decreased	*P* = 0.003	Prakasam[20]
IL-10	Chronic Periodontitis	Decreased	*P* = 0.009	Prakasam[20]
sCD14	1. Chronic Periodontitis 2.Chronic Periodontitis 3.Aggressive periodontitis	1. Elevated 2. Elevated 3. Elevated	*1. P* = 0.018, 2. P = 0.011, 3. P = 0.008	1. Prakasam[20], Isaza-Guzma´n Isaza-Guzma´n 2. Isaza-Guzman[44], 3. Isaza-Guzman[44]
IL-4	Chronic Periodontitis	Elevated	*P* = 0.0028	Prakasam[20]
IL-6	1. Chronic Periodontitis	Elevated	*P* = 0.004	Prakasam[20]
MMP-9	Periodontitis	Elevated	P < 0.001	Lahdentausta[23]
TIMP-1	Periodontitis	Decreased	P = 0.001	Lahdentausta[23]
MPO	Periodontitis	Elevated	P < 0.001	Lahdentausta[23]
MUC4	Periodontitis	Decreased	*P* < 0.01	Lundmark[24]
MMP7	Periodontitis	Elevated	*p* < 0.05	Lundmark[24]
sCD44	Chronic Periodontitis	Elevated	*P* ≤ 0.001	Ghallab[21]
PGE_2	Gingivitis	Elevated	*P* = 0.019	Syndergaard[9]
pH	Chronic Gingivitis	Elevated	*P* = 0.001	Baliga[45]
pH	Chronic Periodontitis	Decreased	*P* = 0.001	Baliga[45]
ANXA1	Gingivitis	Elevated	*P* < 0.05	Hassan[31]
IL-1β	Periodontitis	Elevated	*P* < 0.05	Hassan[31]
IL-1β	Gingivitis	Elevated	*P* < 0.05	Hassan[31]

Table 2: Proteomic Salivary Biomarkers for Gingivitis and Periodontitis

to decrease significantly after periodontal therapy (SRP) in the periodontitis group. Another important study analyzed salivary biomarkers in periodontitis which are involved in extracellular matrix degradation in periodontal tissues, these included matrix metalloproteinase (MMP)-8, MMP-9, myeloperoxidase (MPO), and TIMP-1. MMP-8, MMP-9, and MPO were found to be significantly different in periodontitis subjects versus the healthy control, with area under the curve (AUC) values of 0.69, 0.66, and 0.68, respectively. The use of salivary TIMP-1 was complicated by cardiac status [23].

Several other proteins have been shown to be expressed in significantly different levels in periodontitis versus healthy subjects, these include: mucin 4 [24], MMP-7 [24], beta-2-glycoprotein I [25], α-fibrinogen [25], hemopexin [25], plasminogen [25], arginase [13], monocyte chemoattractant protein (MCP)-1 [26], B-glucuronidase [13], IL-35 [13], IL-18 [13], pentraxin3 [13], neutrophil elastase [13], MCP-2 [27], macrophage derived chemokine [27], type I collagen [28], osteocalcin [28], osteonectin [28], and cystatin [29].

During the inflammatory response of periodontal tissue, numerous cytokines such as IL-1β, IL-6, and prostaglandin E2 (PGE2) are released. Following this, MMP enzymes such as MMP-8 deteriorate alveolar bone and connective tissue components [19]. In a study conducted by Syndergaard et al., participants diagnosed clinically with gingivitis demonstrated significantly higher macrophage inflammatory protein-1α and PGE2 levels compared to the healthy group[9]. Another study by Lee et al. showed that patients with high baseline levels of

IL-6 and MMP-1 have an increased risk of developing gingivitis compared to those with low baseline levels (AUC of 0.89; odds ratio of 17.0; 95% confidence interval, 1.7 to 171.7) [30]. In a study by Hassan et al., participants were split into the three following groups: healthy, gingivitis, and periodontitis. They found that Annexin-1 and IL-1β levels were significantly higher in gingivitis versus healthy patients. They also demonstrated these enzymes to be significantly higher in periodontitis compared with health [31]. IL-1β is considered one of the most common and useful biomarkers for periodontal disease [13] due to its close association with gingivitis and periodontitis. It initially fights infection, but contributes to periodontal destruction as infection progresses [13]. A study by Hong et al. found that MPO and MMP-8 levels were strongly correlated with gingivitis, with AUC larger for MMP-8 (.814) than MPO (.793) [32]. Lee et al. measured subgingival bacterial proportions to discriminate between high and low responders when treated for gingivitis and found that IL-6 and IL-8 levels provided the best distinction [30]. Zhou et al. examined changes of proinflammatory proteins during experimental gingivitis and showed that the levels of IL-6, IL-1β, and calprotectin reflect the severity of gingival inflammation [33]. Lastly, in a study conducted by Henskens et al., salivary albumin concentration was increased in both the gingivitis and periodontitis groups compared to healthy subjects [29].

Genomic Biomarkers

Strong evidence suggests that genetics affects an individual's predisposition to the development and progression of periodontal disease (Table 3). Single nucleotide polymorphisms in human lactoferrin have been associated with increased risk of periodontitis [34]. A study by Yoshie et al. demonstrated that one of the IL-1A polymorphisms (IL-1A + 4845 alleles) may influence values of salivary AST and ALT, key enzymes that reflect inflammation and destruction of the periodontium, after periodontal scaling therapy [22]. Additionally, salivary 8 hydroxydeoxyguanosine (8-OhdG), a reactive oxygen species that plays a role in periodontal destruction, is significantly elevated in patients with periodontitis compared to healthy subjects prior to treatment and significantly reduced after periodontal treatment. In another study, Sawamoto et al. found that 8-OhdG was correlated with the bacterial load of *Porphyromonas gingivalis,* a prominent pathogenic species in periodontal disease, suggesting that levels of salivary 8-OhdG reflect periodontal pathogen load [35].

A study [12] conducted by Kaczor-Urbanowicz et al., validated eight extracellular RNA (exRNA) biomarkers for gingivitis. The exRNAs identified include SPRR1A, lnc-TET3-2:1, RP5-965F6.2, LGALS3, GALNT10 AJ156166, SOX4, FAM25A, and CRCT1. The roles of these exRNA biomarkers mainly include response to inflammation, innate immunity, apoptosis, chemotaxis, antimicrobial activity, and bone hemostasis, which is consistent with periodontal disease as it is of an inflammatory origin. These eight exRNAs showed significant regression after tooth brushing treatment was implemented, and significant improvements in clinical measures of gingival and plaque indices were observed [12]. This study also found that four of the eight identified salivary exRNA's (SPRR1A, lnc-TET3-2:1, FAM25A, CRCt1) can detect gingivitis with an AUC of 0.91, 71% sensitivity, and 100% specificity.

Biomarker	Periodontal Disease	Increased/ Decreased	Statistical Significance	Reference
MiRNA-155	Periodontitis	Elevated	P < 0.001	Al-Rawi[46]
MiRNA-146b	Periodontitis	Elevated	P < 0.001	Al-Rawi[46]
MiRNA-146a	Periodontitis	Elevated	P = 0.012	Al-Rawi[46]
MiRNA-203	Periodontitis	Elevated	P = 0.006	Al-Rawi[46]
SPRR1A	Gingivitis	Decreased	P = 0.044	Kaczor-Urbanowicz[12]
LGALS3	Gingivitis	Decreased	P = 0.025	Kaczor-Urbanowicz[12]
FAM25A	Gingivitis	Decreased	P = 0.023	Kaczor-Urbanowicz[12]
CRCT1	Gingivitis	Decreased	P = 0.046	Kaczor-Urbanowicz[12]
lnc- TET3- 2:1	Gingivitis	Elevated	P <0.001	Kaczor-Urbanowicz[12]
RP5-965F6.2	Gingivitis	Elevated	P <0.001	Kaczor-Urbanowicz[12]
GALNT10	Gingivitis	Elevated	P <0.001	Kaczor-Urbanowicz[12]
SOX4	Gingivitis	Elevated	P = 0.020	Kaczor-Urbanowicz[12]

Table 3: Genomic Salivary Biomarkers for Gingivitis and Periodontitis

Epigenomic Biomarkers

In addition to the genetic factors in the immune response against pathogens in the oral microbiome, epigenetic mechanisms offer further regulation in the periodontal inflammatory pathway (Table 4). Epigenetic changes alter gene expression without altering the DNA itself via chemical modifications of DNA. These changes affect chromatin and thus the activation/inactivation of genes [36]. While some epigenetic biomarkers for periodontal disease exist, there are few that have been identified in saliva, thus gingival biopsies and blood samples are currently required to utilize these biomarkers for diagnosis.

Small extracellular vesicles (sEVs), also termed exosomes, are a group of nanoparticles composed of nucleic acids, lipids, and proteins [37]. Current literature has found that periodontitis patients had decreased CD9/CD81+ salivary sEVs and increased sEV programmed death-ligand 1 (PD-L1) mRNA, hsa-miR-140-5p, hsa-miR-146a-5p and hsa-miR-628-5p miRNA when compared to healthy controls [37]. It was also shown that salivary exosomal hsa-miR-125a-3p could be a useful biomarker for chronic periodontitis with an AUC of 1.0 [37]. Han et al. conducted a pilot study to provide insight into global DNA methylation profiles of sEVs and gram-negative bacterial outer membrane vesicles (OMV). They found that OMVs of four periodontal pathogens (*T. denticola*, *E. corrodens*, *P. gingivalis* and *F. nucleatum),* global DNA methylation pattern of 5-methylcytosine, and lipopolysaccharide-positive OMVs were increased in periodontitis sEVs compared to healthy controls. Global 5-methylcytosine DNA methylation is a particularly highly sensitive biomarker (AUC=1.0) [37]. In a follow-up study by Han et al. comparing healthy and gingivitis patients, no significant differences were found in sEV DNA epigenetics between the two groups based on sEV characteristics such as size and DNA methylation of five inflammatory cytokine gene promoters IL−6, tumor necrosis factor (TNF)-α, IL−1β, IL−8, and IL−10 [38]. Xie et al. studied microRNA profiles of gingival tissues, which regulate the immuno-inflammatory response. They found that expression of hsa-miRNA-146a, hsa-miRNA-146b, and hsa-miRNA-155 were significantly different between healthy and inflamed gingiva, making these candidates potential biomarkers for periodontitis [39].

Limitations of Available Studies

Current studies aiming to identify biomarkers for gingivitis and periodontitis have several limitations. Clinical parameters including periodontal probing depth, plaque index, gingival index, and clinical attachment levels are subject to inter-evaluator error [12,32]. The discrepancies in clinical determinations affect the accuracy of biomarkers. Particularly during the early stages of periodontal disease, both mild inflammation and trauma from periodontal probing can cause bleeding upon probing. Thus, it can be difficult to discriminate gingivitis from gingival trauma, a potential false-positive [32]. Although diagnostic biomarkers for periodontitis are relatively well-studied with large sample sizes,

Biomarker	Disease	Findings	Statistical Significance	Reference
IL8 Gene	Chronic Periodontitis	Increased Hypomethylation	$P < 0.0001$	Oliveira[47]
IL8 Gene	Aggressive Periodontitis	Increased Hypomethylation	$P = 0.016$	Andia[48]
IL-8 mRNA	Chronic Periodontitis	Increased Hypomethylation	$P = 0.007$	Oliveira[47]
E-Cadherin	Chronic Periodontitis	Increased Hypermethylation	$P < 0.0001$	Loo[49]
COX-2	Chronic Periodontitis	Increased Hypermethylation	$P < 0.0001$	Loo[49]
SOCS1	Aggressive Periodontitis	Decreased Demethylation	$P < 0.001$	Baptista[50]
LINE-1	Aggressive Periodontitis	Decreased Demethylation	$P < 0.001$	Baptista[50]
IL-6	Chronic Periodontitis	Decreased Methylation	$P = 0.0001$	Ishida[51]
PTGS2	Chronic Gingival Inflammation	Increased Methylation	$P = 0.03$	Zhang[52]

Table 4: Epigenomic Biomarkers for Gingivitis and Periodontitis

current literature on biomarkers for gingivitis is lacking. Because periodontitis and gingivitis differ in inflammation severity and stage, not all biomarkers for periodontitis can apply to the earlier diagnosis of gingivitis [32], posing a need for studies confirming biomarkers specifically for gingivitis.

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