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Review Article

Dentistry Section

Salivary Markers as Diagnostic Tool for Dental Caries, Periodontal Disease and Peri-implantitis: A Literature Review

BRUNO PIRES MIRANDA[1], HELBA PIRES MIRANDA[2], RENATA PARAGUASSU FRIEDRICH CARREIRO[3], ETYENE SCHNURR[4]

ABSTRACT

Biomarkers are molecules that can be used in screening, diagnosing, characterising, and monitoring diseases, or as prognostic indicators. There are many salivary molecules that can be used as biomarkers of oral diseases such as enzymes, specific and non specific proteins, antibodies, and other substances. This study aimed to research the effectiveness of using salivary biomarkers as a means of diagnosis and raised the following question that are salivary biomarkers sufficient to diagnose oral diseases such as caries, periodontal and peri-implant disease, avoiding systemic diseases? Given this question, this study aimed to investigate the topic in recent scientific literature, looking for information that could clarify the issue. Therefore, a bibliographic review on the topic was carried out, and scientific articles were searched in the PubMed database. The findings showed that salivary biomarkers are sufficient to diagnose oral diseases since several biomarkers in saliva have already been identified, which allow the early diagnosis of these conditions, the monitoring of their progression and their response to treatments. This review may be the first to offer a summary classification of existing salivary biomarkers that can be collected by saliva in a simple and non invasive manner, allowing for early diagnosis. The main finding in this regard was the immune molecules β-defensin-2 and LL-37, collagen I, fibronectin, soluble Cluster of Differentiation 14 (sCD14) cells, Interleukin (IL)-4, IL-13, Interleukin-2 Receptor Alpha chain (IL-2 RA) and eotaxin/CCL11 as predictors of dental caries. For periodontal disease, the higher levels of saliva of IL-1β, IL-6, Metalloproteinase-8 (MMP-8), MMP-9, Macrophage Inflammatory Protein-1α (MIP-1α), Osteoprotegerin (OPG), Tissue Inhibitors of Metalloproteinases-1 (TIMP-1), salivary Total Antioxidant Capacity (TAOC), albumins, uric acid, Superoxide Dismutase (SOD) and peroxidase were related to the pathogenesis of the disease. For peri-implantitis, dysbiosis must be associated with the presence of IL-1β, Tumour Necrosis Factor alpha (TNF-α), TIMP-2, Vascular Endothelial Growth Factor (VEGF), OPG and procalcitonin. These findings may provide an easier view of the co-presence of other components in the oral environment, such as proteins/cytokines in saliva, transient microbials, which can contribute to the pathogenesis of the disease and date the multifactorial aetiology of oral diseases. This constitutes a personalised medical approach, reinforcing the power of clinical examination and medical history assessments to form an accurate diagnostic tool.

Keywords: Biomarkers, Dental implants, Inflammation, Interleukins, Periodontitis, Tooth

INTRODUCTION

Biomarkers are objective medical signs and measurable in a reproducible form. They are quantifiable physiological entities in the biological processes [1]. They can be classified according to the biological level in which they occur (molecular, cellular, tissue, organ) and they are related to susceptibility factors, primary or secondary pathology or the disease's complications [2]. Some examples are specific cells, antibodies, molecules, genes, enzymes, growth factors, hormones, among others [2].

A reliable and reproducible biomarker can be called molecular signature and therefore, can be used in risk evaluation, diagnosis, prognosis, and disease monitoring [3]. In the last decade, the development of technologies of mass spectrometry initiated a new era in the discovery of biomarkers that will potentially have a huge impact on future diagnosis and disease therapy [4].

For that matter, saliva which is the peripheral environment of teeth, has a lot of molecular biological information [5] such as components derived from mucosal surfaces, gingival crevices, and the tooth surfaces of the mouth [6]. It also has microorganisms that colonise the oral cavity and other exogenous substances and, therefore, can potentially offer a vision of the relationship between host and environment [6]. At the cellular level, the exposure to bacterial products and lipopolysaccharides induces the activation of monocytes/macrophages that promote the secretion of cytokine and inflammatory mediators like Interleukin (IL)-1B, IL-6 and Tumour Necrosis Factor (TNF) α, resulting in the release of Matrix Metalloproteinases (MMPs), which harm the integrity of gingival tissues [7].

Many of these inflammatory cells have already been detected in oral fluids, which permitted that saliva emerged as an important and easily accessible biological fluid that can provide valuable diagnosis information about oral health and diseases [7]. As explained previously, biomolecules in saliva can be sensitive indicators for dental health since they affect the survival of oral microorganisms by multiple innate defensive mechanisms, modulating oral microflora. Therefore, resistance or susceptibility to certain diseases, as caries, periodontal and peri-implant diseases may be significantly correlated with alterations in these salivary protein components [8].

Considering the information given, this review intends to research the efficacy of the use of salivary biomarkers as a means of diagnosis, and it raises the following question as an issue: What is the importance of salivary biomarkers to diagnose oral diseases such as caries, periodontal and peri-implant diseases?

Thus, the aim of this review was to investigate the subject in recent scientific literature, in search of information that can clarify the importance of salivary biomarkers for the diagnosis of dental caries, periodontal disease and peri-implantitis.

LITERATURE SEARCH

A narrative review about the theme was performed by searching data exclusively in recent scientific articles in the PubMed database,

using the following keywords (in MESH terms): salivary biomarkers; caries; periodontal disease; peri-implantitis. The study was carried out between October 2020 to May 2021.

Inclusion and Exclusion criteria: The inclusion criteria were publications from the last 10 years (between 2011 to 2021); available in full for online reading or downloading, written in English, Spanish or Portuguese, including review articles (comparative studies), and clinical studies. Articles published before 2011 in languages other than the three mentioned and those that were not available in full online were excluded.

Initially, articles were searched using the following descriptors: salivary biomarkers; caries, with the Boolean operator AND. Then, publications were searched using the following descriptors: salivary biomarkers; periodontal disease, with the Boolean operator AND. Subsequently, publications were searched using the following descriptors: salivary biomarkers, peri-implantitis, with the Boolean operator AND.

To select which articles would be used for the study, a subjective approach was used, i.e., initially the researcher made a selection through reading their titles and abstracts, followed by reading the full content of the pre-selected ones, to obtain more indepth information, where choice was directed to articles which presented data closely related to the aim of this research.

Among the researched articles using the descriptors salivary biomarkers; caries, with the Boolean operator AND, 97 publications were retrieved. Using the descriptors salivary biomarkers, periodontal disease, with the Boolean operator AND, 369 publications were retrieved, part of them repeated from the ones previously selected. With the following descriptors salivary biomarkers, peri-implantitis, with the Boolean operator AND, 12 publications were retrieved.

After the content analysis of the articles, 21 were selected for inclusion in this review, all in English, which are detailed in [Table/Fig-1-3], where [Table/Fig-1] presents the eight publications referring to dental caries disease [5,8-14]; [Table/Fig-2] presents the eight publications referring to periodontal disease [7,15-21], and [Table/Fig-3] presents the five publications referring to peri-implantitis [22-26]. It is important to note that another five articles used in the introduction of this review are not included in these Table/Figs, as they were selected only to clarify some aspects related to biomarkers in general.

Author and year	Aim	Main results and conclusion
Gao X et al., 2015 [9]	Present a review of the current understanding of salivary biomarkers for dental caries.	Evidence found in the literature indicates a high prevalence/incidence of caries among individuals with a pathologically low salivary flow rate, compromised buffer capacity and early or high colonisation of mutans streptococci in saliva.
Ao S et al., 2017 [5]	Identify the different salivary peptides that are expressed in the development of Early Childhood Caries (ECC) in children between 3 and 4 years of age.	Three peptides were shown to be promising, they increased as the disease worsened (1346.6-Da, 2603.5-Da and 3192.8-Da). Among them, 1346.6-Da has been identified as rich in salivary histatin.
Hemadi AS et al., 2017 [8]	Identify in the literature the microorganisms that cause caries in children, as well as dental protective salivary proteins and their potential as biomarkers to evaluate the risk of EEC.	A highly significant correlation was found between higher prevalence of caries in children with higher levels of microorganisms such as mutans streptococci, Candida albicans and Prevotella spp., and the following salivary proteins: IgA, IgG immunoglobulins, Proline-Rich Proteins (PRP) and histatin peptides. Therefore, these molecules can be used as biomarkers for EEC.
Mira A et al., 2017 [10]	Identify salivary molecules that can vary in concentration between caries-free and caries-prone individuals, and that can be used as caries risk biomarkers.	They identified that circadian rhythms alter some molecules and to develop a conclusive caries risk test, the saliva sampling time must be standardised and biomarkers from different categories must be included, such as: immune molecules=IgA, LL-37, β-defensin-2; adhesion molecules=Fibronectin, Collagen I, Statherin; pH components=Phosphate, Formate.
Prester L et al., 2017 [11]	Verify if there is an association between the presence of soluble CD14 (sCD14) in saliva with caries activity, and with the saliva collection method.	A clear association was identified between higher levels of salivary sCD14 and dental caries, pointing to salivary sCD14 as a potential biomarker and predictor of future caries events.
Sharma V et al., 2017 [12]	Investigate the salivary rates of inflammatory cytokines in children with EEC to identify their potential as biomarkers.	The salivary levels of IL-6, IL-8 and TNF-α were significantly associated with the severity of dental caries, presenting excellent sensitivity and specificity, being indicated as potential salivary biomarkers for the diagnosis/prognosis of EEC.
Tian C et al., 2017 [13]	Identify differences in the saliva peptide profile of children with and without the occurrence of new caries lesions, to develop a simple way for early diagnosis and prevention of the relapse of severe EEC (s-ECC).	Two peptides (named after their experimental values of m/z: 3162.0-Da and 3290.4-Da) were shown to be associated with the recurrence of s-ECC and can be promising salivary biomarkers for diagnosis.
Paqué PN et al., 2021 [14]	Investigate the potential of salivary biomarkers (bacterial and protein) to assess the state of the disease in healthy patients or those with gingivitis or caries.	Forty four salivary biomarkers were evaluated, of which four were identified for potential use (IL-4, IL-13, IL-2-RA, and eotaxin/CCL11) for the identification of non invasive caries.

[Table/Fig-1]: Selected articles from dental caries [5,8-14].
Interleukin (IL), Tumour Necrosis Factor (TNF), Matrix Metalloproteinases (MMPs), Immunoglobulins (Ig), soluble Cluster of Differentiation 14 (sCD14)

Author and year	Aim	Main results and conclusion
Sexton WM et al., 2011 [15]	Conduct a longitudinal study to evaluate salivary biomarkers of periodontal disease to determine the response to therapy.	The salivary levels of the Interleukin (IL)-1β, MMP-8, Osteoprotegerin (OPG) and MIP-1α biomarkers reflected both the severity of the disease and the response to therapy, indicating its potential to monitor the state of periodontal disease.
Novakovic N et al, 2014 [16]	Evaluate the influence of non surgical periodontal treatment on salivary antioxidants and to identify their capacity as biomarkers.	The non surgical periodontal treatment influenced salivary Total Antioxidant Capacity (TAOC), albumins, uric acid, Superoxide Dismutase (SOD) and peroxidase, and these parameters conclusively reflected the periodontal status and tissue response on treatment, therefore, they are indicated as reliable biomarkers in the periodontal status evaluation and therapy's outcome.
Yang PS et al., 2014 [17]	Investigate existing associations between salivary antioxidants, behaviours and attitudes related to oral health and results of periodontal treatment.	The scaling-stimulated increase in Superoxide Dismutase (SOD) was related to a higher severity of periodontal disease, and the TAOC increase was inversely related to the severity of periodontal disease. The results showed the importance of scaling-stimulated salivary antioxidants as prognostic biomarkers of periodontal treatment.
Ebersole JL et al., 2015 [7]	Find answers using a diagnostic approach based on salivary biomarkers to identify health and periodontal disease.	Salivary concentrations of IL-1β, IL-6, MMP-8, MIP-1α, alone or in combination, can distinguish health conditions, gingivitis, and periodontal disease, being useful as salivary biomarkers.
Nagarajan R et al., 2015 [18]	Investigate the use of saliva as a diagnostic fluid in conjunction with classification techniques in individuals with clinically diagnosed gingivitis and periodontal disease.	Salivary concentrations of IL-6, IL-8, albumin, calprotectin, Prostaglandin E2 (PGE2), MMP-8 and MIP-1α are elevated in patients with gingivitis, and these biomarkers can help distinguish patients who are making the transition from health to gingivitis and further for periodontal disease.
De Lima CL et al., 2016 [19]	Systematically evaluate the accuracy of salivary biomarkers in the diagnosis of periodontal disease.	The molecules MIP-1α, IL-1β and IL-6 emerge as promising salivary biomarkers in the clinical evaluation of periodontal disease.

Alassiri S et al., 2018 [20]	Describe a new mouth rinse for collecting saliva samples and MMP-8 immunoassays, used to predict and monitor the periodontal disease and peri-implantitis.	The tests have been independently and successfully validated in several countries, confirming their effectiveness in differentiating periodontal and peri-implant health and disease.
Bostanci N et al., 2021 [21]	Use the high-sensitivity Enzyme-Linked Immunosorbent Assay (ELISA) test to assess the effectiveness of 10 salivary biomarkers as predictors of periodontal disease.	The biomarkers MMP-8, MMP-9 and TIMP-1 were the most effective, but the combination of multiple biomarkers, instead of using a single one, can offer more accurate precision.

[Table/Fig-2]: Selected articles from periodontal disease [7,15-21].

Author and year	Aim	Main results and conclusion
Acharya A et al., 2016 [22]	Investigate the association of salivary periodontopathogen count and salivary IL-1β level with the IL-1β response of the Peri-Implant Crevicular Fluid (PICF) in peri-implant mucositis sites.	In the group with no history of periodontal disease, IL-1β was a significant predictor (p-value=0.038) of the highest PICF. But in the group with history of chronic periodontitis, no significant association was observed. These results suggest that a greater peri-implant inflammatory cytokine response could be harder to predict in subjects with inherent periodontal disease susceptibility. However, periodontal susceptibility can impact the immune-inflammatory response in sub-peri-implant niches of those with peri-implant mucositis.
Sánchez-Siles M et al., 2016 [23]	Identify whether peri-implantitis causes an increase in the total salivary concentration of oxidative stress biomarkers.	Total salivary malondialdehyde and myeloperoxidase concentration in the peri-implantitis group was slightly higher than the other groups, although the difference was not statistically significant.
Wang H-L et al., 2016 [24]	Determine the profile of PICF biomarkers combined with microbial profiles from implants with healthy peri-implant tissues and with peri-implantitis to assess real-time disease activity.	The mean concentration of IL-1β, TIMP-2, VEGF and OPG was increased in peri-implantitis and an association was found between a threefold or greater increase in total bacterial DNA with the presence of peri-implantitis.
Gomes AM et al., 2019 [25]	To evaluate the levels of salivary biomarkers IL-1β, IL-10, Receptor activator of nuclear factor κ B (RANK), OPG, MMP-2, TG-β and TNF-α in patients with peri-implant mucositis in the absence or presence of periodontal and peri-implant maintenance therapy (TMPP) over 5 years.	The results revealed an increase in the salivary concentration of TNF-α in the GNTP group (that received no TMPP) compared to the GTP group (that underwent TMPP). The other salivary biomarkers that were evaluated did not show statistically significant differences between the two groups.
Algohar A and Alqerban A, 2020 [26]	Evaluate the levels of procalcitonin in saliva and PICF among healthy and peri-implant disease patients.	The results indicated higher levels of procalcitonin are suggestive of a probable salivary biomarker for peri-implant disease.

[Table/Fig-3]: Selected articles from peri-implantitis [22-26].

According to the results of these selected studies [5,8-14], the findings can be summarised as follows: for Early Childhood Caries (ECC), studies indicate that higher levels of microbes, such as *Streptococcus mutans, Candida albicans* and *Prevotella spp.,* salivary proteins as immunoglobulins IgA and IgG, inflammatory cytokines as IL-6, IL-8 and TNF-α, Proline-Rich Proteins (PRP) and peptides named after their experimental values of m/z as 1346.6-Da, 2603.5-Da, 3162.0-Da, 3290.4-Da and 3192.8-Da can be used as biomarkers for the disease. When it comes to caries in general, it is recommended to use the following biomarkers: sCD14, IL-4, IL-13, IL-2-RA, eotaxin/CCL11, β-defensin-2, LL-37, IgA, collagen I, statherin, fibronectin, and pH components as formate and phosphate.

For periodontal disease [7,15-21], IL-1β, IL-6, MMP-8, MMP-9, MIP-1α, OPG, TIMP-1, salivary TAOC, albumins, uric acid, superoxide dismutase and peroxidase can be considered as potential biomarkers for this disease. In addition, the salivary concentrations of IL-6, IL-8, IL-1β, albumin, calprotectin, Prostaglandin E2 (PGE2), MMP-8 and MIP-1α can be used as biomarkers to indicate patients whose health status is changing in terms of gingivitis and later for periodontal disease, as these concentrations increase as the disease progresses.

Regarding peri-implant disease [22-26], there is evidence about the potential use of IL-1β, TNF-α, TIMP-2, VEGF, OPG and procalcitonin as biomarkers for this condition, especially combined with site specific microbial profiles (e.g., *Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola)* [Table/Fig-4].

Subgroups	Markers	Reference
Dental caries		
Early Childhood Caries (ECC)	*Streptococcus mutans*	Gao X et al., 2015 [9]; Hemadi AS et al., 2017 [8]
	Candida albicans	Hemadi AS et al., 2017 [8]
	Prevotella spp.	Hemadi AS et al., 2017 [8]
	IgA	Hemadi AS et al., 2017 [8]
	IgG	Hemadi AS et al., 2017 [8]
	IL-6	Sharma V et al., 2017 [12]
	IL-8	Sharma V et al., 2017 [12]
	TNF-α	Sharma V et al., 2017 [12]
	PRP	Hemadi AS et al., 2017 [8]
	Histatin peptides	Ao S et al., 2017 [5]; Hemadi AS et al., 2017 [8]; Tian C et al. 2017 [13]
Caries in general	sCD14	Prester L et al., 2017 [11]
	IL-4	Paqué PN et al., 2021 [14]
	IL-13	Paqué PN et al., 2021 [14]
	IL-2-RA	Paqué PN et al., 2021 [14]
	eotaxin/CCL11	Paqué PN et al., 2021 [14]
	β-defensin-2	Mira A et al., 2017 [10]
	LL-37	Mira A et al., 2017 [10]
	IgA	Mira A et al., 2017 [10]
	Collagen I	Mira A et al., 2017 [10]
	Statherin	Mira A et al., 2017 [10]
	Fibronectin	Mira A et al., 2017 [10]
	pH components as formate and phosphate	Mira A et al., 2017 [10]
Periodontal disease		
Gingivitis and periodontitis	IL-6	Ebersole JL et al., 2015 [7]; Nagarajan R et al., 2015 [18]
	IL-8	Nagarajan R et al., 2015 [18]
	IL-1β	Ebersole JL et al., 2015 [7]
	Albumin	Nagarajan R et al., 2015 [18]
	Calprotectin	Nagarajan R et al., 2015 [18]
	PGE2	Nagarajan R et al., 2015 [18]
	MMP-8	Ebersole JL et al., 2015 [7]; Nagarajan R et al., 2015 [18]
	MIP-1α	Ebersole JL et al., 2015 [7]; Nagarajan R et al., 2015 [18]
Periodontitis	IL-1β	Sexton WM et al., 2011 [15]; De Lima CL et al., 2016 [19]

	IL-6	De Lima CL et al., 2016 [19]
	MMP-8	Sexton WM et al., 2011 [15]; Bostanci N et al., 2021 [21]
	MMP-9	Bostanci N et al., 2021 [21]
	MIP-1α	Sexton WM et al., 2011 [15]; De Lima CL et al., 2016 [19]
	OPG	Sexton WM et al., 2011 [15]
	TIMP-1	Bostanci N et al., 2021 [21]
	TAOC	Novakovic N et al, 2014 [16]; Yang PS et al., 2014 [17]
	Albumins	Novakovic N et al, 2014 [16]
	Uric acid	Novakovic N et al, 2014 [16]
	Superoxide dismutase	Novakovic N et al, 2014 [16]
	Peroxidase	Novakovic N et al, 2014 [16]
Peri-implant diseases		
	IL-1β	Acharya A et al., 2016 [22]; Wang HL et al., 2016 [24]
	TNF-α	Gomes AM et al., 2019 [25]
	TIMP-2	Wang HL et al., 2016 [24]
	VEGF	Wang HL et al., 2016 [24]
	OPG	Wang HL et al., 2016 [24]
	Procalcitonin	Algohar A; Alqerban A, 2020 [26]
	Aggregatibacter actinomyce-temcomitans	Wang HL et al., [24]
	Prevotella intermedia	Wang HL et al., [24]
	Porphyromonas gingivalis	Wang HL et al., [24]
	Tannerella forsythia	Wang HL et al., [24]
	Treponema denticola	Wang HL et al., [24]

[Table/Fig-4]: Summary of key biomarkers associated with Dental caries, periodontal disease and peri-implantitis [5,7-26].
soluble CD14 (sCD14); IL: Interleukin; TIMP-1: Tissue inhibitors of metalloproteinases -1; TNF-α: Tumour necrosis factor alpha; TAOC: Total antioxidant capacity; MMP-9: Matrix metallopeptidase 9; OPG: Osteoprotegerin; PGE2: Prostaglandin E2; VEGF: Vascular Endothelial growth factor

DISCUSSION

As seen so far, some molecules present in saliva, such as microorganisms and proteins, can be used as biomarkers to predict the risk of these diseases and help in a prognosis [8].

Biomarkers in Dental Caries

Cariogenic microorganisms present in biofilm (also called dental plaque) ferment the carbohydrates in the diet, producing acid, which causes mineral loss from the hard tissues of the teeth and, consequently leads to the destruction of dental structures resulting in dental caries [9]. As saliva constantly bathes the teeth, the constituents and properties of this oral fluid perform an important role in the occurrence and progression of the disease [9].

Dental caries of deciduous teeth in children aged 71 months or younger is known as ECC and is one of the most common oral infections in children affecting 23% of pre-schoolers in the USA and more than 60% of children in China [8]. To investigate differentially expressed salivary peptides in the development of ECC in three to four-year-old children, Ao S et al. monitored 82 caries-free children for one year, and 15 of them developed ECC while another 15 remained healthy [5]. According to the authors, the levels of different salivary peptides increased over time (1346.6-Da, 2603.5-Da and 3192.8-Da) and showed the best capability of classification to establish a model for children with a high risk of caries. The 1346.6-Da was identified as salivary histatin-rich peptide. Results indicated that peptidomic methods can be applied to help identify new candidate biomarkers for the occurrence and development of ECC [5].

Hemadi AS et al., reviewed 136 publications on ECC and found studies that show a highly significant correlation between a higher prevalence of caries in pre-schoolers with higher levels of microbes, such as *streptococcus mutans*, *candida albicans* and *prevotella* spp., and salivary proteins, including IgA, IgG immunoglobulins, PRP and histatin peptides, in comparison with caries-free individuals [8]. Therefore, these salivary components can be used as biomarkers for ECC.

To design a caries risk test based on salivary biomarkers, Mira A et al., used Enzyme-linked Immunosorbent assay (ELISA) and colorimetric tests to measure 25 compounds in individuals with or without dental caries in different moments of dental biofilm formation and time of the day [10]. Based on the analysis of their study, the authors recommended the following salivary metabolites to provide a differentiation value between healthy individuals and caries-active ones: 1) At 6 hrs: immune molecules (LL-37; β-defensin-2); adhesion molecules (Fibronectin; Collagen I); pH components (Phosphate; Formate); 2) At 0.5 hrs: immune molecules (IgA; LL-37); adhesion molecules (Fibronectin; Statherin- this last one, only if saliva is collected after rinsing with sugary solution); pH components (Formate; Phosphate- both only if saliva is collected after rinsing with sugary solution) [10].

Prester L et al., aimed to verify if there is an association between the presence of soluble CD14 (sCD14) in saliva with caries activity, and with the saliva collection method [11]. The researchers included 55 participants whose ages ranged between 20 and 40 years, 30 with dental caries and 25 caries-free controls. One hundred and ten saliva samples were collected, 55 of resting saliva and 55 of mechanically stimulated saliva. The sCD14 levels were higher in the caries-active group than in the caries-free group in either resting or stimulated saliva [11]. The group with dental caries had a lower resting salivary flow rate than the control group (0.61±0.42 versus 0.98±0.52 mL min-1; p-value <0.01*), just as the group with dental caries was the only one that presented hyposalivation (10% and 13% in stimulated and resting saliva, respectively) [11]. Results showed that the highest salivary sCD14 levels and secretion rates were clearly associated with dental caries and resting saliva.

Sharma V et al., also developed a study focused on ECC, where they evaluated salivary levels of inflammatory cytokines in children with the disease using DMF score (Decay Missing Filled teeth) and ROC curve analysis (Receiver Operating Characteristic) [12]. Fifty children were recruited (25 ECC patients and 25 healthy children). Results showed that IL-6, IL-8 and TNF-α levels were higher in patients with significant reduction (p-value <0.05) after intervention, and their levels were significantly associated (p-value <0.05) with severity of dental caries [12]. It was concluded that the significant elevation of IL-6, IL-8 and TNF-α with great sensitivity and specificity may imply their involvement as potential non invasive diagnostic/prognostic markers in ECC [12].

Tian C et al., identified differences in peptide profiles in stimulated whole saliva among children with or without the occurrence of new carious lesions and provided a simple way for early diagnosis and prevention of the relapse of severe s-ECC [13]. In their study, 26 children between three to four-year-old were evaluated, among them 13 were diagnosed with s-ECC and underwent treatment, and another 13 were matched by age and sex as a control [13]. According to the results, 15 peptides showed significant differences in the group without occurrence of new carious lesions. It was concluded that different saliva peptide peaks can be detected in s-ECC [13]. Besides, two specific peptides with m/z 3162.0-Da and 3290.4-Da values could be promising biomarkers for the diagnosis of s-ECC recurrence [13].

Biomarkers in Periodontal Diseases

More recently, Paqué PN et al., investigated the potential of 44 salivary biomarkers (bacterial and protein) to assess disease status in healthy

patients and patients with gingivitis or caries [14]. They collected saliva samples from 18 patients free of caries and gingivitis, 17 patients with gingivitis, and 38 patients with deep caries lesions. The analysis showed that four of these biomarkers (IL-4, IL-13, IL-2-RA and eotaxin/CCL11) are promising for the identification of noninvasive caries [14]. To confirm the discovery, 10 patients (five free of caries and gingivitis and five with caries) were followed for a period of six months. The results revealed a high correlation between these biomarkers and the clinical classification of the disease [14]. These results indicate IL-4, IL-13, IL-2-RA and eotaxin/CCL11 as potential salivary biomarkers for the identification of noninvasive caries.

Periodontal diseases are a group of inflammatory conditions induced by the infection of periodontal structures. It can be described as a chronic inflammatory and microbial process that is characterised by the presence of pathogenic bacteria associated with an impaired host immune response and the destruction of connective periodontal tissue [15]. This destruction of periodontal tissue is mainly a consequence of an imbalance between the production of free radicals and local antioxidants [16].

This disease has become a serious public health problem worldwide, with periodontal disease severe enough to result in tooth loss being found in 5% to 15% of most populations [17]. In this scenario, gingivitis which affects most of the population if it is not treated, may develop into periodontal disease [18]. Periodontal diseases affect most of the population, reaching approximately 80% of adults in the USA [7] and about 70.6% of Germany's population [19]. Periodontal disease is initiated by an imbalanced interaction between the oral microbial community and the host's inflammatory response or dysbiosis [19]. In susceptible individuals, this dysbiosis triggers a cascade of inflammatory events, which inturn, promotes the loss of periodontal insertion, alveolar bone destruction and lastly, dental loss [19].

The biochemical signalling that occurs in the affected tissues during periodontal disease involves three biological phases (inflammation, degradation of the connective tissue and remodelling of the alveolar bone), contributing to the clinical morbidity observed in the disease [15]. Thus, the molecules circulating in the saliva during these phases become specific biomarkers of the disease [15].

Sexton WM et al., longitudinally assessed salivary biomarkers of periodontal disease to determine response to therapy [15]. A controlled study (6 months) of adults with periodontal disease was performed, with 33 participants only receiving Oral Hygiene Instructions (OHI) and 35 with Scaling and Roots Planning (SRP) + OHI. Saliva samples collected at week 0, 16 and 28 were analysed for the following biomarkers: IL-1β, IL-8, MIP-1α, MMP-8, OPG and TNF-α. All parameters of periodontal health significantly improved in both groups at week 16 with the SRP group showing greater benefit at week 16 and 28. Salivary levels of IL-1β, MMP-8, OPG e MIP-1α reflected disease severity and response to therapy suggesting their potential utility for monitoring periodontal disease status [15].

According to Novakovic N et al., polymorphonuclear cells and monocytes, as well as other inflammatory cells such as endothelial cells, fibroblasts, osteoclasts, produce free radicals in the bacterial challenge, and the reactive free radicals are the host's main response tool to fight infection, playing an important role in the elimination of peri-pathogens [16]. Therefore, the authors investigated the influence of non surgical periodontal treatment on salivary antioxidants to assess their capacity as biomarkers, reflecting periodontal tissue condition and therapy outcome [16].

Sixty-three healthy and non smoking patients were used: out of which, 21 with a healthy periodontium and 42 with periodontal disease; half of them received scaling and root planning and the other half received only OHIs [16]. The study identified that non surgical periodontal treatment influenced salivary Total Antioxidant Capacity (TAOC), albumins, uric acid, superoxide dismutase and peroxidase [16]. Besides, these biochemical parameters conclusively reflected the periodontal status and tissue response in the treatment therefore, they are indicated as reliable biomarkers in the periodontal status evaluation and therapy's outcome [16].

Yang PS et al., investigated associations among scaling-stimulated changes in salivary antioxidants, oral-health-related behaviours and attitudes, and periodontal treatment outcome [17]. To do so, 30 patients with periodontal disease were divided into 3 groups: group without treatment (AB), Non Progressive outcome group (NP) and Effective Treatment group (ET). Saliva was collected before and after to determine SOD and the TAOC. According to the results, the scaling-stimulated increase in SOD was related to a higher severity of periodontal disease in the NP group, and the TAOC increase was inversely related to the severity of periodontal disease in the AB group. The results showed the importance of scaling-stimulated salivary antioxidants as prognostic biomarkers of periodontal treatment [17].

Ebersole JL et al., sought answers using a diagnostic approach based on saliva to identify health and periodontal disease, based on salivary molecules coinciding with the disease and with the significant progress that has already been achieved in the identification of salivary biomarkers related to periodontal disease [7]. To do so, the researchers evaluated biomarkers representing several phases of periodontal disease initiation and progress (IL-1β, IL-6, MMP-8, macrophage inflammatory protein-MIP-1α) in whole saliva from 209 individuals categorised with periodontal health, gingivitis, and periodontal disease [7]. The evaluation of salivary analytes demonstrated utility for individual biomarkers to differentiate periodontal disease from health. They concluded that salivary concentrations of IL-1β, IL-6, MMP-8, MIP-1α, alone or in combination, can distinguish health conditions, gingivitis, and periodontal disease, being useful as salivary biomarkers [7].

Nagarajan R et al., investigated the use of saliva as a diagnostic fluid in conjunction with classification techniques to discern biological heterogeneity in individuals with clinically labelled gingivitis and periodontal disease [18]. Eighty individuals were used (40 per group), where a battery of classification techniques was investigated as traditional single classifier systems. Salivary expression profiles of IL-1β, IL-6, MMP-8 e MIP-1α from 80 patients were analysed. When comparing the results found with the literature, Nagarajan R et al., indicated that the salivary concentrations of IL-6, IL-8, albumin, calprotectin, PGE2, MMP-8 e MIP-1α are elevated in patients who have gingivitis [18]. These salivary analytes appear to serve as biomarkers of gingivitis and may help to discriminate the patients who are transitioning from health to gingivitis and further to periodontal disease [18].

After conducting a systematic assessment of studies on the accuracy of salivary biomarkers in the diagnosis of periodontal disease, De Lima CL et al., found limited evidence to confirm the diagnostic capacity of salivary biomarkers in the clinical evaluation of periodontal disease [19]. However, it is already possible to recognise the growing importance of this diagnostic method, and that the molecules MIP-1α, IL-1β and IL-6 emerge as promising biomarkers for future studies [19].

As already seen, a possible biomarker that has been studied is the MMP-8, which has already been identified and characterised as an important collagenolytic enzyme that causes periodontal and peri-implant degeneration in periodontal disease and peri-implantitis [20]. Thus, Alassiri S et al., explain that the key feature of active periodontal and peri-implant diseases is the sustained pathological elevation and MMP-8 activation in periodontal and peri-implant tissues, which reflect themselves in oral fluids [20]. Consequently, increased levels of MMP-8 are a promising candidate for biomarker to diagnose and assess the progression and evolution of these destructive and degenerative diseases in oral inflammatory tissue [20].

However, to test salivary biomarkers in the clinical routine, it is necessary to develop simple and effective tests. Therefore, Alassiri S et al., describe a new mouth rinse for collecting saliva sample and PISF/GCF/chair side/Point of Care (PoC) lateral-flow aMMP-8 immunoassays

(PerioSafe and ImplantSafe/ORALyser), used to predict and monitor the course, treatment and prevention of periodontal disease and peri-implantitis [20]. The tests have been independently and successfully validated in several countries (Finland, Germany, Netherland, Sweden, Turkey, Nigeria, Malawi, and USA), confirming their effectiveness in differentiating periodontal and peri-implant health and disease [20]. However, the clinical routine use of salivary/oral fluid biomarkers to identify oral and systemic conditions still needs additional studies utilising these non invasive screening, diagnostic, and preventive aMMP-8 PoC/chair-side technologies [20].

In another study, Bostanci N et al., reinforced the importance of developing tests for routine clinical use with salivary biomarkers and used the highly sensitive ELISAs test in a sample of 127 patients divided into three groups: periodontitis (n=60), gingivitis (n=31) and healthy (n=36) [21]. According to their results, the combination of multiple biomarkers, instead of using just one, can offer predictive accuracy above 90% for gingivitis versus healthy groups; and 100% for periodontal disease versus healthy patients, as well as for periodontal disease versus patients with gingivitis [21]. The authors revealed the salivary biomarkers MMP-8, MMP-9 and TIMP-1 as powerful differentiating values compared to a single biomarker [21].

Biomarkers in Peri-implant Disease

Periodontal disease is a risk factor for peri-implantitis since the presence of residual periodontal pockets and periodontal breakdown during maintenance raises the risk of peri-implantitis in patients with susceptible implant [22]. Peri-implantitis can be explained as a bacterial disease that involves inflammation of the soft tissues associated with the progressive loss of supporting bone in dental implants [23]. Peri-implantitis is commonly reported as one of the major contributors of implant failure and associated with both periodontal and non periodontal pathogens [24].

The inflammatory infectious disease that occurs around the implants is known as peri-implant disease and can present as peri-implantitis or peri-implant mucositis [25]. It has already been identified that IL-1β levels in the crevicular fluid of sites with mild peri-implantitis are higher when compared to sites affected by peri-implant mucositis and therefore, have been recommended to improve diagnostic and prognostic effectiveness [22]. Acharya A et al., investigated the association of salivary periodontopathogen count and IL-1β with the Peri-Implant Crevicular Fluid (PICF) IL response at peri-implant mucositis sites among individuals with different periodontal disease susceptibility [22]. Eighty-seven partially edentulous patients were enrolled, presenting at least one implant with peri-implant mucositis, 40 with a history of chronic periodontal disease (P group) and the remaining 47 without a history of periodontal disease (NP group). The counts of saliva IL-1, PICF, IL-1β and pathogens of the red salivary complex were evaluated [22].

Results showed that in the NP group, red complex score and salivary IL-1β were significant predictors of IL-1β PICF; in the P group, no significant associations were noticed [22]. It was concluded that salivary biomarkers can distinguish the “high” pro-inflammatory responders at peri-implant mucositis sites in individuals without inherent periodontal disease susceptibility, and that periodontal susceptibility can impact the immune-inflammatory response in sub peri-implant niches of those with peri-implant mucositis [22].

Sánchez-Siles M et al., sought to identify whether peri-implantitis causes an increase in the total salivary concentration of oxidative stress markers [23]. The authors explained that oxidative stress is defined as the imbalance between oxidisers and antioxidants in the body favouring the first, generating an accumulation of Reactive Oxygen Species (ROS) (pro-oxidants and oxidants). The main target of ROS are polyunsaturated fatty acids in tissue lipids which results in lipid peroxidation and lipid oxidation leads to several secondary products, mainly aldehydes that can aggravate oxidative damage; the main by-product of lipid peroxidation is called malondialdehyde [23]. The polymorphonuclear neutrophils are the first non specific line of defence, with myeloperoxidase being the most abundant protein in neutrophils and it is the only peroxidase that catalyses the peroxide and hydrogen chloride conversion into hypochlorous acid, a powerful oxidising agent involved in defence mechanisms against infectious agents [23]. In their study, Sánchez-Siles M et al., evaluated 70 patients, 28 men and 42 women, 60 of them with dental implants, 30 of which had peri-implantitis and 30 who were healthy [23]. The remaining 10 were the group control: healthy individuals without implants. Periodontal-peri-implant variables were assessed. Saliva samples were assessed for malondialdehyde high chromatography and myeloperoxidase concentrations. Results showed that total salivary malondialdehyde and myeloperoxidase concentration in the peri-implantitis group was slightly higher than the other groups, although the difference was not statistically significant [23].

Wang HL et al., determined the profile of PICF biomarkers combined with the following microbial profiles: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis,* Prevotella *intermedia, Tannerella forsythia* and *Treponema denticola*; present in healthy peri-implant tissues and in the presence of peri-implantitis to evaluated disease activity in real time [24]. The authors conducted a cross-sectional study using 68 patients who were divided into two groups: control group containing 34 patients with atleast one healthy implant; test group with 34 other patients with at least one implant with peri-implantitis. Total DNA content and qPCR analysis (qPCR in real time) for periodontal bacteria obtained from subgingival plaque samples and a PICF analysis for IL-1β, VEGF, MMP-8, TIMP-2, and OPG were performed [24].

Results identified that the mean concentration of IL-1β, TIMP-2, VEGF and OPG was increased in the peri-implantitis patients, and an association was found between a threefold or greater increase in total bacterial DNA of microorganisms with the presence of peri-implantitis, although without a statistically significant difference [24]. The ability to diagnose diseases sites was improved by *T. denticola* combined with IL-1β, VEGF e TIMP-2 PICF levels [24]. Data suggests that the increased levels of the evaluated biomarkers combined with site-specific microbial profiles can be associated with peri-implantitis and may have potential as predictors of peri-implant diseases [24].

In another study, Gomes AM et al., evaluated the levels of salivary biomarkers IL-1β, IL-10, Receptor activator of nuclear factor κB (RANK), OPG, MMP-2, Transforming growth factor beta (TG-β) and TNF-α in individuals with diagnosis of peri-implant mucositis in the absence or presence of periodontal and peri-implant maintenance therapy (TMPP) over 5 years [25]. Eighty patients diagnosed with peri-implant mucositis were evaluated, who were randomised into two groups: GTP group, with 39 individuals, who underwent periodontal and peri-implant maintenance therapy; and GNTP group, with 41 individuals, who did not undergo regular maintenance therapy [25]. Collection of saliva samples and assessment radiographic examination were performed in initial examination (T1) and after 5 years (T2). The ELISA test was used to identify the following markers in the salivary samples: IL-1β, IL-10, MMP-2, OPG, RANK, TGF and TNF-α [25]. The study identified that patients with severe periodontal and peri-implant clinical conditions showed an increase in the salivary concentration of TNF-α, as well as patients with a higher incidence of peri-implantitis, especially those in the GNTP group [25].

Algohar A and Alqerban A enrolled 60 patients to assess the levels of procalcitonin in saliva and PICF and to correlate these findings with the clinical and radiographic parameters of peri-implant disease [26]. Patients were divided into three groups: Group 1 was healthy; Group 2 was peri-implant mucositis; and Group 3 was peri-implantitis. Group 3 showed significant positive correlations between PICF procalcitonin levels and bleeding on probing, probing depth, and crestal bone loss, and Group 2 showed significant positive

correlation between PICF and bleeding on probing [26]. Patients in Groups 2 and 3 had significantly increased procalcitonin levels in saliva and PICF in comparison to Group 1 [26]. These findings indicated that procalcitonin probably has a role in peri-implant inflammation and that higher levels of this molecule can be used as a probable biomarker for peri-implant disease [26].

However, longitudinal studies using larger populations are still needed, not only to confirm these findings, but also to clarify the role of this biomarker in peri-implant disease [25].

CONCLUSION(S)

The present review highlighted the importance of salivary biomarkers in the diagnosis of oral disease. Many biomarkers for these diseases have been identified and can be collected through saliva in a non invasive simple manner thereby allowing early diagnosis of these conditions as well as their progression monitoring and their response to treatment. Still, despite the promising evidence, more studies are needed to confirm these findings, as well as longitudinal studies in larger populations.

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PARTICULARS OF CONTRIBUTORS:
1. Master's Degree in Odontology, Instituto de Saúde de Nova Friburgo - Universidade Federal Fluminense, Nova Friburgo, Rio de Janeiro, Brazil.
2. Degree in Odontology from Universidade Salgado de Oliveira, Niterói, Rio de Janeiro, Brazil.
3. Master's Degree in Odontology from Universidade Veiga de Almeida, Rio de Janeiro, Brazil.
4. Professor, Swiss Biohealth Member Academy, Kreuzlinger, Switzerland; Professor of Graduate Program of Odontology from Instituto de Saúde de Nova Friburgo-Universidade Federal Fluminense, Nova Friburgo, Rio de Janeiro, Brazil.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Bruno Pires Miranda,
Rua Desembar Gador Lima Castro, 19-sobrado-Fonseca,
Niterói, Rio de Janeiro, Brazil.
E-mail: brunopiresmiranda7@gmail.com

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