SALIVARY BIOMARKERS OF PERIODONTAL DISEASE AS A TOOL FOR DIAGNOSIS, PREDICTION, OR PROGNOSIS OF COMPLICATIONS RELATED TO TYPE 2 DIABETES

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ABSTRACT: In type 2 diabetes, there is a general alteration of inflammatory cytokines, suggesting an important role for increased inflammation in the disease's pathophysiology. Diabetes diagnosis primarily relies on various laboratory tests, with glycated hemoglobin (HbA1c) serving as the gold standard. Currently, researchers have explored alternative sources for valuable diagnostic elements, and saliva has emerged as a promising option. Saliva is a cost-effective, bioavailable, non-invasive, and rapid resource that could serve as a reservoir of useful biomarkers for the diagnosis, prognosis, and prediction of type 2 diabetes mellitus. This fluid has been extensively studied in periodontology, where markers of tissue inflammation including osteocalcin, prostaglandin E2 (PGE2), interleukins (IL-1, IL-6, IL-8, IL-11, IL-1), and tumor necrosis factor (TNF) have been identified. However, the potential relationship between salivary biomarkers and type 2 diabetes mellitus has not yet received comprehensive attention in the literature (1,2).

This literature review aimed to assess salivary biomarkers studied in periodontology within the available literature from the past 8 years and explore their potential utility for diagnosing, prognosticating, or predicting complications related to type 2 diabetes.

Methods: A literature review was conducted across various indexed databases, including PubMed, Cochrane, and Medline, using articles published within the last 8 years and of scientific significance.

Results: In the existing literature, nine primary types of salivary biomarkers have been identified that could potentially serve as diagnostic, predictive, and prognostic indicators for type 2 diabetes. These biomarkers encompass salivary glucose,

salivary amylase, interleukins IL-4, IL-6, IL-8, and IL-10, as well as tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and antigen 1.5 (Ag1.5).

Conclusion: The evidence presented in this literature review suggests that there are salivary biomarkers shared between periodontal disease and T2DM, which could serve as bioavailable and minimally invasive tools for aiding in the diagnosis, prognosis, and prediction of diabetes-related complications. However, it is imperative to establish standardized protocols for sample collection and processing, conduct studies across diverse population groups, and mitigate biases to ensure that the results are more consistent and comparable. Further research is required to explore the expression of these biomarkers in saliva and establish comparisons with serum samples from individuals with periodontitis and T2DM, yielding more robust findings. A deeper investigation into biomarkers in periodontics could pave the way for precision medicine approaches, seeking personalized methods for diagnosing, prognosticating, and predicting treatment responses in patients.

Keywords: saliva biomarkers, periodontitis, type 2 diabetes mellitus.

Diabetes Mellitus (DM) is a disease associated with carbohydrate, fat, and protein metabolism alterations. In 2021, it was estimated that approximately 537 million people, 45% of the global population, were suffering from undiagnosed diabetes (3). In Colombia, during the same year, the Ministry of Health and Social Protection estimated that there were 1,676,885 individuals living with diabetes (4). There are two main forms of diabetes: type 1 diabetes (T1DM), which most commonly occurs in children and young adults and is caused by a lack of insulin secretion by the pancreas; and type 2 diabetes (T2DM), on the other hand, is a chronic metabolic disorder characterized by the progressive loss of β-cell function in relation to insulin resistance, resulting in persistent hyperglycemia. This resistance is often accompanied by inflammatory processes that can lead to complications, including blindness, kidney failure, heart attacks, strokes, lower limb amputations, and periodontal disease (5).

In T2DM, inflammatory cytokines are typically altered, suggesting that increased inflammation plays a crucial role in the pathophysiology of the disease (6). The diagnosis of diabetes can be made through various laboratory tests, with glycated hemoglobin (HbA1c) being considered the gold standard. This test measures the average blood glucose level over the past two to three months. According to the American Diabetes Association, HbA1c levels should be maintained below 7% to prevent most diabetes-related complications, except for cardiovascular disease. Therefore, it is established that well-controlled diabetic patients typically have fasting blood glucose levels up to 110 mg/dL and HbA1c levels less than 7% (7). On the other hand, the oral glucose tolerance test (OGTT) analyzes blood glucose levels two hours before and two hours after consuming a sugary drink provided by the laboratory. This test helps assess how the body processes sugar. Finally, the random blood glucose test can be conducted at any time of the day when there are severe symptoms of diabetes (8).

Diabetes diagnosis methods can sometimes be challenging to implement due to limitations such as the need to transport the patient to specific locations for sampling, high costs, especially if done privately, required preparations or extended periods of fasting, a minimum processing time of one day, and discomfort during the procedure. Currently, alternative approaches have been explored to gather biological samples useful for disease diagnosis, with one of them being saliva.

Saliva is a low-cost, readily available, non-invasive, and rapidly obtainable fluid that holds potential as a source of valuable biomarkers for the diagnosis, prognosis, and prediction of T2DM. This has been explored primarily in the field of periodontics, where it is possible to identify inflammation markers in this fluid, such as osteocalcin, prostaglandin E2 (PGE2), interleukins IL-1, IL-6, IL-8, IL-11, IL-1, and tumor necrosis factor (TNF). However, this potential relationship has not been extensively addressed in the existing literature (1,2).

According to the National Institute of Health (NIH), biomarkers are defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a

therapeutic intervention.” (9). Various diagnostic tests use biomarkers for different diseases. There are different types of biomarkers, including diagnostic, predictive, response, and prognostic biomarkers.

Diagnostic biomarkers are used to detect or confirm the presence of a specific disease or condition or to identify individuals with a particular disease subtype, predictive biomarkers identify individuals who are more likely to respond differently to a factor compared to those without the biomarker, response biomarkers help determine whether patients experience a favorable or unfavorable effect after exposure to a medical product or environmental agent, and prognostic biomarkers are employed to assess the likelihood of a clinical event, recurrence, or disease progression in individuals who already have the disease or medical condition of interest (10).

Over the past decade, research on biomarkers associated with type 2 diabetes has seen a notable increase, particularly those found in plasma and serum. Numerous biomarkers have exhibited significant positive correlations with type 2 diabetes (11), and these parameters have been exclusively derived from blood samples. More recently, as saliva samples have been introduced in research, their role as potential indicators of type 2 diabetes risk has been substantiated. Saliva contains many molecular components that are detectable; nevertheless, its effectiveness and utility in clinical and diagnostic settings have been relatively underexplored. The objective of this literature review was to assess, using literature from the last eight years, the salivary biomarkers studied in the field of periodontics that could serve as diagnostic, prognostic, or predictive tools for complications related to T2DM (12).

Saliva as a diagnostic source

Saliva is an acidic fluid with a pH ranging from 6 to 7. It comprises water, inorganic and organic substances, including hormones, antibodies, proteins, enzymes, and cytokines, which can be valuable for diagnosing, prognosticating, or predicting T2DM, as outlined in Table 1. Saliva is produced by three pairs of major salivary glands and thousands of minor salivary glands. It possesses various components and essential physicochemical properties crucial for maintaining oral health. Saliva

plays a vital role in processes such as chewing, swallowing, digestion, speech, and perception, and contributes to maintaining a balanced environment for protecting and facilitating the proper functioning of the human body and overall health (13).

Table 1. Salivary Biomarkers Potentially Useful for Type 2 Diabetes

Type	Biomarker	References
Enzymes	Salivary glucose, salivary amylase	(14), (15)
Cytokines or chemokines	IL-4, IL-6, IL-8, IL 10, TNF- α	(16), (17),(18),(19)
Plasma protein	C-reactive protein	(14), (20), (21),(22)
Monosaccharide	1,5-Anhydroglucitol (1,5Ag)	(23), (24)

Saliva is a complex fluid that exists in a mixture of oral fluids, including serum, bacteria, bacterial products, viruses, fungi, desquamated epithelial cells, other cellular components, and food debris (25). Saliva can be collected with or without stimulation. Stimulated saliva is typically obtained through actions such as chewing or gustatory stimulation, which can modify the volume of saliva collected, alter the concentrations of certain constituents, and change the pH of the fluid. In the literature, two methods for saliva collection are reported: the drain method, where saliva drips naturally from the lower lip, and the spit method, where the individual actively expels saliva into a test tube (Navazesh, 1993) (25).

Early attempts to use saliva as a diagnostic fluid faced several challenges, including the difficulty in detecting certain markers, high costs, lack of standardization in sample collection, and the complexity of sample storage and processing methods. These obstacles have largely been overcome thanks to comprehensive studies of salivary gland physiology, the development of sensitive amplification techniques like enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain

reaction (qRT-PCR), and increased education within the scientific community regarding proper methodologies for collecting and handling salivary samples.

Recent advancements in oral fluid biomarker diagnostics have been driven by innovative molecular approaches, such as proteomics, transcriptomics, and genomics, as well as metagenomic analyses that have expanded our understanding of microbial pathogens associated with both oral and systemic diseases (14). However, it's important to note that saliva collection and analysis have yet to be standardized. Typically, unstimulated saliva is collected, subjected to centrifugation, and stored at -80°C, followed by analysis using ELISA or commercial enzyme immunoassays(11).

The application of salivary diagnostics for systemic diseases received a major boost in 2002 as a result of a program initiated by the National Institute of Dental and Craniofacial Research (NIDCR), "Development and Validation Technologies for Saliva-Based Diagnostics"; which fostered the growth of interest in the use of saliva and other oral samples for the diagnosis of oral and systemic diseases depending on the biochemical nature of the marker, the source, the type of sample taken and the mechanism by which the marker enters the oral cavity (14).

Salivary biomarkers and type 2 diabetes mellitus

To date, the literature has identified nine primary types of salivary biomarkers that may serve as diagnostic, predictive, and prognostic indicators for type 2 diabetes, potentially helping to prevent various complications associated with this condition. These biomarkers include salivary glucose, salivary amylase, IL-4, IL-6, IL-8, IL-10, TNF-α, C-reactive protein, and 1,5 Ag. They can be categorized into subgroups based on their respective functions, as summarized in Table 2.

Table 2. Salivary Biomarkers and Their Utility in Type 2 Diabetes

BIOMARKER	BIOMARKER FUNCTION		
	Diagnostic	Prognostic	Predictive

Salivary glucose	x	x	
Salivary amylase		x	x
IL-4			x
IL-6	x	x	
IL-8			x
IL-10		x	x
TNF-α	x	x	x
C-reactive protein	x	x	x
1,5 Ag		x	x

Salivary glucose is found in concentrations of 0.5 - 1 mg/dL, it increases after consuming food and drinks or depending on the concentration of glucose in the blood, evidencing a positive correlation between the saliva glucose test and HbA1c (26)(15). Although it is relatively easy to measure salivary glucose, due to the multiple sources of this material in the oral cavity, salivary glucose levels do not correlate with blood glucose levels in some studies. However, ongoing research is exploring alternative approaches. In a 2006 study by Rao et al., a distinctive proteomic signature was observed in saliva collected from individuals with T2DM compared to control saliva. This analysis revealed that 65 proteins exhibited more than a two-fold change, many of which were associated with regulatory metabolic and immunological pathways. While further research is required for validation, these findings suggest the potential existence of a unique salivary biomarker linked to diabetes (14).

On the other hand, **salivary amylase** plays a crucial role in initiating the hydrolysis of approximately 30% of the food in the mouth through acinar cells innervated by both sympathetic and parasympathetic pathways. Activation of the sympathetic nervous system increases amylase synthesis, leading to an elevation in its concentration, while parasympathetic activity raises saliva flow. Salivary amylase also plays a significant role in glucose processing, making it a promising biomarker for the assessment and monitoring of DM (15).

In a review by Pérez-Ros P and Navarro-Flores, it was observed that glucose levels in saliva were statistically higher in diabetic patients, ranging from 1.26 to 11 mg/dL, compared to the control group, which reported values between 0.5 and 4.8 mg/dL. Furthermore, it was noted that the concentration of glucose in saliva under fasting conditions or 1-2 hours after eating led to a three-fold or greater increase in the concentration of glucose in the saliva of diabetic patients compared to its levels in blood samples (15). Elevated levels of salivary amylase were also evident in unstimulated samples from diabetic patients. This can be attributed to the altered expression of amylase and cyclic adenosine monophosphate (cAMP) receptors in the parotid gland of diabetic patients, resulting in changes in the production of salivary proteins. Additionally, there is an increase in the permeability of the basement membrane, which could potentially allow the leakage of proteins into saliva through the salivary glands (15).

Acharya et al. conducted a meta-analysis in 2022 of 15 cross-sectional studies, which identified two interleukins (IL) with direct associations to both T2DM and periodontitis. In their first finding, they observed that **IL-10** levels were significantly lower in the group of patients with periodontitis compared to the DM2 + periodontitis group ($p = 0.003$). IL-10 is known for its anti-inflammatory properties and this observation may explain the altered immune response in these patients or how it can progress to chronic periodontitis when levels are low. The second discovery revealed a significantly lower concentration of **IL-4** in the diabetic periodontitis group compared to the non-diabetic periodontitis group ($p < 0.001$). This finding could potentially explain the progression of periodontitis in diabetic patients and their increased susceptibility to the condition. However, it is important to exercise caution, as higher levels of IL-4 may be influenced by other mediators present in either of the pathologies(16). Furthermore, **IL-8** levels exhibited an association between DM2 and periodontitis. However, in this systematic review, the results did not reveal statistically significant differences between the groups with periodontitis plus diabetes and those with periodontitis only. On the other hand, **TNF-α**, a pro-inflammatory mediator, showed a direct link between periodontitis and DM2. This

cytokine has the potential to induce insulin resistance and contribute to chronic systemic inflammation (16).

IL-6 has the potential to induce insulin resistance and may play a role in the process of bone resorption in periodontitis. This marker is synthesized in response to interactions with infectious agents, triggering the acute immune response. Consequently, the levels of IL-6 in saliva may reflect its concentrations in the bloodstream. In this systematic review, higher IL-6 values were observed in patients with both diabetes and periodontitis. However, quantitative analyses did not reveal statistically significant differences between the groups with periodontitis + T2DM and those with periodontitis only (16). Additionally, Costa et al. conducted an evaluation of IL-6 in the saliva of 90 patients with periodontitis and T2DM. Notably, all patients in the untreated periodontitis or T2DM groups were receiving insulin and/or oral hypoglycemic agents. The untreated periodontitis and untreated periodontitis + T2DM groups exhibited higher IL-6 concentrations than the control and diabetic groups ($P<0.01$). Consequently, elevated salivary IL-6 concentrations were identified in patients with periodontitis, regardless of their diabetic status. However, as Costa et al. examined patients with untreated periodontitis with or without T2DM, there remains a possibility that salivary IL-6 concentrations were overestimated. Additional studies are warranted to determine the utility of IL-6 as a biomarker (17).

TNF-α is a pro-inflammatory cell signaling protein, that plays a role in apoptosis, cell differentiation, and oxidative stress (27). Elevated concentrations of this protein have been linked to insulin resistance, with studies reporting an increase of 17% to 39% in concentrations compared to healthy controls (28). Tvarijonavicute et al. conducted an examination of several salivary cytokines in patients with T2DM based on the criteria of the American Diabetes Association and compared them with healthy individuals. Their findings revealed that salivary TNF-α was 1.9 times higher in patients with T2DM compared to non-diabetics ($P<0.049$). However, in another study by Srinivasan et al., a comparison of salivary concentrations of pro-inflammatory adipokines and anti-inflammatory adipokines in self-reported patients with T2DM versus healthy individuals showed minimal differences between the groups (29)

Therefore, further research is necessary to fully explore the potential of TNF-α as a salivary biomarker for T2DM and to assess its clinical utility (18).

C-reactive protein (CRP) is a biomarker produced by the liver and is widely used for predicting the risk of T2DM, regardless of factors such as race, gender, or age (20); Elevated levels of CRP may contribute to inflammatory responses by activating complement proteins or increasing the production of proinflammatory cytokines, including IL-6 and TNF-α, cytokines that are also elevated in patients with DM2. Additionally, CRP can potentially contribute to insulin resistance by promoting the production of thrombogenic substances (21). Several studies have reported that individuals with DM2 exhibit a 41% to 125% increase in serum CRP concentrations compared to non-diabetic individuals. Notably, non-diabetic participants who later developed T2DM had elevated baseline CRP levels, suggesting that CRP could serve as a potential biomarker for T2DM (22). There are published reports demonstrating that CRP can be monitored in saliva samples. However, it is important to note that CRP is a non-specific marker of inflammation that can increase in various conditions, including periodontal diseases(14,21,22).

On the other hand, **1,5-AG** is a monosaccharide similar to glucose found in high concentrations in the bloodstream under normal conditions. It serves as an indicator of short-term glucose fluctuations and hyperglycemia (23). Studies have reported a significant decrease of 35% to 58% in 1,5-AG concentrations in individuals with T2DM compared to healthy subjects (24). Currently, there is limited information available regarding 1,5-AG in saliva and its relationship with DM2. However, it has been detected in measurable concentrations in saliva, making it a potential non-invasive marker for individuals at risk of diabetes.

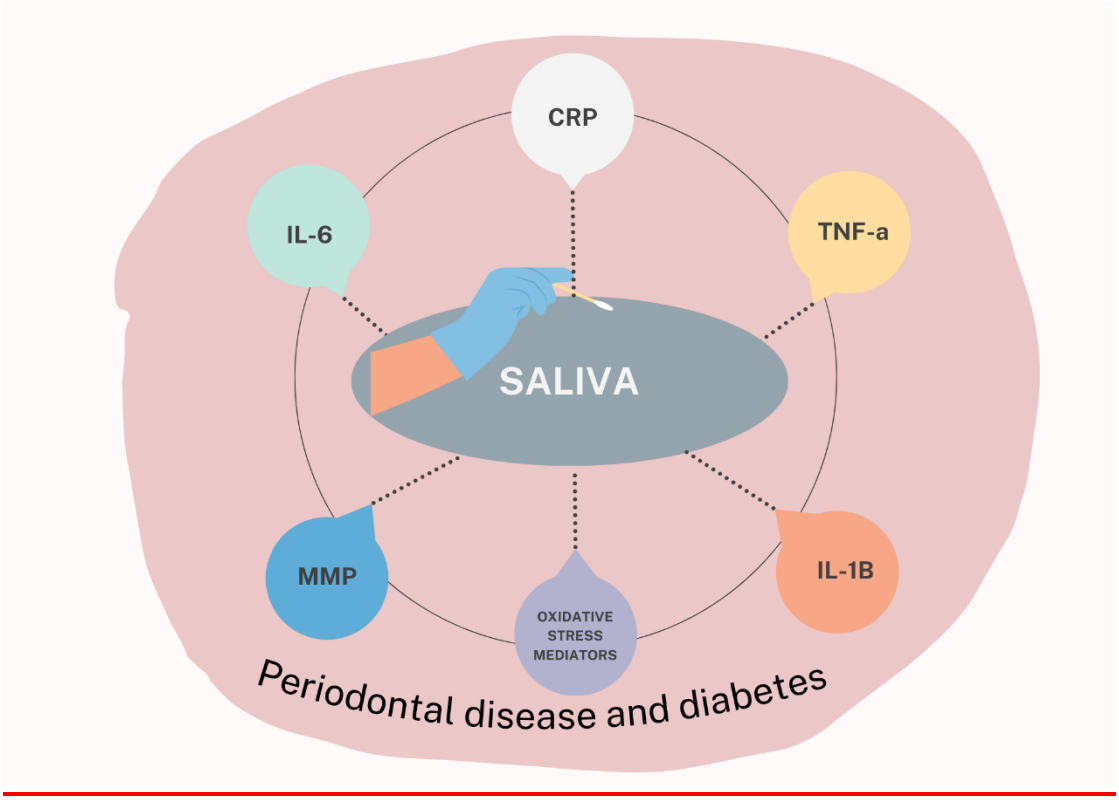
In a cross-sectional study conducted by Mook-Kanamori et al., salivary metabolic biomarkers of T2DM were evaluated, and 1,5-AG was one of the three detectable metabolites ($P = 3.6 \times 10{-}13$). Salivary 1,5-AG demonstrated a positive correlation with serum 1,5-AG ($r=0.74$), but not with serum glucose ($r=-0.51$) or HbA1c ($r=-0.59$). Similarly, Asha et al. assessed 1,5-AG as a salivary biomarker in patients with DM2 and found a moderate positive correlation between serum and saliva levels

of 1,5-AG in both groups ($P < 0.03$ and 0.009, respectively). Despite the limited number of studies, the consistent results suggest the potential use of salivary 1,5-AG as a biomarker for increased risk of T2DM (24).

Common Salivary Biomarkers in Diabetes and Periodontal Disease

Pro-inflammatory and anti-inflammatory cytokines regulate the processes executed by the immune system and the response of each individual. Anti-inflammatory cytokines control the inflammatory process, while pro-inflammatory cytokines amplify and sustain the response. Measuring mediator levels in patients with periodontitis and DM2 provides insight into the inflammatory cascade processes in both conditions, their roles, and their potential to either repair or damage tissues. However, quantifying inflammatory markers has been challenging (12). Diagram 1 illustrates some salivary biomarkers that may exhibit elevated indicators in the presence of periodontal disease or diabetes, including matrix metalloproteinases (MMPs), interleukin 6 and 1B (IL-6 and IL-1B), Tumor Necrosis Factor-alpha (TNF-α), C-reactive protein, and oxidative stress mediators.

Diagram 1. Salivary biomarkers shared by periodontal disease and diabetes.

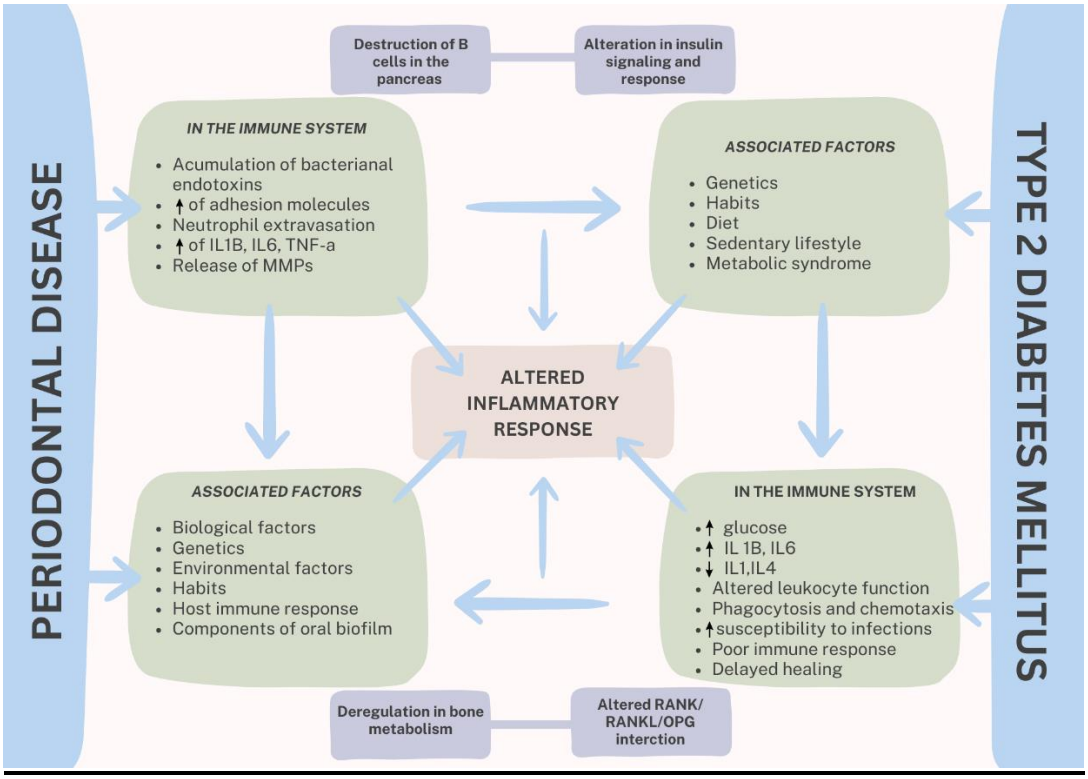


In 1970, AR Feinstein coined the term "comorbidity," which refers to the occurrence of one or more additional diseases or disorders alongside a primary disease or disorder. Diseases that share a comorbidity relationship are more likely to coexist because the genes associated with these diseases are indirectly regulated by common biological mechanisms(30). For instance, both periodontitis and T2DM are multifactorial diseases. Periodontitis can affect individuals irrespective of their health condition, but it is notably more prevalent and severe in individuals with diabetes (31).

Diabetes can lead to disruptions in inflammatory and immune responses, as well as impaired healing. On the other hand, periodontitis is characterized by an imbalanced inflammatory and immunological response, often associated with dysbiotic oral flora. Currently, the biological evidence supporting their comorbidity is grounded in the

biological plausibility of observing complications or advanced periodontitis in patients with poorly controlled diabetes, as illustrated in Diagram 2 (32).

Diagram 2. Relationship between periodontal disease and type 2 diabetes mellitus



The interaction between RANK and RANKL is one of the most potent triggers for osteoclast formation and activity. Conversely, osteoprotegerin (OPG) acts as a decoy receptor, effectively inhibiting the binding of osteoclast precursors to RANK, thereby blocking RANKL activity. Animal studies have provided compelling evidence that the interplay between osteoblast-expressed proteins, namely RANK, RANKL, and osteoprotegerin (OPG), plays a crucial role in promoting osteoclastogenesis in individuals with both periodontitis and diabetes. Consequently, both T1DM and T2DM have a modulating effect on alveolar bone homeostasis. In studies involving rats with diabetes, including T1DM137 and T2DM2138, experimental ligations and the induction of pathogen-induced periodontitis resulted in a 2-4-fold increase in the

number of osteoclasts compared to control rats, which reinforces the previously mentioned ideas (33,34).

The challenge of reducing glycated hemoglobin (HbA1c)

The Diabetes Control and Complications Trial (DCCT) and its 6.5-year follow-up study evaluating Diabetes Interventions and Complications in the United States and Canada revealed a 76% reduction in retinopathy, a 34% decrease in the development of early nephropathy, and a 69% reduction in neuropathy development. Additionally, there was an observed decrease in cardiovascular events and cardiovascular disease-related deaths with the implementation of intensive treatment(35).

According to a 2018 meta-analysis, the prevalence of diabetes is 13.1% among individuals with periodontitis, while it is 9.6% among those without periodontitis. This suggests the importance of managing hyperglycemia over time, regardless of the type of diabetes, as it can significantly impact the progression of periodontitis (36,37).

In a randomized controlled trial, the United Kingdom Prospective Diabetes Study (UKPDS), 5102 patients with newly diagnosed T2DM were studied between 1977 and 1991. Patients were followed for an average of 10 years. Intensive therapy (insulin or oral agents) was compared with conventional therapy (diet with or without pharmacological therapy), and they found that for each percentage point decrease in HbA1c, there was a reported: 35% reduction in the risk of microvascular complications, 25% reduction in diabetes-related deaths, 7% reduction in all-cause mortality, and 18% reduction in combined fatal and non-fatal myocardial infarction. Taken together, DCCT and UKPDS, along with other studies, demonstrate that glycemic control is the key factor in controlling systemic complications related to DM (38,39).

Periodontitis is the sixth complication of diabetes, as poor glycemic control can lead to the worsening of periodontal conditions. There are also cases in which periodontal disease becomes a predisposing factor for diabetes (31), It is crucial to mention that there are environmental, genetic, or behavioral factors that may be related to the onset and development of the disease in susceptible individuals (16).

In 2004, Yoon et al. discovered advanced glycation end-product proteins in the saliva of diabetic patients, which were related to the amount of dental biofilm (40), In another study, conducted by Takeda et al. in 2007, it was demonstrated that serum levels of advanced glycation end products (AGEs) were associated with the severity of periodontitis in individuals with T2DM (41). Delving deeper into the microbiological aspect, a study conducted in mice revealed that alveolar bone loss induced by P. Gingivalis was greater in diabetic animals compared to non-diabetic ones. This was accompanied by increased expression of the receptor for AGEs and MMPs, which had a detrimental effect on gingival tissues. However, when researchers blocked the receptor for AGEs in diabetic mice, levels of inflammatory cytokines and MMPs decreased, effectively suppressing bone loss (42).

When there is an increased expression of IL1β, IL6, and TNF-α in the periodontal tissue and the crevicular fluid, the response of the periodontal tissues can be altered, leading to tissue damage (19). Part of this mechanism of periodontal destruction occurs as follows: an increase in blood glucose levels generates AGEs in cells and tissues. Subsequently, there is a mediated interaction between AGEs and their receptor (RAGE), resulting in cellular oxidative stress. This is followed by an imbalance in the RANKL/OPG relationship, favoring the formation and activation of osteoclasts, which in turn leads to an increase in MMPs and, finally, the resorption of alveolar bone and the destruction of collagen in tissues (16).

Various studies have examined inflammatory markers in saliva and gingival crevicular fluid. In a study conducted by Cutler et al. in 1997, it was found that IL1β levels in the crevicular fluid were elevated in periodontal patients, regardless of whether they had well-controlled diabetes or not (43). When investigating the impact of glycemic control, other studies have also emerged. For instance, Santos et al. in

2010 reported elevated levels of IL17 and IL4 in the crevicular fluid of periodontal patients with poor glycemic control (44). Similarly, Costa et al. in 2010 discovered increased IL6 levels in the saliva of untreated periodontal patients with poorly controlled diabetes (45). Additionally, Ribeiro et al. in 2011 found higher levels of IL17, IL23, and IL4, as well as decreased IFN-γ, in the crevicular fluid of patients with inadequate diabetic control (46). In a 2014 study by Duarte et al., a greater quantity of inflammatory proteins, including macrophages, IL12, IL6, and TNF-α, was observed (47).

Additional findings have been reported by Ozturk et al., who observed higher levels of substance P, a pro-inflammatory peptide, in both serum and crevicular fluid among patients with poor glycemic control (48). Similarly, Kim et al. demonstrated elevated expression of CRP, MMP 14, and tissue inhibitor of metalloproteinase 2 in the periodontal tissues of individuals with inadequate glycemic control (49).

As a result, if periodontitis certainly plays a role in diabetes, it stands to reason that periodontal therapy could impact the levels of circulating inflammatory cytokines (50). Research indicates that periodontal treatment can have a positive effect on diabetes control, including improvements in glycemic control, lipid profiles, and insulin resistance. Additionally, it has been shown to reduce serum levels of inflammatory cytokines and increase serum levels of adiponectin in patients with T2DM (50).

Sol et al. conducted a study involving 190 patients with moderate to poorly controlled T2DM (HbA1c levels between 7.5% and 9.5%) who also had periodontitis. They observed that, following 3 months of periodontal therapy, there were significant reductions in serum levels of CRP, TNFα, IL-6, fasting plasma glucose, HbA1c, fasting insulin, and the HOMA-IR index. The latter is a method used to assess insulin resistance based on fasting blood glucose and insulin concentrations. Additionally, adiponectin levels showed a significant increase in the treated group compared to the untreated group (50).

The positive impact of surgical and non-surgical periodontal therapy on HbA1c levels was also evident in a recent study conducted by D'Aiuto et al. In this 12-month randomized clinical trial, 264 participants were assigned to either receive intensive periodontal treatment (IPT), which included full-mouth subgingival scaling, surgical periodontal treatment, and supportive periodontal care every 3 months until the study's completion or control periodontal treatment (CPT), consisting of supragingival periodontal treatment. The study found that after 12 months, HbA1c levels in the IPT group were 0.6% lower (95% CI: 0.3% to 0.9%) compared to the CPT group, relative to baseline HbA1c levels. The question that remains unanswered is whether these observed benefits are sustained beyond the 12-month period (51).

In a meta-analysis conducted by Engebretson and Kocher, they pooled data from seven of the most comprehensive meta-analyses of randomized controlled trials published between 2013 and 2017. Their analysis demonstrated that periodontal therapy led to a significant reduction in HbA1c levels within 3 to 4 months after treatment, with a range of 0.27% to 1.03% (95% CI: -0.54 to -0.19) (52). In the most recent update, Madianos and Koromantzos confirmed the positive impact of non-surgical periodontal therapy on HbA1c in diabetic patients. They found reductions in HbA1c levels 3 to 4 months after treatment, ranging from -0.27% (95% CI: -0.46 to -0.07) to -1.03% (95% CI: 0.36 to -1.70) and even 6 months after treatment, with reductions ranging from -0.02% (95% CI: -0.20 to -0.16) to -1.18% (95% CI: -0.72 to -1.64) (53).

In contrast, a multicenter randomized clinical trial conducted by Engebretson et al. found that non-surgical periodontal therapy did not lead to improvements in glycemic control of patients with T2DM. However, several authors argue that the periodontal therapy provided in this study may have been insufficient in effectively managing periodontal infection, as patients still exhibited high levels of residual biofilm (72%) and bleeding scores (42%) following the treatment. Additionally, the mean HbA1c

value at the beginning of the study was already close to the therapeutic target, making it challenging to achieve a significant improvement in HbA1c through periodontal intervention alone (54). Lastly, the ongoing debate regarding the impact of periodontal treatment on glycemic control may be attributed to the heterogeneity of trial designs, emphasizing the need for more studies with rigorous methodology.

Discussion

Understanding the expression of proinflammatory cytokines, enzymes, or proteins provides insight into the functioning of the immune system. It can also aid in comprehending the distinct patterns of immune responses in individuals and offer valuable information regarding susceptibility to periodontal disease or T2DM. Some of these inflammatory mediators play a crucial role in either exacerbating or mitigating inflammation during the immune response, ultimately, influencing tissue damage (25).

Different studies show that some inflammatory markers evaluated in the periodontal field, related to tissue inflammation, could be valuable in the medical field by showing evidence that periodontal patients could be more susceptible to presenting T2DM and complications derived from it or vice versa (6,12,15,16,30,55). On the other hand, some studies indicate that rigorous periodontal control could provide stability in diabetic patients for up to a year. However, there are no long-term studies that support this evidence (50,51).

The present literature review has identified that the implementation of an individualized and decisive treatment plan can be facilitated through the utilization of salivary biomarkers as a valuable tool. Salivary biomarkers offer a low-cost, bioavailable, non-invasive, and rapid resource that can be analyzed in the medical field, benefiting patients in terms of diagnosis, prediction, and prognosis of complications associated with T2DM (6,12,15,16,30,55). However, studies are still needed that analyze the behavior of systemic mediators at the local level, studies with high methodological rigor and high clinical evidence that confirm this information

collected over the years, to finally understand the role of these mediators at the salivary level, with their consequent oral and systemic impact.

Conclusion

The evidence presented in this literature review suggests that there are salivary biomarkers shared between periodontal disease and T2DM, which could serve as bioavailable and minimally invasive tools for aiding in the diagnosis, prognosis, and prediction of diabetes-related complications. However, it is imperative to establish standardized protocols for sample collection and processing, conduct studies across diverse population groups, and mitigate biases to ensure that the results are more consistent and comparable. Further research is required to explore the expression of these biomarkers in saliva and establish comparisons with serum samples from individuals with periodontitis and T2DM, yielding more robust findings. A deeper investigation into biomarkers in periodontics could pave the way for precision medicine approaches, seeking personalized methods for diagnosing, prognosticating, and predicting treatment responses in patients (56).

REFERENCES

1. Kc S, Wang XZ, Gallagher JE. Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: Systematic review. J Clin Periodontol. marzo de 2020;47(3):289-308.

2. Chen M, Cai W, Zhao S, Shi L, Chen Y, Li X, et al. Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: A systematic review and meta-analysis. J Clin Periodontol. junio de 2019;46(6):608-22.

3. 20200302_133352_2406-IDF-ATLAS-SPAN-BOOK.pdf [Internet]. [citado 25 de marzo de 2023]. Disponible en: https://www.diabetesatlas.org/upload/resources/material/20200302_133352_2406-IDF-ATLAS-SPAN-BOOK.pdf

4. Día mundial de la diabetes 2021 [Internet]. Cuenta de Alto Costo. 2021 [citado 25 de marzo de 2023]. Disponible en: https://cuentadealtocosto.org/site/general/dia-mundial-de-la-diabetes-2021/

5. Mohamed HG, Idris SB, Mustafa M, Ahmed MF, Åstrøm AN, Mustafa K, et al. Impact of Chronic Periodontitis on Levels of Glucoregulatory Biomarkers in Gingival Crevicular Fluid of Adults with and without Type 2 Diabetes. Bencharit S, editor. PLOS ONE. 20 de mayo de 2015;10(5):e0127660.

6. Longo PL, Artese HPC, Rabelo MS, Kawamoto D, Foz AM, Romito GA, et al. Serum leveis of inflammatory markers in type 2 diabetes patients with chronic periodontitis. J Appl Oral Sci. abril de 2014;22(2):103-8.

7. American Diabetes Association. Standards of Medical Care in Diabetes—2009. Diabetes Care. 1 de enero de 2009;32(Supplement_1):S13-61.

8. Petersmann A, Müller-Wieland D, Müller UA, Landgraf R, Nauck M, Freckmann G, et al. Definition, Classification and Diagnosis of Diabetes Mellitus. Exp Clin Endocrinol Diabetes. diciembre de 2019;127(S 01):S1-7.

9. Strimbu K, Tavel JA. What are biomarkers?: Curr Opin HIV AIDS. noviembre de 2010;5(6):463-6.

10. BEST (Biomarkers, EndpointS, and other Tools) Resource.

11. Desai P, Donovan L, Janowitz E, Kim JY. The Clinical Utility of Salivary Biomarkers in the Identification of Type 2 Diabetes Risk and Metabolic Syndrome. Diabetes Metab Syndr Obes Targets Ther. octubre de 2020;Volume 13:3587-99.

12. Desai P, Donovan L, Janowitz E, Kim JY. The Clinical Utility of Salivary Biomarkers in the Identification of Type 2 Diabetes Risk and Metabolic Syndrome. Diabetes Metab Syndr Obes Targets Ther. octubre de 2020;Volume 13:3587-99.

13. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. J Oral Rehabil. septiembre de 2018;45(9):730-46.

14. Malamud D. Saliva as a Diagnostic Fluid. Dent Clin North Am. enero de 2011;55(1):159-78.

15. Pérez-Ros P, Navarro-Flores E, Julián-Rochina I, Martínez-Arnau FM, Cauli O. Changes in Salivary Amylase and Glucose in Diabetes: A Scoping Review. Diagnostics. 6 de marzo de 2021;11(3):453.

16. Mattos MC de O, Chagas LGA de A, Stefani CM, Damé-Teixeira N, Grisi DC, Salles LP, et al. Expression of inflammatory mediators in periodontitis and T2D patients: a systematic review and meta-analysis. Braz Oral Res. 2022;36:e098.

17. Costa PP, Trevisan GL, Macedo GO, Palioto DB, Souza SLS, Grisi MFM, et al. Salivary Interleukin-6, Matrix Metalloproteinase-8, and Osteoprotegerin in Patients With Periodontitis and Diabetes. J Periodontol. marzo de 2010;81(3):384-91.

18. Tvarijonaviciute A, Castillo C, Ceron JJ, Martinez-Subiela S, Tecles F, López-Jornet P. Leptin and NGF in saliva of patients with diabetes mellitus type 2: A pilot study. J Oral Pathol Med. octubre de 2017;46(9):853-5.

19. Sonnenschein SK, Meyle J. Local inflammatory reactions in patients with diabetes and periodontitis. Periodontol 2000. octubre de 2015;69(1):221-54.

20. Pan A, Wang Y, Yuan JM, Koh WP. High-sensitive C-reactive protein and risk of incident type 2 diabetes: a case–control study nested within the Singapore Chinese Health Study. BMC Endocr Disord. diciembre de 2017;17(1):8.

21. Aryan Z, Ghajar A, Faghihi-Kashani S, Afarideh M, Nakhjavani M, Esteghamati A. Baseline High-Sensitivity C-Reactive Protein Predicts Macrovascular and Microvascular Complications of Type 2 Diabetes: A Population-Based Study. Ann Nutr Metab. 2018;72(4):287-95.

22. Vinagre I, Sánchez-Quesada J, Sánchez-Hernández J, Santos D, Ordoñez-Llanos J, De Leiva A, et al. Inflammatory biomarkers in type 2 diabetic patients: effect of glycemic control and impact of ldl subfraction phenotype. Cardiovasc Diabetol. 2014;13(1):34.

23. Kim WJ, Park CY, Lee KB, Park SE, Rhee EJ, Lee WY, et al. Serum 1,5-Anhydroglucitol Concentrations Are a Reliable Index of Glycemic Control in Type 2 Diabetes With Mild or Moderate Renal Dysfunction. Diabetes Care. 1 de febrero de 2012;35(2):281-6.

24. Mook-Kanamori DO, Selim MMED, Takiddin AH, Al-Homsi H, Al-Mahmoud KAS, Al-Obaidli A, et al. 1,5-Anhydroglucitol in Saliva Is a Noninvasive Marker of Short-Term Glycemic Control. J Clin Endocrinol Metab. 1 de marzo de 2014;99(3):E479-83.

25. Kaufman E, Lamster IB. THE DIAGNOSTIC APPLICATIONS OF SALIVA — A REVIEW. Crit Rev Oral Biol Med. marzo de 2002;13(2):197-212.

26. Satish BNVS, Srikala P, Maharudrappa B, Awanti SM, Kumar P, Hugar D. Saliva: A tool in assessing glucose levels in Diabetes Mellitus. J Int Oral Health JIOH. abril de 2014;6(2):114-7.

27. Virkamäki A, Ueki K, Kahn CR. Protein–protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest. 1 de abril de 1999;103(7):931-43.

28. Lainampetch J, Panprathip P, Phosat C, Chumpathat N, Prangthip P, Soonthornworasiri N, et al. Association of Tumor Necrosis Factor Alpha, Interleukin 6, and C-Reactive Protein with the Risk of Developing Type 2 Diabetes:

A Retrospective Cohort Study of Rural Thais. J Diabetes Res. 8 de agosto de 2019;2019:1-9.

29. Srinivasan M, Meadows ML, Maxwell L. Assessment of Salivary Adipokines Resistin, Visfatin, and Ghrelin as Type 2 Diabetes Mellitus Biomarkers. Biochem Res Int. 2018;2018:1-5.

30. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory Cytokines and the Risk to Develop Type 2 Diabetes. Diabetes. 1 de marzo de 2003;52(3):812-7.

31. Polak D, Sanui T, Nishimura F, Shapira L. Diabetes as a risk factor for periodontal disease—plausible mechanisms. Periodontol 2000. junio de 2020;83(1):46-58.

32. Polak D, Sanui T, Nishimura F, Shapira L. Diabetes as a risk factor for periodontal disease—plausible mechanisms. Periodontol 2000. junio de 2020;83(1):46-58.

33. Wu YY, Xiao E, Graves DT. Diabetes mellitus related bone metabolism and periodontal disease. Int J Oral Sci. 26 de junio de 2015;7(2):63-72.

34. Pacios S, Kang J, Galicia J, Gluck K, Patel H, Ovaydi-Mandel A, et al. Diabetes aggravates periodontitis by limiting repair through enhanced inflammation. FASEB J Off Publ Fed Am Soc Exp Biol. abril de 2012;26(4):1423-30.

35. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 30 de septiembre de 1993;329(14):977-86.

36. Jepsen S, Caton JG, Albandar JM, Bissada NF, Bouchard P, Cortellini P, et al. Periodontal manifestations of systemic diseases and developmental and

acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. junio de 2018;89 Suppl 1:S237-48.

37. Ziukaite L, Slot DE, Van der Weijden FA. Prevalence of diabetes mellitus in people clinically diagnosed with periodontitis: A systematic review and meta-analysis of epidemiologic studies. J Clin Periodontol. junio de 2018;45(6):650-62.

38. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet Lond Engl. 12 de septiembre de 1998;352(9131):837-53.

39. Genuth S, Eastman R, Kahn R, Klein R, Lachin J, Lebovitz H, et al. Implications of the United kingdom prospective diabetes study. Diabetes Care. enero de 2003;26 Suppl 1:S28-32.

40. Yoon MS, Jankowski V, Montag S, Zidek W, Henning L, Schlüter H, et al. Characterisation of advanced glycation endproducts in saliva from patients with diabetes mellitus. Biochem Biophys Res Commun. octubre de 2004;323(2):377-81.

41. Takeda M, Ojima M, Yoshioka H, Inaba H, Kogo M, Shizukuishi S, et al. Relationship of Serum Advanced Glycation End Products With Deterioration of Periodontitis in Type 2 Diabetes Patients. J Periodontol. enero de 2006;77(1):15-20.

42. Lalla E, Lamster IB, Feit M, Huang L, Schmidt AM. A murine model of accelerated periodontal disease in diabetes. J Periodontal Res. 14 de junio de 2010;33(7):387-99.

43. Cutler CW, Machen RL, Jotwani R, Iacopino AM. Heightened Gingival Inflammation and Attachment Loss in Type 2 Diabetics With Hyperlipidemia. J Periodontol. noviembre de 1999;70(11):1313-21.

44. Santos VR, Ribeiro FV, Lima JA, Napimoga MH, Bastos MF, Duarte PM. Cytokine levels in sites of chronic periodontitis of poorly controlled and well-controlled type 2 diabetic subjects: Cytokines in diabetic periodontitis subjects. J Clin Periodontol. diciembre de 2010;37(12):1049-58.

45. Costa PP, Trevisan GL, Macedo GO, Palioto DB, Souza SLS, Grisi MFM, et al. Salivary Interleukin-6, Matrix Metalloproteinase-8, and Osteoprotegerin in Patients With Periodontitis and Diabetes. J Periodontol. marzo de 2010;81(3):384-91.

46. Vieira Ribeiro F, de Mendonça AC, Santos VR, Bastos MF, Figueiredo LC, Duarte PM. Cytokines and Bone-Related Factors in Systemically Healthy Patients With Chronic Periodontitis and Patients With Type 2 Diabetes and Chronic Periodontitis. J Periodontol. agosto de 2011;82(8):1187-96.

47. Duarte PM, Bezerra JP, Miranda TS, Feres M, Chambrone L, Shaddox LM. Local levels of inflammatory mediators in uncontrolled type 2 diabetic subjects with chronic periodontitis. J Clin Periodontol. enero de 2014;41(1):11-8.

48. Ozturk A, Bilgici B, Odyakmaz S, Konas E. The relationship of periodontal disease severity to serum and GCF substance P levels in diabetics. Quintessence Int Berl Ger 1985. 2012;43(7):587-96.

49. Kim JB, Jung MH, Cho JY, Park JW, Suh JY, Lee JM. The influence of type 2 diabetes mellitus on the expression of inflammatory mediators and tissue inhibitor of metalloproteinases-2 in human chronic periodontitis. J Periodontal Implant Sci. 2011;41(3):109.

50. Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type 2 diabetes and chronic periodontitis - PubMed [Internet]. [citado 28 de marzo de 2023]. Disponible en: https://pubmed.ncbi.nlm.nih.gov/21804283/

51. D'Aiuto F, Gkranias N, Bhowruth D, Khan T, Orlandi M, Suvan J, et al. Systemic effects of periodontitis treatment in patients with type 2 diabetes: a 12 month, single-centre, investigator-masked, randomised trial. Lancet Diabetes Endocrinol. diciembre de 2018;6(12):954-65.

52. Engebretson S, Kocher T. Evidence that periodontal treatment improves diabetes outcomes: a systematic review and meta-analysis. J Clin Periodontol. abril de 2013;40:S153-63.

53. Madianos PN, Koromantzos PA. An update of the evidence on the potential impact of periodontal therapy on diabetes outcomes. J Clin Periodontol. febrero de 2018;45(2):188-95.

54. Engebretson SP, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W, et al. The Effect of Nonsurgical Periodontal Therapy on Hemoglobin A_{1c} Levels in Persons With Type 2 Diabetes and Chronic Periodontitis: A Randomized Clinical Trial. JAMA. 18 de diciembre de 2013;310(23):2523.

55. Mohamed HG, Idris SB, Mustafa M, Ahmed MF, Åstrøm AN, Mustafa K, et al. Impact of Chronic Periodontitis on Levels of Glucoregulatory Biomarkers in Gingival Crevicular Fluid of Adults with and without Type 2 Diabetes. Bencharit S, editor. PLOS ONE. 20 de mayo de 2015;10(5):e0127660.

56. König IR, Fuchs O, Hansen G, Von Mutius E, Kopp MV. What is precision medicine? Eur Respir J. octubre de 2017;50(4):1700391.