The potential use of salivary cytokines in diagnosis of periodontal diseases

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Abstract

The aim of this review is to highlight the potential of saliva as a diagnostic tool for periodontal diseases. Periodontitis is a major public health problem due to its high prevalence and it significantly decreases the quality of life. It has been shown that a massive number of cytokines play a crucial part in the pathogenesis of periodontitis, which causes the destruction of soft tissue and the resorption of bone**.** The release of inflammatory mediators and cytokines into the periodontal tissues, caused by periodontal bacteria, leads to periodontal tissue breakdown. To avoid consequences that could have a negative impact on a patient's quality of life, early diagnosis of diseases is essential. Salivary biomarkers have the potential to detect periodontal disease and determine the disease stage. Salivary diagnostics provide higher level of care and lessen the need for unnecessarily intrusive treatments by serving as an easily accessible and non-invasive primary test for diseases.

Keywords: *Saliva, Diagnostics, Periodontal disease, Cytokines*

1. Introduction

Periodontitis is a chronic multifactorial inflammatory disease associated with dental plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus [1]. Periodontitis is a major public health problem due to its high prevalence and it significantly decreases the quality of life [2]. Clinical indicators like bleeding on probing, probing pocket depths, and clinical attachment loss are used in the traditional diagnosis of periodontal diseases. Clinical attachment loss evaluation utilizing the periodontal probe requires a 2 to 3 mm threshold change before a site with considerable breakdown may be identified, while being simple to use, affordable, and relatively non-invasive. This is the most common method for determining whether a patient is experiencing periodontal tissue loss, which results in irreversible dental loss[3].

To avoid consequences that could have a negative impact on a patient's quality of life, early diagnosis of diseases is essential [4]. New technology has been developed by dental research to assist dentists in identifying individuals who are going through pathological processes. Numerous studies have been conducted in the area of inflammatory research to examine the development of diagnostic techniques whose benefits include early identification, a non-invasive approach, high sensitivity, and specificity. As a result, this scenario motivates researchers to develop a technique that may detect periodontal problems early, bringing in a time of individualized clinical care and personalized medicine. In this regard, it becomes apparent that salivary-based diagnosis, which is currently receiving a lot more attention, is used [3].

Saliva, an exocrine secretion of the salivary glands that contains 99 percent water, electrolytes, proteins, and enzymes, aids in chewing, swallowing, and digestion of food in addition to providing sensory perception of the food[5]. Saliva contains hormones, antibodies, growth factors, enzymes, microbes, and their products[6]. Through passive diffusion, active transport, or extracellular ultrafiltration, these components enter saliva from blood. Therefore, it is often possible to consider saliva to be a reflection of how the body functions physiologically [7,8]. There are some benefits to using saliva as a diagnostic tool instead of blood. Saliva is a desirable diagnostic fluid for the detection and monitoring of various biomarkers in infants, children, adults, and reluctant patients since it is non-invasive, simple to use, affordable, low risk of cross-contamination, generally stress-free ,and safer to administer than serum sampling[9,10]. Even when several samples are required, the collection procedure is manageable because saliva is easily accessible. Its collection is noninvasive, which increases patient acceptance of the treatment and provides a stress-free appointment. Saliva is also easier to handle because it does not clot[11].

Systemic alterations have been seen to have an impact on salivary composition, making it possible to identify disease-related biomarkers. Saliva has been studied as a potential diagnostic tool due to how simple and non-invasive it is to acquire, and the number of biomarkers it contains, such as genetic information and proteins [6,9]. Two conditions must be met before saliva-based diagnostics can achieve the objective mentioned above: (1)

finding biomarker(s) for various diseases among the complex components of saliva; and (2) improving the sensitivity and specificity of the biomarker(s) through continued technological advancement[9].

Collection of Saliva

Expectorated whole saliva, a mixture mostly made up of the secretions from the major salivary glands with slight contributions from the minor salivary glands and gingival crevicular fluid (GCF), is the fluid that is typically collected for salivary diagnostic purposes. Resting or unstimulated saliva is collected by passive drooling into a graduated tube or preweighed vial so that the flow rate per unit time may be calculated. Saliva can be collected on cotton swabs, cotton rolls, gauze, or strips of filter paper when volume measurement is not necessary, and then it can be eluted, centrifuged, or aspirated straight from the mouth's floor using plastic pipettes [12].

Saliva is stimulated by a masticatory or gustatory stimulation, expectorated, and handled similarly to the unstimulated fluid when significant amounts of saliva are needed for analytical purposes. The typical masticatory stimulus is softened paraffin wax or a cleaned rubber band, while the typical gustatory stimulus is 2 percent citric acid administered straight to the tongue [12].

Oral swabs or the passive drool technique can be used to collect saliva. In healthy people, the unstimulated salivary flow rate ranges from 0.1-2 mL/min, depending on age and gender[13].

Salivary markers of periodontal soft tissue inflammation

The salivary biomarkers may have diagnostic value for detecting periodontal disease and evaluating the response to periodontal therapy.Various salivary biomarkers have been investigated for the diagnosis and prognosis of periodontal diseases. These include salivary ions, enzymes, immunoglobulins, epithelium keratins, hormones, and inflammatory mediators [14].

The primary role of cytokines, which are highly significant peptide mediators, is cell signaling and communication. Cytokines serve a variety of purposes, including regulating immunological reactions, inflammatory reactions, cell proliferation, and cell differentiation. The term "cytokines" refers to small soluable proteins (5-20 kDa) that bind to certain receptors on particular cells, start some internal cellular changes, and affect numerous chemical and genetic processes. Numerous other cells' behavior is influenced by cytokines, which are produced by particular cells.[15] The release of inflammatory mediators and cytokines into the periodontal tissues, caused by periodontal bacteria, leads to periodontal tissue breakdown.[16]. Inflammation can be caused by a variety of conditions, including oxidative stress, overweight/obesity, poor oral hygiene, and nutritional deficiencies[5,17]. These possible indicators have been identified in periodontics using saliva and GCF.

It has been shown that a massive number of cytokines play a crucial part in the pathogenesis of periodontitis, which causes the destruction of soft tissue and the resorption of bone [18]. The inflammatory process of periodontitis is accompanied by the presence of elevated levels of cytokines like interleukin (IL)-1, IL-6, IL-10, IL-12, interferon (IFN), induced protein (IP)-10, IL-1 receptor antagonist (RA), IL-4, and tumor necrosis factor-alpha (TNF-α). Additionally, there is increased expression of regulatory cytokines, including IL-4, IL-1RA, IL-10, and IP-10 as well as proinflammatory cytokines like IL-1, IL-6, IL-12, and TNF-α [19].

Williamson et al. reported the presence of 27 cytokine biomarkers, including IL-1, IL-1 receptor agonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, eotaxin, basic fibroblast growth hormone, growth-colony stimulating factor, granulocyte-macrophage colony-stimul A commercially available cytokine multiplex test kit that combines the use of fluorescence flow cytometry and enzyme-linked immunoassay (ELISA) technologies were used to measure these cytokines. Only three cytokines, IL-6, IFN-γ, and macrophage inflammatory protein (MIP)-1, identified in saliva samples obtained by passive drooling, showed a significant connection ($p<0.05$) with plasma levels out of the 27 cytokines studied, according to these investigators[20].

Proinflammatory cytokines are generated by cells of the junctional epithelium, connective tissue fibroblasts, macrophages, and polymorphonuclear leukocytes. These cytokines include prostaglandin E2 (PGE2), IL-1β, IL-6, and TNF-α. Osteoclasts and polymorphonuclear leukocytes both generate the enzyme matrix metalloproteinase (MMP)-8, MMP-9, and MMP-13, which all contribute to the breakdown of alveolar bone and connective tissue collagen. PGE2 has been demonstrated in studies to have significant vasodilatory effects and to enhance capillary

permeability, both of which lead to the clinical symptoms of redness and edema. Additionally, it induces fibroblasts and osteoclasts to produce more MMPs [21]

MMP-8: The most common MMP in GCF and diseased periodontal tissue. Recently, a quick point-of-care microfluidic device was used to show that the amount of MMP-8 in saliva from patients with periodontal disease was significantly raised [22]. Periodontitis progression has been positively correlated with the level of MMP-8, an enzyme responsible for tissue destruction, in GCF[22,23].

As IL-1 levels demonstrated a high correlation with the several clinical indicators examined, they would be linked to the onset, severity, and progression of periodontal disease. They may also be a good indicator of how a patient will respond to treatment for periodontal disease. Being detectable in human oral fluids makes it a powerful candidate biomarker for periodontal diseases, predicting a person's risk of developing such a disease[24].

TNF-α may be able to help us identify patients who are more likely to develop periodontitis, such as diabetes patients or smokers. TNF-α would also be helpful as a measurable salivary inflammatory mediator whose levels could be related to the severity of the disease and demonstrate its progression, as this possibility is a potent tool to give patients the best care based on their disease stage[24].

Salivary IL-1 and many oral pathogens showed a connection with periodontitis when elastase, lactate dehydrogenase, IL-1, IL-6, and TNF-α concentrations and the presence of five pathogens were compared in patients with advanced periodontal disease and healthy controls [25]. Additionally, the validation study demonstrated a significant connection between moderate to severe periodontitis and salivary MMP-8, IL-1, and *Porphyromonas gingivalis*[26].

IL-4 and salivary soluble toll-like receptor-2 have recently been found to be positively correlated with the progression of periodontal disease[27].

IL-6, a cytokine involved in the death of periodontal tissue, can be genotypically analyzed in a patient's salivary DNA at an authorized laboratory. Recent research has confirmed that among Caucasians, hereditary IL-6 gene variants represent a substantial risk factor for chronic periodontitis [28].

Salivary biomarkers can be utilized to assess the host reaction to bacterial invasion, according to research on gingivitis that used a multiplex protein array for specific biomarkers linked to host defense, inflammation, tissue damage, and angiogenesis. Salivary IL-6 and IL-8 levels have been found to be the most accurate indicators of high and low responders[29]. Increasing salivary IL-6 levels were also shown to correspond with the severity of dysplasia in a study on dysplastic oral leukoplakia in connection to tobacco use and periodontitis [30].

These findings indicate that salivary biomarkers have the potential to detect periodontal disease and determine the disease stage.

2. Materials and Methods

We used PubMed, Scopus, Embase, Web of Science, and Google Scholar databases for keywords: saliva, periodontal disease, periodontitis, biomarkers, and inflammatory cytokines and retrieved only human clinical research papers and reviews that were written in English. Observational studies were excluded from analyses, as well as those that did not meet these criteria after reading the abstract.

3. Conclusion

Saliva is rich in protein and nucleic acid molecules, indicators of physiological status.Salivary biomarkers have the potential to detect periodontal disease and determine disease stage. Compared to blood sample, saliva offers a viable diagnostic alternative for identifying inflammatory, metabolic, and cardiovascular risk factors, especially in pediatric and geriatric populations where blood sampling may be challenging. Salivary diagnostics provide an easy, affordable, safe, and noninvasive alternative for disease identification, and they have a significant potential to change the next generation of diagnostics.

Although there are still obstacles to overcome, the use of saliva-based oral fluid diagnostics appears to have the potential for future application to identify periodontal disorders and forecast the effectiveness of periodontal therapy.

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