RECENT APPROACHES IN SALIVA AS A CREDIBLE PERIODONTAL DIAGNOSTIC AND PROGNOSTIC MARKER

Priyanka N*, Nitish Kalra*, Namitha Shanbhag†, Kiran Kumar‡, Seema‡, Biji Brijet‡, Uma S.R.‡, A.R. Pradeep*

ABSTRACT:

Objective: Saliva is used to monitor the general health and the onset of specific diseases. Biomarkers, whether produced by normal healthy individuals or by individuals affected by specific systemic diseases, are tell-tale molecules that could be used to monitor health status, disease onset, treatment response and outcome. It has been a great challenge in periodontology to determine biomarkers for screening and predicting the early onset of disease (prognostic tests) or evaluating the disease activity and the efficacy of therapy (diagnostic tests).

Material and Methods: A Google/Medline search was conducted and relevant literature concerning the applications of saliva for periodontal diagnosis was reviewed. Based on the literature, advanced technologies for measuring salivary biomarkers, that have been studied as potential diagnostic tests for periodontal diseases and various commercially available diagnostic aids and their uses were analyzed and compiled.

Conclusion: Traditional diagnostic measures, such as periodontal pocket depth, attachment level, plaque index, bleeding on probing and radiographic assessment of alveolar bone loss, are not necessarily the most efficient method for early diagnosis. A simple and non-invasive diagnostic tool that allows rapid screening provides accurate predictive information and enables reliable evaluation of periodontal disease status would be of great value to both dentists and patients. This review presents an overview of the value of saliva as a credible diagnostic tool in periodontal diseases, and the development of advanced salivary diagnostic tools.


Key Words: Salivary Biomarkers, Periodontal disease, Saliva.

* Department of Periodontics, Government dental College and Research Institute, Bangalore, India.
† Department of Public Health Dentistry, Government dental College and Research Institute, Bangalore, India.
‡ Department of Endodontics & Conservative Dentistry, Government dental College and Research Institute, Bangalore, India.

INTRODUCTION:

Saliva is said to be a “mirror of the body” because it is an indicator of health not just in the oral cavity but throughout the body. The molecular composition of saliva includes therapeutic, hormonal, immunologic, and toxicological molecules, which can provide vital clues to systemic health. Saliva has been used informally as a clinical diagnostic medium for more than 2,000 years. Historically, the viscosity and odor of saliva were used as diagnostic symptoms for certain diseases. A good diagnostic method should have the characteristics of high sensitivity, specificity, and functionality, and meet the requirements of high throughput, portability, and low cost for subsequent clinical application. For salivary diagnostics, many of these goals have been met through engaging diverse technology fields including biology, chemistry, physics, and engineering. Today, the improved efficiency and accuracy of genomic and proteomic biomarker discovery technologies are turning salivary diagnostics into a clinical and commercial reality.

Saliva in comparison with serum:

In diagnostics, saliva is an excellent alternative to serum since it contains sufficient quantities of disease biomarkers, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA), and the collection method is noninvasive, safe, and easy. Saliva is also easier to handle during diagnostic procedures than blood because it does not clot, thus reducing the
number of manipulations required. Collecting saliva also has a reduced potential for accidental transmission of infectious diseases compared to blood samples. Serum components of saliva are derived primarily from the local vasculature that originates from the carotid arteries, saliva has a prodigious fluid source that provides many, if not most, of the same molecules found in the systemic circulation. This makes saliva a potentially valuable fluid for the diagnosis of various systemic diseases.

Saliva in comparison with gingival crevicular fluid (GCF):

Saliva collection is less technique-sensitive than GCF collection. Assessing salivary biomarkers for diagnosing periodontal diseases, will overcome procedural disadvantages of GCF assessing biomarker analysis, like excessive time consumption since each periodontal site need not be individually tested and analyzed.

There are compelling reasons for exploring saliva as a diagnostic tool. It clearly meets the demands for an inexpensive, noninvasive, and easy-to-use screening method. As a diagnostic specimen in the clinic, saliva has many advantages in terms of collection, storage, shipping, and voluminous sampling, all of these processes can be carried out very economically.

Collection of saliva:

Whole saliva is most frequently studied because its collection is easy, noninvasive and rapid to obtain without the need for specialized equipment. It can also be collected with or without stimulation. Unstimulated whole saliva is commonly collected by the ‘draining’ method where the subject's head is tilted forward so that saliva moves towards the anterior region of the mouth and the pooled saliva is drooled into a wide-bore sterile vessel. Stimulated whole saliva is generally obtained by masticatory action (i.e., from a subject chewing on paraffin) or by gustatory stimulation (i.e., use of citric acid or sour candy drops on the subjects tongue) and is expectorated into a tube. Stimulated whole saliva is less suitable for diagnostic applications because the foreign substances used to stimulate saliva tend to modulate the fluid pH and generally stimulate the water phase of saliva secretion, resulting in a dilution in the concentration of proteins of interest. To date, unstimulated whole saliva has been used in the majority of diagnostic studies.

Over the last few years other promising devices have emerged that are based upon modifications to the traditional expectoration technique.

---

* DNA Ginotek Inc, Canada
† Malvern Medical Developments Ltd., Worcester, England
‡ Oasis Diagnostics Corporation, Vancouver, WA, USA

Diagnostic and Prognostic Markers in Saliva-Recent Approaches

OraGene: It is a more sophisticated way to collect saliva into a vessel to which is attached a screw-on cap. In the screw-on cap is a mixture of preservative buffers. Upon completion of the expectoration process, the cap is screwed onto the device releasing the preservative buffer, which drops into the saliva, is mixed by shaking and then acts to protect the integrity of the sample until processing and extraction can take place. It is the most widely used collection device.

Saligene: It is an alternative “spit-in-a-cup” technology, which has additional application as a collector for stool or swab specimens (when coupled with specific extraction kits for these alternate specimen types). In the Saligene device, subjects expectorate into a modified collection tube until a pre-determined volume has been reached. A screw-cap with attached plunger is screwed in place and the plunger depressed causing a preservative/lysis buffer to flow into the collected saliva specimen. The sample of mixed preservatives and saliva is gently shaken then sent to a laboratory for further processing.

Oracol: This test kit has been designed to collect saliva from patients. The kit consists of an absorbent foam swab (designed to collect up to 1 ml of saliva), centrifuge tube and cap. It is supplied sterile in batches of 500. This kit is universally used to collect data on measles, human immuno deficiency virus (HIV), hepatitis A and B, mumps and rubella.

Verofy: Verofy is a unique platform technology that incorporates rapid and standardized saliva collection with high quality immunochromatographic test strips providing a system for delivery of immediate results in field or point-of-care locations. Verofy collects saliva from under the tongue by means of a proprietary absorbent material connected to either one or two immunochromatographic test strips located in the device housing and in fluid communication with the test strips. After approximately 1 to 2 minutes of saliva collection time, a sample volume adequacy indicator built into the device changes appearance, signifying that sufficient sample has been collected for testing. The device can then be removed from the mouth and allowed to run for an additional time (3-15 minutes depending upon the specific test). As for standard immunochromatographic tests, a line or series of test lines will appear on the test strips depending on the diseases or analytes being tested. In addition a control line will appear confirming the validity of the test and the appropriate function of reagents used in the tests. If a positive test result is obtained and a confirmation specimen is required, this can be collected by squeezing the absorbent collection pad through a plastic compression
Table 1: DEMONSTRATES VARIOUS SALIVARY BIOMARKERS OF PERIODONTAL DISEASE

<table>
<thead>
<tr>
<th>DENTAL BIOFILM</th>
<th>INFLAMMATORY</th>
<th>COLLAGEN BREAKDOWN</th>
<th>BONE REMODELLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulins (IgA, IgM, IgG)</td>
<td>β-glucuronidase</td>
<td>α2-macroglobulin</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>Mucins</td>
<td>C-reactive protein</td>
<td>MMP-8</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>IL-1β</td>
<td>MMP-9</td>
<td>Osteocalcin</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>IL-6</td>
<td>Aspartate aminotransferase</td>
<td>SPARC/osteoneectin</td>
</tr>
<tr>
<td>Histatin</td>
<td>MIP 1α</td>
<td>Alanine aminotransferase</td>
<td>RANKL</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Tumor necrosis factor-α</td>
<td>TIMPs</td>
<td>β C-terminal type I collagen telopeptide</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C-telopeptide pyridinoline cross-links of type I collagen</td>
</tr>
</tbody>
</table>

IL: Interleukin; MIP: Macrophage inflammatory protein; MMP: Matrix metalloproteinase; RANKL: Receptor activator of NF-κB ligand; SPARC: Secreted protein, acidic, rich in cysteine; TIMP: Tissue inhibitors of metalloproteinase.

tube provided and into a standard 2 ml eppendorf centrifuge (or equivalent) collection tube. Once collected this sample is capped then archived or sent to a laboratory for suitable confirmation testing.

Recent Advances in salivary diagnostics:

Through the work of the human salivary proteome project, more than 1,000 proteins in saliva have been identified. Advancements in analytical techniques have enabled scientists to discover the specific proteins associated with human diseases. These proteins are referred to as biomarkers.

Currently, biologists, engineers, and dental practitioners are developing “lab-on-a-chip” platforms that use oral fluids in rapid tests to accelerate clinical decision making. Ultimately, these types of tests will lower the cost of health care because they eliminate the need for trained phlebotomists to draw blood. A useful diagnostic application involving a variety of bodily fluids in which the analyte concentration may be extremely low. Specifically, a multiplex platform designated ‘cardiac arrest rapid diagnostic information using saliva’ which uses four matched pairs of highly-specific antibodies each recognizing target antigens myoglobin, C-reactive protein (CRP), myeloperoxidase and IL-1β have demonstrated excellent acute myocardial infarction screening capabilities.

The vision of the technology is to provide the simultaneous assessment of multiple aspects of oral health. The assessment will then help clinicians determine the most appropriate preventive and therapeutic approaches, all during the same office visit. Lab-on-a-chip platforms will be able to perform multiple operations in non laboratory settings such as satellite clinics, field sites, or at home. Furthermore, these testing methods use smaller amounts of sample and reagents (substances used in chemical reactions), further decreasing costs. Current diagnostic approaches that take days or weeks to obtain results are less effective than this new realm of point-of-care diagnostics that provide results within minutes or hours so the treatment plan can begin immediately.

Nano-Biochip technology:

The tools of nanomaterials and microelectronics for the practical implementation of miniaturized sensors are suitable for a variety of important applications. There are two types of systems been created, the first is based on a microbead array, wherein micro-pits within a silicon wafer are populated with a variety of chemically sensitized bead ‘microreactors’. This sensor system is based on a bio-microelectromechanical systems platform, and may be described as a ‘chemical processing unit’ in analogy to the central processing unit that serves as the brains for a computer chip. Instead of handling electrical signals passing through conductors, as in the case of traditional circuits, the Nano-Biochip technology processes fluids so as to provide a digital fingerprint that can be correlated with the local chemical environment, detecting pH, electrolytes, metal cations,
sugars, toxins, proteins and antibodies.7,11-15 Building on this technology, a second class of miniaturized sensor system has been pioneered that contains beads within etchings of stainless steel plates and utilizes a membrane capture element integrated into a fluidics structure.16,17 These membrane microchip ensembles have been adapted to service cell, spore, and bacteria separation and biomarker identification applications.18 Importantly, the performance metrics of these miniaturized sensor systems have been shown to closely correlate with established macroscopic gold-standard methods, making them suitable for use as subcomponents of highly functional detection systems for the analysis of complex fluid samples, such as saliva, for a variety of analyte systems.9,11-17,19-23 The development of a point of care (POC) device that contains a modular and miniaturized sensor system, universal analyzer with functional integrated mechanical/optical interfaces, and flexible microchip architecture can service the future needs of clinicians and the research communities.

In this POC device, saliva (100–300 μl) is placed into the salivary collection/delivery module, and then delivered into the Nano-Biochip. The injection-molded cartridge is ‘creditcard’ size and encloses the array Nano-Biochip where complex fluorescent immunoassays are performed. Here, a network of fluidic components ensures the complete transfer and process of saliva samples to the multiplex bead array to provide quantitative information of target biomarkers of disease. The sample introduction requirements are consistent with the use of saliva or finger-prick quantities of blood that can be directly introduced into the sample introduction port. Detection reagents are stored dry on a conjugate pad embedded within the biochip, and are reconstituted as needed, through the release of a prepacked buffer contained in biochip-integrated pouches. All processing steps are conducted within the microfluidic network of the biochip via actuation inside the analyzer without human intervention. These features eliminate the need for external fluidics, such as pumps, tubing and connectors. Therefore, the integrated system has the potential to reduce cost and reduce the risk for leaks and contamination. The assay is processed entirely through a 5–15 min sequence that is programmed in the main controller board. The flexibility of the control software allows for modifications to be made through an assay builder interface. Control over the flow rate, incubation time and reagent wash, is achieved by the actuation of stepping motors that direct the fluid flow through the depression of the fluid pouches. The sample is directed to an on-chip waste reservoir, which provides a safe containment of biohazardous fluids. The entire biochip can be discarded as solid waste after the assay, facilitating biohazard waste management. Together, these essential features serve to facilitate the transition from chips-in-a-laboratory to a lab-on-a-chip, and offer significant opportunities for POC technology needs.

Table 2: DEMONSTRATES VARIOUS PRODUCTS AND THEIR USES FOR MEASURING SALIVARY BIOMARKERS

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>PURPOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyPerioID</td>
<td>identifies the type and concentration of the specific bacteria that cause periodontal diseases.</td>
</tr>
<tr>
<td>My PerioPath</td>
<td>determines the cause of periodontal infections.</td>
</tr>
<tr>
<td>Oral Fluid NanoSensor Test</td>
<td>simultaneous and precise detection of multiple salivary proteins and nucleic acids.</td>
</tr>
<tr>
<td>Electronic Taste Chips</td>
<td>detects multiple biomarkers for early diagnosis of periodontal disease</td>
</tr>
<tr>
<td>OraQuick</td>
<td>an antibody test that provides results in 20 minutes, usually detects HIV 1 and HIV 2</td>
</tr>
<tr>
<td>Integrated Microfluidic Platform for Oral Diagnostics</td>
<td>rapidly (3–10 min) measures the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva</td>
</tr>
</tbody>
</table>
Newer aids for measuring salivary biomarkers:

Significant advances are in development for the screening of periodontal diseases. The current method of diagnosing periodontitis is through assessment of clinical parameters and radiographs, however, this is not necessarily the most efficient method for early diagnosis. If periodontal diseases are detected early, treatment can be easier and less painful for the patient. Left untreated, periodontal diseases may lead to systemic problems such as cardiovascular disease and diabetes. Therefore, early screening for periodontal diseases is essential during dental examinations.

MyPerioID and My PerioPath:

Two DNA-based saliva tests are available. MyPerioPath and MyPerioID. My PerioPath uses a saliva sample to identify the type and concentration of the specific bacteria that cause periodontal diseases. MyPerioID test also uses saliva to determine a patient’s genetic susceptibility to periodontal diseases and which patients are at higher risk of more serious periodontal infections. Both tests require the shipping of saliva samples to a laboratory for results.

Oral Fluid Nano Sensor Test:

The University of California, Los Angeles (UCLA) Collaborative Oral Fluid Diagnostic Research Center, partnered with engineers at the UCLA School of Engineering, developed a micro electromechanical system based electrochemical detection platform that is capable of real-time, ultrasensitive, ultraspecific multiplex detection of salivary protein and RNA biomarkers. This envisioned product has been labeled the Oral Fluid Nano Sensor Test (OFNASET). It is a point-of-care, automated, and easy-to-use integrated system that will enable simultaneous and precise detection of multiple salivary proteins and nucleic acids. In addition, this system is portable and could be used not only in the doctor’s office, but also in any other healthcare station to perform an instant point-of-care diagnosis. The OFNASET technology platform combines cutting-edge technologies, such as self-assembled monolayers bionanotechnology, cyclic enzymatic amplification, and microfluidics, with several well-established techniques including microinjection molding, hybridization-based detection, and molecular purification. The intended use of the OFNASET is for the point of care multiplex detection of salivary biomarkers for oral cancer. It analyzes saliva for the presence of four salivary mRNA biomarkers (SAT, ODZ, IL-8, and IL-1b) and two salivary proteomic biomarkers (thioredoxin and IL-8).

Electronic Taste Chips:

Several study groups have reported elevated serum CRP levels in periodontitis patients. The higher the levels of CRP in periodontitis patients, the more severe the disease, even with adjustments for external factors. The biomarker CRP is an acute phase reactant and a well-accepted indicator of inflammation. Numerous clinical studies have established elevated serum CRP as a strong, independent risk factor for the development of cardiovascular disease (CVD). CVD has also been associated with oral infections (i.e., periodontal diseases) and there is evidence that systemic CRP may be a link between the two. Clinical measurements of CRP in serum are currently performed with "high sensitivity" CRP (hsCRP) enzyme-linked immunosorbent assay (ELISA) tests that lack the sensitivity for the detection of this important biomarker in saliva. Salivary CRP may represent a novel approach for diagnosing and monitoring chronic inflammatory disease, including CVD and periodontal diseases.

Chemically sensitized bead microreactors within the lab-on-a-chip system were recently applied for measurement of CRP and other biomarkers of inflammation in saliva, demonstrating significantly lower detection level (by > 3 decade orders of magnitude) for CRP than high-sensitivity CRP ELISA methods, allowing for measurement of inflammatory biomarkers related to select disease states. Currently, researchers at Rice University in Houston, Tex, are developing a lab-on-a-chip system using a new detection system for measuring analytes in saliva based on an electronic taste chip (ETC). The ETC methodology was compared with the standard laboratory technology (ELISA) for measuring CRP in saliva, and displayed a 20-fold lower limit of detection than the ELISA. With this technique it was possible to quantitate the difference in CRP levels between healthy individuals and patients with periodontal diseases. Additional studies confirmed the ability of the ETC platform to simultaneously monitor several additional biomarkers. The prospect of a commercially available ETC lab-on-a-chip platform that can detect multiple biomarkers for early diagnosis of periodontal disease is promising.

Oraquick:

It is an antibody test that provides results in 20 minutes. The blood, plasma or oral fluid is mixed in a vial with developing solution, and the results are read from a sticklike testing device. Usually detects HIV 1 and HIV 2. The Oraquick rapid saliva HIV test is definitely the way to go. It is a toothbrush-like device with a fabric swab on the head and a small plastic stem. The stem naps off to avoid contamination, while the swab is inserted into a tube of testing fluid. The testing tube is easily sealed and ready for transport to a testing laboratory. By using a toothbrush-like device that is inserted into the mouth instead of drawing blood, this testing method makes an already high-anxiety

---

§ Oral DNA® Labs Inc, Brentwood, Tennessee, USA
ǁ Ora Sure Technologies, Inc, Bethlehem, USA
screening a little more comfortable.

**Integrated Microfluidic Platform for Oral Diagnostics (IMPOD):**

A clinical point-of-care diagnostic test that enables rapid quantification of an oral disease biomarker in human saliva by using a monolithic disposable cartridge designed to operate in a compact analytical instrument was reported. This microfluidic method facilitates hands-free saliva analysis by integrating sample pretreatment (filtering, enrichment, mixing) with electrophoretic immunoassays to quickly measure analyte concentrations in minimally pretreated saliva samples. Rapid (< 10 min) measurement of levels of the collagen cleaving enzyme MMP-8 in saliva from healthy and periodontally diseased subjects can be achieved using 20 µl of saliva. Based on this, a portable diagnostic device called the IMPOD was developed. An early clinical study in which the hand-held IMPOD was used to rapidly (3–10 min) measure the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva has been reported.

**CONCLUSION:**

Saliva is being accepted as an excellent diagnostic medium and an indispensable tool in the field of diagnostics. It is likely that the development of saliva based methods will impact and expand the role of dentists. Integrating these new salivary diagnostics methods into clinical practice is important to aid dental professionals in making essential health-related decisions for patients. In the near future, taking a saliva sampling in a dental clinic will become as routine as obtaining a urine or serum sample at a physician’s office.

**REFERENCES:**

46

CORRESPONDENCE:

Dr. Priyanka N
Post Graduate Student,
Dept of Periodontics,
Govt. Dental College & Research Institute,
Bangalore 560002, INDIA.
Ph: +919880474837
E mail: priyanka.n7@gmail.com