REVIEW ARTICLE

Advancements in Salivary Biomarkers: Transforming Diagnostic Landscape in Dentistry

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ABSTRACT

Introduction: Clinicians and scientists are actively pursuing diagnostic methodologies that are timely, cost-effective, accurate, and non-invasive. The early detection of pathology plays a crucial role in influencing patient discomfort, prognosis, therapeutic intervention, survival rates, and recurrence. However, traditional diagnostic methods often involve painful and invasive procedures, such as repeated biopsies and blood draws, contributing to patient stress during an already unpleasant experience. **Objective**: The objective of this study is to explore alternative diagnostic approaches that leverage saliva-based microbial, immunological, and molecular biomarkers. This investigation aims to assess the feasibility of using oral fluids to evaluate the health status of individuals, offering a non-invasive and patient-friendly alternative to traditional diagnostic methods. **Methods**: To achieve our objective, we conducted a comprehensive review of existing literature and research related to saliva-based diagnostics. We focused on exploring microbial, immunological, and molecular biomarkers present in saliva and their potential applications in diagnostic procedures. The research methodology involved an in-depth analysis of studies addressing the use of saliva for diagnostic purposes. **Results**: The findings reveal promising opportunities in the discovery of saliva-based biomarkers. These biomarkers provide unique insights into the health status of individuals and offer a non-invasive means of diagnosis. The results of our literature review suggest that saliva has the potential to serve as a valuable diagnostic fluid, presenting advantages over traditional invasive methods. **Conclusion**: In conclusion, the exploration of saliva-based microbial, immunological, and molecular biomarkers presents a compelling avenue for non-invasive diagnostics. Leveraging these biomarkers offers a potential paradigm shift in diagnostic procedures, reducing the need for invasive techniques that contribute to patient discomfort. The integration of saliva-based diagnostics into clinical practices, particularly in dentistry, holds the promise of improving patient experience and overall diagnostic efficacy.
Keywords: *Saliva; Saliva Diagnostics; Saliva Test; Dentistry.*

1. INTRODUCTION

As a diagnostic tool, saliva has exhibited significant promise in the early detection of various diseases. This promise is underscored by the identification of specific biomarkers intricately associated with oral and systemic pathologies. The clinical applications of saliva analysis have been further enhanced by its comparative advantages over traditional blood tests, offering a more convenient and cost-effective approach to diagnostics. The evolution of nanotechnology has played a pivotal role in advancing saliva-based diagnostics, contributing to notable improvements in sensitivity and specificity [1].

The dynamic nature of saliva, with its continuous interaction with the oral environment, presents a wealth of opportunities for exploring its diagnostic potential. Salivary diagnostics not only offer insights into oral health but also extend their reach to systemic conditions, providing a holistic perspective on an individual's well-being. The identification and characterization of salivary biomarkers have become integral to understanding the intricate interplay between oral and systemic health.

The saliva, as a scientifically robust and clinically relevant biofluid, continues to evolve as a valuable asset in diagnostics. The ongoing advancements in technology, particularly in nanotechnology, further underscore its potential for revolutionizing diagnostic practices. Embracing saliva-based diagnostics holds the promise of not only improving the convenience and cost-effectiveness of diagnostic procedures but also contributing to a more comprehensive understanding of health and disease.

2. MATERIELS AND METHODS

1. Literature Search Strategy

To comprehensively review the advancements in salivary biomarkers in dentistry, a systematic literature search was conducted using prominent academic databases, including PubMed, Scopus, and Google Scholar. The search encompassed articles published up to the present date, with a focus on studies exploring the diagnostic landscape of salivary biomarkers in dentistry.

2. Inclusion and Exclusion Criteria

To maintain the relevance and specificity of the collected data, inclusion and exclusion criteria were rigorously defined. Articles were included if they specifically addressed salivary biomarkers in the context of dentistry. Exclusion criteria ensured the exclusion of studies that did not align with the primary focus of this review.

3. Systematic Review Approach

A systematic review methodology was adopted to ensure a structured and unbiased synthesis of the available literature. This involved a meticulous evaluation of study design, methodology, and outcomes reported in each selected article.

4. Data Extraction

A structured data extraction form was employed to systematically extract pertinent information from each selected article. Key data points included types of salivary biomarkers studied, diagnostic applications, methodologies utilized, and reported outcomes. This process facilitated consistency in data extraction and analysis.

5. Citation Tracking

Citation tracking was employed to identify additional relevant articles beyond the initial search. References from selected articles were reviewed to identify sources that might contribute valuable insights to the review.

3. DISCUSSION

3.1 Saliva, a unique biofluid

At the heart of oral hemostasis, saliva is a unique fluid that consists of a mixture of the primary and secondary salivary glands. It also includes constituents of non-salivary origin derived from gingival crevicular fluid (GCF), sputum bronchial secretions, serum, blood cells from oral wounds, bacteria, viruses, fungi, food debris and desquamated epithelial cells [1]. In addition, saliva as a mirror of oral and systemic health serves as an attractive vehicle capable of communicating the overall health status of the individual as well as detecting the onset and progression of morbidity by monitoring therapeutic outcomes after treatment [2].

It is often considered an attractive diagnostic fluid because of its low invasiveness, minimal cost and easy collection of samples. As clinical information, saliva is useful for new approaches to prognosis, laboratory and clinical diagnosis, and monitoring and management of patients with diseases. Saliva diagnostics is thus a subset of the broader field of molecular diagnostics, which is now recognized as a central player in a wide variety of basic and clinical biomedical fields [3].

3.2. Salivary stimulation

Several techniques can be used to stimulate salivary secretion, the simplest of which involves movements of the tongue, cheek or lip. Alternatively, stimulation can be done mechanically by chewing on something like kerosene wax, Parafilm®, Teflon, rubber bands or chewing gum [4].

Again, a drop of lemon juice or citric acid placed in the mouth can provide a taste stimulus for saliva production. After this stimulation, it can then be spit, aspirated, absorbed, or swabbed for collection [4]. It should be noted that salivary composition is not constant and changes depending on the degree of gland stimulation, time of day, diet as well as health status. This reinforces the fact that at the time of sample collection, all Supporting metadata is captured and standardized. As a general rule, when conducting a biomarker discovery study, samples should not be collected within 45 minutes of tooth brushing, within 24 h of dental care, in cases of injury or oral health problems, or in cases of blood contamination [19].

Granger et al., (2014) also advise avoiding the use of stimulants, as they may introduce biases in assay concentrations and dilutions [5]. In addition, prior to sampling, subjects should avoid sweet or acidic foods, as they have observed that they lower saliva Ph and increase bacterial growth. Alcohol, caffeine, nicotine, and prescription / over-the-counter medications should also be avoided (for approximately 12 h) before sampling to avoid biasing the resulting data [19].

3.3. Saliva collection procedure

Depending on the type of saliva considered, the collection protocol differs (Table 1) [6]. Saliva collection methods require careful examination [7], and patients should refrain from eating, drinking, and oral hygiene for at least 1 hour before collection. Thus, the optimal time for collection is 8 to 10 a.m. Before proceeding with the collection, the mouth should be rinsed for one minute with distilled water and then, after 5 minutes, about 5 ml of saliva should be collected. The collected sample should be processed in the laboratory within one hour [8].

3.3.1 Collection of saliva

3.3.1.1 Collection of total saliva

The most commonly collected oral fluid is sputtered whole saliva, which is a mixture of major and minor salivary gland secretions, with a modest contribution from gingival crevice fluid. Two key methods for collection have been developed: stimulated saliva collection and unstimulated whole saliva collection (UWS) [10].

UWS is collected by the passive drooling method in a pre-weighed and calibrated vial to ensure that salivary flow can be estimated [9], simply lowering the head and allowing saliva to flow from the lower lip into the plastic vial [4]. Stimulated whole saliva is collected by sputum technique: the patient is asked to spit into a collection bottle. With this method, there is a fourteen-fold increase in the risk of bacterial contamination being introduced into the sample [4].
The use of UWS is preferred over stimulated saliva because it avoids potential differences generated by various reflex stimuli. However, a limitation of UWS includes the low volume collected, especially from non-compliant groups [children), geriatric patients, and diabetic patients with xerostomia (low saliva production] [9,10]. Over time, various modifications to the saliva collection process have been implemented that have improved many potential problems with UWS collection, including avoiding contamination, obtaining a pure sample, and increasing the volume collected [9].

3.3.1.2 Collection of parotid saliva

To obtain it the method introduced by Carlson and Crittenden (1910) is used. In this method, a double chambered metal cup with two outlet tubes is used. One end holds the cup using vacuum suction. The second end serves as a collection vessel for the saliva. Sample collection can be improved by applying citric acid (10%; 1 ml) to the back of the tongue every 30 seconds. However, it is essential to discard the first 1.5 ml of saliva before collecting the samples [8].

3.3.1.3 Submandibular / sublingual saliva collection

Truelove, Bixler and Merrit (1967) used a "V" shaped collector. Saliva can be collected by placing the end of the collection device at the orifice of Wharton's canal, after placing sterile cotton sponges in the floor of the mouth and on the oral mucosal areas to obstruct the submandibular and sublingual canals [4]. Regardless of the salivary sampling procedure, standardization is necessary for pre-analytical and analytical variables, such as collection and storage methods, circadian variation, sample recovery, sample contamination prevention, and analytical procedures. Despite the challenges, the use of saliva as a diagnostic or screening tool has grown exponentially over the past decade [10].

Table 1: Description of human saliva collection methods [8].

Type of whole oral fluid	Collection method and type of collection device
Whole Saliva	Patients should refrain from eating, drinking, and performing oral hygiene procedures for at least 1 h before saliva collection (Optimal collection time is 8:00-10:00 am). Prior to collection, perform a 1 min oral rinse with distilled water, then after 5 min, collect approximately 5 ml of saliva. The collected sample should be processed in the laboratory within 1 hour.
Unstimulated whole saliva	Passive drooling: in this method, limit mouth movements and drain saliva from the lower lip into a plastic bottle. Spit method: ask the subject to spit into a collection bottle. In this method, 14 times more bacterial contamination is introduced into the sample.
Stimulated whole saliva	For gland stimulation, different things were used such as natural gum, a piece of kerosene wax, citric acids and powdered drink crystals.
Parotid gland	Method introduced by Carlson and Crittenden (1910). In this method, a double-chambered metal cup with two outlet tubes is used. One end holds the cup in place using vacuum suction. The second half acts as a saliva collection vehicle. Sample collection can be enhanced by applying citric acid (10%; 1 ml) to the back of the tongue every 30 s. Discard the first 1.5 ml of saliva before sample collection [8].
Submaxillary/sublingual gland	Truelove, et al., (1967) used a "V" shaped collector. This method is similar to the parotid gland collection method, but in this case the initial 2 ml is discarded [8].
Minor glands	Kutscher et al., (1967) used capillary tubes to collect saliva from minor glands located on the flared surface of the lower lips [8]

3.3.2. Commercial devices

To begin a diagnostic procedure based on total saliva, the necessary samples must first be collected. The standard method is to drain saliva using several special devices such as [10]:

The Salivette®: This device has been widely used by the research community for a wide range of applications from steroid hormone detection to HIV antibody detection, and oxidative stress markers. The Salivette® device is available in rolls or sponges made of cotton or polyester. To collect a sample, it is placed in the mouth for 2 minutes, chewed; the swab is placed back in its holder and closed. The saliva sample is then recovered from the swab by centrifugation and placed in the transport tube for shipment to a testing laboratory [8].

Quantisal® (Immunoassay): A culture swab collection and transport system [8]. SCS® (Greiner-BioOne): a whole saliva collection device by trained professional users that includes a series of tubes, steps, reagents and a general purpose sample cup [8]. Despite the attractiveness of saliva, its use must be carefully evaluated with respect to standardization of pre-analytical and analytical variables, such as accurate choice of collection methods (stimulated or unstimulated], possibility of direct volume quantification, proper sample recovery, and prevention of contamination by blood or food debris [9].

It is also important to check for possible circadian variation of the analyte to define collection schedules and have high sensitivity and reproducibility. In addition, saliva is a hypotonic fluid compared to plasma, some components are found at lower concentrations (sodium, magnesium, chloride), others higher (potassium, calcium, bicarbonate, phosphate) and similar concentrations (uric acid - UA, urea). Salivary flow, composition and protein concentration vary between individuals depending on several factors such as age, oral microbial enzymes, sample processing, storage, protease activity, etc. [11].

3.3.3. SALIVARY BIOMARKERS

3.3.3.1. Definition

The key to salivary diagnosis is the proper identification, validation and detection of disease-related biomarkers. A biomarker refers to a quantifiable biological parameter that is objectively measured as an indicator of normal biological, pathogenic or pharmacological responses to a therapeutic intervention [12]. According to the National Institutes of Health (NIH): they are anatomical features and measurable naturally occurring molecules that encompass a variety of classes: DNA, RNA, metabolites, proteins, and microbes; they are also referred to as "molecular signatures" that can indicate the physiological or pathological state of the individual and predict disease at an early stage, which is particularly beneficial for the rapid management of common complications of disease.

Early diagnosis, prognosis and subsequent treatment is the primary focus of contemporary medicine. Saliva is a fluid that has aroused great interest among researchers because of its multiple advantages over other body fluids. The multitude of its components that can act as a biomarker influenced existing technologies to develop protocols, methods and tests that add substantially to the diagnostic arsenal, and provide information important to oral and general health. The diagnostic applications of saliva have expanded and evolved rapidly due to advances in salivomics [13]. On the other hand, the discovery of discriminatory biomarkers in oral fluids embodies an almost unparalleled breakthrough in clinical and translational science. Continuation in this area could make saliva an acceptable means of diagnosis, an endpoint with the potential to cause a paradigm shift in the discipline of molecular diagnosis [14].

3.3.3.2 Development of salivary biomarkers

The biomarker development process is a challenging task with several levels of evaluation before final approval by the Food and Drug Administration (FDA) (Fig. 1). Early-stage samples are analyzed using one or more of 6 "omics" libraries: proteome, transcriptome, immunome, metabolome, microbiome, and epigenome. Each library contains a vast collection of information potentially useful in determining an individual's current health status. After discovery, verified biomarkers go through successive levels of validation using independently designed prospective randomized open label studies (PROBE]. FDA approval is obtained after substantial evaluations have been performed using large patient populations and advanced statistical algorithms [14]. Biomarkers for oral and systemic diseases are discovered using one or more of the "omics" libraries. Verified biomarkers are subjected to increasing scrutiny and larger independent cohorts until they reach pivotal multicenter validation [14].

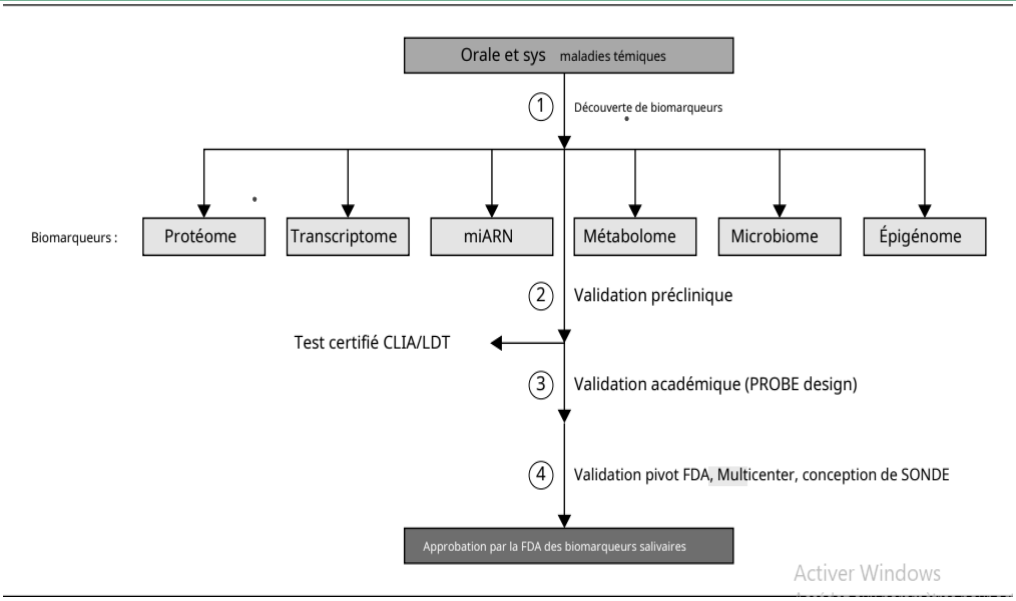

Figure 1: Salivary biomarker development and roadmap to US Food and Drug Administration (FDA) approval [14]. *(**PRoBe**: Prospective sample collection, retrospective blinded evaluation; **CLIA**: Clinical Laboratory Improvement Amendments; **LDT**: Laboratory Development Test).*

3.3.3.3. The five toolboxes of salivary diagnosis "omics"

The term "salivaomics" was coined in 2008 to highlight the various "omics" present in saliva including: the genome, transcriptome, proteome, metabolome and microbiome. Salivomics has been widely studied in recent decades with the advent of more advanced analytical techniques [11]. These are the diagnostic and signaling alphabets whose discovery has positioned saliva for translational and clinical applications, including dental and personalized medicine [9].
Omics have continued to generate megadata, which are analyzed using advanced machine learning approaches to identify biomarkers, potentially leading to a paradigm shift in medical diagnosis and therapeutics [9].

3.3.3.3.4. Proteomics

Proteomics is a powerful approach to biomedical research, directly studying the key functional components of biochemical systems, namely proteins [1]. Wilkins et al., (1996) introduced the term "proteome", which was an amalgam of two words: "protein" and "genome". The proteome is thus the protein complement of the genome, and proteomics is the analysis of the expressed part of the DNA.

The value of biomarkers has been recognized and widely explored using proteomic methods. These approaches should provide a useful microarray array compatible with low cost point-of-care devices [1]. They are valuable in body fluids because of their high clinical potential as sources of disease markers. In principle, a comprehensive analysis of human salivary proteomes can provide a full spectrum of oral and general health. In addition, analysis of salivary proteomes during complications can reveal signatures of morbidity at an early stage and track disease progression [15]. The most important and revealing components of saliva are proteins. Human saliva has specific proteomic content that allows researchers to perform noninvasive tests to discover new salivary biomolecules associated with general and dental health. Proteomic studies of saliva aid in the identification of novel proteins and peptides that can help quantify the biological activity of disease states [13]. Techniques used by researchers and biochemists to perform proteome work from saliva include gel electrophoresis, capillary electrophoresis, nuclear magnetic resonance, and immunoassay [13].

A systemic study of all salivary secretory proteomes was initiated when the National Institute of Dental and Craniofacial Research (NIDCR) began work on a comprehensive catalog of the human salivary proteome [12]. In 2007, a collection of 1166 diverse proteins was recorded in a study that was conducted using protein mass spectroscopy and 2D gel electrophoresis. In investigating the differences between salivary and plasma protein composition, Schulz et al., in 2013 examined a protein dataset as part of the international Human Plasma Proteome project, and found that approximately 30% of whole saliva proteins are found in plasma. This overlap shows reasonable connections that can be made between the salivary proteome and different parts of the body [12].

As further studies have shown, the salivary proteome can be used to make connections between saliva and cystic fibrosis, diabetes, periodontitis, dental caries, and acquired immune deficiency syndrome (AIDS). Proteomic analysis salivary biomarkers can even be useful for health safety applications such as radiation exposure. A particularly interesting application of proteomic biomarkers is the detection of Oral Squamous Cell Carcinoma (OSC) which accounts for 90% of all head and neck cancer cases and although most cases of OSC are not detected until the cancer has

reached an advanced stage [12]. Downstream, a comprehensive analysis of salivary proteomes can contribute to the understanding of pathophysiology and provide a basis for the recognition of potential biomarkers for different human pathologies [3].

3.3.4. Transcriptomics

Salivary Transcriptomics is based on the analysis of the oral transcriptome, which is the set of all mRNA and miRNA molecules present in the salivary medium capable of transmitting instructions carried by DNA for the subsequent production of proteins [12]. This discovery presented a second diagnostic alphabet in saliva and opened the door to another pathway for salivary transcriptomic diagnosis [15].

The presence of mRNA in the extracellular medium was highlighted in 2008 by Gilad [16]. It constitutes an important source of potentially relevant diagnostic information. MiRNAs are small (19-21 nucleotides) regulatory molecules that functionally interact with and potentially inhibit the translation of complementary antisense RNA sequences [10]. Like messenger RNAs, miRNAs can be aberrantly expressed in irregularly functioning or tumorigenic cell lines [11]. These attributes suggest that transcriptomic assessment can reveal highly specific discriminatory indicators [14].

Interestingly, both whole saliva and saliva supernatant harbor both miRNA and total RNA. In support of this claim, researchers probing the salivary transcriptome have identified more than 1,000 miRNAs and more than 3,000 species of messenger RNA [12]. Suggesting that oral fluids contain viable biochemical data, these findings support the idea that saliva is an informative biofluid that may be useful in discerning health and disease. Along these lines, research at the UCLA School of Dentistry has discovered and is continuing to identify salivary biomarkers for cancers of the oral cavity, pancreas, lung, stomach, Sjogren's syndrome, diabetes, as well as periodontal disease [14].

3.3.3.1 Metabolomics

The collection of small molecules present in cells, tissues, organs and biofluids is known as the metabolome [17]. The science of searching for unique chemical fingerprints of these molecules in specific biological samples is metabolomics [14].

The metabolome validates the parallel assessment of a group of endogenous and exogenous metabolites, including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols, and carbohydrates that represent interesting candidates for understanding disease phenotypes in biological systems [17]. Analysis of these key metabolites in body fluids could not only help determine diagnosis, but also facilitate selection, design, and modification of care plans. [14] Like the transcriptome and proteome, the metabolome is continually changing and is a dynamic picture of cellular and organ function, reflecting gene and protein expression and the environment. Metabolomic studies generate quantitative data for many metabolites to elucidate metabolic dynamics related to disease state and drug exposure [18]. As a new strategy attempting to find markers of interest, metabolomic analysis of saliva has been successfully used in the fields of physiology, diagnostics, functional genomics, pharmacology, toxicology, nutrition and personalized medicine [3].
As such, salivary metabolomics is an emerging and promising field that offers another unique perspective on the use of oral fluids in molecular diagnostics. In fact, researchers are now reporting significant discrepancies in several classes of metabolites [dipeptides, amino acids, carbohydrates, lipids, and nucleotides) when comparing those with periodontitis to healthy subjects [34]. Similar studies analyzing metabolic profiles have discerned individuals with oral cavity, pancreatic or breast cancers from their respective healthy controls (35-37]. This suggests that disease-specific metabolic signatures may be embedded in salivary secretions [14].

Furthermore, because of its sensitivity and quantitative reproducibility, this noninvasive, efficient, and inexpensive approach can be considered a promising method for the development of robust, sensitive, and reproducible diagnostic tests for the separation, detection, characterization, and quantification of the oral metabolome [19].
Although the application of this approach is still in its preliminary stages, its influence continues to grow each year [14]. Emerging as a promising biofocus, metabolomics will drive salivary analyses and offer great public health benefits in the long term [3].

5.3.4. Microbiomics

The microorganisms in the human oral cavity have been referred to as oral microflora, oral microbiota, and more recently oral microbiome [14]. Following the Human Microbiome Project, established by the NIH, numerous studies have investigated the amount of bacterial flora, some estimating approximately 500 to 1,000 species and others reporting up to 10,000 species are present in the oral hemostasis [12]. Characterization of the enormous diversity of the human salivary microbiome will facilitate the diagnosis of oral and systemic infectious diseases. [20]. According to, Belstrom et al., in 2017 saliva is an appropriate source for comprehensive genotyping. Genomic, metagenomic, and meta-transcriptomic analyses have been used to characterize the diversity and community composition of the salivary microbiota [20].

Common techniques used by researchers include bacterial microarrays, DNA hybridization, PCR, next-generation sequencing, and quantitative 16S rRNA gene sequencing. The most recent and promising technique is the use of a 16S rRNA-based oligonucleotide microarray, commonly referred to as Human Microbe Identification Microarray (HOMIM) [12]. HOMIM has been used in studies comparing samples from sick people to controls, in order to detect species variations in the microbiota. The objective is to identify pathogen profiles, monitor changes and map alterations identified in patients, many of which are correlated with the microbiota [12].

Sampling of the salivary microbiome may also provide unique data as the oral cavity is the first part of the gastrointestinal system and thus offers a unique opportunity to examine the symbiotic relationship between individuals and the colonizing microbiota. The balance between bacterial families in the salivary microbiome may also indicate more global outcomes beyond oral health. Next-generation sequencing of microbial (or viral) genetic material in oral fluids can serve as a unique and independent biomarker of infectious disease. Bacterial DNA can also be used to identify oral bacterial species or taxa resistant to culture and other isolation methods. This emerging research reveals the unique role of human saliva in systemic immune defense and new insights into the integrity of the biological system in individuals [7].

Continued efforts in this area could distinguish the oral microbiome as a reservoir of microbial constituents with the ability to identify both local and systemic disease, a truly unexpected but extremely important feature of the oral cavity [14]. Collectively, this research highlights that the salivary microbiome deserves greater attention as it represents both a challenge for salivary analysis and a measure of the delicate balance that exists between microbiota-dependent health and pathology [7].

5.3.5. Genomics

It has been established that high quality, high molecular weight DNA molecules can be purified from human saliva, which will be collectively referred to as the salivary genome. Using an increasing range of DNA collection kits, stabilizing reagents and purification procedures developed for oral fluid samples [7]. The quality of the salivary genome is good: 72% to 96% of samples can be genotyped, 84% can be amplified, and 67% can be sequenced and stored long-term without significant degradation [21]. Known for its crucial role in cell differentiation, saliva genomic DNA has been shown to be highly informative and discriminatory, allowing cells to maintain unique characteristics by controlling and modulating gene expression [10,14]. DNA patterns obtained from saliva have recently been reported as a good tool for investigating oral and systemic disorders [9]. While its application in forensic and clinical investigations is enhanced by high-throughput technology platforms such as genome-wide microarrays, the oral microbiota (commensal and pathogenic), food scraps and desquamated oral mucosal cells (source of the human genome) [10]. The DNA yield from saliva ranges from 11 to 46µg per mL with a percentage of human DNA of 37-77%. Using commercially available kits, salivary DNA collection is a simple process that yields good and sufficient quality and quantit [9].

Remarkably, it is not uncommon to find DNA in salivary secretions [14]. Since the collection procedures are based on kits that allow the isolation of high quality DNA without contamination, this can be used in many diagnostic laboratories for mutations and polymorphisms associated with susceptibility to diseases such as cancer, periodontal diseases, viral diseases etc [10]. Therefore, several researches have focused on elucidating promoter hypermethylation patterns in salivary DNA. One such study found gene panels capable of discerning oral squamous cell carcinoma patients from controls with a sensitivity of 62-77% and specificity of 83-100%. These reports suggest that salivary DNA may be an effective analyte for the detection and monitoring of oral squamous cell carcinomas of the head of the neck and oral cavity [14]. Ongoing and future analyses in this area could serve as a precursor for the development of saliva-based predictive, pre-symptomatic, carrier, and prenatal screenings for local and systemic diseases [14,10]. To this end, a common ambition among clinicians and scientists to credibly create an approach based on the exploration of oral biofluid containing analytes sensitive to our overall health status [14].

4. CONCLUSION

Envision a future where your health status is accessible without the discomfort, inconvenience, and pain associated with physical examinations. Is this a distant dream or an imminent reality with profound potential impacts? Recent advancements in saliva diagnostics suggest that this future is not only plausible but rapidly approaching. Ongoing discoveries and substantial progress underscore that saliva contains real-time information reflecting an individual's overall and oral health status. Researchers assert that, akin to blood and tissue biopsies, oral fluids can serve as a source of biochemical data capable of detecting specific diseases.

What adds to the intrigue is that this phenomenon extends beyond local disorders like oral cancer, Sjögren's syndrome, caries, and periodontal disease to encompass distant pathologies such as autoimmune diseases, cardiovascular and metabolic diseases, viral/bacterial infections, and certain cancers. These revelations form the cornerstone of the

burgeoning field of salivary diagnostics, prompting investigations and research among oral health clinicians to unravel the complexities of this salivary milieu.

The future trajectory of this field hinges on further validation of disease-specific biomarkers and their integration into state-of-the-art multiplex tests. These tests should be versatile, quantitative, reliable, sensitive, specific, rapid, robust, cost-effective, and efficient, allowing for broad implementation in diagnostic, prognostic, and patient management programs. As we embark on this scientific journey, the realization of a comprehensive and accessible diagnostic platform through salivary diagnostics appears not only promising but poised to redefine the landscape of healthcare.

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