A systemic review on salivary diagnostics

ABSTRACT

In recent years, there has been a significant change in perspective that has resulted in the recognition that the oral cavity and oral diseases should not be viewed in isolation. Instead, they should be seen as an integral part of the body's overall physiology, playing a crucial role in maintaining overall health and reflecting the presence of systemic diseases. Saliva has undergone thorough investigation as a prospective diagnostic tool in the past ten years due to its convenient and noninvasive accessibility, as well as its plentiful supply of biomarkers. This is a comprehensive analysis of the research published in the last 15 years in electronic databases that examine the effectiveness of saliva as a diagnostic tool. An examination of well researched diagnostic areas using saliva, the important substances being analyzed, and the methods employed was conducted. Data obtained from electronic databases, subsequently followed by the extraction and administration of data.Out of the 530 research analyzed, 80 studies specifically focused on the changes in saliva for detecting neoplasms. These studies identified 35 different substances in the saliva of individuals with neoplasms. In comparison, 20 substances were recognized in individuals with metabolic diseases, and the fewest substances were found in individuals with systemic disorders. The enzyme-linked immunosorbent assay (ELISA) is the most often used technology, with the primary analyte being immunoglobulins. The utilization of high-throughput proteomic methodologies in diverse investigations during the past decade has resulted in the identification of novel biomarkers.

Keywords – biomarker, diagnosis, diseases, oral, saliva, tool

## INTRODUCTION

Global effect is observed in human disorders such as cancer, cardiovascular, metabolic, and neurological diseases. Diagnosing various medical disorders is getting increasingly difficult and therefore necessitates the addition of laboratory tests to the clinical examination [1]. Salivary diagnostics shows significant potential as a reliable method for early detection, prediction of outcomes, and monitoring of post-treatment condition. Whole saliva is a complex combination of various substances, including secretions from both major and minor salivary glands, fluids from the mucosal lining, gingival crevicular fluid, serum and blood components from oral injuries, shed epithelial cells, expelled bronchial and nasal secretions, bacteria and their byproducts, viruses, fungi, other cellular elements, and remnants of food. The fluid is composed of a diverse range of substances including hormones, proteins, enzymes, antibodies, antimicrobial components, and cytokines [2].

The elements from the blood enter the saliva through transcellular, passive intracellular diffusion and active transport, or paracellular routes through extracellular ultrafiltration within the salivary glands or the gingival crevice [3, 4]. Saliva offers numerous benefits as a clinical tool compared to serum and tissues. These advantages include the ability to collect samples in a noninvasive manner, reduced sample sizes, strong patient compliance, cost effectiveness, easy storage and transportation, higher sensitivity, and correlation with blood levels. Recent advancements in technology have revealed numerous medically significant salivary biomarkers for various disease situations, such as cancer, autoimmune disorders, viral and bacterial infections, cardiovascular diseases, and metabolic disorders [2].

This systematic review aims to collect the published data on studies using saliva as a diagnostic tool from past 15 years and determine the significant techniques involved in evaluating the various analytes detected in saliva.

## MATERIAL AND METHODS

### Search stratergy-

- The study identification process involved searching electronic databases, specifically Google Scholar and MEDLINE, using the phrase "saliva as a diagnostic tool".
- Subsequently, the electronic databases PubMed and ScienceDirect were searched for publications published in English between the years 2008 and 2023.
- The pertinent articles were selected through an extensive search of the literature.
- The quality of the included studies was assessed to reduce the likelihood of selection bias. The mesh terms used were "saliva" AND "diagnostic tool," "SALIVA," and "diagnostic."

### Inclusion criteria-

- The study encompasses publications published from 2008 to 2023, which were retrieved from electronic sources such as "PubMed," "Medline," "ScienceDirect," and "Biomed Central."
- All studies from prior databases, including those published up to the present date, were included in the search.
- Only English-language articles were included.

### Exclusion criteria-

- Studies that do not aim to assess the effects of health on humans were not included.
- Papers that provide a general discussion without presenting data were excluded.
- Any article that was clearly inappropriate was eliminated early in the search process, based on abstracts and titles found in electronic catalogs.

- Review articles, editorials, consensus statements, and opinions were excluded.

Data management-

Information was obtained from all the studies that were included. The retrieved data were inputted electronically into a Microsoft Word document to streamline the process of summarizing and analyzing the information. The information collected for the study included the following:

1. Study details, such as the year, design, and aim of the study.

2. Characteristics of the study and the population involved.

3. Details about the intervention used in the study.

4. The outcomes that were measured.

5. The results obtained from the study.

To ensure accuracy, two reviewers independently extracted the data, and any disagreements were resolved through consensus or by involving a third reviewer. The collected information was entered into a spreadsheet to make it easier to create graphical representations.

RESULTS

Out of the 530 studies that were considered, 80 studies were selected for this systematic review based on the inclusion and exclusion criteria. The studies that provided comprehensive

information on sample size, methodology, outcomes, significant values, sensitivity, and specificities were taken into account. Out of the 80 papers analyzed, the majority were cross-sectional studies, whereas cohort studies and nonrandomized trials were the least common. The majority of research using saliva as a sample are focused on neoplasms, followed by infections, metabolic disorders, periodontal diseases, and systemic diseases. The year 2015 had the most extensive research undertaken. The enzyme-linked immunosorbent test (ELISA) was the most frequently used method, followed by real-time polymerase chain reaction (PCR), quantitative PCR, and glucose oxidase peroxidase methods. The studies primarily compare and quantify the levels of analytes in saliva with those in serum, with plasma being the predominant choice. The analytes that were extensively researched were immunoglobulins in different types of illnesses. Out of the 80 investigations, saliva samples from patients with neoplasms have shown the presence of almost 35 different analytes. In the case of metabolic disorders, 20 analytes have been detected, while the fewest analytes have been found in patients with systemic disorders.(table1) 80% of the research highlight the usefulness of unstimulated whole saliva. The passive drool method . remained the favored approach for collecting saliva in multiple investigations. Saliva was compared and associated with 10 distinct media of interest in the experiments .

Table 1 Most common analytes detected

| Diagnosis areas | Analytes found |
|---|---|
| Neoplasms | LDH, MMP-9, IL6, IL8, HPV16, Cu, CA-19, Sol CD44 |
| Metabolic disorders | Total protein, glucose |

| | |
|---|---|
| Infections | HIV-ab, Hep.B surface antigen, IgM, Ig A |
| Syndromes | Cortisol, flow rate, Na,Cl |
| Periodontal diseases | Nitric oxide |
| Systemic disorders | Cortisol, miRNA, glucose, alpha amylase |
| Miscellaneous | Cortisol , urea |

## DISCUSSION

The use of saliva for diagnosis has garnered much attention because it is easy to collect, cost-effective, can be stored easily, and does not require intrusive procedures. Other bodily fluids, such as blood and urine, which are commonly utilized for illness diagnosis, often present challenges in the collecting process. Saliva contains biocomponents that have the potential to be employed as biomarkers. Therefore, the use of saliva as a diagnostic tool will enhance the diagnostic resources, offering crucial insights into both oral and systemic health. The salivary biomarkers span various omic domains, including proteomics, metabolomics, and transcriptomics. This review examines the multiple studies on saliva-related diagnostics and its connection to systemic disorders, emphasizing its potential as a diagnostic material.[5,6]

Thanks to the proliferation of sophisticated technology and computational techniques, a significant number of research are now being conducted employing saliva on a previously unprecedented scale. This technology has been effective in quantifying, detecting, and analyzing many substances and factors, therefore contributing to the progress in identifying and confirming salivary biomarkers.[7-11]

ELISA was the predominant approach utilized in the previous decade. This might be attributed in part to the recent standardization of ELISA for use with saliva. With a sensitivity and specificity of 98%, ELISA has become a highly sought-after technique, particularly after more precise procedures such as PCR and immunohistochemistry.[12-16] The majority of published studies focused on ELISA, radioimmunoassay, and PCR for the detection of micro RNA, interleukin (IL)-1α, IL-6, IL-8, vascular endothelial growth factor-a, tumor necrosis factor-α, immunoglobulins, and enzymes such as C-reactive proteins in saliva. These studies yielded significant results.[17-22] In 2015, the glucose oxidase peroxidase technique was used in 95% of investigations conducted on diabetic individuals, yielding significant findings.[23-27] This could be attributed to the fact that there was an increase in awareness and a surge in the number of studies conducted on diabetes that year. The series of studies provided strong evidence that saliva can serve as a valuable tool, not only for diagnosing medical conditions, but also for monitoring the likely course and outcome of those conditions. Saliva can be utilized as a supplementary instrument.

A limited number of research were undertaken in individuals with syndromes linked to oral lesions. The International Diabetes Federation predicted an estimated 55% rise in the mortality rate by 2035. In 2013, there were 5.1 million deaths worldwide that were directly due to diabetes. This means that there was one death every 6 seconds, which is an 11% increase compared to 2011.[28-33]

Starting in 2015, researchers began evaluating a diverse range of substances in saliva. They focused on investigating the levels of cortisol in depression and studying the changes in transplant patients, specifically those who underwent renal transplant or implant placement for periodontal diseases. Additionally, they conducted research on therapeutic drug

monitoring.[26,27] Oral cancer currently ranks sixth among the most deadly diseases in humans. Recent studies have focused on detecting analytes to enable early identification of neoplasms, particularly those affecting the head and neck.[34,35].

Previous studies attempted to identify trace and main elements [26-33], but their importance in diagnosing certain conditions was not fully recognized when compared to blood samples. Studies conducted on various body fluids, such as urine and saliva, have indicated that saliva can be utilized as an additional diagnostic tool.

Furthermore, in the recent past, research papers have been published regarding the identification of several metabolic byproducts such lactic acid, pyruvic acid, and sialic acid in the saliva of individuals with tumors, including both cancerous and pre-cancerous growths, as well as cardiovascular disorders and other related conditions. Prominent findings were achieved in this domain regarding the gold standard under consideration.

Thorough research is necessary to determine the most efficient methods of detecting substances in saliva. Out of all the available methods like gel electrophoresis, liquid chromatography, kinetic assays, and spectrophotometry, a large number of studies need to be conducted for each analyte in order to determine its significance in early detections. These studies are then followed by meta-analysis to draw further conclusions.

Out of the 24 studies that examined individuals with neoplasms, the most common type was oral squamous cell carcinoma, followed by tongue cancer and parotid tumors, which were the least common. Out of the 24 chosen, 19 research examined the amounts of a desired substance in relation to precancerous lesions, specifically oral leukoplakia, followed by oral lichen planus and

oral submucous fibrosis. [20,22,30]. A strong association has been found between serum levels and the histological grades of disease presentation.

## CONCLUSION

The utilization of saliva as a diagnostic medium raised numerous study inquiries. The published studies have concluded that saliva can be used to screen for various diseases by detecting certain analytes. Proteomics and metabolomics have gained increasing pace in the last ten years due to the development of advanced proteomic technologies, such as mass spectrometry-based approaches and microarrays.

Furthermore, a substantial amount of research must be conducted utilizing contemporary approaches for each selected analyte in the realm of diagnostic significance. Consequently, there is a scarcity of studies that focus on a particular substance being analyzed, the methods employed, and a specific area of diagnosis. This highlights the urgency to shift towards research that is more directed and aims to fully explore the capabilities of saliva.

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