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Non-Invasive Immunological Monitoring Using Saliva in Dental and Oral Health Research

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ABSTRACT
Saliva plays vital roles in oral and systemic health and is emerging as a valuable tool in biomedical research. It contains bioactive components such as cytokines, enzymes, and immunoglobulins that reflect immune status, making it a promising non-invasive diagnostic fluid. A literature review was conducted using PubMed and Google Scholar Science Direct (2015–2025) with keywords related to saliva, immunological markers, and oral health. Studies included were original research on human subjects. Articles were screened following PRISMA guidelines. Salivary biomarkers such as IL-6, TNF-α, IL-17A/F, IFN-γ, CRP, calprotectin, IP-10, MCP-1, NT-proBNP, IL-1β, IL-2, IL-8, and sTNFRII have been linked to oral cancer, periodontitis, diabetes, autoimmune disorders, cardiovascular disease, and viral infections. Studies showed that these markers reflect both local inflammation and systemic immune responses. Saliva captures immune activity through pathways like Th17, TNF, and JAK/STAT signaling. Its non-invasive nature, molecular stability, and compatibility with modern analysis techniques (e.g., transcriptomics and proteomics) make it ideal for monitoring disease progression and immune function. The presence of stable biomarkers like cytokines, acute-phase proteins, and cardiovascular indicators in saliva supports its diagnostic relevance.Saliva offers a practical, non-invasive approach to immunological monitoring. With proven biomarkers for various diseases, it holds strong potential for future diagnostic and research applications.

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INTRODUCTION

Saliva within the oral cavity plays various critical roles in maintaining health, both locally in the oral environment and systemically throughout the body (Dawes *et al.*, 2015; Pedersen *et al.*, 2018). Among its primary functions, saliva serves as a natural lubricant and cleansing agent that helps preserve oral hygiene. It also acts as a pH buffer, maintaining a neutral pH in the mouth and thereby preventing tooth demineralization while supporting antimicrobial activity (Dawes *et al.*, 2015). Additionally, saliva contributes to bolus formation, taste percep-

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tion, and the initiation of carbohydrate digestion through enzymatic activity, making it essential in the processes of mastication and swallowing. Another important role of saliva is in speech, as it aids in proper articulation (Pedersen *et al.*, 2018).

Saliva is secreted by three pairs of major salivary glands—namely, the parotid, submandibular, and sublingual glands—as well as numerous minor salivary glands. These glands produce a complex fluid containing a variety of biological biomarkers (Gug *et al.*, 2019; Zakiawati & Sufiawati, 2021). An increasing number of recent studies have employed saliva as a research specimen, especially in dental science. Its use is frequently aligned with the selected research methodology and the particular health problems under investigation. As a diagnostic medium, saliva offers distinct advantages due to its non-invasive collection method, ease of sampling, and minimal risk, making it ideal for a wide range of biomedical and clinical research applications (Zakiawati & Sufiawati, 2021; Zhou & Liu, 2023). Its rich composition of biological molecules, particularly proteins, supports its use as a biological indicator for detecting oral abnormalities. The unique structure of salivary proteins further enhances its diagnostic potential (Gug *et al.*, 2019; Al Shehhi *et al.*, 2024). Saliva contains a wide range of molecular components that can be analyzed, including cytokines and other proteins. This literature review aims to explore the role of saliva in molecular research, with a particular emphasis on its relevance to immunological aspects and overall oral health.

METHODS

This study conducted a literature review and article search using the PubMed database. The search utilized specific keywords, including [salivary, immunology markers, oral health, and research]. For the specific keyword “saliva AND (immunological markers OR cytokines) AND (oral health OR dental research) AND non-invasive diagnostics”. The article selection process followed the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. The process involved removing duplicate articles and further refining the selection to include studies published between 2019 and 2024, and those published in English. Book sections, studies involvoing animals, review articles, and conference proceedings were excluded. Data extraction encompassed a range of variables such as author names, article titles, publication years, study designs.

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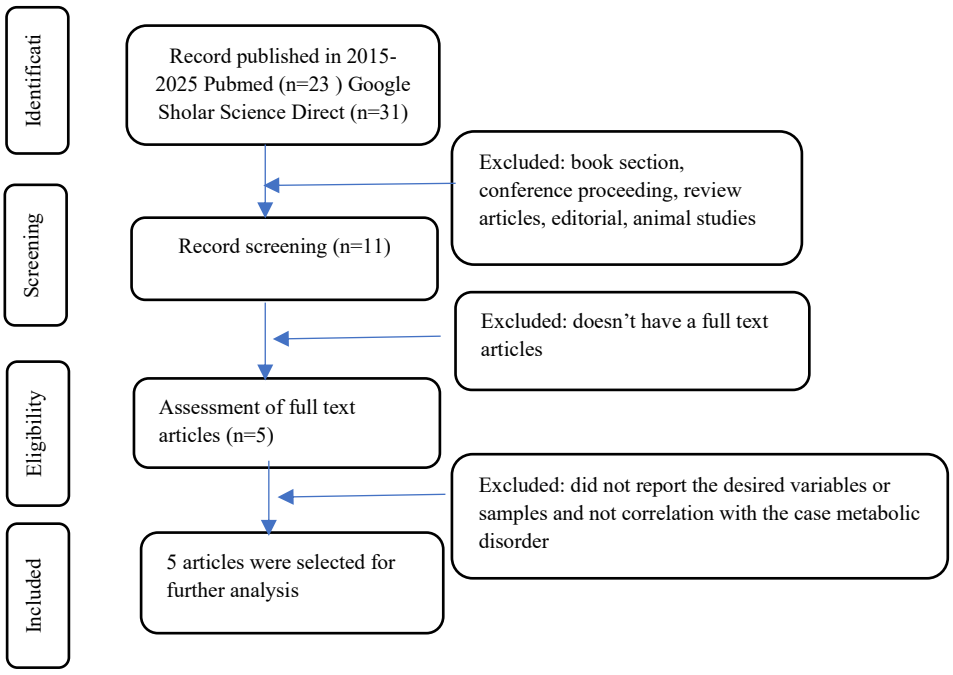

Figure 1. The article selection process flow diagram

RESULTS AND DISCUSSION

The article selection process is outlined in Figure 1. a total of 54 articles were initially identified through the designated keywords in PubMed and Google scholar Science Direct from 2015-2025. After meticulous removal of duplicate articles and application of the inclusion and exclusion criteria, 5 articles met the study's eligibility criteria. Table 1 provides a summary of the extracted data from the selected studies.

Table 1 Data extracted from included studies

No	Authors/ year	Title	Methods	Result
1	Ruacho et al. / 2022	Inflammatory markers in saliva and urine reflect disease activity in patients with systemic lupus erythematosus	A total of 84 patients with SLE selected to represent high and low general DA, and 21 controls were included. participants were fasting for 12 hours before collection of blood, urine and saliva samples. CSF-1, IL-34 and	CSF-1, TNF-α, IP-10 and MCP-1 in saliva, serum and urine, as well as calprotectin in saliva and urine were increased in patients with SLE as compared with controls ($p<0.05$). Saliva is a novel alternative body fluid, with potential for surveillance of general DA in patients with SLE, but urine is more informative in patients with

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No	Authors/ year	Title	Methods	Result
			calprotectin levels were analysed by ELISA	SLE with predominantly renal DA
2	Bachtiar et al. / 2024	The utility of salivary CRP and IL-6 as a non-invasive measurement evaluated in patients with COVID-19 with and without diabetes	quantitative real-time PCR to measure salivary levels of C-reactive protein (CRP) and interleukin-6 (IL-6) in saliva obtained from patients diagnosed with mild COVID-19, in a diabetic group (DG; n = 10) and a non-diabetic group (NDG; n = 13). All participants were diagnosed with periodontitis, while six participants with periodontitis but not diagnosed with COVID-19 were included as controls	Salivary CRP and IL-6 levels were comparable. the levels of salivary CRP were significantly correlated with total proteins, in which a strong and moderate positive correlation was found between DG and NDG, respectively. A linear positive correlation was also noted in the relationship between salivary IL-6 level and total proteins, but the correlation was not significant
3	Sanggapa et al.,/ 2024	Salivary Interleukin-6 as a Non-Invasive Biomarker for Chronic Periodontitis and Tooth Loss in Type 2 Diabetes	240 subjects aged 30-69 years with minimum of 15 natural teeth. Fasting, unstimulated whole saliva was collected, full-mouth intra-oral examination. Salivary levels of IL-6 were assessed with the commercially available Human Interleukin-6 ELISA	Average SIL-6 levels were significantly elevated in Group 4 (T2DM with CP and tooth loss) (P = 0.001) and in severe periodontitis (P = 0.001). Karl Pearson Correlation found a significant association between average SIL-6 and average periodontal pocket depth (APPD) (r = 0.180), average clinical attachment loss ≥3 mm (ACAL3) (r = 0.289) and severity of periodontitis (r = 0.3228). The receiver operating characteristic (ROC) curve

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				depicted an overall sensitivity of 53.3%, specificity of 68.6% and accuracy of 60% in the detection and assessment of CP in T2DM with tooth loss. IL-6 in saliva is a valuable, non-invasive biomarker in the detection and evaluation of CP in T2DM with tooth loss
4	Mohite et al., 2024	Assessment and Comparison of N-Terminal-Probrain Natriuretic Peptide (NT-proBNP) in Saliva and Serum of Healthy Subjects, Periodontitis Patients, and Periodontitis Patients With Myocardial Infarction	90 patients, 30 in each group i.e., healthy group, periodontitis patients and patients suffering from periodontitis with myocardial infarction, were enrolled. Salivary and serum samples were collected from the participants after obtaining informed consent. The samples were subjected to human NT-proBNP sandwich type enzyme-linked immunosorbent assay (ELISA) for quantitative evaluation	alivary and serum concentrations of NT-proBNP were significantly higher with p-value=0.000 in subjects suffering from periodontitis with MI. The salivary NT-proBNP levels were significantly higher than serum NT-proBNP levels in periodontitis and periodontitis with MI patients. alivary NT-proBNP can be used as a non-invasive diagnostic marker for diagnosing periodontitis and MI. Future research could explore targeted therapies for the shared inflammatory pathways between periodontitis and MI.

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No	Authors/ year	Title	Methods	Result
5	Sanggapa et al., 2024	Diagnostic accuracy of salivary hemoglobin, lactate dehydrogenase and Interleukin-6 to determine chronic periodontitis and tooth loss in type 2 diabetic	240 individuals with at least 15 remaining teeth, ranging in age from 30 to 70, were chosen and Group I controls were defined as follows: healthy (HbA1c levels ≤6.4 %) with no CP; Group II included chronic periodontitis and non-T2DM (HbA1c ≤ 6.4 %); Group III included T2DM (HbA1c ≥ 6.5 %) and CP; and Group IV included T2DM (HbA1c ≥ 6.5 %) with periodontitis-related tooth loss. ELISA colorimetric assay was used to quantify the results using the unstimulated whole saliva of fasting participants	One-way ANOVA comparing Biomarker levels across the four groups revealed a statistically significant difference (F = 68.013) (p = 0.0001). Tukey's multiple post hoc yielded a significant difference between groups with least mean average biomarker levels observed among the controls (Group1) and maximum with group IV. Estimates of Salivary Hemoglobin can assume an important role in comparison to SIL-6 & SLDH in determining the degree of periodontitis, including tooth loss, and identifying elevated glycemic levels

Saliva is often promoted as the diagnostic fluid of the future due to its advantages as a biological specimen. This is largely based on the perception that saliva collection is quick, simple, and non-invasive. While this perception is generally accurate, in certain situations and specific populations, collecting saliva can prove to be more challenging than expected, time-consuming, and may result in insufficient sample volume for proper testing. Extraction of the data shows in table 1. Describe that use of saliva as a biological specimen in clinical and molecular research is increasingly gaining attention due to its non-invasive nature, ease of collection, and its ability to reflect both physiological and pathological states of the body, including immune responses at both local and systemic levels. Several studies have highlighted the role of saliva as a potential diagnostic medium for measuring various

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immunological markers. Ruacho et al. (2022) demonstrated that proinflammatory biomarkers such as CSF-1, TNF-α, IP-10, MCP-1, and calprotectin were significantly elevated in the saliva of patients with systemic lupus erythematosus (SLE) compared to healthy controls. This finding indicates that saliva can serve as an alternative body fluid for monitoring disease activity in systemic conditions such as SLE, although urine was found to be more informative in cases with predominantly renal involvement.

Furthermore, a study by Bachtiar et al. (2024) evaluated salivary concentrations of CRP and IL-6 in COVID-19 patients with and without diabetes. The results showed a positive correlation between salivary CRP and IL-6 levels and total salivary proteins, particularly in the diabetic group. These findings support the involvement of systemic immune responses reflected in saliva and suggest its potential use for monitoring systemic inflammation. On the other hand, Sanggapa et al. (2024), in two separate studies, investigated salivary IL-6, hemoglobin, and LDH as non-invasive indicators in patients with type 2 diabetes mellitus (T2DM) with periodontitis and tooth loss. Salivary IL-6 was significantly correlated with periodontal pocket depth and clinical attachment loss, making it a promising biomarker for detecting and evaluating chronic periodontitis, especially in patients with metabolic comorbidities. Another study by Mohite et al. (2024) reported significantly elevated levels of NT-proBNP—a well-known cardiovascular biomarker—in the saliva of patients with periodontitis and myocardial infarction. Interestingly, salivary NT-proBNP levels were higher than those in serum, further supporting the role of saliva in reflecting systemic inflammatory status associated with periodontal disease and other systemic complications.

Overall, these studies affirm that saliva is an effective and practical medium for monitoring immunological biomarkers. Various molecules such as IL-6, CRP, TNF-α, calprotectin, and NT-proBNP detected in saliva demonstrate strong diagnostic potential not only for oral diseases like periodontitis but also for systemic conditions including diabetes, viral infections, and autoimmune diseases. These advantages position saliva as a promising tool for the development of non-invasive disease screening and monitoring methods in the future. Saliva, with its adaptable physical characteristics, is composed of approximately 98% water and 2% organic and inorganic compounds. This complex biological fluid includes various components such as DNA, RNA, microRNAs, proteins, hormones, growth factors, inhibitors, enzymes, minerals, electrolytes, buffers, immunoglobulin A (IgA), antimicrobial agents, cytokines, mucins, nitrogen-containing substances, and other glycoproteins. These constituents collectively contribute to essential functions like maintaining electrolyte and ecological balance, regulating oral cavity pH, cleansing and protecting oral tissues, exhibiting antimicrobial effects, and supporting digestion. Additionally, saliva is involved in key physiological activities such as chewing, swallowing, digestion, taste perception, and speech. Certain salivary proteins—including mucins, lactoferrin, lysozyme, cystatins, histatins, α-amylase, and immunoglobulins—demonstrate strong diagnostic value for monitoring and detecting oral diseases and cancers. Mucins and amylase, in particular, possess antimicrobial properties, promote adherence to the dental pellicle, and help preserve proteins by forming protective complexes that guard against enzymatic degradation(Kumar et al., 2023; Zhang et al., 2022; K. Al-Manei (2020).

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Saliva has increasingly gained attention as a research specimen in the medical and biomedical fields due to its non-invasive nature, ease of collection, and richness in biological information. It contains various important biomarkers such as proteins (e.g., EGF, VEGF, CEA, and c-erbB-2), RNA (both mRNA and microRNA), and metabolites, which have been associated with a wide range of diseases, including oral, pancreatic, and lung cancers, as well as systemic and periodontal conditions. Moreover, these molecules remain stable in saliva and can be analyzed using modern techniques such as transcriptomics and metabolomics. Another advantage is that saliva collection does not require invasive procedures, making it ideal for early diagnosis, large-scale screening, and continuous disease monitoring. With advancements in analytical technologies and bioinformatics, saliva is now considered a viable alternative to serum and, in some aspects, even holds greater potential for disease diagnosis and monitoring (Kumar et al., 2023). Saliva is increasingly recognized as a promising specimen in immunological research due to its content of bioactive molecules that reflect the immune status of the body, both locally in the oral cavity and systemically (Spielmann & Wong, 2011; Lima et al., 2024). Various immunological pathways are involved in the biological activity of saliva, particularly through components of the innate immune system such as IL-1β, TNF-α, IL-6, and IL-8, which are secreted by epithelial cells, macrophages, and neutrophils. Saliva has proven to be a highly promising medium for detecting and monitoring immunological biomarkers due to its content of various cytokines that reflect both local and systemic immune status. One of the most prominent pathways highlighted is the IL-17 and TNF-α axis. A study by Zielińska et al. (2020) demonstrated that salivary levels of IL-17A, IL-17F, and TNF-α were significantly elevated in patients with oral and oropharyngeal cancer, especially in more advanced stages of the disease. These cytokines also correlated with oral bacterial colonization, reinforcing the potential of saliva as a non-invasive tool for cancer surveillance.

The immunological role of IL-17A and IFN-γ is also evident in the context of food allergies. Yin et al. (2023) reported significantly increased levels of both cytokines in the saliva of children with food allergies compared to healthy controls, with a strong correlation ($r = 0.79$). This suggests that mucosal immune activation can be effectively monitored through saliva, positioning IL-17A and IFN-γ as promising markers for allergic conditions. Furthermore, in the context of systemic viral infections, IL-17 also demonstrates clinical relevance. A study on COVID-19 patients revealed that salivary IL-17 levels mirror systemic conditions and are strongly associated with disease severity, need for mechanical ventilation, and mortality risk within the first 29 days of admission. These findings indicate that the Th17 pathway is not only active at the mucosal level but also reflects systemic immunity detectable in oral fluid. Additionally, a 2021 systematic review identified several pro-inflammatory cytokines—such as IL-1β, IL-2, IL-6, TNF-α, and IFN-γ—present in saliva among patients with conditions like HIV, tuberculosis, mucosal injuries, and graft-versus-host disease. This further confirms saliva's utility as a broad diagnostic tool, particularly in resource-limited settings, owing to the ease and non-invasiveness of sample collection. Taken together, these studies underscore that saliva can effectively reflect both innate and adaptive immune pathways, particularly through key mediators such as IL-17, TNF-α, and IFN-γ. These biomarkers are relevant not

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only for local diseases like oral cancer and periodontitis but also for systemic conditions including allergies, viral infections, and immune disorders. Its practicality and diagnostic potential position saliva as a valuable tool in current and future immunological research.

CONCLUSION

Saliva is increasingly recognized as a promising diagnostic fluid due to its non-invasive collection, molecular stability, and ability to reflect both local and systemic immune responses. It contains key immunological biomarkers, which have been linked to various conditions including oral cancer, periodontitis, autoimmune diseases, and viral infections. Its practical advantages and diagnostic relevance make saliva an effective tool for immunological monitoring in both clinical and research settings.

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