Advances in Salivary Biomarkers for Early Identification of Dental Caries.

Abstract

Dental caries remains one of the most prevalent chronic oral diseases worldwide, often undetected until significant enamel and dentin damage occurs. Saliva, as a non-invasive diagnostic medium, contains proteins, enzymes, microbial components, metabolites, and immunological molecules that reflect oral physiological and pathological states. Recent advances in proteomics, metabolomics, genomics, and biosensor technologies have enabled the identification of multiple salivary biomarkers associated with early caries risk and disease progression. This review synthesizes current evidence on microbial, proteomic, immunological, oxidative stress, and biochemical salivary biomarkers for early caries detection. It also discusses technological innovations, limitations, and future perspectives in salivary diagnostics. Although promising, variability in saliva composition and methodological heterogeneity remain challenges. Standardized protocols and large-scale studies are required to integrate salivary biomarkers into routine clinical practice.

Keywords

Dental caries; Saliva; Salivary biomarkers; Early diagnosis; Proteomics; Caries risk assessment.

Introduction

Dental caries is a multifactorial disease resulting from interactions between fermentable carbohydrates, cariogenic microorganisms, host susceptibility, and time. Globally, dental caries affects billions of people, representing a significant public health concern[1]. Early detection is critical to implement preventive measures and minimize irreversible tooth damage. Traditional diagnostic methods, such as visual-tactile examination and radiography, often detect lesions only after substantial demineralization[2].

Saliva has emerged as an attractive diagnostic fluid due to its non-invasive collection, ease of handling, and rich composition of biologically active molecules. Salivary biomarkers, including

microbial profiles, proteins, metabolites, cytokines, and oxidative stress markers, may reflect oral health status and predict caries susceptibility[3]. Advances in molecular diagnostics, proteomics, and metabolomics have enhanced the detection and quantification of these biomarkers, allowing more accurate early risk assessment[4].

Saliva as a Diagnostic Medium

Saliva maintains oral homeostasis by lubricating tissues, buffering acids, facilitating enamel remineralization, and providing antimicrobial activity. Its composition includes organic components such as immunoglobulins, enzymes, glycoproteins, and antimicrobial peptides, as well as inorganic ions like calcium and phosphate[5].

Alterations in salivary composition may indicate increased caries susceptibility. For example, changes in protein concentration, microbial load, and cytokine levels can serve as early indicators of disease risk[6]. Salivary diagnostics, therefore, present a promising tool for non-invasive, personalized preventive dentistry.

Microbial Salivary Biomarkers

Cariogenic microorganisms are critical in initiating dental caries. *Streptococcus mutans* and *Lactobacillus* spp. produce acids that demineralize enamel, while *Candida albicans* has been implicated in early childhood caries[7].

Recent metagenomic studies have revealed shifts in the oral microbiome of caries-active individuals, including complex interactions among bacterial communities, which may provide more precise diagnostic information than single-species analysis[8]. Salivary microbial profiles are increasingly recognized as potential predictive biomarkers for caries risk assessment.

Protein-Based Salivary Biomarkers

Proteomic analyses have identified numerous salivary proteins associated with caries susceptibility. Key proteins include:

- **Alpha-amylase** – influences carbohydrate breakdown and biofilm formation.
- **Mucins (MUC5B, MUC7)** – involved in microbial adhesion and lubrication.
- **Histatins** – antimicrobial peptides that inhibit fungal and bacterial growth.
- **Lactoperoxidase** – contributes to antimicrobial activity.
- **Proline-rich proteins & statherin** – modulate mineral homeostasis and bacterial adhesion[9].

Systematic reviews report significant differences in these proteins between caries-active and caries-free individuals, supporting their potential as diagnostic markers[5].

Immunological and Inflammatory Biomarkers

Host immune response in the oral cavity can be reflected through cytokines, chemokines, and growth factors present in saliva. Salivary cytokines such as IL-4, IL-13, IL-2RA, TNF-α, and eotaxin (CCL11) have been linked with caries activity[10]. These markers indicate immune modulation in response to microbial colonization and may serve as early indicators of disease development[11].

Oxidative Stress Biomarkers

Oxidative stress plays a role in dental caries by modulating host defense mechanisms. Salivary biomarkers of oxidative stress include malondialdehyde (MDA), superoxide dismutase (SOD), uric acid, and total antioxidant capacity. Elevated oxidative stress markers have been reported in children with active caries, suggesting their potential utility as adjunctive diagnostic markers[12].

Biochemical and Physicochemical Salivary Parameters

Salivary flow rate, pH, buffering capacity, and mineral content (calcium, phosphate) are classical predictors of caries risk. Reduced flow or buffering capacity facilitates acidogenic microbial proliferation and enamel demineralization, while higher mineral concentrations support remineralization[13]. Assessment of these parameters alongside molecular biomarkers may improve early risk stratification.

Emerging Technologies in Salivary Diagnostics

Advances in **proteomics, metabolomics, genomics, and transcriptomics** have enhanced the identification of novel biomarkers[14]. **Biosensor-based technologies**, including electrochemical and nanotechnology platforms, allow rapid, chairside detection of salivary proteins, metabolites, and nucleic acids[15]. Integrating **artificial intelligence** with multi-omics data can improve predictive modeling for individualized caries risk assessment[16].

Limitations and Challenges

Despite promising research, several challenges impede clinical translation:

- **Biological variability**: Salivary composition varies with age, diet, medications, systemic conditions, and circadian rhythms[13].
- **Methodological heterogeneity**: Differences in collection methods, analytical techniques, and small sample sizes reduce comparability[2].
- **Standardization**: Protocols for saliva collection, storage, and biomarker quantification are not yet uniform, limiting reproducibility[10].

Future Perspectives

Future studies should focus on **multi-biomarker panels**, combining microbial, proteomic, immunological, oxidative stress, and biochemical markers. Such integration may improve diagnostic sensitivity and specificity[14].

High-throughput **omics approaches** and **machine learning algorithms** can model complex biomarker interactions to predict individual caries risk and personalize preventive strategies[16]. **Biosensor miniaturization and point-of-care platforms** may soon enable rapid, chairside screening for caries risk in clinical and community settings[15].

Conclusion

Salivary biomarkers offer a promising non-invasive approach for early detection and risk assessment of dental caries. Microbial profiling, proteomic analysis, immune and inflammatory markers, oxidative stress indicators, and physicochemical parameters have demonstrated associations with caries activity[14,16]. Advances in biosensor technologies and AI-based analysis enhance translational potential. However, biological variability, methodological heterogeneity, and lack of standardized protocols remain barriers to routine clinical application. Large-scale validation studies are required before salivary diagnostics can become a standard tool in preventive dentistry[10,15].

Table 1: Summary of Salivary Biomarkers for Early Caries Detection

Biomarker Type	Specific Biomarkers	Role/Significance	Reference
Microbial	*Streptococcus mutans*, *Lactobacillus spp.*, *Candida albicans*	Acid production, enamel demineralization, caries risk	7,8
Proteomic	Alpha-amylase, MUC5B, MUC7, Histatins, Lactoperoxidase, Statherin	Host defense, microbial adhesion, biofilm regulation	5,6
Immunological / Inflammatory	IL-4, IL-13, IL-2RA, TNF-α, CCL11	Immune response modulation, early caries indicator	10,11

Oxidative Stress	Malondialdehyde (MDA), Superoxide dismutase (SOD), Total antioxidant capacity, Uric acid	Oxidative stress marker, adjunctive caries indicator	12
Biochemical / Physicochemical	Salivary flow rate, pH, buffering capacity, calcium, phosphate	Acid-base balance, remineralization, caries susceptibility	13

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