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Salivary Biomarkers for Early Childhood Caries: Current Evidence, Clinical Applications and Future Perspectives

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ABSTRACT

Early childhood caries (ECC) is one of the most prevalent chronic diseases affecting children worldwide and remains a significant public health concern despite advances in preventive dentistry. Conventional caries diagnosis primarily relies on clinical examination and radiographic assessment, which often identify lesions after irreversible demineralization has occurred. Consequently, there is growing interest in identifying reliable biological markers capable of detecting disease susceptibility before the appearance of clinical manifestations. Saliva has emerged as an attractive diagnostic medium because it can be collected non-invasively, repeatedly, economically, and without causing discomfort to young children. Saliva contains a diverse array of biological constituents including microorganisms, proteins, enzymes, cytokines, antibodies, metabolites, nucleic acids, and electrolytes that reflect the physiological and pathological status of the oral cavity. Numerous studies have demonstrated significant alterations in salivary microbial profiles, inflammatory mediators, antimicrobial proteins, oxidative stress markers, and metabolomic signatures among children with early childhood caries. Advances in proteomics, metabolomics, microbiomics, transcriptomics, and point-of-care diagnostic technologies have further expanded the potential of saliva as a source of clinically useful biomarkers. However, variability in saliva collection methods, analytical techniques, patient characteristics, and study designs continues to limit the translation of these biomarkers into routine clinical practice. This review summarizes the current evidence regarding salivary biomarkers associated with ECC, discusses their biological significance, evaluates their diagnostic potential, highlights recent technological developments, and explores future directions for precision-based risk assessment and preventive pediatric dentistry.

Keywords: Early childhood caries; saliva; biomarkers; proteomics; microbiome; pediatric dentistry; metabolomics; diagnosis.

INTRODUCTION

Early childhood caries (ECC) is one of the most prevalent chronic diseases affecting infants and preschool-aged children worldwide and continues to represent a major public health challenge despite substantial advances in preventive dentistry[1]. The American Academy of Pediatric Dentistry (AAPD) defines ECC as the presence of one or more decayed (non-cavitated or cavitated), missing due to caries, or filled tooth surfaces in any primary tooth of a child younger than 71 months[2]. Severe early childhood caries (S-ECC) is characterized by extensive destruction of primary dentition, rapid disease progression, pain, infection, impaired mastication, altered nutrition, speech difficulties, reduced quality of life, and increased treatment costs[2,3]. Untreated ECC may also negatively affect physical growth, psychosocial development, school attendance, and the eruption and health of the permanent dentition[3].

Dental caries is a multifactorial, biofilm-mediated, sugar-driven disease resulting from the dynamic interaction between cariogenic microorganisms, fermentable dietary carbohydrates, susceptible tooth surfaces, host factors, and time[1]. Repeated consumption of dietary sugars promotes acid production by cariogenic microorganisms such as Streptococcus mutans, Streptococcus sobrinus, Lactobacillus species, Scardovia wiggsiae, and Candida albicans, leading to repeated episodes of enamel demineralization that eventually progress to cavitation if left untreated[4,5]. Although microbial biofilms play a central role in disease initiation, host defense mechanisms,particularly saliva,are equally important in maintaining oral homeostasis and protecting against the development of dental caries[5].

Saliva is a complex biological fluid secreted primarily by the major and minor salivary glands. Approximately 99% of saliva consists of water, while the remaining fraction contains electrolytes, proteins, glycoproteins, enzymes, immunoglobulins, antimicrobial peptides, cytokines, nucleic acids, metabolites, microorganisms, and exfoliated epithelial cells[6]. These constituents contribute to numerous physiological functions including lubrication of oral tissues, facilitation of mastication and swallowing, buffering of plaque acids, maintenance of oral pH, remineralization of enamel, antimicrobial defense, and regulation of the oral microbiome[6,7]. Alterations in salivary flow rate, buffering capacity, antimicrobial activity, or biochemical composition may compromise these protective mechanisms and increase an individual's susceptibility to dental caries[7].

Conventional methods for diagnosing dental caries rely predominantly on visual-tactile examination, radiographic assessment, laser fluorescence, fiber-optic transillumination, and other adjunctive diagnostic techniques[8]. While these methods are effective for detecting existing lesions, they generally identify disease after structural demineralization has already occurred. Consequently, they provide limited information regarding an individual's biological susceptibility or future risk of developing caries[8]. Modern pediatric dentistry has therefore shifted from a restorative approach toward a preventive, risk-based model that emphasizes early identification of susceptible children and implementation of personalized preventive strategies before irreversible tissue destruction develops[9].

In recent years, saliva has attracted considerable attention as a non-invasive diagnostic medium because it reflects both local oral and systemic physiological conditions. Compared with blood collection, saliva sampling is painless, inexpensive, easy to perform, repeatable, and particularly suitable for infants and young children who may not tolerate invasive procedures[10]. Furthermore, saliva can be collected in community settings without sophisticated equipment or highly trained personnel, making it an attractive tool for large-scale screening and epidemiological studies[10]. These advantages have stimulated extensive research into salivary diagnostics and their application in pediatric oral health.

Advances in molecular biology and high-throughput analytical technologies,including proteomics, metabolomics, microbiomics, transcriptomics, genomics, and next-generation sequencing,have substantially expanded the understanding of salivary composition and its relationship with oral diseases[5,11]. Numerous investigations have identified significant differences in salivary microbial profiles, inflammatory cytokines, antimicrobial proteins, immunoglobulins, enzymes, oxidative stress markers, metabolites, and microRNAs between children with ECC and caries-free controls[5,11,12]. These biomarkers have shown potential not only for detecting active disease but also for predicting caries susceptibility, monitoring disease progression, and evaluating preventive or therapeutic interventions.
Among the various categories of salivary biomarkers, microbial biomarkers such as Streptococcus mutans and Scardovia wiggsiae have consistently demonstrated strong associations with ECC[4,5]. Likewise, host-derived biomarkers including secretory immunoglobulin A (sIgA), lactoferrin, lysozyme, defensins, histatins, mucins, matrix metalloproteinases, inflammatory cytokines, oxidative stress markers, and salivary metabolites have emerged as promising indicators of disease activity and host response[5,11–13]. Integration of multiple biomarker classes rather than reliance on a single marker may improve diagnostic accuracy and facilitate individualized caries risk assessment.

Despite encouraging findings, several challenges continue to hinder the clinical implementation of salivary biomarkers. Considerable heterogeneity exists among published studies regarding saliva collection protocols, stimulated versus unstimulated samples, collection timing, storage conditions, laboratory methodologies, patient age, dietary habits, oral hygiene practices, and analytical techniques[11,12]. Moreover, many reported biomarkers require validation in large multicenter longitudinal studies before they can be incorporated into routine pediatric dental practice. The absence of standardized reference values and universally accepted diagnostic thresholds also limits their current clinical applicability.

Given the growing interest in precision dentistry and minimally invasive preventive care, salivary biomarkers represent a promising avenue for improving the early detection and management of ECC. A comprehensive understanding of these biomarkers may facilitate earlier diagnosis, individualized risk prediction, targeted preventive interventions, and improved long-term oral health outcomes in children. Therefore, this review critically summarizes the current evidence regarding salivary biomarkers associated with early childhood caries, discusses their biological significance and diagnostic potential, evaluates recent advances in salivary diagnostic technologies, highlights existing limitations, and explores future directions for translating salivary biomarker research into routine pediatric dental practice.

BIOLOGY OF SALIVA AND ITS ROLE IN CARIES PREVENTION

Saliva is a specialized exocrine secretion that plays a fundamental role in maintaining oral homeostasis and preserving the integrity of hard and soft oral tissues. It is continuously secreted by the three pairs of major salivary glands,the parotid, submandibular, and sublingual glands,as well as numerous minor salivary glands distributed throughout the oral mucosa. In healthy individuals, approximately 0.5–1.5 L of saliva is produced daily, although secretion varies according to age, circadian rhythm, hydration status, diet, medications, autonomic stimulation, and systemic health[6,14]. Whole saliva represents a mixture of glandular secretions together with gingival crevicular fluid, microorganisms, desquamated epithelial cells, leukocytes, food debris, and their metabolic products, making it a rich source of biological information for diagnostic purposes[10,13].

Approximately 99% of saliva consists of water, while the remaining 1% contains a complex mixture of inorganic ions and organic molecules. The inorganic fraction includes calcium, phosphate, bicarbonate, sodium, potassium, chloride, magnesium, and fluoride ions, whereas the organic fraction contains enzymes, glycoproteins, mucins, antimicrobial peptides, immunoglobulins, cytokines, hormones, nucleic acids, metabolites, and thousands of proteins that participate in maintaining oral health[6,14]. Advances in proteomic technologies have identified more than 3,000 proteins and peptides in human saliva, many of which are directly involved in immune regulation, tissue repair, mineral homeostasis, and antimicrobial defense[13].

Saliva provides multiple protective functions against the initiation and progression of dental caries. One of its most important roles is mechanical cleansing of the oral cavity. Continuous salivary flow removes food particles, unattached microorganisms, and fermentable carbohydrates from tooth surfaces, thereby reducing bacterial colonization and limiting substrate availability for acid production[7,14]. Reduced salivary flow, as observed in dehydration, systemic diseases, or medication-induced xerostomia, prolongs sugar clearance time and significantly increases caries susceptibility[14].

The buffering capacity of saliva is another major determinant of caries resistance. Following carbohydrate ingestion, cariogenic microorganisms metabolize sugars to produce organic acids that lower plaque pH below the critical threshold for enamel dissolution. Salivary bicarbonate serves as the principal buffering system in stimulated saliva, whereas phosphate and protein buffers contribute predominantly in unstimulated saliva. These buffering mechanisms neutralize bacterial acids, restore plaque pH, and reduce the duration of demineralization episodes[6,15]. Several clinical studies have reported significantly lower salivary pH and buffering capacity in children with early childhood caries compared with caries-free controls, supporting their role in disease susceptibility[15].

Saliva also serves as the primary source of calcium, phosphate, and fluoride ions required for enamel remineralization. During acid attacks, hydroxyapatite crystals dissolve, releasing calcium and phosphate from enamel. Once plaque pH returns to neutral, these ions, together with fluoride, are redeposited onto partially demineralized enamel surfaces, promoting remineralization and increasing resistance to future acid challenges[16]. Salivary proteins such as statherin and proline-rich proteins further stabilize calcium and phosphate ions in solution, preventing their spontaneous precipitation and facilitating mineral homeostasis[16].

An equally important function of saliva is its contribution to innate and adaptive immune defense. Saliva contains numerous antimicrobial proteins and peptides that inhibit microbial colonization and regulate the composition of the oral microbiome. These include lysozyme, lactoferrin, histatins, cystatins, defensins, cathelicidins, peroxidases, agglutinins, and mucins, each possessing distinct antimicrobial mechanisms[5,7]. Lysozyme hydrolyzes bacterial cell walls, lactoferrin sequesters iron essential for bacterial growth, while histatins and defensins exhibit broad-spectrum antimicrobial activity against bacteria and fungi, including Candida albicans[5,17].

Secretory immunoglobulin A (sIgA) is the predominant immunoglobulin in saliva and represents the principal component of adaptive mucosal immunity. Secretory IgA inhibits bacterial adhesion to tooth surfaces, promotes agglutination of microorganisms, neutralizes bacterial toxins, and interferes with biofilm formation without inducing excessive inflammatory responses[17]. Several investigations have demonstrated altered salivary sIgA concentrations in children with ECC, although reported findings remain inconsistent because of differences in study populations, disease severity, saliva collection methods, and laboratory techniques[5,15].

Saliva also contributes to regulation of the oral microbial ecosystem by maintaining a balanced relationship between commensal and pathogenic microorganisms. Under healthy conditions, salivary antimicrobial factors help preserve microbial diversity and suppress excessive proliferation of acidogenic and aciduric species. Frequent sugar consumption and repeated acid exposure disrupt this ecological balance, favouring cariogenic microorganisms such as Streptococcus mutans, Streptococcus sobrinus, Scardovia wiggsiae, and Lactobacillus species. This ecological shift results in sustained acid production, prolonged reductions in plaque pH, and progressive demineralization of enamel[1,5].

Recent advances in salivary proteomics, metabolomics, microbiomics, and transcriptomics have demonstrated that saliva reflects dynamic molecular changes occurring during the earliest stages of caries development[5,11]. Alterations in protein expression, inflammatory mediators, oxidative stress markers, microbial composition, metabolites, and extracellular nucleic acids may precede clinically detectable lesions, making saliva an attractive medium for early disease prediction[11,13]. High-throughput analytical techniques, including liquid chromatography–mass spectrometry, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, nuclear magnetic resonance spectroscopy, and next-generation sequencing, now permit simultaneous evaluation of hundreds of potential biomarkers from small salivary samples[13].

Evidence from recent systematic reviews indicates that several physical and chemical characteristics of saliva,including salivary flow rate, pH, buffering capacity, calcium concentration, phosphate concentration, and selected immune components,differ significantly between children with ECC and caries-free children. However, considerable methodological heterogeneity among studies currently limits the establishment of universal diagnostic thresholds for these parameters[15]. Consequently, current research increasingly focuses on combining multiple microbial, immunological, proteomic, and metabolomic biomarkers into integrated diagnostic panels rather than relying on a single salivary marker[5].

Collectively, these findings establish saliva as far more than a passive oral secretion. It is an active biological fluid that continuously participates in enamel protection, microbial regulation, immune surveillance, tissue repair, and maintenance of oral homeostasis. The complex molecular composition of saliva provides an excellent foundation for the discovery of reliable biomarkers capable of identifying children at increased risk of early childhood caries before irreversible clinical damage becomes evident.

EARLY CHILDHOOD CARIES: ETIOLOGY AND PATHOGENESIS

Early childhood caries (ECC) is a multifactorial, dynamic, biofilm-mediated disease resulting from the interaction of biological, behavioral, environmental, and socioeconomic factors over time[1,3]. Rather than being caused by a single microorganism or isolated risk factor, ECC develops through a complex imbalance between pathological and protective factors within the oral environment. The contemporary understanding of dental caries is based on the ecological plaque hypothesis, which proposes that frequent exposure to fermentable carbohydrates induces ecological shifts within the dental biofilm, favoring the proliferation of acidogenic and aciduric microorganisms capable of sustaining a cariogenic environment[18]. These microbial and environmental changes lead to repeated episodes of enamel demineralization that, if not adequately counteracted by remineralization, progress to cavitation and tooth destruction.

Microbial Factors

The oral cavity harbors one of the most diverse microbial ecosystems in the human body, containing more than 700 bacterial species. Under healthy conditions, these microorganisms exist in a balanced ecological community that contributes to oral health. However, frequent sugar intake and poor oral hygiene alter the composition of the dental biofilm, increasing the abundance of cariogenic microorganisms[19].

Among these microorganisms, Streptococcus mutans remains the most extensively studied pathogen associated with ECC. S. mutans possesses several virulence characteristics that promote caries development, including strong adherence to enamel, production of extracellular polysaccharides through glucosyltransferase enzymes, efficient metabolism of dietary sugars, acid production (acidogenicity), and the ability to survive in acidic environments (aciduricity)[20]. These properties enable S. mutans to dominate mature cariogenic biofilms and maintain prolonged acidic conditions that favor enamel dissolution.

Although S. mutans is considered a principal cariogenic organism, contemporary molecular studies have demonstrated that ECC is associated with a polymicrobial community rather than a single pathogen. Organisms including Streptococcus sobrinus, Scardovia wiggsiae, Veillonella species, Prevotella species, Actinomyces species, and Lactobacillus species have also been implicated in disease progression[21]. High-throughput sequencing technologies have further revealed that microbial diversity changes considerably during ECC development, supporting the concept that dysbiosis of the oral microbiome, rather than the presence of one specific microorganism, underlies disease initiation.

Increasing evidence also supports the contribution of fungal organisms, particularly Candida albicans, to ECC. C. albicans interacts synergistically with S. mutans, enhancing biofilm formation, extracellular polysaccharide production, and acid tolerance. These interactions increase biofilm virulence and accelerate tooth demineralization, particularly in severe forms of ECC[22].

Dietary Factors

Dietary sugars represent the primary environmental driver of ECC. The World Health Organization recommends limiting free sugar intake because frequent consumption significantly increases caries risk[23]. Sucrose is considered

the most cariogenic carbohydrate because it serves as a substrate not only for acid production but also for synthesis of extracellular glucans that strengthen bacterial adhesion and biofilm architecture[24].

Repeated intake of sugary foods and beverages maintains a low plaque pH for prolonged periods. Each episode of sugar consumption initiates bacterial fermentation, producing organic acids such as lactic acid, acetic acid, and formic acid. When plaque pH falls below the critical threshold of approximately 5.5 for enamel, hydroxyapatite crystals dissolve, initiating mineral loss[16]. Frequent sugar exposure prevents adequate remineralization between acid attacks, resulting in cumulative enamel destruction.

Night-time bottle feeding with milk or sugar-containing beverages, prolonged breastfeeding combined with frequent sugar exposure after tooth eruption, inappropriate feeding practices, and frequent snacking have all been associated with increased ECC prevalence[3,23]. These behaviors are particularly detrimental because salivary flow decreases during sleep, reducing the natural buffering and cleansing capacity of saliva.

Host Factors

Host susceptibility plays an equally important role in ECC development. Salivary flow rate, buffering capacity, antimicrobial activity, enamel composition, tooth morphology, eruption timing, and immune responses all influence an individual's resistance to dental caries[6,16].

Children with reduced salivary flow or impaired buffering capacity experience prolonged acid exposure, delayed clearance of dietary sugars, and diminished remineralization potential. Similarly, developmental enamel defects, enamel hypoplasia, and hypomineralization increase plaque retention and create surfaces that are more susceptible to acid dissolution[25].

Host immune responses also contribute to disease progression. Salivary antimicrobial proteins such as lactoferrin, lysozyme, histatins, defensins, and secretory immunoglobulin A regulate microbial colonization and biofilm composition[17]. Variations in the concentration or activity of these defense molecules may alter susceptibility to cariogenic microorganisms and influence ECC risk.

Behavioral and Environmental Risk Factors

Behavioral factors strongly influence the development of ECC. Inadequate tooth brushing, delayed initiation of oral hygiene, infrequent use of fluoride toothpaste, irregular dental visits, poor parental oral health knowledge, and unfavorable dietary habits substantially increase disease risk[3,23].

Parental oral health also influences microbial transmission. Vertical transmission of S. mutans from caregivers to infants occurs primarily through saliva-sharing activities such as sharing utensils, cleaning pacifiers with the mouth, or kissing. Early colonization by cariogenic bacteria increases the likelihood of ECC, particularly when accompanied by frequent sugar exposure[20].

Socioeconomic determinants including low household income, limited access to dental care, lower parental education, food insecurity, and inadequate community preventive programs further contribute to disease burden. These social determinants frequently interact with biological risk factors, explaining the disproportionately high prevalence of ECC among disadvantaged populations[3].

Demineralization–Remineralization Balance

Dental enamel is continuously subjected to alternating cycles of demineralization and remineralization. Under physiological conditions, these processes remain in equilibrium. Following sugar consumption, bacterial acid production lowers plaque pH and promotes dissolution of hydroxyapatite crystals. Once salivary buffering restores neutral pH, calcium, phosphate, and fluoride ions facilitate remineralization of partially dissolved enamel[16].

ECC develops when repeated acid challenges outweigh the protective capacity of saliva and fluoride, producing a persistent net loss of mineral. Initially, this process manifests clinically as non-cavitated white spot lesions, which remain reversible if appropriate preventive measures are instituted. Continued acid exposure eventually results in cavitation extending into dentin and, ultimately, pulpal involvement[18].

Molecular Basis of ECC

Recent advances in omics technologies have significantly enhanced understanding of ECC pathogenesis. Genomic, transcriptomic, proteomic, metabolomic, and microbiomic analyses demonstrate that disease progression is accompanied by coordinated alterations in microbial gene expression, host inflammatory pathways, protein secretion, metabolic products, and immune responses[5,13].

Inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), matrix metalloproteinases, oxidative stress markers, and specific metabolites have been found at

altered concentrations in saliva from children with ECC[5]. These molecular alterations often precede clinically detectable lesions and provide the biological rationale for investigating salivary biomarkers as tools for early diagnosis and individualized caries risk assessment.

A comprehensive understanding of ECC pathogenesis is fundamental for interpreting salivary biomarker research. Because dental caries arises from complex interactions between microorganisms, host defense mechanisms, diet, environmental influences, and immune responses, no single biomarker is likely to provide sufficient diagnostic accuracy. Consequently, current research increasingly focuses on identifying multimarker salivary panels that reflect multiple aspects of disease biology and improve the prediction of ECC susceptibility.

Saliva as a Diagnostic Fluid

The concept of saliva as a diagnostic fluid has evolved considerably over the past two decades owing to advances in molecular biology, analytical chemistry, and high-throughput "omics" technologies. Traditionally regarded merely as a lubricant for the oral cavity, saliva is now recognized as a valuable biological fluid capable of reflecting both local oral conditions and systemic physiological changes[10,13]. Because saliva contains numerous proteins, nucleic acids, microorganisms, metabolites, hormones, enzymes, antibodies, cytokines, and electrolytes, it has emerged as an attractive medium for disease diagnosis, prognosis, therapeutic monitoring, and personalized medicine[26].

Unlike blood collection, saliva sampling is non-invasive, painless, inexpensive, and can be performed repeatedly without causing significant discomfort or anxiety. These advantages are particularly important in pediatric dentistry, where patient cooperation is often limited and invasive procedures may negatively influence future dental behaviour[27]. Saliva collection generally requires minimal equipment, poses virtually no risk of needle-stick injuries or transmission of blood-borne infections, and can be performed in community settings, schools, and epidemiological surveys without specialized personnel[10]. Consequently, salivary diagnostics have gained increasing attention as an alternative to conventional laboratory investigations, especially in children.

Human saliva represents a complex mixture of secretions from the major salivary glands (parotid, submandibular, and sublingual glands) and numerous minor salivary glands, together with gingival crevicular fluid, oral microorganisms, exfoliated epithelial cells, leukocytes, food debris, and microbial metabolites[6,13]. This diverse composition enables saliva to provide comprehensive information regarding host immune responses, microbial ecology, metabolic activity, inflammatory status, and tissue homeostasis. Changes occurring during the development of dental caries are therefore reflected in salivary composition, making saliva an ideal source for biomarker discovery[5].

One of the principal advantages of saliva is that it contains biomarkers derived from both the host and the oral microbiome. Host-derived biomarkers include cytokines, immunoglobulins, antimicrobial peptides, enzymes, hormones, oxidative stress markers, proteins, extracellular vesicles, messenger RNA (mRNA), microRNA (miRNA), and DNA fragments[13,26]. Simultaneously, saliva contains microbial biomarkers such as cariogenic bacteria, fungi, bacterial DNA, microbial proteins, and metabolic products that directly reflect alterations within the oral biofilm[5]. The simultaneous evaluation of host and microbial biomarkers provides a comprehensive understanding of disease activity and has the potential to improve diagnostic accuracy compared with individual biomarkers.

Saliva collection methods are broadly classified as unstimulated and stimulated saliva sampling. Unstimulated whole saliva is obtained without external stimulation and primarily reflects basal glandular secretion. Stimulated saliva is collected following mechanical stimulation (chewing paraffin wax or gum base) or gustatory stimulation using acidic solutions such as citric acid[28]. Unstimulated saliva is generally preferred for biomarker studies because it more closely represents the physiological oral environment and avoids dilution or compositional changes induced by stimulation[29]. Nevertheless, stimulated saliva provides larger sample volumes and may be useful when greater quantities of biological material are required for laboratory analysis.

Standardization of saliva collection is essential because multiple physiological and environmental factors influence salivary composition. Circadian rhythm, age, sex, hydration status, dietary intake, physical activity, medications, emotional stress, oral hygiene practices, systemic diseases, and recent food consumption may significantly alter salivary flow rate and biomarker concentrations[30]. Consequently, most research protocols recommend collecting saliva in the morning after an overnight fast or at least one to two hours after eating, drinking, tooth brushing, or using mouthwash. Participants are also instructed to avoid vigorous physical activity immediately before sample collection to minimize biological variability[29,30].

Following collection, appropriate sample processing and storage are critical for preserving biomarker integrity. Saliva samples are typically centrifuged to remove cellular debris and microorganisms before aliquoting and storage at −80°C until analysis[26]. Repeated freeze-thaw cycles should be avoided because they may degrade proteins, enzymes, cytokines, and nucleic acids, potentially affecting analytical accuracy. Standard operating procedures for sample collection, transport, storage, and processing are therefore essential to ensure reproducibility among studies[29].

Recent technological advances have substantially expanded the diagnostic capabilities of saliva. Proteomic technologies such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and two-dimensional gel electrophoresis enable identification and quantification of thousands of salivary proteins[13]. Similarly, metabolomic analyses using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry characterize numerous low-molecular-weight metabolites associated with bacterial metabolism and host physiological responses[31]. Next-generation sequencing technologies have revolutionized microbiome research by enabling comprehensive characterization of bacterial, fungal, and viral communities present within saliva, while transcriptomic analyses permit evaluation of messenger RNA and regulatory microRNAs associated with disease progression[32].

In addition to laboratory-based techniques, considerable progress has been made in developing chairside salivary diagnostic devices. Point-of-care biosensors, microfluidic platforms, lab-on-a-chip systems, electrochemical sensors, optical biosensors, and nanotechnology-based diagnostic devices are increasingly capable of detecting multiple biomarkers rapidly using very small saliva samples[33]. These technologies have the potential to facilitate real-time caries risk assessment during routine dental appointments and may ultimately support personalized preventive strategies for children at high risk of developing ECC.

Despite these advantages, several limitations continue to restrict widespread clinical implementation of salivary diagnostics. Biomarker concentrations vary considerably between individuals and may be influenced by biological variability, sample contamination, collection techniques, storage conditions, and analytical methodologies[30]. Furthermore, many biomarkers lack standardized reference ranges, and differences among published studies frequently arise from heterogeneous patient populations, varying disease definitions, and inconsistent laboratory protocols[5]. These methodological challenges complicate comparison of results across studies and limit translation into routine clinical practice.

An additional challenge is that ECC is a multifactorial disease involving complex interactions between host susceptibility, oral microbiota, dietary habits, environmental influences, and socioeconomic determinants. Consequently, reliance on a single salivary biomarker is unlikely to provide sufficient diagnostic accuracy. Increasing evidence suggests that combining microbial, immunological, proteomic, metabolomic, and inflammatory biomarkers into integrated diagnostic panels offers superior sensitivity and specificity compared with individual biomarkers[5,11]. Such multimarker approaches align with the principles of precision dentistry, enabling individualized prediction of disease risk and targeted preventive interventions.

Overall, saliva fulfills many characteristics of an ideal diagnostic fluid. It is readily accessible, non-invasive, economical, biologically informative, and compatible with modern molecular technologies. Continued advances in biomarker discovery, analytical techniques, and point-of-care diagnostics are expected to further strengthen the role of salivary diagnostics in pediatric dentistry. However, rigorous validation through large multicentre prospective studies remains essential before salivary biomarker panels can be routinely incorporated into clinical management of early childhood caries.

SALIVARY BIOMARKERS ASSOCIATED WITH EARLY CHILDHOOD CARIES

Saliva contains a diverse array of biological molecules that reflect the dynamic interactions between the host, oral microbiota, and the surrounding environment. During the development of early childhood caries (ECC), significant alterations occur in the concentration and activity of various salivary constituents, including microorganisms, antimicrobial proteins, immunoglobulins, cytokines, enzymes, oxidative stress markers, metabolites, and nucleic acids. These alterations provide valuable information regarding disease initiation, progression, and host response, making saliva an attractive medium for non-invasive biomarker discovery[5,11]. However, because ECC is a multifactorial disease, no single biomarker has demonstrated sufficient sensitivity and specificity for routine clinical diagnosis. Current evidence suggests that combining multiple biomarkers into integrated diagnostic panels may provide greater diagnostic accuracy than reliance on individual markers alone[5,11].

1. Microbial Biomarkers

Among all salivary biomarkers, microbial biomarkers have been investigated most extensively because dental caries is fundamentally a biofilm-mediated disease. Salivary microbial analysis provides information regarding the composition of the oral microbiome and the abundance of cariogenic organisms before obvious clinical manifestations appear[5].

Streptococcus mutans

Streptococcus mutans remains the principal microbial biomarker associated with ECC. Its strong ability to adhere to enamel, synthesize extracellular glucans from sucrose, produce organic acids, and survive in acidic environments enables it to play a central role in the initiation and progression of dental caries[20]. Numerous longitudinal and cross-sectional studies have consistently demonstrated significantly higher salivary counts of S. mutans in children with ECC than in caries-free children[5]. Moreover, early colonization by S. mutans, particularly during the first two years of life,

has been associated with increased caries incidence in the primary dentition[20]. Nevertheless, S. mutans alone cannot accurately predict disease because some caries-free children also harbor substantial bacterial loads, whereas certain children with ECC exhibit relatively low bacterial counts[5].

Streptococcus sobrinus
Streptococcus sobrinus frequently coexists with S. mutans and contributes to increased biofilm virulence through enhanced acid production. Several investigations have shown that simultaneous colonization by both organisms is associated with more severe forms of ECC than colonization by S. mutans alone[5]. Consequently, combined detection of these two microorganisms may improve the predictive value of salivary microbial analysis.

Scardovia wiggsiae
Recent molecular studies have identified Scardovia wiggsiae as an emerging cariogenic microorganism strongly associated with severe ECC. This anaerobic bacterium demonstrates remarkable acid tolerance and has been detected frequently in deep dentinal lesions and advanced carious lesions, even in the absence of detectable S. mutans. These observations suggest that S. wiggsiae may contribute to disease progression through mechanisms complementary to those of traditional cariogenic streptococci[5].

Candida albicans
Increasing evidence indicates that Candida albicans is not merely an opportunistic commensal but may actively participate in ECC pathogenesis. Laboratory and clinical studies demonstrate synergistic interactions between C. albicans and S. mutans, resulting in enhanced extracellular polysaccharide production, greater biofilm biomass, increased acidogenicity, and greater resistance to environmental stress[22]. Salivary detection of C. albicans is therefore increasingly considered an adjunctive microbial biomarker for severe ECC.

2. Immunological Biomarkers
Host immune responses are reflected in saliva through changes in immunoglobulins, cytokines, and inflammatory mediators. These biomarkers represent the biological response of the host to microbial colonization and tissue destruction rather than the microorganisms themselves.

Secretory Immunoglobulin A (sIgA)
Secretory immunoglobulin A (sIgA) is the predominant immunoglobulin in saliva and constitutes the first line of adaptive mucosal immunity. It inhibits bacterial adhesion to enamel, promotes microbial agglutination, neutralizes bacterial enzymes and toxins, and interferes with biofilm maturation[17]. However, studies evaluating salivary sIgA in ECC have reported conflicting findings. Some investigators observed significantly higher sIgA concentrations in children with ECC, suggesting a compensatory immune response to increased microbial challenge, whereas others reported reduced concentrations in caries-active children, implying impaired mucosal immunity[5]. These inconsistencies likely reflect differences in age, disease severity, saliva collection methods, and analytical techniques.

Pro-inflammatory Cytokines
Inflammatory cytokines have emerged as promising biomarkers because dental caries induces a local inflammatory response within oral tissues. Elevated salivary concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) have been reported in children with ECC compared with caries-free controls[5]. IL-6, in particular, has shown a strong positive association with caries severity, and recent clinical evidence suggests that salivary IL-6 concentrations increase progressively with increasing numbers of active carious lesions. Nevertheless, larger multicentre studies are required before cytokines can be recommended for routine diagnostic use.

3. Antimicrobial Salivary Proteins
Human saliva contains numerous antimicrobial proteins that regulate the composition of the oral microbiome and protect against microbial colonization. Alterations in these proteins may increase susceptibility to ECC.

Lactoferrin
Lactoferrin is an iron-binding glycoprotein with broad-spectrum antibacterial, antiviral, and antifungal activity. By sequestering iron, lactoferrin inhibits bacterial growth and suppresses proliferation of cariogenic microorganisms such as S. mutans. Experimental studies also demonstrate that lactoferrin disrupts bacterial membranes and interferes with biofilm formation[5]. Although some investigations report lower salivary lactoferrin concentrations in children with ECC, others have observed increased levels, possibly reflecting an adaptive immune response to bacterial challenge. Therefore, lactoferrin appears more valuable as part of a multi-biomarker panel than as an isolated diagnostic marker.

Lysozyme
Lysozyme hydrolyzes peptidoglycan within bacterial cell walls and constitutes an important component of innate oral immunity. Several studies have demonstrated altered salivary lysozyme concentrations in ECC; however, findings

remain inconsistent. Current systematic reviews conclude that although lysozyme shows potential diagnostic value, methodological heterogeneity limits definitive conclusions regarding its clinical utility.

Histatins, Defensins, and Cystatins
Histatins are histidine-rich salivary peptides possessing potent antimicrobial and antifungal properties, particularly against Candida albicans. Defensins exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, whereas cystatins regulate protease activity and contribute to maintenance of enamel integrity. Recent proteomic studies have reported increased defensins and altered histatin expression in severe ECC, while reduced cystatin S concentrations have emerged as one of the more consistently reported proteomic findings in affected children. However, current evidence remains insufficient to establish these proteins as independent diagnostic biomarkers.

4. Enzymatic Biomarkers
Several salivary enzymes have been investigated as indicators of tissue destruction and disease activity. Matrix metalloproteinases (MMPs), particularly MMP-8 and MMP-9, participate in degradation of extracellular matrix proteins and dentinal collagen following bacterial invasion. Elevated salivary MMP concentrations have been reported in children with severe ECC and may reflect ongoing destruction of mineralized dental tissues[2]. Carbonic anhydrase VI, an enzyme involved in salivary buffering, and α-amylase have also been evaluated, although current evidence regarding their diagnostic performance remains inconsistent.

5. Oxidative Stress Biomarkers
Oxidative stress represents an imbalance between reactive oxygen species and antioxidant defense mechanisms. During ECC progression, bacterial metabolism and host inflammatory responses increase oxidative stress within the oral cavity. Elevated salivary malondialdehyde concentrations together with alterations in total antioxidant capacity, superoxide dismutase, glutathione peroxidase, and catalase activity have been reported in children with ECC[2]. Although these biomarkers provide insight into disease pathophysiology, their diagnostic specificity remains limited because oxidative stress is common to numerous inflammatory oral conditions.

6. Metabolomic and Emerging Biomarkers
Advances in metabolomics have enabled comprehensive characterization of small-molecule metabolites present in saliva. Organic acids, amino acids, peptides, and microbial metabolic products differ significantly between children with ECC and healthy controls, reflecting alterations in bacterial metabolism and host physiology[31]. More recently, salivary microRNAs, extracellular vesicles, exosomes, and cell-free nucleic acids have emerged as novel candidate biomarkers because of their stability and regulatory roles in immune responses and tissue homeostasis. While preliminary findings are promising, these biomarkers remain largely experimental and require extensive validation before clinical application[13,26].

Overall, current evidence indicates that salivary biomarkers provide valuable insights into the biological processes underlying ECC. However, systematic reviews consistently conclude that no single biomarker demonstrates sufficient diagnostic performance for routine clinical use. Future research should therefore focus on standardized methodologies and multimarker diagnostic panels integrating microbial, immunological, proteomic, and metabolomic biomarkers to improve the early prediction and prevention of ECC.

CLINICAL APPLICATIONS, CURRENT CHALLENGES, AND FUTURE PERSPECTIVES

The growing body of evidence supporting salivary biomarkers has shifted the focus of pediatric dentistry from disease detection toward disease prediction and prevention. Traditional diagnostic methods identify carious lesions only after visible demineralization or cavitation has occurred. In contrast, salivary biomarkers have the potential to identify children at increased risk of developing early childhood caries (ECC) before irreversible structural damage becomes clinically evident[5,11]. Such an approach aligns with the principles of predictive, preventive, personalized, and minimally invasive dentistry, which emphasize early intervention and individualized patient care[9].

One of the most promising clinical applications of salivary biomarkers is **caries risk assessment**. Current caries risk assessment models rely primarily on clinical examination, dietary history, fluoride exposure, oral hygiene practices, socioeconomic factors, and previous caries experience. Although these factors remain valuable, they provide limited information regarding the underlying biological processes driving disease development. The incorporation of salivary microbial, immunological, and biochemical biomarkers may improve the accuracy of risk prediction by identifying biological changes that precede the appearance of clinical lesions[5].

Salivary biomarker analysis may also facilitate early diagnosis of ECC. Elevated levels of cariogenic microorganisms, inflammatory cytokines, and oxidative stress markers may indicate an active disease process before radiographic or clinical evidence becomes apparent[5,15]. Early identification of high-risk children would allow clinicians to

implement preventive interventions such as dietary counselling, fluoride therapy, fissure sealants, oral hygiene reinforcement, and regular recall visits before extensive restorative treatment becomes necessary.

Another important application is monitoring disease progression and treatment outcomes. Changes in salivary biomarker profiles following preventive or restorative treatment may reflect reductions in cariogenic bacterial load, improvement in oral homeostasis, or decreased inflammatory activity. Consequently, serial salivary analysis could potentially serve as an objective method for evaluating treatment effectiveness and monitoring patient compliance with preventive recommendations[11].

Advances in biosensor technology have further accelerated the translation of salivary diagnostics into clinical practice. Point-of-care diagnostic devices, microfluidic platforms, electrochemical biosensors, and lab-on-a-chip technologies are capable of detecting multiple salivary biomarkers rapidly using very small sample volumes[33]. These portable devices offer several advantages in pediatric dentistry, including rapid analysis, minimal patient discomfort, reduced laboratory dependence, and the potential for chairside decision-making. Although many of these technologies remain under investigation, continued improvements in sensitivity, specificity, and affordability may facilitate their routine clinical use in the near future[26].

Artificial intelligence (AI) and machine learning are emerging as valuable tools for analyzing complex salivary biomarker datasets. Because ECC is influenced by multiple interacting biological and environmental factors, AI algorithms can integrate microbial profiles, protein expression patterns, metabolomic data, clinical findings, dietary habits, and demographic variables to generate individualized caries risk predictions. Preliminary studies suggest that machine learning models may outperform conventional statistical methods when multiple biomarkers are evaluated simultaneously. However, larger prospective studies are required before AI-assisted salivary diagnostics can be integrated into routine pediatric dental practice.

Despite these encouraging developments, several important challenges continue to limit the clinical application of salivary biomarkers. One of the greatest limitations is the lack of standardized saliva collection and processing protocols. Variations in stimulated versus unstimulated saliva, collection time, fasting status, sample handling, storage conditions, and analytical methods contribute to significant differences in reported biomarker concentrations across studies[28–30]. Establishment of internationally standardized protocols is therefore essential to improve reproducibility and facilitate comparison between studies.

Another limitation is the biological variability of saliva. Salivary composition is influenced by age, sex, circadian rhythm, hydration status, dietary intake, oral hygiene practices, medications, systemic diseases, and developmental stage[6,30]. These variables may affect biomarker concentrations independently of caries activity, thereby reducing diagnostic specificity. Future investigations should therefore incorporate appropriate adjustment for potential confounding factors and include larger, well-characterized study populations.

Current evidence also indicates that no single salivary biomarker possesses sufficient diagnostic accuracy for independent clinical use. Although biomarkers such as Streptococcus mutans, IL-6, secretory IgA, lactoferrin, matrix metalloproteinases, and oxidative stress markers demonstrate significant associations with ECC, their reported sensitivity and specificity vary considerably among studies[5,15]. Consequently, most experts advocate the development of multimarker diagnostic panels that combine microbial, immunological, proteomic, and metabolomic biomarkers to improve diagnostic performance.

Future research should prioritize large multicentre longitudinal studies involving diverse pediatric populations to validate candidate biomarkers and establish clinically relevant reference ranges. Integration of high-throughput omics technologies,including proteomics, metabolomics, microbiomics, transcriptomics, and genomics,with artificial intelligence and advanced bioinformatics may facilitate the identification of robust biomarker signatures capable of accurately predicting ECC before clinical disease develops. Furthermore, the development of inexpensive chairside biosensors capable of simultaneously detecting multiple biomarkers may enable routine biological caries risk assessment during pediatric dental examinations.

In summary, salivary biomarker research has significantly advanced our understanding of the biological mechanisms underlying ECC and offers promising opportunities for improving early diagnosis, individualized risk assessment, disease monitoring, and preventive care. However, further standardization, rigorous clinical validation, and technological refinement are required before salivary biomarker testing can become an integral component of routine pediatric dental practice.

CONCLUSION

Early childhood caries remains one of the most prevalent chronic diseases affecting children worldwide and continues to impose substantial clinical, social, and economic burdens despite advances in preventive dentistry. The multifactorial

nature of ECC, involving complex interactions among cariogenic microorganisms, host immune responses, dietary habits, saliva, and environmental factors, necessitates the development of more sensitive and predictive diagnostic approaches than conventional clinical examination alone. In this context, saliva has emerged as an ideal diagnostic medium because of its non-invasive collection, ease of sampling, cost-effectiveness, and rich molecular composition.
Current evidence demonstrates that numerous salivary biomarkers,including microbial species, immunoglobulins, inflammatory cytokines, antimicrobial proteins, enzymes, oxidative stress markers, metabolites, and nucleic acids,are associated with the initiation and progression of ECC. Among these, Streptococcus mutans, Streptococcus sobrinus, Scardovia wiggsiae, secretory immunoglobulin A, interleukin-6, lactoferrin, matrix metalloproteinases, and oxidative stress markers have shown particularly promising diagnostic potential. Nevertheless, considerable heterogeneity among published studies regarding patient characteristics, saliva collection protocols, analytical methodologies, and biomarker quantification has limited their translation into routine clinical practice.

Current research increasingly supports the concept that ECC cannot be accurately predicted by any single biomarker. Rather, multimarker diagnostic panels integrating microbial, immunological, proteomic, metabolomic, and genetic biomarkers are likely to provide greater diagnostic accuracy and improve individualized caries risk assessment. Furthermore, recent advances in omics technologies, biosensor development, microfluidic devices, and artificial intelligence have created new opportunities for rapid chairside salivary diagnostics that may facilitate earlier intervention and more personalized preventive strategies.

Future investigations should focus on large-scale multicentre longitudinal studies to validate candidate biomarkers, establish standardized saliva collection and analytical protocols, and determine clinically meaningful diagnostic thresholds. Integration of salivary biomarker analysis into evidence-based caries risk assessment models may ultimately transform pediatric dental practice from a predominantly restorative discipline to one centered on prediction, prevention, and precision oral healthcare.

In conclusion, salivary biomarkers represent one of the most promising advances in contemporary pediatric dentistry. Although further validation is required before routine clinical implementation, continued progress in biomarker discovery and diagnostic technologies has the potential to significantly improve the early detection, prevention, and management of early childhood caries, thereby enhancing long-term oral health outcomes for children.

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