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SALIVARY MicroRNAs AS EMERGING BIOMARKERS IN PEDIATRIC HEALTH AND DISEASE: A REVIEW

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Abstract

The identification of reliable, non-invasive biomarkers is a priority in pediatric healthcare, where early diagnosis and prevention play a decisive role in long-term outcomes. MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression and participate in multiple physiological and pathological processes. Recent studies have demonstrated that miRNAs are present in saliva in a stable form, making them attractive candidates for pediatric biomarkers, particularly where invasive sampling is ethically and practically challenging. This review summarizes the biological basis of microRNAs, their origin and stability in saliva, methodological considerations for salivary miRNA detection, and their potential applications in pediatric oral and systemic diseases. Ethical concerns, limitations, and future research directions are also discussed. Salivary microRNAs represent a promising tool for non-invasive pediatric diagnostics; however, further validation and standardization are required before routine clinical application.

Keywords: Salivary microRNAs; Pediatric biomarkers; Non-invasive diagnostics; Saliva-based diagnostics; Oralhealth, Systemic health

1. Introduction

Early diagnosis and prevention are central goals of pediatric healthcare. Children represent a biologically distinct population characterized by continuous growth, immune maturation, and developmental plasticity, all of which influence disease expression and biomarker profiles[1]. As a result, biomarkers validated in adults cannot be directly extrapolated to pediatric populations, emphasizing the need for child-specific diagnostic approaches.

MicroRNAs (miRNAs) are short, endogenous, non-coding RNA molecules, approximately 18–25 nucleotides in length, that regulate gene expression at the post-transcriptional level[2]. They play essential roles in cellular differentiation, immune regulation, metabolism, and organ development. Dysregulation of miRNA expression has been associated with inflammatory disorders, metabolic diseases, malignancies, and neurodevelopmental conditions[3]. These characteristics have positioned miRNAs as promising molecular biomarkers.

Advances in molecular biology have demonstrated that miRNAs are detectable not only within cells but also in extracellular body fluids such as blood, urine, and saliva[4]. Among these, saliva has gained increasing attention in pediatric research due to its non-invasive, painless, and cost-effective collection, making it suitable for repeated sampling[5]. Importantly, salivary miRNAs exhibit remarkable stability despite the presence of degradative enzymes, supporting their feasibility as diagnostic biomarkers[6]. This review focuses on the emerging role of salivary microRNAs as biomarkers in pediatric health and disease.

2. Biology of microRNAs and Their Presence in Saliva

MicroRNAs are generated through a multistep biogenesis pathway involving transcription of primary miRNA transcripts, nuclear processing into precursor miRNAs, and cytoplasmic cleavage to form mature miRNAs[2]. Mature miRNAs bind to target messenger RNAs and regulate gene expression by inhibiting translation or promoting degradation.

Extracellular miRNAs demonstrate high stability, which is primarily attributed to their encapsulation within extracellular vesicles such as exosomes or their association with RNA-binding protein complexes[6]. This protection enables miRNAs to persist in harsh extracellular environments, including saliva.

Saliva is a complex biological fluid composed of salivary gland secretions, gingival crevicular fluid, oral epithelial cells, immune cells, and microorganisms. Salivary miRNAs originate from multiple sources, including salivary glands, epithelial cell turnover, inflammatory exudates, and systemic circulation[7]. Consequently, salivary miRNAs may reflect both local oral conditions and systemic physiological or pathological processes. Studies have demonstrated concordance between salivary and circulating miRNA profiles, supporting saliva as a surrogate diagnostic medium[8].The biological sources and pathways contributing to salivary microRNAs are outlined in Table 1.

Source	Mechanism of Entry into Saliva	Diagnostic Significance
Salivary gland cells	Active secretion	Reflects glandular and systemic status
Oral epithelial cells	Cell turnover and apoptosis	Indicates local oral pathology
Gingival crevicular fluid	Inflammatory exudate	Marker of gingival inflammation
Blood circulation	Passive diffusion / vesicle transport	Reflects systemic diseases
Immune cells	Exosome-mediated release	Indicates immune activation

TABLE 1. Sources and Biological Origin of Salivary microRNAs

Primary microRNA (pri-miRNA) is processed into precursor miRNA (pre-miRNA) and mature miRNA, which is packaged into exosomes, released into saliva, and protected from enzymatic degradation(Figues 1).

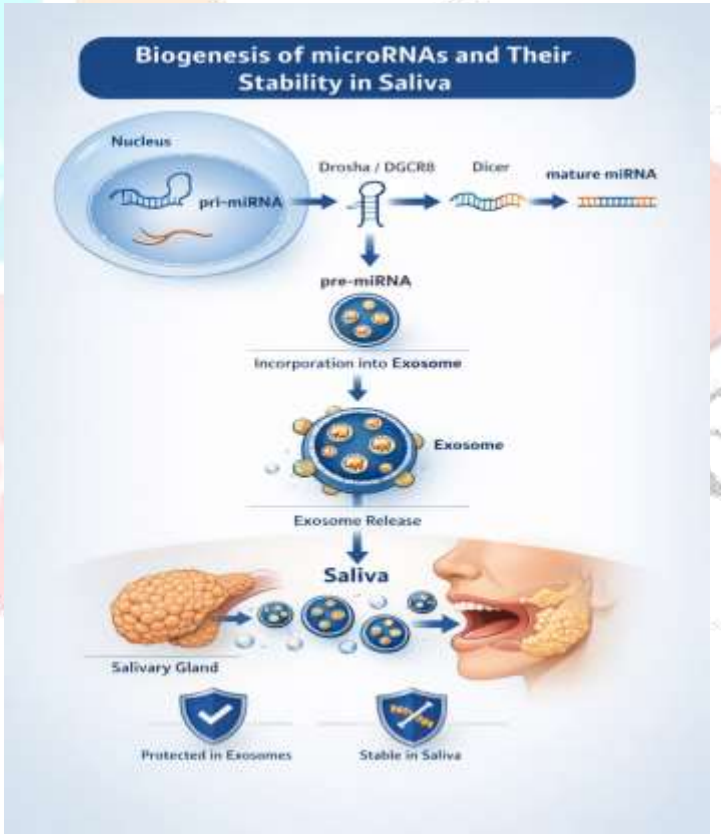

Figure 1. Biogenesis of microRNAs and their stability in saliva

3. Methodological Considerations for Salivary microRNA Detection

Accurate analysis of salivary miRNAs requires standardized protocols for sample collection, processing, RNA extraction, and analysis. Unstimulated whole saliva is generally preferred, as stimulation may alter salivary composition and dilute biomarker concentrations[9]. Factors such as time of collection, fasting status, and recent oral activity should be standardized to minimize variability.

After collection, saliva samples are centrifuged to remove debris and stored at low temperatures to preserve RNA integrity[10]. RNA extraction presents challenges due to low miRNA concentration and the presence of inhibitors; therefore, extraction kits optimized for small RNA isolation are recommended[11].

Common analytical platforms include quantitative reverse transcription polymerase chain reaction (qRT-PCR), microarray analysis, and next-generation sequencing. Among these, qRT-PCR is most widely used due to its sensitivity, specificity, and cost-effectiveness[12]. However, lack of universally accepted endogenous controls for normalization remains a major limitation, particularly in pediatric studies.

4. Clinical Applications of Salivary microRNAs in Pediatric Diseases

4.1 Pediatric Oral Diseases

Dental caries and gingival inflammation are among the most prevalent chronic diseases in children. Conventional diagnostic methods detect these conditions only after clinical damage has occurred. Studies have reported altered salivary miRNA expression in children with early childhood caries, particularly miRNAs involved in immune and inflammatory pathways[13]. Similarly, gingival inflammation has been associated with dysregulated salivary miRNAs related to cytokine signaling and tissue remodeling[14].

These findings suggest that salivary miRNAs may serve as early molecular indicators of oral disease susceptibility and activity, supporting their role in preventive pediatric dentistry.

Salivary microRNAs implicated in pediatric oral diseases are summarized in Table 2.

Pediatric Oral Condition	**Dysregulated miRNAs (examples)**	**Biological Pathway Involved**	**Clinical Utility**
Early childhood caries	miR-21, miR-146a, miR-155	Inflammation, immune regulation	Caries risk assessment
Gingivitis	miR-223, miR-200b	Cytokine signaling	Early inflammation detection
Oral dysbiosis	miR-150, miR-29a	Host–microbe interaction	Preventive monitoring

TABLE 2. Salivary microRNAs Associated with Pediatric Oral Diseases

4.2 Systemic Pediatric Diseases

Beyond oral health, salivary miRNAs have shown potential as biomarkers for systemic pediatric diseases. MiRNAs play a key role in immune regulation, and altered expression has been reported in inflammatory and immune-mediated conditions[3]. In metabolic disorders such as childhood obesity, dysregulated miRNA expression has been linked to insulin resistance and metabolic inflammation[15].

Distinct miRNA signatures have also been identified in pediatric malignancies, and emerging evidence suggests that salivary miRNAs may reflect tumor-associated molecular changes[16]. Additionally, altered miRNA expression has been reported in neurodevelopmental disorders, highlighting the potential of salivary miRNAs as non-invasive biomarkers in conditions where tissue access is limited[17].

Evidence supporting salivary microRNAs in systemic pediatric diseases is summarized in Table 3.

Disease Category	Pediatric Condition	Salivary miRNA Role	Potential Application
Inflammatory	Asthma, allergies	Immune modulation	Disease activity monitoring
Metabolic	Obesity, type 2 diabetes	Metabolic regulation	Early risk identification
Oncology	Leukemia, solid tumors	Tumor-associated signatures	Non-invasive screening
Neurodevelopmental	Autism spectrum disorder	Neural signaling pathways	Early diagnostic aid

TABLE 3. Salivary microRNAs in Systemic Pediatric Diseases

5. Ethical Considerations and Limitations

Ethical considerations are particularly important in pediatric research. Saliva-based sampling offers significant advantages by minimizing physical discomfort and psychological distress compared to invasive procedures[18]. However, challenges remain regarding biological variability related to age, growth, dentition stage, and immune maturation.

Most available studies are cross-sectional and involve small sample sizes, limiting generalizability. The lack of standardized protocols and pediatric-specific reference ranges further restricts clinical translation. Ethical issues related to data privacy and interpretation of predictive biomarker information in children must also be carefully addressed.

6. Discussion

The evidence summarized in this review supports the growing interest in salivary microRNAs as non-invasive biomarkers in pediatric health and disease. The biological characteristics of miRNAs, including their regulatory functions, disease-specific expression patterns, and stability in saliva, provide a strong rationale for their diagnostic potential. In pediatric populations, where invasive sampling is often impractical or ethically challenging, saliva represents a particularly advantageous diagnostic medium.

A key strength of salivary miRNAs lies in their ability to reflect both local oral pathology and systemic disease processes. Altered salivary miRNA expression has been associated with pediatric oral diseases such as early childhood caries and gingival inflammation, as well as systemic conditions including inflammatory disorders, metabolic dysfunction, malignancies, and neurodevelopmental abnormalities. This dual representativeness reinforces the concept of saliva as a mirror of overall health.

Despite these promising findings, several limitations hinder clinical implementation. Methodological heterogeneity across studies, including differences in saliva collection, RNA extraction, analytical platforms, and normalization strategies, limits reproducibility and comparability. These challenges are amplified in pediatric research due to age-related biological variability. Furthermore, the predominance of cross-sectional studies restricts understanding of the predictive and prognostic value of salivary miRNAs over time.

Overall, while salivary microRNAs represent a promising diagnostic avenue, their routine clinical use in pediatric healthcare will depend on standardized methodologies, longitudinal validation, and ethical integration.

7. Future Perspectives and Conclusion

Salivary microRNAs represent a promising frontier in pediatric diagnostics due to their non-invasive nature, biological relevance, and stability. Advances in molecular technologies and data analysis are expected to enhance their diagnostic accuracy and clinical applicability. However, before routine clinical implementation, further research is required to establish standardized protocols, pediatric reference ranges, and longitudinal validation. With continued investigation, salivary microRNAs may contribute significantly to early diagnosis, prevention, and personalized pediatric healthcare.

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