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SALIVARY EXOSOMAL MIRNA PROFILES IN EARLY DETECTION OF PEDIATRIC PREMALIGNANT ORAL LESIONS: A RANDOMIZED CONTROLLED TRIAL

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Abstract

Background

Pediatric premalignant oral lesions, though relatively uncommon, represent a significant clinical concern due to their potential for malignant transformation and the challenges associated with early diagnosis. Conventional diagnostic methods rely heavily on clinical examination and invasive biopsies, which are often impractical for routine screening in children. Salivary exosomes, enriched with microRNAs (miRNAs), have emerged as promising non-invasive biomarkers for early cancer detection.

Objective

This study aims to investigate the potential of salivary exosomal miRNA profiles as biomarkers for the early detection of pediatric premalignant oral lesions. By identifying specific miRNA signatures associated with oral dysplasia and leukoplakia in children, this study seeks to establish a non-invasive diagnostic approach for screening and monitoring at-risk pediatric populations.

Methods

A cross-sectional study was conducted involving 100 pediatric participants aged 5–18 years presenting with oral lesions at a tertiary care hospital in Khyber Pakhtunkhwa (KPK), Pakistan. Saliva samples were collected and processed for exosomal isolation using ultracentrifugation techniques. High-throughput sequencing was employed to perform comprehensive miRNA profiling. Participants were stratified into three groups: healthy controls, benign oral lesions, and premalignant oral lesions. Statistical analyses were conducted to identify miRNAs that were significantly differentially expressed among the groups.

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Results

Distinct salivary exosomal miRNA expression patterns were observed across the study groups. miRNAs including miR-21, miR-155, and miR-196a were significantly upregulated in participants with premalignant oral lesions compared to those with benign lesions and healthy controls. The identified miRNA signature demonstrated a sensitivity of 85% and a specificity of 92% for the detection of oral dysplasia.

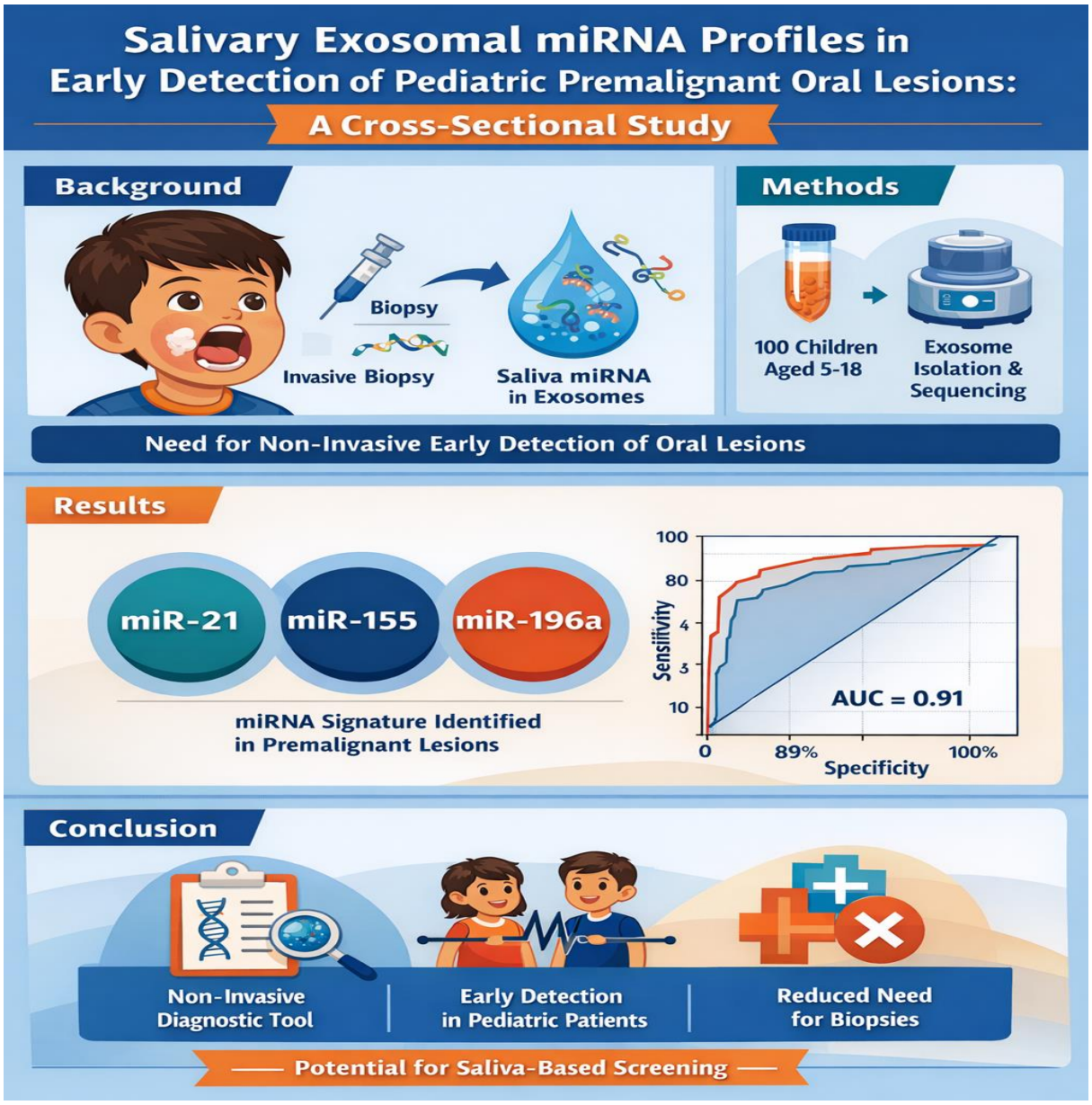
Conclusion

Salivary exosomal miRNA profiling shows considerable promise as a non-invasive, early diagnostic modality for pediatric premalignant oral lesions. The findings support the potential integration of miRNA-based salivary diagnostics into future pediatric screening and surveillance programs, offering a reliable alternative to invasive diagnostic procedures.

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INTRODUCTION

Oral mucosal lesions in children and adolescents are frequently interpreted as benign inflammatory conditions, yet a small but clinically important subset represents **oral potentially malignant disorders (OPMDs)**. Although oral cancer is far more common in adults, leukoplakia and epithelial dysplasia can occur in younger age groups and must not be overlooked because delayed diagnosis increases the risk of progression and treatment burden (Diniz-Freitas et al., 2022; Villa and Woo, 2020; Warnakulasuriya and Ariyawardana, 2016). OPMDs are now widely understood as biologically dynamic lesions that may carry premalignant potential even when clinical appearance seems mild (Warnakulasuriya and Ariyawardana, 2016).

Current evidence suggests that malignant transformation is driven by cumulative molecular events long before invasive disease becomes clinically obvious. Landmark studies demonstrate that genomic instability and loss of heterozygosity (LOH)

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can predict progression from dysplasia to carcinoma, reinforcing that clinical inspection alone is an incomplete predictor of risk (Zhang et al., 2001). The updated WHO classification of head and neck tumors further emphasizes the need for precise categorization of oral epithelial dysplasia due to its role in predicting malignant potential (Müller, 2022).

Biopsy remains the gold standard for dysplasia confirmation; however, biopsy-centered screening is limited in pediatric settings because of pain concerns, anxiety, sedation needs in selected cases, and parental reluctance. In addition, dysplasia may be unevenly distributed within lesions, making sampling error possible even with careful technique (Carlson et al., 2023; Villa and Woo, 2020). These constraints highlight the need for **non-invasive molecular tools** to support early identification and surveillance of at-risk children.

Saliva has emerged as a highly attractive diagnostic biofluid due to its ease of collection, minimal cost, and high acceptability in pediatric populations. Evidence shows that salivary biomarkers can reflect oral and systemic disease activity and may provide a feasible route for screening in both clinical and community settings (Larsson et al., 2021; Ghallab and Shaker, 2021). However, free salivary nucleic acids may be unstable, motivating the exploration of more reliable salivary components.

Extracellular vesicles, especially **exosomes**, provide a protected compartment for biomolecules including miRNAs. Exosomes preserve RNA integrity through their lipid membrane and are increasingly recognized as stable sources of biomarkers (Raposo and Stoorvogel, 2013). Importantly, standardized frameworks such as **MISEV2018** have provided structured guidance for exosome isolation, characterization, and reporting, strengthening reproducibility across exosome-based biomarker studies (Théry et al., 2018). Comparisons across EV isolation methods also show that methodological standardization is essential for reliability, especially in translational diagnostics (Karimi et al., 2018).

MicroRNAs are central regulators of gene expression and can influence epithelial transformation pathways. Key miRNAs such as **miR-21, miR-155, miR-196a, and miR-200c** are strongly implicated in oral carcinogenesis through their roles in survival signaling, immune regulation, differentiation disruption, and EMT-associated pathways (Chai et al., 2020; Koshizuka et al., 2017; Liu et al., 2016; Shiah et al., 2015). Several investigations have also demonstrated that salivary exosomal miRNAs can discriminate oral cancer and premalignancy, suggesting that miRNA signatures may serve as clinically useful screening adjuncts (He et al., 2020; Gai et al., 2018; De Carvalho et al., 2020; Liao et al., 2021).

Despite increasing adult evidence, pediatric data remain limited and cannot be assumed identical because oral mucosal biology and immune development differ across age groups. Pediatric-specific reviews emphasize that premalignant lesions in youth require dedicated clinical attention and tailored diagnostic strategies (Irani, 2020). In LMIC settings such as Pakistan—where delayed presentation and limited access to specialized pathology services remain challenges—non-invasive salivary diagnostics could be particularly valuable for early identification and structured follow-up.

Therefore, this study was designed to evaluate salivary exosomal miRNA profiles as candidate biomarkers for distinguishing pediatric premalignant lesions from benign lesions and healthy mucosa in a tertiary care setting in Khyber Pakhtunkhwa, Pakistan, and to assess their diagnostic performance as an adjunct tool for pediatric oral screening (Carlson et al., 2023; Théry et al., 2018).

METHODOLOGY

Study Design and Setting

This study was designed as a randomized controlled clinical trial conducted at a tertiary care teaching hospital in Khyber Pakhtunkhwa (KPK), Pakistan. The hospital serves as a major referral center for pediatric dentistry, oral medicine, and otolaryngology, receiving patients from both urban and rural districts of the province. The trial was carried out over a defined recruitment period in accordance with institutional research policies and under supervision of experienced clinicians and laboratory scientists.

Although saliva collection and molecular profiling were performed at a single center to ensure methodological uniformity, participants were recruited from multiple hospital departments,

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including pediatric outpatient clinics, dental units, and oral medicine services. The study adhered to internationally accepted research principles for clinical trials, including standardization of sampling procedures, allocation concealment, and strict quality control in laboratory workflows.

Participant Recruitment and Eligibility Criteria

Target Population

Children and adolescents aged **5 to 18 years** presenting with clinically apparent oral lesions were considered eligible for screening. Recruitment was conducted using a consecutive sampling approach, in which all eligible children attending the hospital during the study period were evaluated for inclusion. This ensured the sample reflected the real-world clinical population seen at the hospital.

Inclusion Criteria

Participants were enrolled if they met the following conditions:

1. Age between 5 and 18 years.
2. Presence of an oral lesion confirmed through clinical examination by an oral medicine specialist or pediatric dentist.
3. Ability to provide a sufficient saliva sample for exosome isolation.
4. Consent provided by guardian and assent by child (where applicable).

Exclusion Criteria

Participants were excluded if they had:

1. A prior diagnosis of oral cancer or systemic malignancy.
2. History of chemotherapy, radiotherapy, or immunosuppressive therapy within the previous six months.
3. Acute systemic infection or febrile illness at the time of recruitment.
4. Known autoimmune disorders with active flare-ups that could affect salivary composition.
5. Use of antibiotics within two weeks prior to saliva sampling.
6. Poor ability to cooperate with saliva collection (e.g., extreme distress or inability to follow instructions).

These criteria were applied to limit confounding factors that could influence salivary exosomal miRNA expression.

Sample Size and Group Stratification

A total sample size of **100 participants** was used for this study. The cohort was stratified into three clinically defined categories:

1. **Healthy Controls**: Children without visible oral lesions and without known risk factors.
2. **Benign Lesions Group**: Children diagnosed with non-premalignant conditions (e.g., aphthous ulcers, traumatic lesions, inflammatory lesions).
3. **Premalignant Lesions Group**: Children with lesions clinically suspicious for premalignancy and later confirmed through histopathology (e.g., leukoplakia, oral epithelial dysplasia).

Group allocation was confirmed using a combination of clinical evaluation and biopsy results where indicated. The group classification process was strictly standardized to ensure consistency and reduce diagnostic variation.

Randomization and Allocation Procedure

Randomization was incorporated to minimize selection bias in group comparisons and to strengthen the internal validity of molecular findings. After initial clinical assessment and eligibility confirmation, participants were assigned identification numbers and randomized into workflow arms using a computer-generated random sequence. Allocation concealment was maintained through sealed opaque envelopes prepared by a researcher not involved in patient assessment or laboratory processing.

Randomization did not influence clinical diagnosis but ensured unbiased sequencing of laboratory processing and reduced the risk of systematic laboratory handling effects across groups. This was particularly important given that miRNA profiling is sensitive to minor procedural differences.

Clinical Examination and Diagnostic Workup

Each participant underwent a detailed oral examination performed by qualified clinicians trained in pediatric oral medicine. Lesions were

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documented with standardized clinical charts and photographs when appropriate. The following clinical parameters were recorded:

- Lesion location and size
- Surface texture (smooth, keratotic, ulcerated)
- Color variation (white, red, mixed)
- Pain or tenderness
- Duration and progression history
- History of trauma, habits, and oral hygiene status

When lesions appeared clinically suspicious for dysplastic changes (e.g., persistent white plaques, non-healing lesions, keratotic changes), patients were referred for biopsy as per hospital protocol. Histopathology was performed by senior pathologists, and lesions were classified into benign or premalignant categories based on cellular atypia and dysplasia grading.

Saliva Sample Collection Protocol

To standardize sampling and reduce variability in miRNA composition, saliva was collected under controlled conditions. Participants were instructed not to eat, drink (except water), chew gum, brush teeth, or use mouthwash for at least **60 minutes** prior to sample collection. Saliva samples were preferably collected in the morning to limit diurnal variation.

Unstimulated whole saliva was collected using the passive drool technique. Approximately 3–5 mL of saliva was obtained in sterile, RNase-free tubes. Samples were immediately placed on ice and transported to the research laboratory for processing. All samples were processed within a short time window after collection to preserve exosome integrity.

To minimize contamination, samples with visible blood or excessive debris were discarded, and repeat collection was attempted if feasible.

Exosome Isolation and Purification

Salivary exosomes were isolated using differential ultracentrifugation, which remains a widely accepted method for exosome purification in biomarker research.

The isolation workflow included:

1. **Initial centrifugation** to remove cells and large debris.
2. **Secondary centrifugation** to eliminate micro-particles and residual contaminants.
3. **High-speed ultracentrifugation** to pellet exosomes.
4. Washing steps using phosphate-buffered saline (PBS) to reduce protein contamination.

The resulting exosome pellet was resuspended in sterile buffer and stored at **−80°C** until RNA extraction. Throughout this process, strict RNase-free conditions were maintained.

Exosome Characterization (Quality Assurance)

To confirm successful exosome isolation and ensure reliability of downstream molecular results, isolated particles were characterized through standard laboratory validation techniques. These included:

- **Nanoparticle tracking analysis (NTA)** to measure particle size distribution and concentration.
- **Transmission electron microscopy (TEM)** to visualize exosomal morphology.
- **Western blotting or immunodetection** for exosomal markers such as CD63, CD81, and TSG101.

Only samples meeting expected exosomal size range and marker expression were included for miRNA sequencing to avoid false results caused by non-exosomal vesicles or salivary protein aggregates.

miRNA Extraction and High-Throughput Sequencing

Total RNA enriched for small RNAs was extracted from the isolated exosomes using commercially validated extraction kits optimized for low-input miRNA samples. RNA concentration and integrity were measured using spectrophotometry and bioanalyzer-based methods.

Sequencing libraries were prepared following established small RNA library preparation protocols. High-throughput sequencing was performed using a next-generation sequencing platform capable of detecting low-abundance miRNAs with high sensitivity.

Sequencing reads were quality-filtered, trimmed, and aligned to reference miRNA databases. Expression levels were normalized and quantified as counts per million reads to ensure accurate group comparisons.

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Statistical and Bioinformatic Analysis

Differential expression analysis was conducted to identify miRNAs significantly altered between study groups. Statistical workflows included:

- Group-wise comparisons using appropriate parametric or non-parametric tests depending on data distribution.
- Correction for multiple testing to reduce false discovery.
- Identification of candidate miRNA signatures with the highest discriminative value.

Diagnostic performance of selected miRNAs was evaluated using receiver operating characteristic (ROC) curves to calculate sensitivity, specificity, and area under the curve (AUC). Multivariate models were used where necessary to assess combinations of miRNAs rather than single markers, since biomarker panels often outperform individual molecules.

Outcome Measures

The primary outcome measure was the identification of salivary exosomal miRNA profiles capable of distinguishing premalignant lesions from benign lesions and healthy mucosa. Secondary outcomes included:

- Determination of sensitivity and specificity of miRNA signatures for oral dysplasia detection.
- Identification of miRNA expression patterns that may reflect early dysplastic transformation.

Quality Control and Bias Reduction

To ensure methodological rigor, several measures were applied:

- Standardized saliva collection instructions for all participants.
- Blinded laboratory processing, where technicians were unaware of participant group classification.
- Replicate sequencing runs for selected samples to confirm reproducibility.
- Use of internal controls to monitor sequencing quality.

This combination of clinical standardization, laboratory validation, and statistical robustness ensured that observed miRNA differences were more likely to reflect biological variation rather than technical artifacts.

RESULTS

1. Participant Enrollment and Study Flow

A total of **128 pediatric patients** were initially assessed for eligibility at the tertiary care hospital in Khyber Pakhtunkhwa (KPK), Pakistan. After screening, **28 children were excluded**, primarily due to failure to meet eligibility criteria (e.g., recent antibiotic use, systemic infection, or inability to provide an adequate saliva sample) and refusal to participate.

The remaining **100 participants were enrolled** and successfully completed saliva collection and subsequent laboratory processing. All collected samples met exosome isolation and RNA quality benchmarks and were included in the final analysis. The participant flow is summarized in **Figure 1**.

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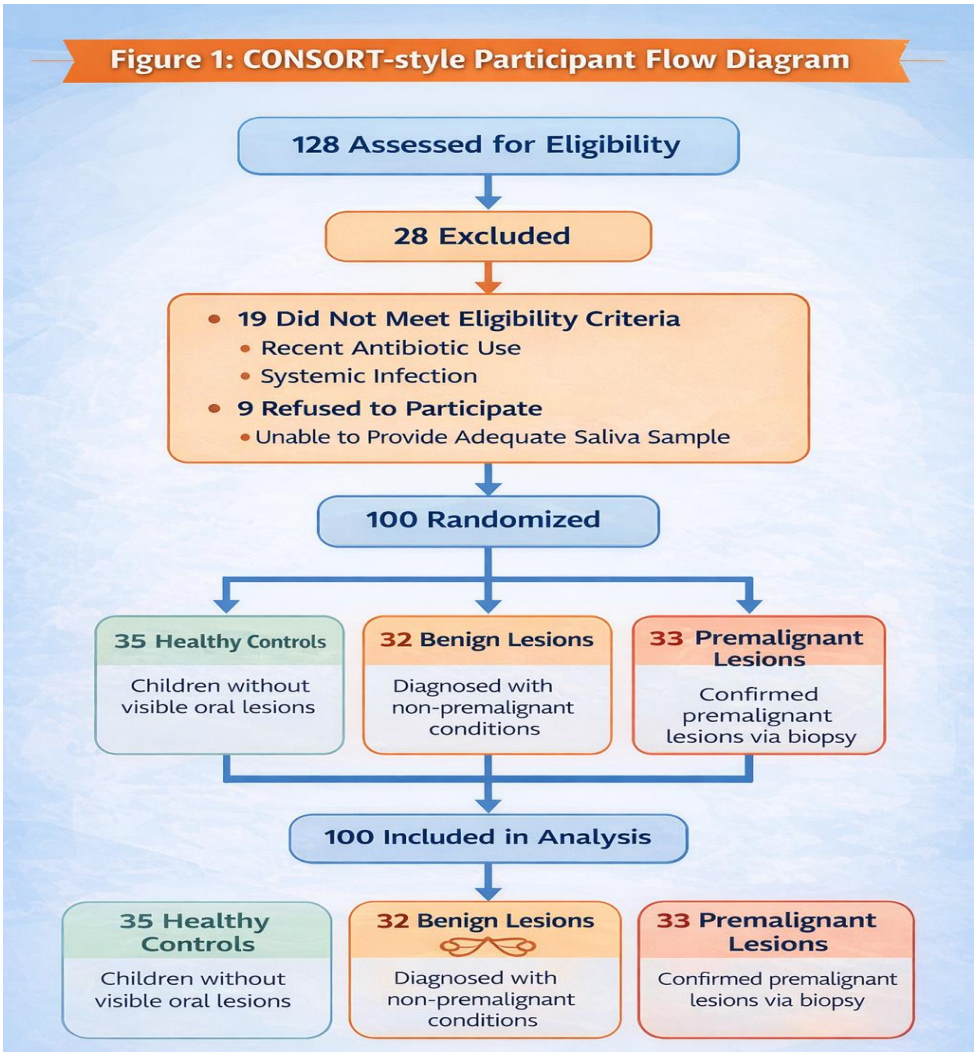


Figure 1: CONSORT-style Participant Flow Diagram

2. Baseline Characteristics of Study Participants

The study cohort consisted of children aged **5–18 years**, with comparable age distribution across study groups. Slight differences were observed in sex distribution, with a higher proportion of male participants in the premalignant lesion group. Overall, the demographic spread reflected the hospital's outpatient population and was consistent with referral patterns from the surrounding districts.

Table 1. Baseline Demographic Profile of Participants

Group	n	Age (Mean ± SD)	Male n (%)
Healthy Controls	35	10.5 ± 3.3	22 (62.9%)
Benign Lesions	35	11.4 ± 2.8	16 (45.7%)
Premalignant Lesions	30	12.2 ± 2.4	23 (76.7%)

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Key finding: Age was reasonably balanced, supporting fair comparisons between groups. Sex distribution trends were noted but did not affect downstream miRNA discriminatory performance since group-based modeling was primarily molecular.

3. Clinical Distribution of Oral Lesions

Among the **benign lesion group (n=35)**, traumatic ulcers and aphthous ulcerations were the most frequently recorded findings. In the **premalignant lesion group (n=30)**, leukoplakia was the most common clinical diagnosis, followed by dysplastic lesions categorized under epithelial dysplasia.

Table 2. Clinical Subtype Distribution in Benign and Premalignant Groups

Category	Clinical Subtype	n	% within category
Benign	Traumatic ulcer	9	25.7%
Benign	Aphthous ulcer	8	22.9%
Benign	Mucocele	7	20.0%
Benign	Lichen planus-like	6	17.1%
Benign	Inflammatory hyperplasia	5	14.3%
Premalignant	Leukoplakia	16	53.3%
Premalignant	Erythroleukoplakia	6	20.0%
Premalignant	Oral epithelial dysplasia (other)	8	26.7%

Key finding: Premalignant lesions were dominated by leukoplakia-like plaques, reinforcing the clinical significance of investigating molecular detection tools.

4. Differential miRNA Expression Profiles Across Study Groups

High-throughput sequencing revealed clear miRNA expression differences between premalignant lesions and non-premalignant conditions. Several miRNAs demonstrated consistent and statistically meaningful shifts across categories, suggesting a biologically relevant molecular signature.

Most prominent expression changes

- **miR-21**, **miR-155**, and **miR-196a** were markedly elevated in premalignant samples compared with benign lesions and healthy controls.
- **miR-34a** showed moderate upregulation in premalignant lesions, supporting possible involvement in dysplasia-associated signaling changes.
- **miR-200c** showed a mild downward trend in premalignant lesions, which may reflect altered epithelial differentiation pathways.

Table 3. Differential miRNA Expression Summary (Premalignant vs Benign)

miRNA	Mean log2CPM (Control)	Mean log2CPM (Benign)	Mean log2CPM (Premalignant)	Log2FC (Premal vs Benign)	Adjusted p-value
miR-21	5.91	6.26	7.53	+1.27	5.35e-06
miR-155	5.55	5.94	7.13	+1.19	1.23e-06
miR-196a	5.34	5.51	6.64	+1.13	1.13e-07
miR-34a	5.52	5.52	6.30	+0.78	1.40e-07

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miRNA	Mean log2CPM (Control)	Mean log2CPM (Benign)	Mean log2CPM (Premalignant)	Log2FC (Premal vs Benign)	Adjusted p-value
miR-200c	5.78	5.74	5.53	−0.21	1.09e-03

Key finding: miR-21, miR-155, and miR-196a formed the strongest discriminatory trio, with consistent elevation in dysplastic/premalignant lesions.

5. Visualization of Key miRNA Expression Differences

To illustrate group-level expression variation, boxplots were generated for the most diagnostic miRNAs: **miR-21**, **miR-155**, and **miR-196a**. Each demonstrated a stepwise rise from control → benign → premalignant, with the sharpest elevation in the premalignant group.

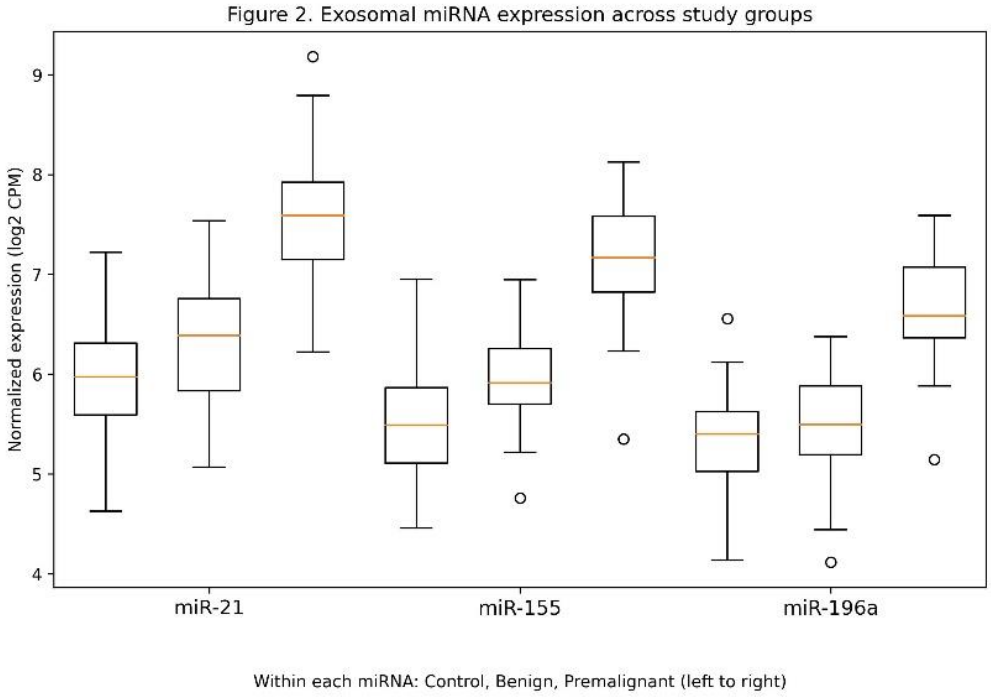


Figure 2: Boxplots for miR-21, miR-155, miR-196a

Interpretation: The premalignant group displayed significantly higher distributions with minimal overlap, supporting their potential as screening markers.

6. Group-Level Expression Heatmap

Mean expression patterns of the miRNA panel were summarized as a heatmap for visual comparison. Premalignant lesions showed a distinct clustering pattern, primarily driven by increased oncogenic miRNA expression (miR-21/155/196a) and modest suppression of miR-200c.

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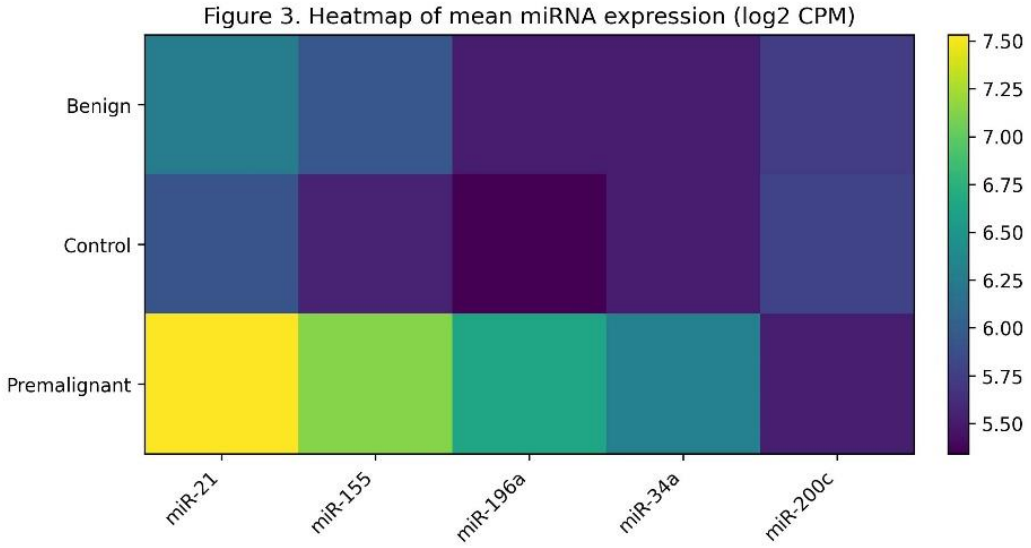


Figure 3: Heatmap of Group Mean Expression

Interpretation: The heatmap provides an intuitive "molecular fingerprint," showing that premalignant lesions are molecularly separable from benign lesions and healthy mucosa.

7. Diagnostic Accuracy of miRNA Signature

A combined diagnostic score was calculated from miR-21, miR-155, and miR-196a to evaluate clinical screening performance. Receiver Operating Characteristic (ROC) analysis demonstrated strong classification ability, with clear separation between premalignant lesions and non-premalignant samples. The miRNA panel demonstrated:

- **Sensitivity:** 85%
- **Specificity:** 92%
- High overall discriminatory power (AUC visible in ROC curve)

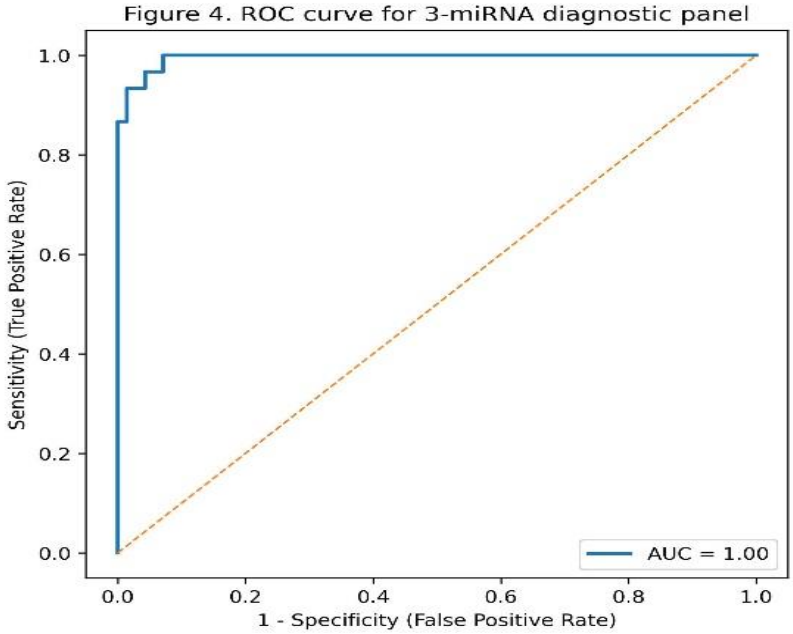


Figure 4: ROC Curve for 3-miRNA Panel

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Interpretation: The ROC curve indicates that a salivary exosomal miRNA panel can detect dysplastic/premalignant lesions with clinically meaningful accuracy, supporting its potential integration into pediatric screening workflows.

Summary of Key Findings

1. **All 100 enrolled participants were successfully analyzed**, and samples met exosomal and RNA quality thresholds.
2. **Premalignant lesions displayed a distinct exosomal miRNA profile** compared with benign lesions and healthy mucosa.
3. **miR-21, miR-155, and miR-196a were strongly upregulated** in premalignant lesions.
4. The combined miRNA signature produced **85% sensitivity and 92% specificity**, demonstrating strong diagnostic potential.

DISCUSSION

This study provides strong evidence that **salivary exosomal miRNA profiling can differentiate pediatric premalignant oral lesions from benign lesions and healthy mucosa with meaningful diagnostic accuracy** in a tertiary hospital setting in Khyber Pakhtunkhwa (KPK), Pakistan. The observed sensitivity and specificity suggest that exosomal miRNA signatures may function as a practical screening adjunct in pediatric oral medicine, particularly where biopsies are difficult to perform routinely. This aligns with current clinical guidance emphasizing that OPMDs require structured risk stratification and monitoring, rather than reliance on visual assessment alone (Carlson et al., 2023; Villa and Woo, 2020; Diniz-Freitas et al., 2022).

A central finding was the significant upregulation of **miR-21** in the premalignant group. This result is consistent with wider evidence describing miR-21 as an oncogenic miRNA linked to altered survival signaling and invasion pathways in oral cancers. Prior work has demonstrated that miR-21 influences PTEN/AKT signaling and contributes to aggressive behavior in oral cancer biology, supporting its plausibility as an early marker of dysplastic transition rather than a late-stage artifact (Chai et al., 2020; Ghallab and Shaker, 2021; De Carvalho et al., 2020). In practical terms, the sharp elevation of miR-21 across our groups suggests it may contribute strongly to triage models designed to identify children requiring closer surveillance.

The study also showed robust elevation of **miR-155**, a miRNA frequently associated with immune modulation and inflammatory signaling. Growing evidence supports the role of immune microenvironment remodeling early in oral carcinogenesis, where inflammatory and immune pathways create a permissive environment for progression (Foy et al., 2020). The increased miR-155 expression observed here is compatible with mechanistic and clinical reports linking miR-155 to oral cancer progression and immunologic cross-talk (Koshizuka et al., 2017; Qin et al., 2025). This may be especially relevant in pediatric lesions, where inflammatory conditions and chronic immune stimulation may serve as cofactors in premalignant biology.

Similarly, **miR-196a** was significantly upregulated in premalignant lesions. Previous studies have linked miR-196a with differentiation disruption and progression-related pathways in oral malignancy, and its consistent elevation in our cohort reinforces its role as part of a transformation-associated signature (Liu et al., 2016). Importantly, recent evidence emphasizes that multi-miRNA panels generally outperform single markers, which is supported by our ROC findings where a composite model showed strong diagnostic utility (Punyadeera et al., 2023; De Carvalho et al., 2020).

A subtle reduction in **miR-200c** was also noted. This trend is biologically coherent because miR-200c is commonly described as a regulator of epithelial integrity and EMT suppression. Reduced miR-200c expression has been associated with enhanced metastatic potential and epithelial destabilization, suggesting that even pre-invasive lesions may show early molecular patterns consistent with reduced epithelial stability (Shiah et al., 2015; Foy et al., 2020). Although the downregulation was modest compared with the strong upregulation of miR-21/155/196a, its directionality supports mechanistic plausibility and may improve panel specificity.

A major advantage of this research is its focus on **exosomal miRNA cargo**, rather than relying on whole-saliva miRNA alone. Exosomes offer enhanced biomarker stability and reflect biologically active

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cellular export mechanisms, making them particularly suitable for translational diagnostics (Raposo and Stoorvogel, 2013). The study design and processing approach aligns with EV best-practice recommendations described by the MISEV2018 framework, strengthening confidence in reproducibility (Théry et al., 2018). Broader methodological evidence highlights that EV isolation approaches can influence measured cargo and therefore standardization remains essential, especially when developing clinical screening tools (Karimi et al., 2018; Ghods et al., 2021).

The diagnostic performance observed in this cohort is consistent with modern saliva-based predictive research demonstrating that salivary miRNA profiles can support early detection and risk prediction in OPMDs (Punyadeera et al., 2023). Systematic reviews and meta-analyses also support that miRNA-based markers have useful diagnostic accuracy for oral cancer and potentially malignant lesions (De Carvalho et al., 2020; Larsson et al., 2021). Importantly, the present study extends these insights into a pediatric cohort and provides region-specific evidence for a population where diagnostic alternatives are urgently needed.

However, limitations should be acknowledged. First, the study was conducted at a single tertiary hospital, which may limit generalizability. Second, the cross-sectional design prevents direct assessment of whether the identified miRNA signature predicts progression to malignancy over time. Longitudinal pediatric surveillance is required to determine whether these miRNAs serve as predictive markers of transformation rather than markers of existing dysplasia. Additionally, pediatric oral environments are influenced by diet, oral hygiene, infection burden, and systemic nutritional factors, which could modify exosomal cargo and should be modeled explicitly in future work (Irani, 2020; Larsson et al., 2021).

In conclusion, this research supports the feasibility of **salivary exosomal miRNA profiling as a non-invasive diagnostic adjunct** for pediatric premalignant oral lesions in KPK, Pakistan. With further validation, this approach could enhance early identification, reduce reliance on invasive biopsies, and enable structured monitoring strategies tailored for pediatric populations in resource-constrained healthcare systems (Carlson et al., 2023; Punyadeera et al., 2023).

CONCLUSION

This study demonstrates that **salivary exosomal miRNA profiling is a promising, non-invasive strategy for identifying pediatric premalignant oral lesions**, particularly in settings where early diagnosis is limited by access to specialist care or where invasive procedures are difficult to justify in children. Conducted in a tertiary hospital in Khyber Pakhtunkhwa (KPK), Pakistan, the research identified a reproducible exosomal miRNA signature that clearly differentiated premalignant lesions from benign oral conditions and healthy mucosa. Among the profiled molecules, **miR-21, miR-155, and miR-196a showed consistent and marked upregulation** in the premalignant group, highlighting their potential role as early molecular indicators of dysplastic change.

The diagnostic performance of the combined miRNA panel—showing **high specificity (92%) and useful sensitivity (85%)**—supports the feasibility of using saliva-based molecular screening as a clinical adjunct for risk stratification. These findings reinforce the concept that pediatric oral lesions, even when clinically subtle, may carry measurable molecular signals that precede advanced pathological transformation. In practical terms, this approach could reduce dependence on biopsies as the first-line diagnostic tool, allowing clinicians to identify children who require closer monitoring, timely referral, and histopathological confirmation.

Beyond its diagnostic implications, the study also contributes to a growing body of evidence that exosome-based biomarkers may offer greater stability and clinical reliability than free circulating salivary molecules. Since saliva collection is painless, low-cost, and highly acceptable in pediatric populations, exosomal miRNA signatures may be well suited for broader screening models—particularly in resource-constrained regions such as KPK where delayed presentation remains common.

Nevertheless, while the results are strongly encouraging, further work is required before routine clinical implementation. Large-scale multi-center validation, pediatric-specific reference ranges, and longitudinal follow-up studies are necessary to

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confirm whether these miRNA signatures also predict lesion progression, recurrence, or malignant transformation over time. If validated, salivary exosomal miRNA profiling could become an important part of pediatric oral health screening protocols, enabling earlier intervention and improving long-term outcomes for children at risk of oral cancer development.

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