Salivary miR-324-5p as a Novel Biomarker of Periodontal Status Among People with HIV Undergoing ART

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BACKGROUND

Periodontitis is a prevalent inflammatory disease of tooth-supporting tissues. microRNA (miRNA) expression in extracellular vesicles (EVs) can reflect periodontal status. People with human immunodeficiency virus (PWH) often have higher prevalence and greater severity of periodontal disease than HIV-negative populations (Buwembo et al., 2024). However, the specific miRNAs associated with periodontal status in PWH under Antiretroviral therapy (ART) remain unclear.

The aim of this study was to investigate the salivary miRNAs in EVs among PWH with periodontitis.

METHODS

- Study conducted from July 2024 to April 2025 on Japanese male and female participants (26-80 years) at Okayama University Hospital.
- PWH with periodontitis undergoing ART were a case group (n=12). A control group (n=12) was matched based on age and periodontal status.
- Saliva samples and periodontal status data were collected. EVs were isolated from saliva samples, followed by total RNA extraction.
- In the screening phase, the samples were pooled from each group, and miRNAs were identified by next-generation sequencing (NGS).
- In the validation phase, based on the NGS result, the top one miRNAs were selected from case group for Real-time quantitative PCR (RT-qPCR) analysis, followed by t-test and spearman correlation analyses to investigate the association between miRNA expression and the periodontal status of PWH (n=15).
- In the bioinformatics phase, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted.
- *T*-test and spearman correlation analysis were performed. Significance level of 5% was set for all statistical analyses.

miR-324-5p can be a biomarker of periodontal status among PWH

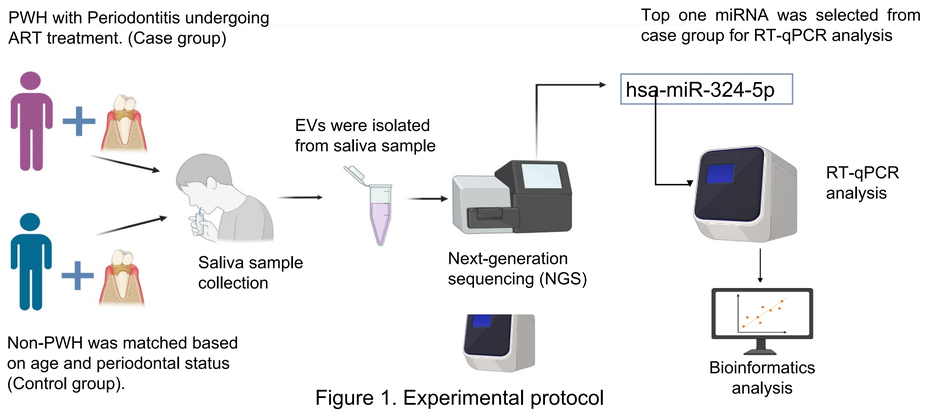


Figure 1. Experimental protocol

RESULTS

- In our study, we observed that salivary EV-derived miR-324-5p expression in the case group was significantly associated with periodontal inflamed surface area (PISA) (p = 0.028).
- KEGG pathway analysis revealed that miR-324-5p targets mRNAs, including *calcium voltage-gated channel auxiliary subunit gamma 8* (*CACNG8*), *insulin-like growth factor 1 (IGF1*), *mitogen-Activated Protein Kinase Kinase Kinase 9* (*MAP3K9*), and *ras-related protein 1* (*RAP1A*) which are involved in mitogen-activated protein kinases (MAPK) signaling pathway.

Table 2. Association between salivary miR-324-5p expression and other factors.

Variable	Cases (n=15)	
	Spearman Correlation Coefficient	*P*-value¶
Age	0.059	0.834
BOP	0.262	0.346
PISA	0.564	**0.028***
PPD	0.368	0.177
CD4 count	-0.121	0.681
Duration of ART intake	0.271	0.328

PISA, periodontal inflamed surface area. ¶ Spearman correlation analysis, *P < 0.05; significant

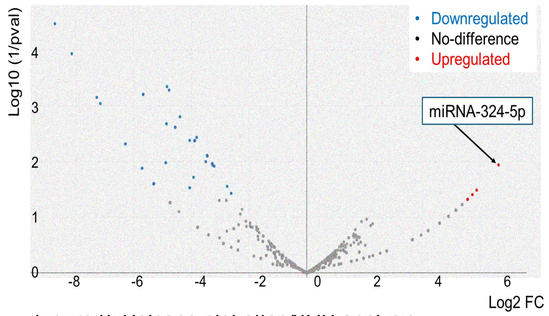


Figure 2: Volcano plot after NGS analysis

Table 1. Characteristics of the participants

Variablex	Total (n=24)*	Case group (n=12)	Control group (n=12)	P value†
Age (years)	62.6 ± 16.3	64.3 ± 15.3	61 ± 17.8	0.636
Number of teeth present	21.3 ± 8.0	18.8 ± 8.1	23.7 ± 7.3	0.141
Mean PPD (mm)	2.6 ± 0.4	2.7 ± 0.5	2.5 ± 0.4	0.325
BOP (%)	21.5 ± 18.2	25.0 ± 21.4	18.0 ± 14.5	0.359
BMI (kg/m²)	23.5 ± 3.2	24.7 ± 3.6	22.3 ± 2.3	0.068
Plaque control record(%)	50.3 ± 28.6	53.9 ± 33.2	46.7 ± 23.9	0.546
Salivary flow rate (ml/min)	0.5 ± 0.3	0.5 ± 0.4	0.5 ± 0.2	0.859

PPD, probing pocket depth; BOP, bleeding on probing; BMI, body mass index. *Mean ± SD; † t-test

CONCLUSIONS

- The miR-324-5p can be a specific biomarker in PWH and reflect severity of periodontal status.
- miR-324-5p has previously been reported to be significantly upregulated in PWH compared to HIV-negative controls (Francesca et al., 2018). Moreover, this miRNA has been implicated in inflammatory regulation.
- Bioinformatics analysis identified CACNG8, IGF1, MAP3K9, and RAP1A as potential mRNA targets of miR-324-5p.
- Notably, these genes are involved in the MAPK signaling pathway, which is well recognized for its role in the pathogenic mechanisms of periodontitis (Travan et al., 2013). IGF1 has been shown to potentiate pro-inflammatory responses in human mononuclear cells through MAPK pathway activation (Thalijn et al., 2017), and MAP3K9 contributes to inflammatory and apoptotic signaling within the same pathway (Kangning et al., 2024).
- The MAPK signaling pathway may be an important target to seek the mechanisms of periodontitis in PWH undergoing ART.

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PLAIN LANGUAGE SUMMARY

The salivary miR-324-5p in EVs can be a potential novel biomarker for of periodontal status in PWH undergoing ART. The miR-324-5p may offer new insights into the molecular mechanisms underlying HIV-associated periodontitis.