Check for updates

Received: 15 April 2022 | Revised: 13 October 2022 | Accepted: 29 November 2022

DOI: 10.1111/jre.13081

Journal of PERIODONTAL RESEARCH WILEY

ORIGINAL ARTICLE

Six miRNA expressions in the saliva of smokers and non-smokers with periodontal disease

Figen Öngöz Dede[1] | Ceren Gökmenoğlu[1] | Emrah Türkmen[2] | Şeyma Bozkurt Doğan[3] | Burhanettin Sertaç Ayhan[4] | Kubilay Yildirim[5]

[1]Faculty of Dentistry, Department of Periodontology, Ordu University, Ordu, Turkey

[2]Faculty of Dentistry, Department of Periodontology, İstanbul Medipol University, İstanbul, Turkey

[3]Faculty of Dentistry, Department of Periodontology, Yıldırım Beyazıt University, Ankara, Turkey

[4]Department of Pharmaceutical Chemistry, Anadolu University, Health Sciences Institute, Eskişehir, Turkey

[5]Karadeniz Advanced Technology Research and Application Center, Ondokuzmayis University, Samsun, Turkey

Correspondence
Figen Öngöz Dede, Department of Periodontology, Faculty of Dentistry, Ordu University, 52100 Altınordu, Ordu, Turkey.
Email: figen_ongoz@hotmail.com

Funding information
The Scientific and Technological Research Council of Turkey, Grant/Award Number: 318S106

Abstract

Background: It has been stated that microRNA (miRNA) plays an important role in development, homeostasis, and immune functions, and abnormal miRNA expression may cause faster disease progression.

Objective: The aim of this study was to determine miR-203, miR-142-3p, miR-146a, miR-146b, miR-155, and miR-29b gene expressions in the saliva of smokers and non-smokers with the periodontal disease before and after non-surgical periodontal therapy (NSPT).

Methods: A total of 90 individuals, 30 with periodontitis, 30 with gingivitis, and 30 periodontally healthy (control group), were included. These three groups were divided into subgroups as smoking and non-smoking individuals, with 15 people in each group. NSPT was applied to patients with periodontitis and gingivitis. Saliva samples and clinical parameters were obtained at baseline and repeated 6 weeks after NSPT.

Results: Saliva miR-203, miR-142-3p, miR-146a, miR-146b, and miR-155 gene expressions were significantly upregulated in patients with periodontal disease compared to the control group both in smokers and non-smokers, and also these miRNAs' gene expressions were significantly higher in the periodontitis group than in the gingivitis group at baseline ($p < .05$). A significant increase in saliva miR-142-3p expression was detected in all groups of smokers compared to non-smokers ($p < .05$). Although there was a decrease in salivary miRNAs gene expressions with the treatment, it was not statistically significant ($p > .05$).

Conclusions: These results suggest that salivary miR-146a, miR-146b, miR142-3p, miR-155, and miR-203 gene expressions increased with the progression of periodontal disease, but unchanged after periodontal treatment. Moreover, smoking may contribute to an increase in the levels of salivary miR-142-3p in the periodontal health and disease.

KEYWORDS
gingivitis, miRNA, periodontal therapy, periodontitis, smoking

© 2022 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Periodontal disease is a chronic and multifactorial inflammatory disease that develops with the interactions between the dysbiotic dental plaque biofilm and the host immuno-inflammatory response.[1] Early diagnosis of periodontal diseases will prevent irreversible loss of periodontal tissues as well as teeth.[2] Recently, molecular approaches identifying genetic and epigenetic conditions will clarify which state of inflammatory condition is present and determine who is at risk for future destruction of the periodontium.[3] One of the approaches in this area has been micro-RNA (miRNA).[4]

MiRNA is a type of single-stranded small non-coding RNA molecule with a length of approximately 22 nucleotides.[5,6] It has been reported that miRNAs affect important cellular biological functions such as proliferation, migration, invasion, signal transduction, autophagy, and apoptosis.[7] Different miRNA expression has been shown to regulate cell growth and function in cells involved in innate and acquired immunity.[8] MiRNAs have been shown to play an important role in development, homeostasis, and immune functions, including diseases such as cancer, developmental abnormalities, neuromuscular disorders, and cardiovascular diseases.[4] It has been stated that abnormal miRNA expression may cause more severe and faster disease progression.[9]

A review reported the potential roles of miRNAs in periodontal inflammation.[10] Xie et al.[9] examined miRNA profiles, which they thought to be associated with periodontal disease, in the inflamed and healthy gingival tissue and determined that expressions of hsa-miR-126, hsa-miR-20a, hsa-miR-142-3p, hsa-miR-19a, hsa-let-7f, hsa-miR-203, hsa-miR-17, hsa-miR-223, hsa-miR-146b, and hsa-miR-146a increased and the expressions of hsa-miR-155 and hsa-miR-205 decreased. On the other hand, with the understanding of the importance of analysis of saliva miRNAs in oral cancers for non-invasive diagnostic purposes,[11] a review indicated that miRNAs may serve as saliva markers for periodontitis.[2] It has been suggested that there may be early diagnoses in periodontitis with saliva miRNA analysis, which may help prevent bone and tooth loss.[2] Moreover, Schmalz et al.[2] stated that miR-203, miR-142-3p, miR-146a, miR-155, and miR-29b play a role in oral cancers and their roles in periodontal disease should be investigated.

Smoking is a major risk factor for periodontal diseases and it is known that smoking affects the occurrence, severity, and recovery of periodontal diseases after periodontal treatment.[12] A recent review suggested that smoking can modulate the expression of miRNAs by various signaling pathways such as apoptosis, angiogenesis, and inflammatory pathways.[13] An experimental study revealed that miRNA expression in oral fibroblasts and oral keratinocytes was dysregulated by exposure to tobacco condensate.[14] Additionally, smoking has been reported to reduce miR-203a expression in rectal cancer.[15] Another study reported that smoking has an effect on downregulation of miR-200b and miR-92a expressions on oral mucosa and there was a negative correlation between the number of cigarettes and the expression level of miR-203a.[16] Because of this knowledge, this study hypothesized that smoking can dysregulate miRNA expression profiles in periodontal disease.

Recently, interest in studies investigating the relationship between periodontal disease and miRNA has been increasing. To date, few studies have examined various miRNA gene expression analyses in various body fluids and gingival tissues for periodontitis,[2,17,18] but no studies have evaluated the effects of smoking on miRNA levels in the individuals with periodontal disease before and after periodontal treatment. The authors theorize that expressions of these miRNAs would increase with exacerbation of periodontal disease, then periodontal therapy might improve these levels, and that smoking might also raise the expression of these miRNAs. The objectives of this study were (1) to identify the levels of miR-203, miR-142-3p, miR-146a, miR-146b, miR-155, and miR-29b gene expressions in the saliva of the patients with periodontal health and disease, (2) to assess the impact of smoking on saliva miR-203, miR-142-3p, miR-146a, miR-146b, miR-155, and miR-29b gene expressions in smokers and non-smokers with periodontal disease and health, and (3) to explore the effect of non-surgical periodontal treatment on saliva miR-203, miR-142-3p, miR-146a, miR-146b, miR-155, and miR-29b gene expressions in smokers and non-smokers with periodontal disease.

2 | MATERIAL AND METHODS

2.1 | Study population and study design

Participants of the present study were recruited from the Department of Periodontology, Ordu University between September 2019 and November 2020 to the study. The study protocol was confirmed by the Clinical Research Ethics Committee of Ordu University, Ordu, Turkey, according to the Helsinki Declaration of 1975, as revised in 2008 (Protocol ID: 2018\212, Clinical Trial. org-NCT05046678). Informed consent was provided by individuals included in the study.

At the beginning of the study, the periodontal conditions of all participants were defined according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[19] Periodontal healthy groups included individuals with clinically gingival health on an intact periodontium who had bleeding on probing (BOP) < 10% and probing depth (PD) ≤ 3 mm, no attachment, and alveolar bone loss, and no history of periodontitis. Gingivitis groups included patients without attachment and alveolar bone loss who had gingival inflammation, PD ≤ 3 mm, and BOP > 30% in the entire mouth. Periodontitis patients consisted of generalized stage 3-grade B and were selected from subjects who had interdental clinical attachment levels (CAL) ≥ 5 mm, PD ≥ 6 mm on at least two non-adjacent teeth, and radiographic bone loss extending to the middle or apical third of the root. These patients had attachment and bone loss at ≥30% of the teeth and loss of ≤4 teeth due to periodontitis. Periodontitis grade has been determined by radiographic bone loss/age.[20] Accordingly, patients between 0.25-1.00 were categorized as Grade B. In addition, participants were included as smokers if they had smoked ≥10 cigarettes/day for ≥5 years[21] and smokers with periodontitis were categorized as Grade C, whereas participants were included as non-smokers if they had no previous history of smoking. Additionally, the participants

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

were excluded who have any systemic disease, pregnancy or lactation, history of radiotherapy or chemotherapy, had received antibiotic or any anti-inflammatory drug, or any periodontal treatment within the past 6 months. Individuals with at least 20 teeth (excluding third molars) and older than 18 years were evaluated clinically and radiographically.

After clinical evaluation, a total of 90 subjects (47 males and 43 females, mean age: 42.86 ± 7.49) including individuals with periodontitis ($n = 30$), gingivitis ($n = 30$), and periodontal healthy ($n = 30$) were enrolled in this study. All groups were divided into smokers and non-smokers. Study participants were classified into six groups as follows: (1) smokers with periodontitis (SP, $n = 15$, 8 males and 7 females; age: 42.87 ± 10.04 years); (2) non-smokers with periodontitis (NP, $n = 15$, 8 males and 7 females; age: 45.60 ± 6.87 years); (3) smokers with gingivitis (SG, $n = 15$, 8 males and 7 females; age: 42.33 ± 7.18 years); (4) non-smokers with gingivitis (NG, $n = 15$, 8 males and 7 females; age: 44.73 ± 7.00 years); (5) smokers with periodontal healthy (SH, $n = 15$, 8 males and 7 females; age: 38.33 ± 5.53 years); and (6) non-smokers with periodontal healthy (NH, $n = 15$, 7 males and 8 females; age: 43.27 ± 6.61 years).

2.2 | Clinical measurements and intra-examiner reproducibility

The periodontal status of each individual was examined with full mouth plaque index (PI),[22] gingival index (GI),[23] BOP,[24] PD, CAL, and radiographical evaluation. All measurements were recorded at six sites around each tooth with a Williams periodontal probe (Hu-Friedy, Chicago, IL) by a single calibrated examiner who was blinded to the whole study (ET). Before the actual measurements, 10 randomly selected participants were used to calibrate the researcher. Each clinical parameter was measured by the investigator at two simultaneous intervals 48 h apart. If there was >90% similarity at the millimeter level between the two measurements, the calibration of the investigator was recorded as acceptable.[25]

2.3 | Periodontal treatment

Mechanical plaque control (instructions on tooth brushing and the use of interdental cleaning tools (interproximal brushes or/and dental floss)) was given to all individuals participating in the study. Non-surgical periodontal treatment (NSPT) was applied to patients with periodontitis and gingivitis by the same investigator (ET). The treatment of periodontitis patients was accomplished within 2 weeks, depending on the periodontal status of each patient. Subgingival instrumentation [debridement, scaling, and root planning (SRP)] using manual scalers, curettes, and ultrasonic instruments was performed on all 4 quadrants of patients with periodontitis and maintenance and a repeat of oral hygiene. The treatment of gingivitis patients was performed with only scaling by using scalers or ultrasonic instruments. Clinical measurements were repeated from both groups (gingivitis and periodontitis) 6 weeks after NSPT.[26,27]

2.4 | Saliva sampling

The samples were collected at baseline from all groups and 6 weeks after NSPT from gingivitis and periodontitis groups. Saliva sampling was performed 1 day after clinical periodontal measurements, in the morning after 10–12 h of fasting because the complex structure of saliva can be affected by any situation. Unstimulated saliva samples were collected. Each individual was instructed to rinse with tap water to remove the deposits in the mouth. Then saliva samples were collected by instructing the individuals to spit every 60 s for 5 min. The samples were put into 50-ml CELLSTAR tubes (#227261, Greiner Bio-One, Kremsmünster, Austria) by spitting and were kept on ice. The volume of saliva was recorded and the saliva flow rate was calculated which was expressed as ml/min.[28] Immediately after saliva collection, samples were pretreated with sonication for the experiment as described in Iwai et al.[29] The agitated saliva samples were then centrifuged at 5000 rpm for 15 min at 4°C to precipitate the cell debris. The supernatant part of the samples was removed and the remaining part was frozen in liquid nitrogen and stored in −80°C.

2.5 | RNA preparation and the miRNA isolation

Prior to miRNA isolation, the frozen saliva samples were thawed on ice and total RNA was firstly extracted using a protocol described in the Oragene®•RNA Self-Collection Kit (DNA Genotek). In brief, saliva samples were mixed with 300 µl of Oragene®•RNA solution. The mix was incubated for 1 h at 50°C, then heated at 90°C for 15 min and allowed to cool to room temperature. Afterward, 48 µl of the Oragene®•RNA Neutralizer solution was added. Samples were mixed, incubated on ice for 10 min, and then centrifuged at 10000 × g for 3 min at room temperature. The supernatant was collected and the isolation of total RNA was completed after its quantification on NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). An Agilent 2100 Bioanalyzer (Santa Clara, CA) was used to detect the size distribution of total RNA, as well as determine the quality of the RNA. miRNA isolation was performed using the microRNA Purification Kit (NORGEN Biotek Corporation, Thorold, ON, Canada) according to the manufacturer's instructions. The isolation was started with the addition of 250 µl of frozen saliva samples into Buffer PBS (150 µl) included in the kit. Then the solution was vortexed and 400 µl of 96% ethanol was added. After vortexing, 600 µl of the solution was transferred into the spin column and centrifuged at 14000 rpm for 1 min. The residual solution was taken into the collection tube and an equal amount of ethanol was added. After the vortex, the solution was loaded into a microRNA enrichment column for binding the small RNA molecules to the silica from flow-through. Centrifugation enabled the washing solution to pass through the column and the same process was repeated two more times. To obtain RNA molecules bound to the column, 50 µl of ES-A solution was lastly added to the column and centrifugation enabled the RNA molecules to be available in the

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

tube. Quantification and quality of RNA in the samples were determined using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, ABD). The integrity of the RNA samples was tested with 2100 Bioanalyzer (Agilent Technologies) using the small RNA assay. All the RNA samples were then stored at -80°C until they were used in expression analysis.

2.6 | Real-time quantitative PCR analysis of miRNAs for gene expression

RT-qPCR-based miRNA gene expression analyses were made with 2-Step qRT-PCR Kits (MyBioSource, San Diego, California). This kit includes miRNA and U6 high specific reverse transcribe (RT) and PCR primer set and contains SYBR Green dye for gene expression quantification. In the study, miRNA samples were firstly converted to cDNA using 54 mer RT primers as recommended by the manufacturer. The 48-mer region of these primers forms a stem-loop structure, while the 6-mer region was designed according to the targeted miRNA (Table 1). The reaction was carried out in 20 µl solution containing 4 µl 5X RT Master Mix, 0.75 µl dNTP (10 mM), 1.20 miR-RT Primers Mix (1 µM), 0.2 µl MMLV Reverse Transcriptase (200 U/µl), and ~2 ng RNA sample. The remaining part of the solution was completed to 20 µl with nuclease-free sterile water. The reverse transcription was run at 25°C for 30 min, at 42°C for 30 min, and finally 5 min at 85°C in a Thermocycler (Applied Bio-systems, Waltham, Massachusetts) to obtain cDNAs.

miRNA gene expression analyses were performed according to the MyBioSource SYBR-based RT-qPCR protocol (MyBioSource, San Diego, California). In the study, U6 small nuclear RNA included in miRNA kits was used as an internal control. Each reaction was carried out in 20 µl solution constitutes from 10 µl 2X Real-time PCR Master Mix, 0.4 µl miRNA specific Primer set (10 µM), 0.4 µl ROX reference dye, 1 µl PCR enhancer, 0.2 µl Taq DNA polymerase (5 U/µl), 2 µl miRNA RT product, and 6 µl dd H2O. ROX dye was provided as an internal passive reference for the SYBR Green signal normalization during data analysis to correct well-to-well fluorescent fluctuations. RT-qPCR analyses were performed using the Applied Biosystems 7900HT Sequence Detection System (Applied Bio-systems, Waltham, Massachusetts). The reaction was carried out under 95°C for 3 minutes hold, 40 cycles at 95°C, and at 62°C for 12 s. SYBR and ROX signal was selected in the analysis program for the gene detection and reference, respectively. To confirm the specificity of amplification, the samples were subjected to melt curve analysis, which involved a ramp step that ranged between 60 and 95°C with an initial hold for 90 s followed by a rise of 1°C at each step with a 5 s hold to enable the melting process. Analysis of melt curve showed distinct peak intensities for each of the miRNAs between 75 and 85°C. Relative expression levels of each miRNA were calculated using the $2^{-\triangle\triangle Ct}$ method.

2.7 | Statistical analysis

The primary outcome variable (change in miR-146a expressions between periodontitis and healthy groups before periodontal treatment) was used to decide the sample size calculation.[30] We estimated that a sample size of 13 patients in each group would allow for a type II error level of $\beta = 0.95$ (95% power) and a type I error level of $\alpha = 0.05$ (5% probability). To protect from possible dropouts, the sample size was increased to 15 patients in each group.

Statistical analysis was carried out using the statistical package software (SPSS 20.0 V, SPSS Inc., Chicago, IL). The data obtained from the study were first evaluated with Levene's test to check whether the homogeneity of variances was assumed or not, and with the Shapiro-Wilks test to check whether it satisfies the assumption of normality. The descriptive statistical analyzes of the data were evaluated with one-way ANOVA and the mean and standard deviation values of the groups were calculated. Multiple comparisons of the mean values of the groups were performed using Tukey HSD, and intragroup comparisons before and after treatment were performed using the paired *t*-test. Statistically significant differences were considered at $p < .05$ for all analyses.

3 | RESULTS

3.1 | Clinical findings

Clinical periodontal findings of the study groups are shown in Table 2. There were no significant differences in the age and gender distribution among groups ($p > .05$).

TABLE 1 Oligonucleotide sequences of target mature miRNAs and specific forward PCR primers (5′–3')

Target miRNA	Mature miRNA sequence	PCR Primer
hsa-miR-142-3p	TGTAGTGTTTCCTACTTTATGGA	GGGGTGTAGTGTTTCCTACT
hsa-miR-203	GTGAAATGTTTAGGACCACTAG	GGGGTGAAATGTTTAGGAC
hsa-miR-146b	TGAGAACTGAATTCCATAGGCT	GGGTGAGAACTGAATTCCA
hsa-miR-146a	TGAGAACTGAATTCCATGGGTT	GGGTGAGAACTGAATTCCA
hsa-miR-155	TTAATGCTAATCGTGATAGGGGT	GGGTTAATGCTAATCGTGAT
hsa-miR-29b	TTTAGATTTAAATAGTGATTGTCT	GGGGGTTCTTGTGACTAAAG
U6 small nuclear RNA	U6-forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′	U6-reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′
RT primer	5′-GTCGTATCCAGTGCGT- GTCGTGGAGTCGGCAATTGCACTGGATACGAC-3	

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Clinical periodontal findings of the study groups before (T_0) and after periodontal treatment (T_t)

		Control		Gingivitis		Periodontitis	
		Non-smokers ($n = 15$)	Smokers ($n = 15$)	Non-smokers ($n = 15$)	Smokers ($n = 15$)	Non-smokers ($n = 15$)	Smokers ($n = 15$)
PI	T_0	0.28 ± 0.06	0.27 ± 0.07	1.87 ± 0.57†,‡	1.79 ± 0.47†,‡	1.72 ± 0.47†,‡	1.99 ± 0.47†,‡
	T_t			0.28 ± 0.11	0.21 ± 0.08	0.58 ± 0.30	0.64 ± 0.27
	p			<.001	<.001	<.001	<.001
GI	T_0	0.12 ± 0.17	0.06 ± 0.08	1.54 ± 2.03†,‡	0.60 ± 0.95†,‡,§	1.36 ± 1.83†,‡,¶	0.94 ± 1.23†,‡,§,††
	T_t			0.66 ± 0.34	0.19 ± 0.08	0.56 ± 0.32	0.20 ± 0.20
	p			<.001	<.001	<.001	<.001
BOP (%)	T_0	0.00 ± 0.00	0.00 ± 0.00	57.22 ± 16.67†,‡	28.57 ± 8.16†,‡,§	64.68 ± 19.99†,‡,¶	38.02 ± 10,78†,‡,§,††
	T_t			18.35 ± 7.39	10.88 ± 2.24	18.15 ± 11.52	13.14 ± 2.92
	p			<.001	<.001	<.001	<.001
PD (mm)	T_0	1.25 ± 0.06	1.39 ± 0.13	1.73 ± 0.46	1.80 ± 0.44	3.22 ± 0.54†,‡,§,¶	3.09 ± 0.43†,‡,§,¶
	T_t			1.49 ± 0.40	1.65 ± 0.46	2.23 ± 0.48	2.45 ± 0.43
	p			=.147	=.397	<.001	<.001
% sites PD 4–5 mm	T_0	–	–	–	–	42.6 ± 12.6	46.3 ± 8.52
	T_t	–	–	–	–	24.6 ± 10.8	29.5 ± 8.41
	p	–	–	–	–	<.001	<.001
% sites PD ≥ 6 mm	T_0	–	–	–	–	21.7 ± 6.24	26.4 ± 5.38
	T_t	–	–	–	–	19.4 ± 5.82	24.8 ± 6.89
	p	–	–	–	–	=.068	=.052
CAL (mm)	T_0	1.25 ± 0.06	1.39 ± 0.13	1.73 ± 0.46	1.80 ± 0.44	5.04 ± 0.92†,‡,§,¶	4.57 ± 0.99†,‡,§,¶
	T_t			1.49 ± 0.40	1.65 ± 0.46	4.38 ± 0.93	3.90 ± 0.79
	p			=.147	=.397	=.062	=.054
% sites CAL 4–5 mm	T_0	–	–	–	–	47.4 ± 0.89	45.62 ± 13.4
	T_t	–	–	–	–	40.5 ± 6.82	39.4 ± 7.23
	p	–	–	–	–	<.001	<.001
% sites CAL ≥ 6 mm	T_0	–	–	–	–	25.1 ± 5.51	24.9 ± 4.71
	T_t	–	–	–	–	22.9 ± 6.73	21.5 ± 4.69
	p	–	–	–	–	=.083	=.071
SFR (ml/min)	T_0	3.00 ± 0.86	2.95 ± 1.49	3.50 ± 1.87	3.43 ± 1.37	3.18 ± 1.55	3.51 ± 1.24
	T_t			3.05 ± 1.15	3.26 ± 0.70	2.91 ± 1.06	3.90 ± 1.60
	p			=.435	=.679	=.589	=.471
Time of smoking habit/years		–	28 (10–40)	–	24 (10–40)	–	30 (10–50)
Number of cigarettes/day		–	25 (10–40)	–	20 (10–30)	–	26 (10–40)

Note: Data are expressed as mean ± standard deviation. $p < .05$ was considered statistically significant.

Abbreviations: BOP, Bleeding on Probe; CAL, Clinical attachment level; GI, Gingival index; PD, Probing depth; PI, Plaque index; SFR, Saliva flow rate.

†Significant difference with non-smokers periodontally healthy group ($p < .05$).

‡Significant difference with smokers periodontally healthy group ($p < .05$).

§Significant difference with non-smokers gingivitis group ($p < .05$).

¶Significant difference with smokers gingivitis group ($p < .05$).

††Significant difference with non-smokers periodontitis group ($p < .05$).

For both smokers and non-smokers, PI, GI, and BOP values were statistically significantly higher in all groups with periodontal disease (NG, NP, SG, SP) than in periodontal healthy groups at baseline (NH, SH) ($p < .05$) and a significant reduction was observed after the NSPT ($p < .001$). GI and BOP values in patients with periodontal disease were found to be significantly lower in smokers than in non-smokers ($p < .001$). PD and CAL scores in periodontitis groups with smokers and non-smokers were significantly higher than gingivitis and periodontal healthy groups with smokers and non-smokers at baseline ($p < .05$), but there was no statistically significant difference between periodontitis groups in the PD and CAL ($p > .05$). When PD and CAL values were examined after NSPT in periodontitis and gingivitis groups, there was a statistically significant decrease in PD only in the periodontitis groups with smokers and non-smokers ($p < .001$). In

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

terms of saliva flow rates, no statistically significant difference was found among all groups at baseline and values before and after NSPT in the periodontitis and gingivitis groups ($p > .05$).

3.2 | Gene Expressions of miRNAs

The Log2 fold change results of the saliva miR-203, miR-142-3p, miR-146a, miR-146b, miR-155, and miR-29b gene expressions and comparisons of the study groups before and after periodontal treatment for smokers and non-smokers are shown in Figure 1.

miR-146a, miR-146b, miR-142-3p, miR-155, and miR-203 gene expressions were significantly upregulated in periodontitis groups with smokers and non-smokers (NP, SP) compared to gingivitis and periodontal healthy groups (NG, SG, NH, SH), and miR-29b gene expressions were also significantly upregulated in NP group than in NH group at baseline ($p < .05$). miR-146a, miR-146b, miR-142-3p, miR-155, and miR-203 gene expressions were significantly upregulated in gingivitis groups with smokers and non-smokers (NG, SG) compared to the periodontal healthy group with non-smokers (NH) at baseline, excluding miR-155 gene expressions in NG group ($p < .05$). Only miR-142-3p gene expressions in patients with periodontitis and gingivitis were found to be significantly upregulated in smokers than in non-smokers at baseline ($p < .05$). On the other hand, it was found that there was a statistically significant upregulation in miR-146a, miR-146b, and miR-142-3p gene expressions in smokers compared to non-smokers with the periodontally healthy group at baseline ($p < .05$). Moreover, there were no significant differences in saliva miRNA expressions before and after NSPT of patients with periodontitis and gingivitis for smokers and non-smokers ($p > .05$).

4 | DISCUSSION

Recently, there has been increasing interest in examining miRNAs in periodontal disease. In clinical studies, mostly, miRNAs have been studied in gingival tissue by an invasive method.[9,31–34] Although miRNAs in periodontal diseases have been investigated, the miRNAs examined in our study have not been adequately discussed in other studies. In a few studies, the effect of periodontal treatment on miR-146a, miR-155, and miR-203 gene expressions was examined for periodontitis.[17,30,34–36] However, the gingivitis form of periodontal disease and the effect of periodontal treatment and smoking on other miRNAs expressions were not investigated in any study. Thus to the best of our knowledge, this is the first study evaluating the effect of smoking on salivary miR-203, miR-142-3p, miR-146a, miR-146b, miR-155, and miR-29b gene expressions in patients with the periodontal disease before and after non-surgical periodontal treatment. At the same time, we know that previous studies[2,11] have indicated that salivary miRNA levels are important in the diagnosis and prognosis of oral cancers and can also be used in the diagnosis of periodontal disease. Recently, there has been increasing interest in the analysis of miRNA levels in periodontal health and disease. As the evidence in these studies increases, we think that saliva miRNA analysis can help clinicians in the diagnosis and prognosis of periodontal disease.[17,31,34,35]

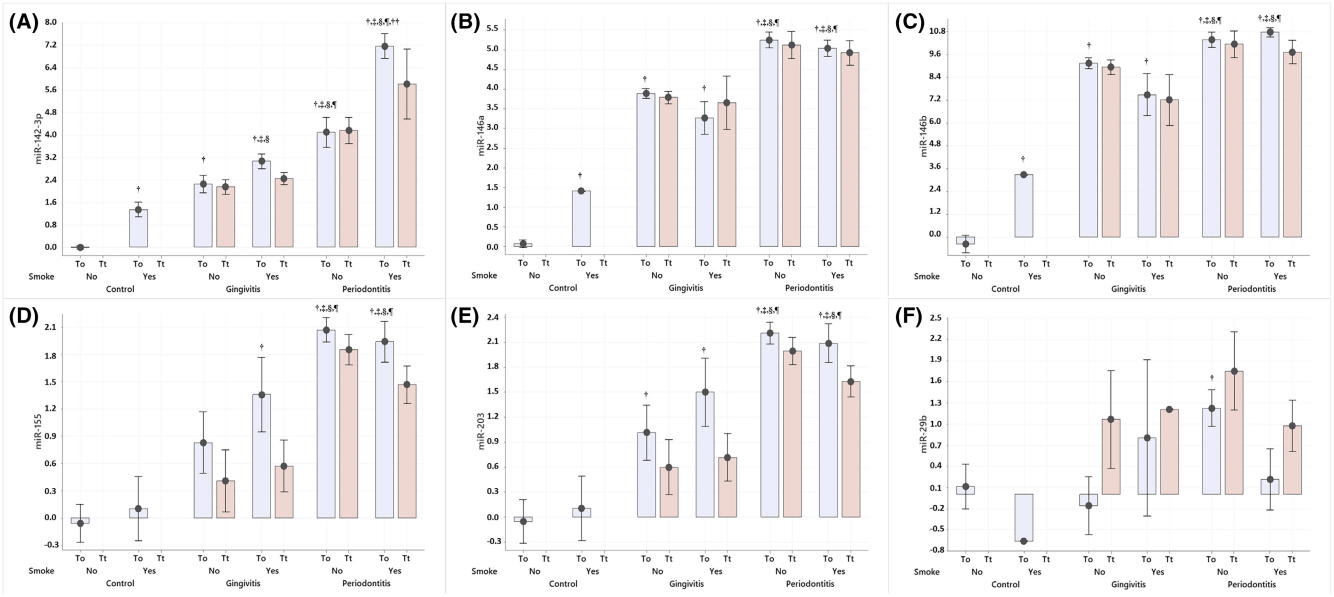

FIGURE 1 Log 2 gene expression changes of miRNAs in the saliva of study groups before (T_0) and after periodontal treatment (T_t). Expressions of miRNA-142-3p (A), miRNA-146a (B), miRNA-146b (C), miRNA-155 (D), miRNA-203 (E), and miRNA-29b (F) in saliva were measured by real-time qPCR. YES: smoking individuals; NO: non-smoking individuals. Data are expressed as mean ± standard deviation. $p < .05$ was considered statistically significant. †Significant difference with non-smokers periodontally healthy group ($p < 0.5$). ‡Significant difference with smokers periodontally healthy group ($p < 0.5$). §Significant difference with non-smokers gingivitis group ($p < .05$). ¶Significant difference with smokers gingivitis group ($p < .05$). ††Significant difference with non-smokers periodontitis group ($p < .05$).

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

In this study, for both smokers and non-smokers, it was shown that the expressions of miR-142-3p, miR-146a, miR-146b, miR-155, and miR-203 upregulated in the presence of periodontal disease and increased during the transition from gingivitis to periodontitis. Xie et al.[9] reported that miR-203 expression was upregulated in inflamed gingiva. Besides, Moffatt & Lamont[37] reported that expression of miR-203 increased in gingival epithelial cells stimulated with *P. gingivalis*. Stoecklin-Wasmer et al.[31] showed the overexpression of miR-155 in the gingival tissue of individuals with periodontitis compared to healthy individuals. Honda et al.[38] reported that the expression of miR-155 increased after stimulation of THP-1 cells with *P. gingivalis* LPS, but this increase was lower than that of miR-146a. A recent study found that miR-146a levels in gingival tissues increased in patients with generalized periodontitis stages II, III and grades A, B, and stages III, IV, and grade C compared to healthy subjects.[34] Interestingly, these levels were significantly higher in stages II, III, and grades A, B periodontitis compared to stages III, IV, and grade C periodontitis.[34] In addition to these studies, the increased saliva and gingival crevicular fluid (GCF) miR-146a, miR-146b, and miR-155 levels have been reported in periodontitis.[30,35] Similar to these studies, Al-Rawi et al.[17] detected higher rates of miR-146a, miR-146b, miR-155, and miR-203 expressions in saliva samples of individuals with periodontitis than healthy individuals, and also reported that diabetes causes increase expressions of miRNA. Similarly, our findings demonstrated high expression levels of these miRNAs in the saliva of patients with periodontal disease and suggest that increased expressions of these miRNAs may be associated with activity and severity of periodontal disease. But, contrary to the above studies, Stoecklin-Wasmer et al.[31] showed lower expression of miR-203 in the gingival tissue of individuals with periodontitis compared to healthy individuals. Additionally, Sipert et al.[33] showed that miR-155 was downregulated in the gingiva after induction of gingival cells with LPS. These conflicting results may be due to changes in different cell and disease types, analysis techniques, and severity of inflammation.

miR-29b expressions in periodontitis with non-smokers showed upregulation compared to healthy individuals with non-smokers in the present study. A recent review reported that miR-29b expressions upregulated in the gingival tissue of both human and mouse as a result of periodontal disease.[3] However, it has been stated that miR-29b has been identified as a positive regulator of osteoblastogenesis and also acts as a decreaser of collagen synthesis in mature osteoblasts.[39] The results of our study are unexpected, as osteoclastic cells are stimulated in bone loss, which is a hallmark of periodontitis. The present study is the first clinical study to show associate periodontitis with miR-29b. These results may imply that miR-29b might play a different role in periodontitis and that measuring the quantification of miR-29b in saliva can provide data on the pathological process of periodontitis.

Smoking is thought to be the major factor affecting the biosynthesis and maturation of miRNAs.[13] Several reports have stated that smoking causes changes in the expression of miRNAs, and miRNA profiles that are sensitive to smoking have been reported.[13,40] Conickx et al.[41] determined that miR-142-3p was upregulated in lung tissues in cigarette smoke-exposed mice. Contrary to this study, miR-142-3p was observed to be downregulated in the normal bronchial mucosa of smokers compared to never-smokers.[42] It is not possible to make a direct comparison with these studies above, since the present study material is different. The present study determined that smoking upregulated miR142-3p, miR-146a, and miR-146b expressions in periodontally healthy individuals. Also, it was observed that the expression of miR-142-3p increased in smokers with periodontitis and gingivitis groups. Therefore, we suggest that the effect of smoking on miR142-3p expression may be the independent presence of periodontal disease. The fact that smoking causes higher miR-146a and miR-146b expression in periodontally healthy individuals, while there is no difference in individuals with periodontal disease can be interpreted as the periodontal disease affects the expression of these miRNAs more than smoking.

Radović et al.[35] indicated that patients with periodontitis showed a decrease in miR-146a and miR-155 expressions in GCF after 6 weeks of the NSPT. In contrast, a recent study showed no statistically significant difference in miR-146a levels in gingival tissue 1 month after NSPT.[34] Our study determined that there was a decrease in miR-142-3p, miR-146a, miR-146b, miR-155, and miR-203 gene expressions in saliva after periodontal treatment and an increase in miR-29b, but no statistically significant. There is no literature available to discuss our study data. The discrepancy may be due to the different sample sources used and the short evaluation period of the treatment.

Our study has some limitations. Firstly, GCF and gingival tissue compared with saliva can better reflect the immuno-inflammatory processes in periodontal diseases, and saliva may also be more affected by systemic conditions. Therefore, the assessment of GCF/gingival tissue samples would be better to test the hypothesis on the possible involvement/association of the miRNAs with periodontal diseases. In the evaluation of the effect of periodontal treatment on miRNA, there is a need for further studies showing correlations between the expressions of these genes in saliva, GCF, and gingival tissue, which present the biological processes in periodontal disease. The second limitation, smoking status was based on the self-reporting by patients and we did not evaluate the cotinine levels in the serum and/or saliva samples in our study. The long-term cumulative measure of exposure, such as pack-year, can be transformed from self-reported smoking history. However, reported smoking habits can be particularly unreliable.[43] It has been indicated that the measurement of cotinine levels in the serum and/or salivary samples is more reliable for the estimation of smoking status.[43] The third limitation is that patients with stage 3 periodontitis are only included in the study. Further studies involving all stage (stage 1, 2, 3, 4) periodontitis groups may better explain the role of miRNA in periodontal disease.

5 | CONCLUSIONS

The present study concluded that gene expressions of miR-146a, miR-146b, miR142-3p, miR-155, and miR-203 in saliva increased in

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

smokers and non-smokers with periodontitis compared to gingivitis and periodontally healthy groups. Our study also showed that periodontal treatment could not provide positive improvement in salivary miR-146a, miR-146b, miR142-3p, miR-155, miR-29b, and miR-203 gene expressions. Moreover, miR-142-3p gene expressions in saliva raised in smoker compared to non-smoker groups and even higher with the progression of periodontal disease. Further studies are needed to better elucidate the mechanisms by which these miRNAs in periodontal disease by eliminating the limitations and to enable the development of potential therapeutic strategies.

AUTHOR CONTRIBUTIONS

Study conception and design: Figen Öngöz Dede, Ceren Gökmenoğlu, and Şeyma Bozkurt Doğan. *Analysis and interpretation of the data*: Burhanettin Sertaç Ayhan and Kubilay Yıldırım. *Data collection*: Figen Öngöz Dede, and Emrah Türkmen. *Drafting of the manuscript*: Figen Öngöz Dede, and Ceren Gökmenoğlu. All authors gave their final approval and agreed to be accountable for all aspects of the work.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Yeliz Kaşko Arıcı, Department of Biostatistics and Medical Informatics, Faculty of Medicine, Ordu University, for assistance in the statistical analysis. This research was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) (Project No: 318S106).

CONFLICT OF INTEREST

The authors report no conflicts of interest related to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Figen Öngöz Dede https://orcid.org/0000-0002-4211-3359
Ceren Gökmenoğlu https://orcid.org/0000-0002-3803-7189
Emrah Türkmen https://orcid.org/0000-0001-5166-2109
Şeyma Bozkurt Doğan https://orcid.org/0000-0001-5670-6430
Burhanettin Sertaç Ayhan https://orcid.org/0000-0002-1915-1306
Kubilay Yildirim https://orcid.org/0000-0003-3834-0396

REFERENCES

1. Slots J. Periodontitis: facts, fallacies and the future. *Periodontol 2000* 2000. 2017;75(1):7-23. doi:10.1111/prd.12221
2. Schmalz G, Li S, Burkhardt R, et al. MicroRNAs as salivary markers for periodontal diseases: a new diagnostic approach? *Biomed Res Int*. 2016;2016:1027525. doi:10.1155/2016/1027525
3. Luan X, Zhou X, Naqvi A, et al. MicroRNAs and immunity in periodontal health and disease. *Int J Oral Sci*. 2018;10(3):24. doi:10.1038/s41368-018-0025-y
4. Sonkoly E, Ståhle M, Pivarcsi A. MicroRNAs and immunity: novel players in the regulation of normal immune function and inflammation. *Semin Cancer Biol*. 2008;18(2):131-140. doi:10.1016/j.semcancer.2008.01.005
5. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 2014;15(8):509-524. doi:10.1038/nrm3838
6. Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. *Transl Res*. 2011;157(4):163-179. doi:10.1016/j.trsl.2011.01.007
7. Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19(1):92-105. doi:10.1101/gr.082701.108
8. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol*. 2010;10(2):111-122. doi:10.1038/nri2708
9. Xie YF, Shu R, Jiang SY, Liu DL, Zhang XL. Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues. *Int J Oral Sci*. 2011;3(3):125-134. doi:10.4248/IJOS11046
10. Kim DH, Sætrom P, Snøve O, Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proc Natl Acad Sci U S A*. 2008;105(42):16230-16235. doi:10.1073/pnas.0808830105
11. Lin X, Lo HC, Wong DTW, Xiao X. Noncoding RNAs in human saliva as potential disease biomarkers. *Front Genet*. 2015;7(6):175. doi:10.3389/fgene.2015.00175
12. Nociti FH, Casati MZ, Duarte PM. Current perspective of the impact of smoking on the progression and treatment of periodontitis. *Periodontol 2000* 2000. 2015;67(1):187-210. doi:10.1111/prd.12063
13. do Amaral NS, Cruz e Melo N, de Melo Maia B, Rocha RM. Noncoding RNA profiles in tobacco- and alcohol-associated diseases. *Genes (Basel)*. 2016;8(1):6. doi:10.3390/genes8010006
14. Bhat MY, Advani J, Rajagopalan P, et al. Cigarette smoke and chewing tobacco alter expression of different sets of miRNAs in oral keratinocytes. *Sci Rep*. 2018;8(1):7040. doi:10.1590/1678-7757-2019-0382
15. Mullany LE, Herrick JS, Wolff RK, Stevens JR, Slattery ML. Association of cigarette smoking and microRNA expression in rectal cancer: insight into tumor phenotype. *Cancer Epidemiol*. 2016;45:98-107. doi:10.1016/j.canep.2016.10.011
16. Taş B, Güre AO. The effect of maras powder and smoking on the microRNA deregulation of oral mucosa. *J Appl Oral Sci*. 2020;7(28):e20190382. doi:10.1590/1678-7757-2019-0382
17. Al-Rawi NH, Al-Marzooq F, Al-Nuaimi AS, Hachim MY, Hamoudi R. Salivary microrna 155, 146a/b and 203: a pilot study for potentially non-invasive diagnostic biomarkers of periodontitis and diabetes mellitus. *PLoS One*. 2020;15(8):e0237004. doi:10.1371/journal.pone.0237004
18. Roganović JR. microRNA-146a and −155, upregulated by periodontitis and type 2 diabetes in oral fluids, are predicted to regulate SARS-CoV-2 oral receptor genes. *J Periodontol*. 2021;92(7):35-43. doi:10.1002/JPER.20-0623
19. G. Caton J, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J Clin Periodontol*. 2018;45(20):S1-S8. doi:10.1111/jcpe.12935
20. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(Suppl 1):159-172. doi:10.1002/JPER.18-0006
21. Varghese J, Bhat V, Chianeh YR, Kamath V, Al-Haj Husain N, Özcan M. Salivary 8-hydroxyguanosine levels in smokers and non-smokers with chronic periodontitis. *Odontology*. 2020;108(4):569-577. doi:10.1007/s10266-020-00496-x
22. Löe H, Silness J. Periodontal disease in pregnancy I Prevalence and Severity. *Acta Odontol Scand*. 1963;21:533-551. doi:10.3109/00016356309011240
23. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121-135. doi:10.3109/00016356408993968
24. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J*. 1975;25(4):229-235.

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

25. İnce G, Gürsoy H, İpçi ŞD, Cakar G, Emekli-Alturfan E, Yılmaz S. Clinical and biochemical evaluation of lozenges containing lactobacillus reuteri as an adjunct to non-surgical periodontal therapy in chronic periodontitis. *J Periodontol*. 2015;86(6):746-754. doi:10.1902/jop.2015.140612
26. Hendek MK, Erdemir EO, Kisa U, Ozcan G. Effect of initial periodontal therapy on oxidative stress markers in gingival Crevicular fluid, saliva, and serum in smokers and non-smokers with chronic periodontitis. *J Periodontol*. 2015;86(2):273-282. doi:10.1902/jop.2014.140338
27. Kanmaz B, Lappin DF, Nile CJ, Buduneli N. Effects of smoking on non-surgical periodontal therapy in patients with periodontitis stage III or IV, and grade C. *J Periodontol*. 2020;91(4):442-453. doi:10.1002/JPER.19-0141
28. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci*. 1993;20(694):72-77. doi:10.1111/j.1749-6632.1993.tb18343.x
29. Iwai K, Minamisawa T, Suga K, Yajima Y, Shiba K. Isolation of human salivary extracellular vesicles by iodixanol density gradient ultracentrifugation and their characterizations. *J Extracell Vesicles*. 2016;5:30829. doi:10.3402/jev.v5.30829
30. Gao Y, di Hao C. Expression of miR-146a in saliva of chronic periodontitis patients and its influence on gingival crevicular inflammation and MMP-8/TIMP-1 levels. *Shanghai Kou Qiang Yi Xue*. 2018;27(3):309-312.
31. Stoecklin-Wasmer C, Guarnieri P, Celenti R, Demmer RT, Kebschull M, Papapanou PN. MicroRNAs and their target genes in gingival tissues. *J Dent Res*. 2012;91(10):934-940. doi:10.1177/0022034512456551
32. Ogata Y, Matsui S, Kato A, Zhou L, Nakayama Y, Taka H. MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients. *J Oral Sci*. 2014;56(4):253-260. doi:10.2334/josnusd.56.253
33. Sipert CR, Morandini AC, Dionísio TJ, Trachtenberg AJ, Kuo WP, Santos CF. MicroRNA-146a and microRNA-155 show tissue-dependent expression in dental pulp, gingival and periodontal ligament fibroblasts in vitro. *J Oral Sci*. 2014;56(2):157-164. doi:10.2334/josnusd.56.157
34. Sattari M, Taheri RA, Aref Nezhad R, Motedayyen H. The expression levels of MicroRNA-146a, RANKL and OPG after non-surgical periodontal treatment. *BMC Oral Health*. 2021;21(1):523. doi:10.1186/s12903-021-01883-8
35. Radović N, Nikolić Jakoba N, Petrović N, Milosavljević A, Brković B, Roganović J. MicroRNA-146a and microRNA-155 as novel crevicular fluid biomarkers for periodontitis in non-diabetic and type 2 diabetic patients. *J Clin Periodontol*. 2018;45(6):663-671. doi:10.1111/jcpe.12888
36. Elazazy O, Amr K, Abd El Fattah A, Abouzaid M. Evaluation of serum and gingival crevicular fluid microRNA-223, microRNA-203 and microRNA-200b expression in chronic periodontitis patients with and without diabetes type 2. *Arch Oral Biol*. 2021;121:104949. doi:10.1016/j.archoralbio.2020.104949
37. Moffatt CE, Lamont RJ. Porphyromonas gingivalis induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells. *Infect Immun*. 2011;79(7):2632-2637. doi:10.1128/IAI.00082-11
38. Honda T, Takahashi N, Miyauchi S, Yamazaki K. Porphyromonas gingivalis lipopolysaccharide induces miR-146a without altering the production of inflammatory cytokines. *Biochem Biophys Res Commun*. 2012;420(4):918-925. doi:10.1016/j.bbrc.2012.03.102
39. Li Z, Hassan MQ, Jafferji M, et al. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J Biol Chem*. 2019;294(25):10018. doi:10.1074/jbc.M809787200
40. Vrijens K, Bollati V, Nawrot TS. MicroRNAs as potential signatures of environmental exposure or effect: a systematic review. *Environ Health Perspect*. 2015;123(5):399-411. doi:10.1289/ehp.1408459
41. Conickx G, Avila Cobos F, Van Den Berge M, et al. MicroRNA profiling in lung tissue and bronchoalveolar lavage of cigarette smoke-exposed mice and in COPD patients: a translational approach. *Sci Rep*. 2017;7(1):12871. doi:10.1038/s41598-017-13265-8
42. Mascaux C, Laes JF, Anthoine G, et al. Evolution of microRNA expression during human bronchial squamous carcinogenesis. *Eur Respir J*. 2009;33(2):352-359. doi:10.1183/09031936.00084108
43. Scott DA, Palmer RM, Stapleton JA. Validation of smoking status in clinical research into inflammatory periodontal disease. *J Clin Periodontol*. 2001;28(8):715-722. doi:10.1034/j.1600-051X.2001.280801.x

How to cite this article: Öngöz Dede F, Gökmenoğlu C, Türkmen E, Bozkurt Doğan Ş, Ayhan BS, Yildirim K. Six miRNA expressions in the saliva of smokers and non-smokers with periodontal disease. *J Periodont Res*. 2023;58:195-203. doi:10.1111/jre.13081

16000765, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jre.13081 by Istanbul Medipol University, Wiley Online Library on [18/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License