Gingival crevicular fluid microRNA associations with periodontitis

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Abstract

Purpose: The present study was performed to assess the associations of gingival crevicular fluid (GCF) microRNAs miR-140-3p, miR-145-5p, miR-146a-5p, and miR-195-5p with periodontitis (PD) and to evaluate the possible influence of rheumatoid arthritis (RA) in this context.

Method: GCF samples were collected from 134 individuals with PD and 76 periodontally healthy individuals, with or without RA. After miRNA extraction from GCF, the levels of miR-140-3p, miR-145-5p, miR-146a-5p, and miR-195-5p were assessed using RT-qPCR.

Results: MI-146a-5p levels were significantly lower among the patients with PD than among the healthy individuals (P < 0.001) and negatively correlated with PD severity based on PD stage and periodontal outcome parameters (P < 0.05). Patients with severe PD had higher GCF levels of miR-140-3p and miR-145-5p than did periodontally healthy individuals (P < 0.05). Significant AUC values for diagnosis of severe PD were revealed for miR-140-3p (AUC = 0.614, P = 0.022), miR-145-5p (AUC = 0.621, P = 0.016) and miR-146a-5p (AUC = 0.702, P < 0.001). Combination of the aforementioned miRNAs increased the diagnostic performance (AUC = 0.709, P < 0.001).

Conclusion: It was demonstrated that miR-140-3p, miR-145-5p and miR-146a-5p were associated with PD and would be potentially effective for GCF-based non-invasive periodontitis diagnostics in patients with and without RA.

Keywords: biomarker, microRNA, periodontitis, rheumatoid arthritis

Introduction

Periodontitis (PD) is a chronic inflammatory disease affecting periodontal tissues with an adverse influence on general health [1]. PD is one of the most common diseases worldwide with an estimated prevalence of up to 50% in the adult population, depending on the age group and diagnostic criteria used in the studies [2,3]. The etiopathogenesis of PD involves a complex interaction between periodontal microbiota and the host immune system. A persistent bacterial load accompanied by prolonged periods of immune system activation result in gradual destruction of tooth-supporting tissues and development of a chronic low-grade inflammatory state that immunosuppression results in gradual destruction of tooth-supporting tissues and development of a chronic low-grade inflammatory state that negatively contributes to a range of systemic conditions [4]. Conversely, systemic diseases that alter immune system function can have a significant impact on the inflammatory response in periodontal tissues [5]. A number of studies have demonstrated significant correlations between rheumatoid arthritis (RA) and PD in terms of prevalence and disease activity, irrespective of confounders such as age, sex and smoking status [5,6]. Moreover, the pathogenesis of both PD and RA involves connective tissue breakdown and bone resorption, driven by altered levels of various inflammatory mediators [7].

Currently, it is widely accepted that epigenetic guidance through microRNAs (miRNAs) has a major role in the regulation of various cellular processes, including immunity and inflammation [8,9]. miRNAs are non-coding, single-stranded ribonucleic acids that have a significant negative impact on gene expression and protein synthesis [9]. Aberrant expression of miRNAs has been proposed as a significant pathological aspect of many diseases, such as various types of cancer, autoimmune diseases (i.e., RA) and PD [8,10]. miRNA deregulation is tissue-specific and miRNAs maintain sufficient stability to be used as diagnostic biomarkers for various diseases [11]. A number of studies have found associations between miRNA expression in gingival tissue and the clinical status of PD and have proposed the use of miRNAs for diagnosis and therapy in this setting [12,13]. Furthermore, a handful of studies have suggested that assessment of miRNA levels in gingival crevicular fluid (GCF) would be useful for PD diagnostics, given that GCF is rich in PD markers and the sampling procedure is non-invasive and can be performed easily [12,13]. Moreover, the composition of GCF represents the intensity of inflammation in periodontal tissues, as the amount of various cellular components (i.e., immune cells and periodontal pathogens) as well as organic molecules (serum proteins, antibodies, inflammatory mediators, miRNAs, etc.) may vary depending on the clinical status of PD [13].

In the present study, authors performed an assessment of GCF levels of four miRNAs: miR-140-3p, -145-5p, -146a-5p, and -195-5p, which were selected based on PD-specificity as detected in previous studies and reported clinical value [14–19]. The study aim was to assess the associations of these GCF miRNAs with PD and to evaluate the possible influence of RA.

Materials and Methods

Study participants and clinical examination

A total of 210 participants were enrolled in the study from two study centers at the Vilnius University Hospital Santaros and Zalgirio Clinics. Patients undergoing specialized treatment at the study centers during the enrollment period (2018-2020) were asked to participate in accordance with inclusion and exclusion criteria. Individuals were enrolled if no exclusion criteria were evident, including edentulism, oral cavity cancer and oral premalignancies, periodontal treatment in the previous 6 months, other comorbidities (autoimmune and endocrine disorders, excluding RA), and medication that might affect periodontal status (calcium channel blockers, antiepileptic drugs, etc.). Only those who agreed to participate and signed an informed consent form were enrolled. Vilnius Regional Biomedical Research Ethics Committee approval (No. 158200-18-992-500) was obtained before the study. The research was carried out in accordance with the principles of the Declaration of Helsinki of 1975, revised in 2013.

The participants were categorized into two groups: patients with diagnosed periodontitis (PD+, n = 134) and periodontally healthy individuals (PD-, n = 76). The clinical status of PD was assessed by one periodontist (A.R.) performing full mouth probing at six points and X-ray examination. The clinical attachment loss (CAL) was considered the primary endpoint for intra-examiner agreement assessment and a Kappa coefficient value of 0.84 was obtained. The following periodontal outcome parameters were documented, excluding third molars and implants: 1) CAL, 2) probing pocket depth (PPD), 3) bleeding on probing (BOP), 4) alveolar bone loss (BL), and 5) the number of missing teeth. PD diagnosis was determined...
according to the 2018 American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) definitions of PD and staging of periodontal disease [20,21]. Accordingly, cases of periodontitis were defined as clinically detectable PD-related interdental CAL at ≥2 non-adjacent teeth or having a buccal or oral CAL of ≥3 mm with ≥3 mm pocketing detectable at ≥2 teeth. The severity of PD was categorized into stages: initial – stage I, moderate – stage II, severe – stage III, advanced severe – stage IV. Participants without periodontal disease (PD− group) presented with clinically healthy gingival tissues (BOP <10 %, PPD ≤3 mm). For mean bone loss value evaluation, a computer-based assessment of digital panoramic X-rays was performed following a protocol described previously [22]. The alveolar BL was indicated as the proportion of the distance from the cementoenamel junction to the alveolar crest compared to the root length for distal and mesial sites of the first and the second molars.

Among 210 participants, 84 additionally diagnosed as having RA were investigated to assess possible associations between RA and GCF miRNA levels. Participants fulfilling the ACR/EULAR 2010 rheumatoid arthritis classification criteria were enrolled [23]. The presence of synovitis in at least 1 joint, no other possible alternative diagnosis, and a total classification criteria score of 6 or greater from the individual scores in 4 domains confirmed the diagnosis of RA [23]. The clinical status of RA was assessed by a rheumatologist (E.P.) using the disease activity score (DAS28) by counting 28 tender and swollen joints, a visual analogue scale (VAS 100 mm), and measurement of the C-reactive protein concentration (CRP, mg/l) [24]. Additionally, the Health Assessment Questionnaire (HAQ), the Rheumatoid Arthritis Impact of Disease (RAID) questionnaire, and any current treatment for RA with glucoctocoids, conventional synthetic disease-modifying antirheumatic drugs (DMARDs) or biologic DMARDs were assessed [23,25].

GCF sampling

Before the sampling procedure, supragingival plaque and saliva were removed and the sampling site was isolated with cotton pellets. A superficial intracrevicular GCF sampling technique was employed. For participants with periodontal disease, GCF sampling was performed from the deepest periodontal pocket during a separate visit. For periodontally healthy participants, GCF collection was performed on the palatal side of the maxillary incisors. For each participant, a total of five sterile absorbent paper points were inserted into the periodontal pocket and left in place for 30 sec. Paper points visibly contaminated with blood were discarded. Following GCF sampling, the paper points were pooled into a 2-ml tube containing 20 µL of aqueous RNAlater Stabilization Solution (Invitrogen, Thermo Fisher Scientific [TFS], Cleveland, OH, USA). All prepared samples were coded, labeled and stored at −80°C.

RNA extraction

A miRNeasy Mini Kit (Qiagen, Hilden, Germany) was used in accordance with the manufacturer’s instructions for RNA extraction from GCF. Samples were incubated for 5 min with added QIAzol Lysis Reagent. Further incubation was performed for 3 min with 200 µL of chloroform. For extraction efficiency control, 25 fmol of cel-miR-39 was utilized (Qiagen). RNA qualitative and quantitative assessment was conducted with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).

cDNA synthesis and quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Extracted RNA was reverse transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems [ABI], TFS) and miRNA-specific stem-loop primers (TaqMan MicroRNA Assay): miR-140-3p (ID: 02234); miR-145-5p (ID: 00278); miR-146a-5p (ID: 000468); miR-195-5p (ID: 000494) and cel-miR-39 (ID: 000200), employing the manufacturer’s protocol.

miRNA abundance was evaluated by RT-qPCR in a final reaction volume (10 µL) containing 2× TaqMan Universal Master Mix II, without UNG, 20× TaqMan MicroRNA Assay (both from ABI, TFS), 1.33 µL of cDNA and RNase-free water. All samples were run in triplicate on a Viia7 Real-Time PCR System using Viia 7 Software v1.2 (ABI, TFS).

Statistical analysis

Power analysis was based on a previous study that had assessed levels of miRNA in GCF (including miR-146a-5p) in relation to periodontal status [18]. Sample size was calculated for correlation analysis with a test power of 80%, an alpha probability of 0.05 and an expected small effect size (r = 0.2), and this revealed that a minimum sample size of 193 participants was needed to demonstrate a significant correlation between clinical variables and GCF miRNA levels. Sample size calculation was performed using GPower 3.1.9 (University of Düsseldorf, Düsseldorf, Germany) statistical software.

Descriptive data were displayed as mean ± standard deviation (SD). The Shapiro-Wilk test was used to verify the normality of continuous variables. Normally distributed data were assessed by Student’s t test whereas the Mann-Whitney U test was used when the distribution of data was not normal. Categorical variables were analyzed by χ² test. A relative quantification method was applied for evaluation of the miRNA level, comprising normalization to spiked-in cel-miR-39 and global miRNA expression, and the values were then recalculated into relative quantities (healthy participants group) and log-transformed using GenEx software, v7.0 (MultiD Analyses AB, Sweden). Multivariate associations of miRNA GCF levels with clinical variables were analyzed by performing logistic regression analysis and calculating the odds ratio (OR) with 95% confidence intervals (CI). Spearman correlation testing was used to determine correlations between miRNA levels and clinical data. The diagnostic performance of miRNAs was evaluated by calculating the area under the receiver-operator characteristic (ROC) curve (AUC). SPSS 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism v8.4.2 (GraphPad Software, San Diego, CA, USA) statistical software was used for data analysis and visualization. Associations were considered statistically significant at P ≤0.05.

Results

Characteristics of the study participants

The study population comprising 210 participants were grouped according to periodontal status: 134 were diagnosed with periodontitis (PD+ group) and 76 were periodontally healthy (PD− group). Among the 210 participants, 84 were additionally diagnosed as having RA and assigned to PD diagnosis groups: 56 as PD+ and 28 as PD−. Details of patient characteristics including anthropometric, sociodemographic, clinical and oral health-related data are provided in Table 1.

Associations of miRNA GCF levels with presence of PD

To assess the associations of miRNA levels in GCF with the presence of PD, the authors performed comparative analysis of the PD+ with PD− groups. This revealed that participants diagnosed as having PD had lower GCF levels of miR-146a-5p (P < 0.001) and close to significantly higher levels of miR-145-5p (P = 0.057) relative to periodontally healthy individuals. Since the PD+ and PD− groups were considerably different in terms of age, sex, BMI and smoking status (Table 1), multivariate analysis with adjustment for the aforementioned variables was performed. This revealed that higher GCF levels of miR-146a-5p were independently associated with reduced odds of having PD (OR = 0.644; 95% CI = 0.45 to 0.97, P = 0.036). None of the other assessed miRNAs showed significant inter-group differences.

Associations between miRNA GCF levels and PD severity

Correlation analysis was performed to assess associations between miRNA GCF levels and clinical parameters of PD. miR-146a-5p demonstrated a negative correlation with periodontal outcome parameters indicative of poorer PD clinical status, including mean values of CAL, PPD, BOP, BL and missing teeth (P < 0.010) (Fig. 1B). On the other hand, miR-140-3p and miR-145-5p were positively correlated with higher mean values of the assessed periodontal parameters, while miR-195-5p revealed no significant correlation (Fig. 1B).

Since PD severity may be represented by PD staging according to the 2018 guidelines, further assessment was performed by analyzing miRNA GCF associations with PD stages. The levels of all the analyzed miRNAs in GCF differed significantly between patients with advanced severe (stage IV) periodontal disease and periodontally healthy individuals (P < 0.05). The associations between other PD stages and levels of miRNA in GCF were less consistent. A detailed representation of miRNA levels relative to PD stages is presented in Fig. 2.
Table 1  Characteristics of the study participants.

| Periodontal status, N | PD−, 134 | RA−, 78 | PD−, 76 |
|----------------------|----------|---------|---------|
| RA status, N         | RA−, 56  | RA−, 78 | RA−, 48 |
| Age (years)          | 54.66 ± 9.84 | 52.21 ± 10.78 | 50.46 ± 13.46* | 43.46 ± 13.56* |
| Female (N)           | 47       | 59      | 28      | 42 |
| PD Stages            |          |         |         | |
| Periodontally healthy n (%) | N.A. | 28 (13.3%) | 76 (36.2%) |
| Stage I              | 14 (6.7%) | 34 (16.2%) | N.A. |
| Stage II             | 20 (9.5%) | 40 (19%) | N.A. |
| Stage III            | 7 (3.3%) | 15 (7.1%) | N.A. |
| Stage IV             | 15 (7.1%) | 23 (11%) | N.A. |
| PD clinical parameters |        |         |         | |
| CAL (mm)             | 2.23 ± 0.91 | 1.99 ± 0.95 | 1.25 ± 0.76* | 0.89 ± 0.57* |
| PPD (mm)             | 2.76 ± 0.61 | 2.57 ± 0.5 | 2.05 ± 0.25 | 1.93 ± 0.32 |
| BOP (%)              | 42.75 ± 16.81 | 43.92 ± 17.74 | 12.64 ± 4.16 | 13.75 ± 6.63 |
| BL (proportion of root length) | 43.43 ± 17.3*** | 13.34 ± 5.83*** |
| Missing teeth (N)    | 25.9 ± 8.25 | 25.13 ± 8.36 | 15.87 ± 4.53* | 13.61 ± 4.44* |
| RA clinical status and treatment |        |         |         | |
| DAS28 (score)        | 4.41 ± 1.28 | N.A. | 3.76 ± 1.66 | N.A. |
| RAID (score)         | 4.79 ± 2.12 | N.A. | 3.89 ± 2.52 | N.A. |
| HAQ (score)          | 0.87 ± 0.65 | N.A. | 0.84 ± 0.59 | N.A. |
| Receiving sDMARDs (N)| 33       | N.A. | 22      | N.A. |
| Receiving bDMARDs (N)| 22*      | N.A. | 19*     | N.A. |
| PD modifying factors |        |         |         | |
| BMI (kg/m²)          | 24.95 ± 4.26 | 25.69 ± 3.92 | 24.12 ± 4.06 | 24.18 ± 4.34 |
| Current smokers (N)  | 14       | 26*     | 12      | 5 |

bDMARDs, biologic disease-modifying antirheumatic drugs; BL, bone loss; BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment loss; DAS28, Disease Activity Score 28-joint count C reactive protein; HAQ, health assessment questionnaire; PD, periodontitis; PPD, periodontal probing depth; RA, rheumatoid arthritis; RAID, rheumatoid arthritis impact of disease; sDMARDs, synthetic disease-modifying antirheumatic drugs. Data represent mean ± standard deviation. Significant differences are indicated (*) in pairs with regard to level of statistical significance: *P < 0.05, **P < 0.01 and ***P < 0.001.

Fig. 1 Correlation matrix analysis. (A) Spearman correlations represented by color as indicated by the scale bar: strong positive correlation (red), strong negative correlation (blue), no correlation (white). (B) Correlation between gingival crevicular fluid (GCF) miRNAs and clinical parameters of periodontitis (PD), including clinical attachment loss (CAL), periodontal probing depth (PPD), bleeding on probing (BOP), and bone loss (BL). (C) Correlation between GCF miRNAs and rheumatoid arthritis clinical status and treatment, including disease activity score using 28 joint counts (DAS28), rheumatoid arthritis impact of disease (RAID) questionnaire, health assessment questionnaire (HAQ), synthetic disease-modifying antirheumatic drugs (sDMARDs), and biologic disease-modifying antirheumatic drugs (bDMARDs). (D) Correlation between GCF miRNAs and possible confounders of PD, including smoking status (non-smokers/current smokers), body mass index (BMI), age and sex (male). Enlarged sections of the correlation matrix (B-D) contain correlation coefficients for each pair indicating significant correlations (*) as follows: *P < 0.05, **P < 0.01.
Associations of miRNA GCF levels with RA and confounding factors
With the aim of examining GCF miRNA associations with rheumatological status, a subgroup comparison of patients having both PD and RA with periodontally healthy individuals was performed. This revealed that miRNA-146a-5p was downregulated \((P = 0.001)\) whereas miR-145-5p was upregulated \((\text{FC} = 1.28, P = 0.036)\) amongst patients with both illnesses. However, multivariate analysis demonstrated that RA was not associated with miRNA GCF levels \((P > 0.05)\). Interestingly, patients treated with bDMARDs had higher levels of miR-146a-5p than individuals who were not receiving biologic therapy \((P = 0.011)\).

Among the possible confounding factors assessed, smoking, age and sex were related to GCF miRNA levels. miR-140-5p showed a negative correlation with age and male sex \((P < 0.05)\) (Fig. 1D), whereas current smokers had higher levels of miR-140-3p than former smokers or non-smokers \((P = 0.041)\).

Diagnostic value of circulating miRNAs
The diagnostic potential of miR-140-3p, -145-5p, -146a-5p, and -195-5p was analyzed using ROC analysis and calculation of AUC values for each miRNA (Fig. 3). miR-146a-5p revealed significant diagnostic performance for distinction between the PD+ versus PD− groups \((P < 0.05)\) and mild to moderate \((\text{AUC} = 0.612, \text{sensitivity} = 60.8\%, \text{specificity} = 60.5\%, P = 0.018)\) or severe PD \((\text{AUC} = 0.709, \text{sensitivity} = 66.7\%, \text{specificity} = 65.8\%, P < 0.001)\) separately. Moreover, increased AUC values were observed among participants with both diseases as compared to participants with PD only, as shown in Fig. 3.

Discussion
The complex pathogenesis of PD and the lack of early, non-invasive diagnostic approaches may be related to the high prevalence of periodontal pathology worldwide. The diagnostic properties of miRNAs have been extensively analyzed and successfully applied in oncology and may also hold promise in the field of periodontology. Therefore, in the present study, levels of miR-140-3p, -145-5p, -146a-5p, and -195-5p in GCF were assessed using RT-qPCR to evaluate their associations with PD and diagnostic performance. These miRNAs were selected on the basis of previous studies indicating that they are significantly deregulated in periodontitis-affected gingival tissues and may be associated with the etiopathogenesis of PD \([15-19]\). To perform a comprehensive assessment of GCF miRNA associations with PD, the authors conducted univariate and multivariate analyses in which the primary outcome variables were PD presence and PD severity represented by periodontal outcome parameters and stages. Additionally, as PD may be influenced by multiple diseases, especially those affecting immune system homeostasis, possible associations with RA were also assessed.

The major finding of the present study was that lower levels of miR-146a-5p in GCF were strongly associated with the presence and severity of PD. It has been proposed that miR-146a-5p has protective properties and could be considered a negative feedback regulator of inflammation \([26,27]\). In the pathogenesis of PD, the expression of miR-146a-5p is increased when a combination of these miRNAs was used and reached significant levels for the diagnosis of PD \((\text{AUC} = 0.655, \text{sensitivity} = 62.7\%, \text{specificity} = 61.8\%, P < 0.001)\) and mild to moderate \((\text{AUC} = 0.612, \text{sensitivity} = 60.8\%, \text{specificity} = 60.5\%, P = 0.018)\) or severe PD \((\text{AUC} = 0.709, \text{sensitivity} = 66.7\%, \text{specificity} = 65.8\%, P < 0.001)\) separately. Moreover, increased AUC values were observed among participants with both diseases as compared to participants with PD only, as shown in Fig. 3.
Higher levels of miR-146a-5p result in inhibition of nuclear factor-κB (NF-κB) activation, which in turn downregulates both tumor necrosis factor-α (TNF-α) and inflammation [28,29]. Long-term exposure to LPS leads to monocytic hyporesponsiveness – a state referred to as LPS tolerance, which is considered an essential aspect of immune system homeostasis as it prevents unnecessary exaggeration of inflammation [30]. Nahid and colleagues have proposed that LPS tolerance is associated with increased levels of miR-146a-5p in human monocytes [28]. Given the protective role of miR-146a-5p, it has been hypothesized previously that downregulation or impaired function of miR-146a-5p may be associated with diseases characterized by a sustained exaggerated inflammatory state, such as the aggressive form of PD [31]. The findings of this study are in line with the above hypothesis, as the decrease in miR-146a-5p levels in GCF was correlated with the increase in PD severity based on stage and periodontal outcome parameters. Interestingly, in contrast to the present findings, Radovic and colleagues reported higher miR-146a-5p levels in the GCF of patients diagnosed as having PD [18]. Moreover, several studies have reported an increase of miR-146a-5p expression in periodontitis-affected gingival tissues as compared to healthy gingiva, most likely representing a futile attempt by the immune system to reduce inflammation in periodontal tissues [12,17].

Another notable finding of the present study was that levels of miR-140-3p and miR-145-5p in GCF were correlated positively with increased PD severity. In accordance with this, Stoecklin-Wasmer and colleagues reported that PD patients had almost double the level of miR-140-3p in GCF relative to healthy individuals [15]. On the basis of an *in vitro* study by Fushimi demonstrating that miR-140-3p modulates osteoblast differentiation through the Wnt3a and transforming growth factor β3 (TGFβ3) signaling pathways, it has been proposed that miR-140-3p may be associated with bone remodulation [32]. Similarly, Cui and colleagues have shown that miR-140-3p inhibits osteogenic differentiation of human periodontal ligament fibroblasts after treatment with an osteogenesis inducer [33]. In the present study, miR-140-3p showed the highest coefficient of correlation with the mean values of BL and CAL, which are parameters directly and indirectly associated with bone resorption. Meanwhile, similarly to miR-140-3p, it has been reported that miR-145-5p is also involved in bone remodulation [34]. Liu and colleagues found that inhibition of miR-145-5p was associated with enhanced osteogenic differentiation of adipose-derived stem cells [35]. Another study has reported that upon induction of osteoblast differentiation, the expression of miR-145-5p decreased [36]. Moreover, it is known that TNF-α induces the expression of miR-145-5p, which in turn leads to the promotion of human gingival epithelial cell apoptosis through BACH2 gene expression [19]. Additionally, Wu and colleagues have found that miR-145-5p was upregulated in inflamed canine gingival tissues [37]. Considering the proposed negative role of miR-145-5p and miR-140-3p in the etiopathogenesis of PD, the present findings appear to be concordant, as PD patients with a poor clinical status showed increased levels of miR-145-5p and miR-140-3p.

Patients diagnosed as having RA were enrolled in this study to assess possible associations with miRNA levels in their GCF. The study rationale was based on a substantial amount of scientific evidence to support significant correlations between the clinical status of PD and RA [5]. The present subgroup analysis that compared participants with both diseases (PD and RA) to periodontally healthy individuals revealed miRNA associations similar to those demonstrated in the PD+ versus PD− analysis. However, as RA was not independently associated with miRNA levels in GCF, the aforementioned finding may only be regarded as an insight for future studies. It might be inferred that the periodontal status of RA patients may...
be aggravated by the autoimmune disease on one hand, but alleviated by RA medication on the other [38]. Although it remains uncertain whether biologic DMARDs may affect the pathogenesis of PD, several studies have reported that bDMARDs reduce inflammation in periodontal tissues and reduce the level of TNF-α in GCF [38,39]. In the present study, participants receiving bDMARDs had significantly higher levels of miR-146a-5p, further supporting the assumption that miR-146a-5p is closely interconnected with TNF-α and that treatment with bDMARDs may affect periodontal tissues.

The EFP and AAP have been encouraging research into new biomarkers of periodontal disease [20]. To diagnose PD at an early stage when only minimal clinical symptoms are evident, a non-invasive point-of-care diagnostic method is necessary. In the present study, ROC analysis was performed on participants categorized into groups based on PD severity. One miR-146a-5p demonstrated any significant diagnostic performance for participants with varying PD severity (stage IV), whereas miR-140-3p and miR-146a-5p showed significant AUC values only for diagnosis of severe PD (stage III-IV), suggesting that the capacity to diagnose the disease non-invasively at an early stage should be improved. One possible solution would be to use a combination of miRNAs to improve diagnostic performance. Currently used diagnostic tools based on this principle employ a combination of a dozen miRNAs [40]. With similar intent, assessment of the diagnostic performance of additional miRNAs should be encouraged, as a combination of multiple miRNAs may be more promising for early non-invasive PD diagnostics.

Despite the considerable sample size utilized in this study and the assessment of the possible influence of RA – an immune-inflammatory disease – the present study had several drawbacks. First, the PD group patients were older than the periodontally healthy individuals, although as PD is an age-related disease, this was perhaps unremarkable. Similarly, sex, BMI and tobacco usage were not matched between the groups. Additionally, as the study design did not allow assessment of the causal relationship between miRNAs and PD, the present findings only reflect correlation-type associations. In conclusion, the level of miR-146a-5p in GCF showed a significant negative correlation with PD in terms of both the presence and severity of periodontitis. Higher levels of miR-140-3p and miR-145-5p in GCF were observed in PD patients with poorer clinical status. miR-140-3p, miR-145-5p and miR-146a-5p demonstrated good diagnostic performance, especially when used in combination. RA was not associated with miRNA GCF levels, but it was evident that patients who had been receiving bDMARDs had higher levels of miR-146a-5p in their GCF. The present findings should contribute to current understanding of GCF-derived miRNA associations with PD, highlighting areas for further research.

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Conflict of interest

No potential conflicts of interest relevant to this article are reported.

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