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MicroRNAs and periodontal disease: a qualitative systematic review of human studies

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ABSTRACT

Purpose: MicroRNAs (miRNAs) are epigenetic post-transcriptional regulators that modulate gene expression and have been identified as biomarkers for several diseases, including cancer. This study aimed to systematically review the relationship between miRNAs and periodontal disease in humans, and to evaluate the potential of miRNAs as diagnostic and prognostic biomarkers of disease.
Methods: The review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (reference number CRD42020180683). The MEDLINE, Scopus, Cochrane Library, Embase, Web of Science, and SciELO databases were searched for clinical studies conducted in humans investigating periodontal diseases and miRNAs. Expression levels of miRNAs across the different groups were analysed using the collected data.
Results: A total of 1,299 references were identified in the initial literature search, and 23 articles were finally included in the review. The study designs were heterogeneous, which prevented a meta-analysis of the data. Most of the studies compared miRNA expression levels between patients with periodontitis and healthy controls. The most widely researched miRNA in periodontal diseases was miR-146a. Most studies reported higher expression levels of miR-146a in patients with periodontitis than in healthy controls. In addition, many studies also focused on identifying target genes of the differentially expressed miRNAs that were significantly related to periodontal inflammation.
Conclusions: The results of the studies that we analysed are promising, but diagnostic tests are needed to confirm the use of miRNAs as biomarkers to monitor and aid in the early diagnosis of periodontitis in clinical practice.

Keywords: Epigenetic biomarker; Humans; miRNA; Periodontal diseases; Periodontitis

INTRODUCTION

Periodontitis is a chronic, multifactorial immunoinflammatory disease, typically caused by anaerobic gram-negative bacteria within dental plaque or biofilm and characterised by the destruction of tooth-supporting tissues; in some cases, this leads to tooth loss [1]. The aetiopathogenesis of this disease is complex. Traditionally, it was considered to be a simple infection caused by different bacterial species that colonised the periodontal pocket. We now know that an inappropriate host immune-inflammatory response against these bacteria and

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

their products drives disease progression in susceptible individuals [2]. Therefore, although bacteria initiate periodontal destruction, disease progression is due to additional factors [3].

For many years, much of the research on periodontics was concerned with the implications of genetic variants or mutations for the aetiopathogenesis of periodontitis, such as the relationship between interleukin (IL)-1 beta gene polymorphism and increased susceptibility to periodontitis [4]. Expanding our knowledge of gene expression modulation by epigenetic regulatory mechanisms is one of the greatest challenges in periodontal research.

Epigenetics is an emerging field of science that investigates changes in gene expression that are not attributed to DNA sequence alterations. Many epigenetic mechanisms, such as those based on microRNAs (miRNAs), are used by cells to activate or inhibit certain genes to produce different proteins [5].

miRNAs constitute a large family of short, non-coding RNA molecules and are ~22 nucleotides in length. These post-transcriptional regulators modulate gene expression either by inducing target messenger RNA (mRNA) degradation or by repressing translation initiation and thus protein synthesis [6]. For this to occur, miRNA must bind to the 3′-untranslated region of target mRNA transcripts, which usually results in gene silencing [6]. However, complete sequence complementarity between a single miRNA and its target mRNA is not required for gene silencing to occur; therefore, a single miRNA has the potential to control the translation of many different genes concurrently [7]. To date, over 2,500 genes encoding miRNAs have been identified in the human genome [8].

Over 2,000 miRNAs have been identified in humans [9]. These single-stranded RNA molecules participate in physiological processes such as cellular development, differentiation, and apoptosis [10]. However, many studies have also reported that miRNA dysregulation may have a substantial impact on the pathophysiology of diseases such as cancer [11], coronary heart disease [12], and diabetes [13], as well as on inflammatory autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus [8].

Furthermore, extracellular miRNAs remain remarkably stable in the bloodstream and other biofluids, such as saliva, urine, and cerebrospinal fluid, making miRNAs ideal candidates as biomarkers for the diagnosis and prognosis of many diseases, including periodontitis [14].

Although studies have shown that miRNAs play important roles in various systemic inflammatory diseases, to date, no systematic review has evaluated the possible association between miRNAs and periodontitis.

This systematic review aimed to analyse the putative relationship between miRNAs and periodontal diseases in humans, and to evaluate their potential as diagnostic and prognostic biomarkers.

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MATERIALS AND METHODS

Review question

A systematic review was carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The study was registered in the PRISMA database (PROSPERO), under reference number CRD42020180683. The following population, intervention/exposure, comparison, outcome question was formulated to address the specific aim of the study with reference to humans, miRNAs, healthy subjects without periodontal disease, and periodontitis, respectively: In humans, is there a relationship between miRNA expression levels and periodontal disease?

Inclusion and exclusion criteria

Cross-sectional, case-control, and cohort studies and randomised clinical trials were included in this review. Both prospective and retrospective investigations were included. Clinical case reports, literature reviews, animal studies, *in vitro* studies, and journal editorials, and studies without a healthy control group were excluded.

Search strategy

Electronic searches of the MEDLINE, Scopus, Cochrane Library, Embase, Web of Science, and SciELO databases were conducted in January 2020 for publications that investigated periodontitis and miRNAs. Detailed search strategies were developed for each database. These were based on a search strategy presented for MEDLINE using the following keywords (MeSH and free terms) combined with the Boolean connectors *AND* and *OR*: ((“periodontal” [All Fields] OR “periodontally” [All Fields] OR “periodontically” [All Fields] OR “periodontics” [MeSH Terms] OR “periodontics” [All Fields] OR “periodontic” [All Fields] OR “periodontitis” [MeSH Terms] OR “periodontitis” [All Fields]) AND (“micrornas” [MeSH Terms] OR “micrornas” [All Fields] OR “mirna” [All Fields] OR “mirnas” [All Fields] OR “mirna’s” [All Fields])). No year restrictions were applied for the electronic database search.

In addition, online manual searching of the following key periodontal journals was conducted, for articles published between 2010 and the present date: *Journal of Clinical Periodontology*, *Journal of Periodontology*, *Journal of Periodontal Research*, and *Journal of Dental Research*. Only articles published in English or Spanish were included.

Two reviewers (PMM and PAP) appraised the titles and abstracts, and reviewed the full texts of the selected articles. The kappa statistic (κ) was calculated to assess inter-rater reliability. In cases of disagreement between the reviewers, a third reviewer was consulted (ALR). Duplicate articles were excluded from the analysis. We also recorded the reasons for rejecting any articles.

Extraction of results and study characteristics

The following information was extracted from each article when available: first author’s surname, publication year, study design, study groups, sample size, mean age of participants, type of sample, type of periodontal disease (chronic or aggressive, following the Armitage classification [15]), analysed miRNAs, and main outcomes. All data were reviewed to consider appropriateness for a meta-analysis.

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Qualitative assessment

The Newcastle-Ottawa Quality Assessment Scale (NOS) was used to assess the methodological quality and risk of bias of the non-randomised studies. This checklist consists of 8 detailed quality items divided into 3 categories (selection, comparability, and outcome). Each item could be awarded 1 star, except for comparability, which was awarded 2 stars. Thus, the total maximum score was 9 stars. A score of 7 stars or more indicated a low risk of bias. This assessment was performed independently by 2 reviewers (PMM and PAP), and by a third (ALR) reviewer when there was no consensus.

RESULTS

Selected studies

The electronic search generated 1,292 references (287 in PubMed, 178 in Embase, one in the Cochrane Library, 735 in Scopus, 87 in Web of Science, and 4 in SciELO), and 7 additional records were identified by manual searches. A total of 71 duplicates were found using the Mendeley® bibliographic citation management software; these were excluded from the analysis. In addition, after screening 1,228 titles and abstracts, a further 1,193 articles were discarded for the following reasons: 1,060 investigated epigenetic mechanisms and pathologies that were different to miRNAs and periodontitis, respectively, 92 were animal models or *in vitro* studies, and 41 were literature reviews. A total of 35 publications were obtained as full-text articles; however, 12 of these were later excluded based on our inclusion/exclusion criteria (not written in English or Spanish, n=3; *in vitro* studies, n=5; literature review, n=1; not relevant to the review objectives, n=1; no control group, n=2). Finally, 23 articles were included in the qualitative analysis. The PRISMA flow diagram in Figure 1 summarizes the study selection criteria.

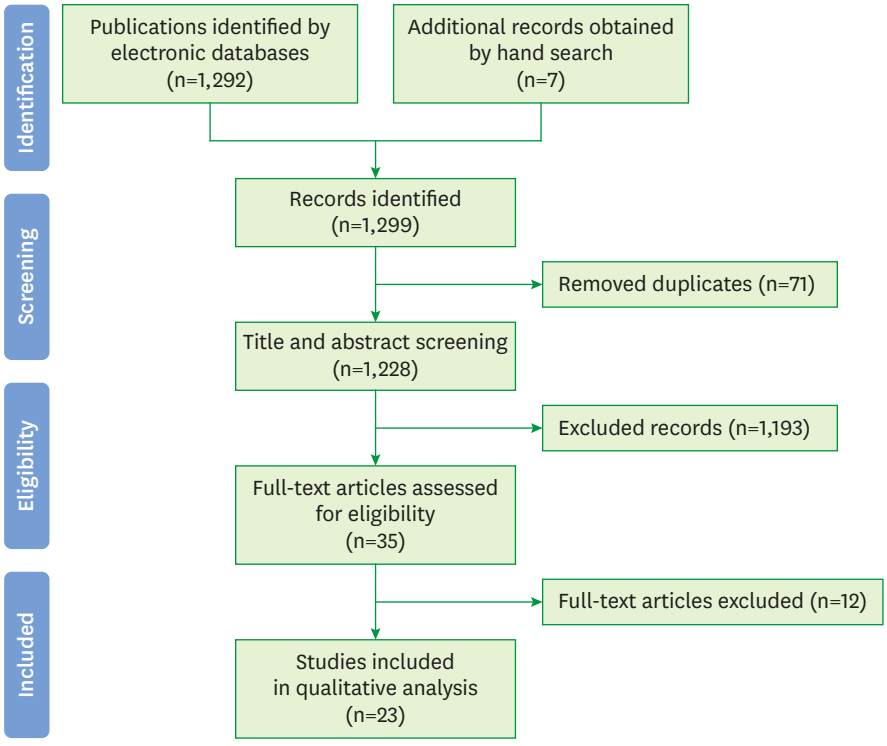

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis flow diagram.

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Inter-rater reliability

Inter-examiner agreement was high for the full-text screening (κ=0.85).

Characteristics and main outcomes of the included studies

Table 1 summarizes the data extracted from each included article. All 23 studies were cross-sectional studies. Most of the studies performed large-scale sequencing to identify potential diagnostic biomarkers, although some focused on specific miRNAs. Most of the investigations (n=13) obtained biological samples from gingival tissue biopsies, but some analysed blood samples (n=4), saliva (n=2), gingival crevicular fluid (n=3), and subgingival biofilm (n=1). The sample size varied among studies and ranged from 6 [16] to 550 [17] patients. Most of the studies compared miRNA expression levels between patients with periodontitis and healthy controls. However, some authors evaluated how systemic diseases such as obesity [18-20], diabetes [21], and coronary heart disease [22,23] influence relative miRNA expression levels. In addition, many studies focused on identifying target genes that were significantly related to periodontal inflammation of the differentially expressed miRNAs.

Table 1. Characteristics and main outcomes of included studies

Main outcomes	Analyzed miRNAs	Type of sample	Sample size mean age: years±SD	Study groups	Author, type of study
- Upregulation of miR-146 in G1, G2, and G3 compared to G4 (OR, 1.43) - No significant differences between G1, G2, and G3	miR-146	Blood sample	264 G1: n=66; 52.14±2.5 G2: n=66; 58.24±1.3 G3: n=66; 43.22±1.9 G4: n=66; 48±4.4	G1: CHD without periodontitis G2: CHD + CP G3: Healthy + CP G4: Healthy without CP	Bagavad Gita *et al.* [22], cross-sectional
- Upregulation of miR-142-3p in G1	miR-142-3p	Gingival biopsy	46 G1: n=26; ND G2: n=20; ND	G1: CP G2: Healthy	Chen *et al.* [24], cross-sectional
- Upregulation of miR-146a in G1 compared to G2 (OR, 17.8) - The higher the PD, the higher the miR-146a expression level - The higher the miR-146a expression level, the lower the PROINF CYT expression level	miR-146a	Gingival biopsy	28 G1: n=18; 27±13 G2: 4n=10; 32±12	G1: AP G2: Healthy	Ghotloo *et al.* [25], cross-sectional
- Upregulation of both miRNAs in G1 and G2 compared to G3	miR-146a, miR-499	Blood sample	197 G1: n=75; ND G2: n=38; ND G3: n=84; ND	G1: CP G2: PI G3: Healthy	Kadkhodazadeh *et al.* [26], cross-sectional
- miR-200b-5p expression levels were 1.6 times higher in G1	miR-323a-3p, miR-200b-5p, miR-188-5p, miR-4721, mir-557, miR-196a	Gingival biopsy	36 G1: ND G2: ND	G1: Obesity + CP or AP G2: Healthy + CP or AP	Kalea *et al.* [18], cross-sectional
- Upregulation of these miRNAs in G1: miR-181b (OR, 4.64), miR-19b (OR, 4.79), miR-23a (OR, 4.76), miR-30a (OR, 4.76), let-7a (OR, 9.48), miR-301a (OR, 8.59)	Massive sequencing: 93 miRNAs	Gingival biopsy	ND G1: ND G2: ND	G1: CP G2: Healthy	Lee *et al.* [27], cross-sectional
- miR-144-5p expression levels were 4.8 times higher in G1	17 miRNAs	Gingival biopsy	32 G1: n=16; 41.25±4.89 G2: n=16; 38.00±4.68	G1:CP G2: Healthy	Li *et al.* [28], cross-sectional
- miR-1226 expression levels were 15.8 times higher in G1	miR-671, miR-122, miR-1306, miR-27a, miR-223, miR-1226	Gingival crevicular fluid	18 G1: n=9; 50.44±8.09 G2: n=9; 33.33±12.05	G1: CP G2: Healthy	Micó-Martínez *et al.* [29], cross-sectional
- miR-146 expression levels were 32.6 times higher in G1 - The higher the PD, the higher the miR-146a expression level	miR-146a	Gingival biopsy	30 G1: n=20; 44±8 G2: n=10; 32±12	G1: CP G2: Healthy	Motedayyen *et al.* [30], cross-sectional
- Upregulation of miR-128 (OR >5), miR-34a (OR >5), miR-381 (OR, 10) in G1 - Downregulation of miR-15b (OR, 1), miR-211 (OR, 1), miR-372 (OR >1), miR-656 (OR >1) in G1	Massive sequencing: 93 miRNAs	Gingival biopsy	ND G1: ND G2: ND	G1: CP G2: Healthy	Na *et al.* [31], cross-sectional

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Table 1. (Continued) Characteristics and main outcomes of included studies

Main outcomes	Analyzed miRNAs	Type of sample	Sample size mean age: years±SD	Study groups	Author, type of study
- Significant differences in miRNA expression levels in tissue with CP compared to healthy tissue (in both groups); OR ≥1.6 - Significant differences in miRNA expression level between G1 and G2	Massive sequencing	Gingival biopsy	28 G1: n=14; ND G2: n=14; ND	G1(i): Obesity (tissue with CP) G1(ii): Obesity (healthy tissue) G2(i): Healthy (tissue with CP) G2(ii): Healthy (healthy tissue)	Naqvi *et al*. [19], cross-sectional
- Upregulation of 40, downregulation of 40 miRNAs in G1 - miR-143-3p expression was 5.82 times higher in G1	Massive sequencing	Blood sample	32 G1: n=16; 43.38±9.92 G2: n=16; 40.56±8.47	G1: CP G2: Healthy	Nisha *et al*. [32], cross-sectional
- miR-150, miR-223, and miR-200b expression levels were 2.72 times higher in G1	Massive sequencing	Gingival biopsy	6 G1: n=3; ND G2: n=3; ND	G1: CP G2: Healthy	Ogata *et al*. [16], cross-sectional
- Obesity led to a hyperinflammatory state, increasing the risk for CP - Highlights the overexpression of miR-106b in G1 (OR, 6.4)	Massive sequencing: 88 miRNAs	Gingival biopsy	24 4 participants per group 45±13.3 (overall mean age of all participants)	G1: Obesity + CP G2: Obesity without CP G3: Healthy + CP G4: Healthy without CP	Perri *et al*. [20], cross-sectional
- Upregulation of both miRNAs in G1 and G3 - After NSPT, miRNA expression levels were similar among groups	miR-146, miR-155	Gingival crevicular fluid	48 G1: n=24; 54.9±25.45 G2: n=24; 33.2±26.93 G3: n=24; 54.7±27.31 G4: n=24; 33.4±26.37	G1: DM2 + CP G2: DM2 without CP G3: Healthy + CP G4: Healthy without CP	Radović *et al*. [21], cross-sectional
- Upregulation of miR-223-3p, miR-203a, and miR-205-5p in G1 and G2 compared to G3	Massive sequencing: 752 miRNAs	Gingival crevicular fluid	20 G1: n=7; 67.57±ND G2: n=2; 37.5±ND G3: n=11; 32.45±ND	G1: CP G2: AP G3: Healthy	Saito *et al*. [33], cross-sectional
- 91 upregulated miRNAs (highlighted: miR-451 [OR, 2.63], miR-223 [OR, 2.53], miR-486-5p [OR, 2.46], miR-3917 [OR, 2.08]) - 68 downregulated miRNAs (highlighted: miR-1246 [OR, 0.33], miR-1260 [OR, 0.44], miR-141 [OR, 0.46], miR-1260b [OR, 0.46], miR-203 [OR, 0.46], miR-210 [OR, 0.47], miR-205 [OR, 0.49])	Massive sequencing: 1,349 miRNAs	Gingival biopsy	198 44.5±ND (overall mean age of all participants) G1: n=158; ND G2: n=40; ND	G1: CP + tissue with CP G2: CP + healthy tissue	Stoecklin-Wasmer *et al*. [34], cross-sectional
- No significant differences in miR-146a expression levels between groups - Downregulation of miR-196a2 in G1 (OR, 0.23) compared to G2	miR-146a, miR-196a2	Blood sample	370 G1: n=190; 38.16±8.4 G2: n=180; 29.64±5.5	G1: CP G2: Healthy	Venugopal *et al*. [38], cross-sectional
- Upregulation of miR-125a (OR, 2.07) and miR-499 (OR, 1.54) in G1 compared to G2	miR-125a, miR-499a	Blood sample	550 G1: n=262; ND G2: n=288; ND	G1: CP G2: Healthy	Venugopal *et al*. [17], cross-sectional
- Upregulation of miR-21 and let-7a in G1 (OR, 2) - Upregulation of miR-100 in G1 (OR, 1.6) - No significant differences in miR-125b expression levels between groups	miR-125b, miR-21, miR-100, let-7a	Gingival biopsy	200 G1: n=100; 48.4±11.6 G2: n=100; 40.4±8.5	G1: CP G2: Healthy	Venugopal *et al*. [35], cross-sectional
- Upregulation of 96 miRNAs (highlighted: miR-126, miR-20a, miR-190, miR-32, miR-362-3p; OR, 5–10; OR, 5–10) and downregulation of 34 miRNAs (highlighted: miR-155, miR-205; OR, 2–5) in G1 compared to G2 - Upregulation of miR-146 in G1 (OR, 2)	Massive sequencing: 1,769 miRNAs	Gingival biopsy	20 G1: n=10; 40.6±ND G2: n=10; 36.5±ND	G1: CP G2: Healthy	Xie *et al*. [36], cross-sectional
- Upregulation of miR-146: G1 > G2 > G3 - G1: 2-fold increase compared to G3 - Positive correlation with BMI, periodontal and cardiac parameters	miR-146a	Subgingival biofilm	90 G1: n=30; 53.07±7.72 G2: n=30; 52.27±7.13 G3: n=30; 51.10±7.90	G1: CP + CHD G2: CP without CHD G3: Healthy	Yagnik *et al*. [23], cross-sectional
- Upregulation of miR-555 (OR, 1.85), miR-130a-5p (OR, 1.71), miR-664a-3p (OR, 1.54), miR-501-5p (OR, 1.57), miR-6770-5p (OR, 0.65), miR-4717-5p (OR, 0.64), miR-21-3p (OR, 0.63) in G1 compared to G2	Massive sequencing: 2,565 miRNAs	Blood sample	60 G1: n=30; 67.0±11.7 G2: n=30; 65.0±13.2	G1: CP G2: Healthy	Yoneda *et al*. [37], cross-sectional

OR: odds ratio, miRNA: microRNA, SD: standard deviation, G: group, CHD: coronary heart disease, CP: chronic periodontitis, ND: no data, PD: probing depth, PI: peri-implantitis, PROINF CYT: pro-inflammatory cytokines, NSPT: nonsurgical periodontal therapy, DM2: type 2 diabetes mellitus, AP: aggressive periodontitis, BMI: body mass index.

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Based on the included studies, most of the evaluated miRNAs were upregulated in periodontally compromised patients [16-337], and miR-146a was the miRNA most-frequently analysed by microarray and reverse-transcriptase polymerase chain reaction (RT-PCR) [21-23,25,26,30,36,38]. There appeared to be a positive correlation between miR-146a levels and disease severity as quantified in terms of probing depth, clinical attachment level, and bleeding on probing [23,25,30]. However, the odds ratios (ORs) differed greatly among studies, ranging from 1.43 [22] to 32.6 [30]. After miR-146 [21-23,25,26,30,36,38], the next most-frequently investigated miRNAs were miR-142-3p [20,24,36], miR-223 [16,29,33,34], miR-155 [21,36], miR-205 [33,34,36], miR-21 [35,37], let-7a [27,35], miR-200b [16,18], and miR-499 [17,26]. Several studies reported contradictory results for miRNA expression levels; for example, miR-155 was upregulated in the study of Radović *et al.* [21], but downregulated in that of Xie *et al.* [36].

The lack of homogeneity between the reports from the different authors prevented a meta-analysis of the data. In addition, some articles included ORs or fold-changes, whereas others solely stated that there were no significant differences between groups without referencing the use of any measure to assess the miRNA expression profiles. For this reason, it was not possible to harmonize the results of the different studies into a single measure or graph.

Assessment of the quality of the included studies

We performed a qualitative analysis of the included studies (Table 2). Since all the studies were observational, the NOS was used. Overall, most studies received ≥7 stars, indicating a low risk of bias (high-quality studies).

Table 2. Quality of included studies

Study	Selection				Comparability		Exposure			Total score/risk of bias
Bagavad Gita *et al.* [22]	*			*	*	*	*	*	*	7/Low
Chen *et al.* [24]	*			*	*	*	*	*	*	7/Low
Ghotloo *et al.* [25]	*			*	*	*	*	*	*	7/Low
Kadkhodazadeh *et al.* [26]	*	*	*	*	*		*	*	*	8/Low
Kalea *et al.* [18]	*	*	*	*	*	*	*	*	*	9/Low
Lee *et al.* [27]		*	*		*		*	*	*	6/High
Li *et al.* [28]	*	*	*	*	*	*	*	*	*	9/Low
Micó-Martínez *et al.* [29]	*	*	*	*	*	*	*	*	*	9/Low
Motedayyen *et al.* [30]	*	*	*	*	*	*	*	*	*	9/Low
Na *et al.* [31]	*		*	*	*	*	*	*		7/Low
Naqvi *et al.* [19]	*	*	*	*	*	*	*	*	*	9/Low
Nisha *et al.* [32]	*	*	*	*	*	*	*	*	*	9/Low
Ogata *et al.* [16]	*		*	*	*	*	*	*		7/Low
Perri *et al.* [20]	*	*	*	*	*	*	*	*	*	9/Low
Radović *et al.* [21]	*	*	*	*	*	*	*	*	*	9/Low
Saito *et al.* [33]	*	*		*	*		*	*	*	7/Low
Stoecklin-Wasmer *et al.* [34]	*	*	*	*	*	*	*	*	*	9/Low
Venugopal *et al.* [35]	*	*	*	*	*		*	*	*	8/Low
Venugopal *et al.* [17]	*	*	*	*	*		*	*	*	8/Low
Venugopal *et al.* [36]	*		*	*	*	*	*	*	*	8/Low
Xie *et al.* [37]	*	*	*	*	*	*	*	*	*	9/Low
Yagnik *et al.* [23]	*	*	*	*	*		*	*	*	8/Low
Yoneda *et al.* [38]	*	*	*	*	*		*	*	*	8/Low

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DISCUSSION

miRNAs play significant roles in various immune processes, and affect both the innate and humoral responses of the host against the bacterial challenges associated with periodontal disease. The current qualitative systematic review examined the relationship in humans between differentially expressed miRNAs and periodontal disease, and aimed to determine the potential value of these miRNAs as diagnostic or prognostic periodontal biomarkers.

We were not able to conduct a meta-analysis due to the methodological differences among studies. For example, samples were obtained from different biological sources, sample sizes differed greatly among the studies, and some authors focused on specific miRNAs. In contrast, others performed large-scale sequencing using various technologies (such as microarray hybridization, quantitative RT-PCR, and next-generation sequencing), and analysed up to 2,565 different miRNAs (Yoneda *et al.* [37]). Furthermore, 6 studies [18-23] evaluated the impact of systemic diseases such as obesity, diabetes, and coronary heart disease on miRNA expression levels in periodontal patients.

One of the most-researched miRNAs in periodontal diseases was miR-146a [21-23,25,26,30,36,38], which is located in the second exon of the LOC285628 gene on human chromosome 5 [36] and belongs to the miR-146 family, along with miR-146b. Despite significant structural similarities between miR-146a and miR-146b, they do not have comparable biological functions. miR-146a serves as a key negative regulator of the innate immune system. Bacterial components of plaque, particularly lipopolysaccharide, stimulate Toll-like receptors (especially TLR-2 and TLR-4), which leads to upregulation in monocytes of miRNAs such as miR-155, miR-21, and miR-146a [39]. Most of the included studies reported that miR-146a expression levels were higher in patients with periodontitis compared to healthy controls (OR, 1.43 [22] to 32.6 [30]) [21-23,25,26,30,36]. There also appeared to be a positive correlation between the miR-146a level and disease severity, as assessed in terms of probing depth, clinical attachment level, and bleeding on probing [23,25,30].

However, 1 study did not find any significant association between miR-146a and chronic periodontitis [38]. Venugopal *et al.* [38] assessed miR-146a single nucleotide polymorphisms (SNPs), which are genetic variants of miRNA that can alter the biogenesis, binding affinity, and specificity to target mRNAs. Meanwhile, Kadkhodazadeh *et al.*, [26] who also evaluated miR-146a SNPs, reported a positive correlation between miR-146a gene polymorphisms and periodontitis and peri-implantitis. Venugopal *et al.* [38] believed that this discrepancy might have been due to differences in environmental and participant lifestyle factors.

To understand the beneficial (or detrimental) effects of each miRNA in periodontal inflammation, most of the included studies performed target gene predictions of significantly expressed upregulated or downregulated miRNAs using various bioinformatics tools and databases, such as TargetScan, miRDB, microRNA.org, PicTar, etc. For instance, several studies analysed the role of miR-146a as a negative feedback regulator of inflammation in periodontitis [25]. Elevated miR-146a levels are reported to suppress the expression of the IL-1 receptor-associated kinase 1 and tumour necrosis factor receptor-associated factor 6 target genes, and thus inhibit nuclear factor-kappa B activation, which is the transcription factor most heavily implicated in the production of many pro-inflammatory cytokines, such as tumour necrosis factor-alpha, IL-1β, IL-6, and IL-8, chemokines, adhesion molecules, and prostaglandins [39]. Several studies demonstrated that overexpression of miR-146a

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was accompanied by a reduction in the levels of these pro-inflammatory cytokines [25,30]. However, these results do not agree with those obtained by Bagavad *et al.*, [22] who observed upregulation of both miR-146a and associated cytokines.

Furthermore, no investigations that screened for multiple candidate periodontitis miRNAs simultaneously (massive sequencing) [16,19,20,27,31-34,36,37] cited miR-146a as the most highly expressed miRNA. For example, Lee *et al.* [27] highlighted upregulation of let-7a (OR, 9.48), Xie *et al.* [36] reported upregulation of miR-126, miR-20a, miR-190, miR-32, and miR-362-3p (OR, 5–10) and downregulation of miR-155 and miR-205 (OR, 2–5), Ogata *et al.* [16] showed upregulation of miR-150, miR-223, and miR-200b (OR, 2.72), and Nisha *et al.* [32] reported upregulation of miR-143-3p (OR, 5.82). This variability among studies could be due to differences in the profiling techniques and sample media used, and reflects the complex nature of a multifactorial disease such as periodontitis, in which different regulatory networks intervene between miRNAs and periodontal inflammation-related genes.

Periodontopathogens in intimate contact with an inflamed and ulcerated crevice or pocket epithelium may gain entry to the bloodstream. The resultant bacteraemia and associated endotoxaemia in patients with untreated periodontitis could initiate the overproduction of destructive inflammatory mediators at distant sites. Therefore, periodontitis patients may be at increased risk of developing a number of systemic conditions associated with similar overactive host responses to external stimuli, such as coronary heart disease, obesity, and diabetes [20-23]. There is substantial evidence of the presence of gram-negative periodontal pathogens in atheromatous plaques [22]. These systemic conditions can also alter the host susceptibility to microbial agents, thus exacerbating periodontal destruction. This was reported by Perri *et al.*, [20] who observed higher levels of miR-106b in chronic periodontitis patients with obesity (OR, 6.4) than in those without obesity (OR, 4.9).

Radović *et al.* [21] compared the expression levels of miR-146a and miR-155 in gingival crevicular fluid before and after nonsurgical periodontal treatment in periodontitis patients with and without type 2 diabetes. They observed significantly higher levels of these miRNAs before treatment compared to periodontally healthy controls; moreover, nonsurgical periodontal therapy significantly reduced the expression of both of these miRNAs [21].

In a recent review on miRNA expression in periodontal and peri-implant diseases, which included animal and human studies, miR-142-3p, miR-155, and miR-146a were cited as potential diagnostic biomarkers for periodontal disease activity. Furthermore, in peri-implantitis studies, most miRNAs were downregulated, except for miR-145, which was significantly upregulated [40].

Despite their promising indications, stability, and straightforward testability, the use of miRNAs as biomarkers for monitoring and early diagnosis of periodontitis has not yet been incorporated into routine clinical practice. This is mainly due to the heterogeneity of existing studies and the lack of diagnostic tests to evaluate the sensitivity and specificity of these miRNAs. Further investigations using standardised sample collection protocols, miRNA sources (saliva, gingival crevicular fluid, etc.) and detection methods are needed to identify specific miRNAs for periodontal diseases with expression levels varying according to disease progression or the response to treatment.

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