

## Original Article

# Comparative evaluation of microRNA-155 expression level and its correlation with tumor necrotizing factor $\alpha$ and interleukin 6 in patients with chronic periodontitis

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## ABSTRACT

**Background:** MicroRNAs are a class of small noncoding ribonucleic acids that perform a critical role in adjustment of gene expression. miRNAs-155 (miR-155) participates in controlling inflammation. Periodontitis is defined as inflammatory disorder of tissues surrounding the teeth. In this study, the expression levels of miR-155 and its target genes, tumor necrotizing factor alpha (TNF- $\alpha$ ), and interleukin-6 (IL-6) were evaluated in a group of Iranian patients.

**Materials and Methods:** This sectional study was performed on 10 healthy controls and 10 individuals with chronic periodontitis by means of polymerase chain reaction (PCR) test. For each individual, clinical parameters including probing depth and clinical attachment loss and blood samples were measured. Levels of miR-155, TNF- $\alpha$ , and IL-6 were quantified using real-time PCR ( $\alpha=0/05$ ) and the results were analyzed by Mann-Whitney U test.

**Results:** The level of miR-155 was significantly higher in patients with chronic periodontitis ( $P < 0.001$ ). A positive correlation was observed between the level of miR-155 and clinical parameters ( $P < 0.05$ ). Level of miR-155 in tissue samples was correlated with blood samples although the expression level was higher in blood samples.

**Conclusion:** As the expression level of miR-155, TNF- $\alpha$ , and IL-6 genes was higher in subjects with chronic periodontitis than healthy individuals, it might suggest a role for miR-155 in patients with chronic periodontitis.

**Key Words:** Interleukin-6, microRNAs, microRNA-155, periodontitis, tumor necrosis factor-alpha

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## INTRODUCTION

Chronic periodontitis is the most common form of periodontal disease which is a multifactorial inflammatory disorder affecting tissue surrounding the teeth, destroying alveolar bone, and tooth loss, eventually. This disease can have a negative effect on general health and it is a

known risk factor for some systemic diseases such as atherosclerosis and diabetes.<sup>[1-5]</sup>

Although this disease is mainly caused by a group of pathogenic microorganisms specifically

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*Porphyromonas gingivalis*, but the progression of disease is under the influence of multiple factors such as systemic, environmental, and genetic factors. Bacterial elements such as lipopolysaccharides (LPS) stimulate different host cells including leukocytes, fibroblasts, and epithelial cells by Toll-like receptors (TLRs). Signaling through these receptors induces proinflammatory cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 beta (IL-1  $\beta$ ), interleukin-6 (IL-6), and other inflammatory mediators.<sup>[6-8]</sup> These inflammatory mediators advocate a number of events that lead to destruction of periodontal tissues. As innate immune response plays an important role in periodontal disease to avoid inflammation, the cells involved in innate immune response should be regulated by endogenous genes.<sup>[2]</sup>

MicroRNAs (miRNAs) are a class of small noncoding ribonucleic acids (RNA) that have a critical role in controlling a few biological processes such as cell division, differentiation, apoptosis, and embryogenesis.<sup>[9]</sup> They participate in the regulation of gene expression by targeting messenger ribonucleic acid (mRNA) which causes mRNA degradation or translation inhibition. MiRNAs are implicated in establishing and maintaining of immune cells and are involved in innate immunity by regulating Toll-like receptor signaling and ensuing a cytokine response.

Recent studies have reported different miRNA expression patterns between healthy tissues and inflamed tissues, suggesting the involvement of miRNAs in regulation of periodontal disease.<sup>[10-12]</sup> MicroRNA-155 (miR-155) is a multifunctional miRNA, which is involved in several biological processes such as hematopoiesis, inflammation, and immunity. Studies define a pro-inflammatory role for miR-155 through deactivation of anti-inflammatory cytokines.<sup>[13,14]</sup> Study showed that in miR-155-deficient mice, an uncontrolled inflammatory response was seen.<sup>[15]</sup>

Considering the suggested role for miR-155 in periodontitis, the purpose of this study was to evaluate miR-155 and its target genes (TNF- $\alpha$  and IL-6) expression level both in gingival tissue and blood sample of the study group.

## MATERIALS AND METHODS

In this sectional study, a total of 20 cases referring to the Department of Periodontology, Isfahan Dental

School, Isfahan, Iran, during October 2017 to June 2018 were selected. Exclusion criteria for all cases were smoking, presence of any systemic disease such as diabetes, allergy, and intake of drugs including corticosteroids and antibiotics within 1 month before the surgery. Pocket depth (PD) and clinical attachment level (CAL) were documented using a calibrated Williams periodontal probe (Fattah Teb, Iran) for each individual. Six sites were examined for each tooth.

Clinically healthy gingival tissues were collected from crown-lengthening procedures with no evidence of periodontal disease. Diseased group samples were collected from periodontal tissues during surgical phase therapy for patients who had an initial uneventful phase I therapy with PD >4 mms and CAL >4 mm.

The mean weight of tissue samples was more than 0.1 g. Blood samples were taken from this group and the samples were stored in tubes with ethylenediaminetetraacetic acid (EDTA) (MT company, Iran).

Samples were stored in nitrogen at -80oc until RNA extraction. For measuring the expression level of proinflammatory cytokines including TNF- $\alpha$ , IL-6, and miR-155 in the real-time polymerase chain reaction (PCR) assay, specific primers have been designed using primer 3 program [Table 1]. Total RNA was extracted from the samples using small RNA extraction kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. The quality and quantity of RNA were assessed using a Gel Electrophoresis (Dentagene, Iran). Complementary deoxyribonucleic acid synthesis kit (Thermoscientific, USA) and OligoDT, Random hexamer primers were used to synthesize cDNA. Afterward for testing the specificity of designed primers, PCR was performed

**Table 1: Designed primers for the required genes**

Gene	
TNF- $\alpha$	Forward: 5- AACAGACCACCCTTCGAAAC-3 Reverse: 5- GATGTCAGGGATCAAAGCTGTAG-3
IL-6	Forward: 5- AGGACATGACAACCTCATCTCATTCC-3 Reverse: 5- GACAGGTTTCTGACCAGAAGAAG-3
miR-155	Forward: 5-TGTCAG GCAACC GTATTCC ACC GTG AGTGGTACCCT-3 Reverse: 5-CGT CAG ATG TCC GAG TAG AGG GGG AAC GGC
Human U6 RNA primer	Forward: 5- TTGGAACGATACAGAGAAGATTAGC-3 Reverse: 5- TCACGAATTTGCGTGTCCATC-3
Universal primers	Forward: 5-TGTCAGGCAACCGTATTCACC-3 Reverse: 5-CGTCAGATGTCCGAGTAGAGG-3

by using the master mix kit (amplicon, Denmark) based on the manufacturer's instruction [Table 2]. Finally, the level of miR-155, TNF- $\alpha$ , and IL-6 expression level was measured by real-time PCR with Master Mix 5x firepolevagreengreen kit (Solis-biodyne, Estonia) and Rotorgene-q (Qiagen, Germany). The expression level of each sample was normalized to its glyceraldehyde 3 phosphate dehydrogenase and human U6 RNA primer. Relative quantification of miR-155 and the cytokines were calculated using 2-JJct and pfaffle method.<sup>[16]</sup> Data gathered were analyzed by Mann-Whitney U test in SPSS software, version 22 (SPSS, Chicago, IL, USA) ( $\alpha = 0.05$ ).

## RESULTS

The aim of this study was to estimate the expression level of miR-155 and its target genes (TNF- $\alpha$  and IL-6) in tissue and blood samples of patients

**Table 2: Master mix quantitative polymerase chain reaction guide**

Components	Quantity
Master mix $\times$ 2x	14 $\mu$ lit
Forward primer	10 $\mu$ M
Reverse primer	1 $\mu$ M
CDNA	1 $\mu$ M
Free water	7 $\mu$ lit

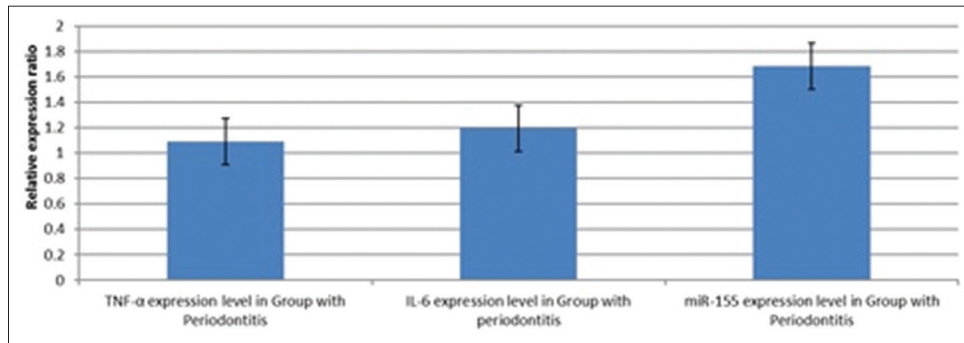
with chronic periodontitis [Figures 1 and 2]. Our results revealed a 64.4, 1.66- and 2.04-fold increase in miR-155, TNF- $\alpha$ , and IL-6 expression in the patients with chronic periodontitis compared to the healthy cases ( $P < 0.001$ ). Our tests could not find a correlation between the expression level of TNF- $\alpha$  and IL-6 ( $P = 0.730$ ).

We also found a correlation between the expression level of miR-155 in tissue samples with blood samples ( $P < 0.001$ ), but the expression level was much higher in the blood samples than in the tissue samples. Correlation between expression level of TNF- $\alpha$  and IL-6 was not observed between blood samples and tissue samples ( $P = 0.25$ ,  $P = 0.43$ ).

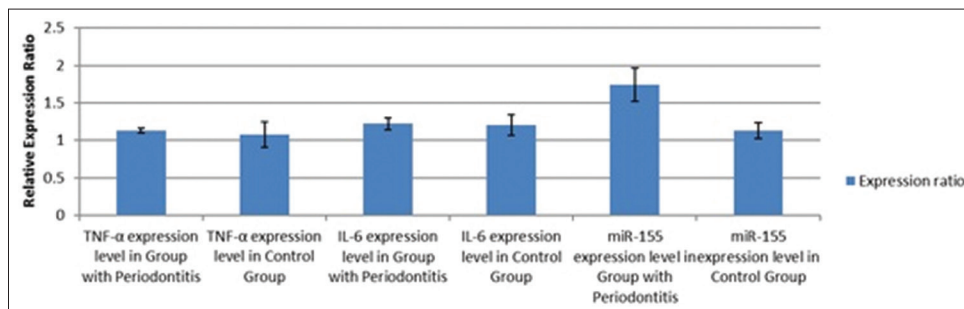
The level of miR-155 was correlated with clinical parameters including PD and CAL in the patients with chronic periodontitis ( $P = 0.001$ ), and there was no correlation between the levels of proinflammatory cytokines and clinical parameters of the patients [Figures 3 and 4].

## DISCUSSION

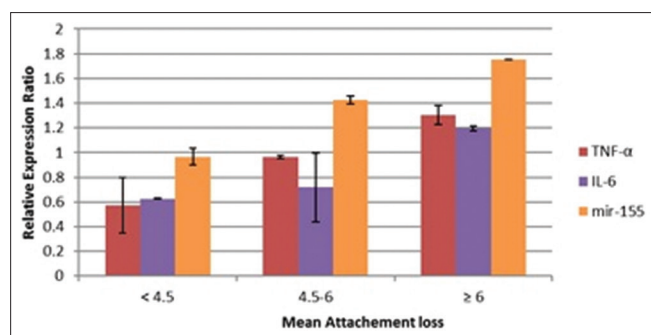
miRNAs have important roles in controlling several biologic processes such as cell division, differentiation, apoptosis, and embryogenesis.<sup>[9,17]</sup> Different studies, before, have had pointed to the



**Figure 1:** Relative expression ratio of three genes in blood samples.



**Figure 2:** Relative Expression ratio of three genes in tissue samples.

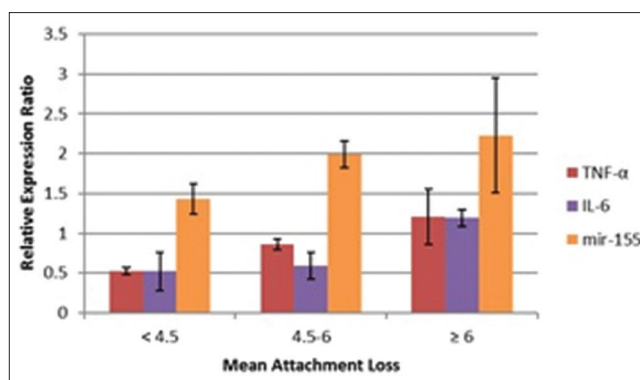


**Figure 3:** Relative expression level ratio of three genes according to Pfaffle test according to mean attachment loss in patients with chronic periodontitis in tissue samples.

role of miRNAs in healthy and inflamed periodontal tissues.<sup>[18]</sup> In 2012, the first miRNA research in chronic periodontitis was accomplished by stoecklin-wasmer, and they announced that miRNAs may have role in both pathogenesis and homeostasis of periodontal diseases.<sup>[13]</sup>

In 2015, Motedayyen *et al.*<sup>[2]</sup> and Gu *et al.*<sup>[19]</sup> have shown during inflammation, the expression level of miRNA 146a increases which in part is followed by a reduction in the expression level of TNF- $\alpha$  and IL-6. To date, no studies have examined the relation between expression level of miR-155, TNF- $\alpha$ , and IL-6, synchronously, in blood and tissues of patients with chronic periodontitis. Thus, the aim of present study was to evaluate the expression level of miR-155, TNF- $\alpha$ , and IL-6 in blood and tissues samples of patients with chronic periodontitis. In this study, the results revealed that the expression level of miR-155 was higher in blood and tissue samples of patients with chronic periodontitis compared to control group with a strong correlation to expression level of TNF- $\alpha$  and IL-6 [Figure 3]. Although expression level of TNF- $\alpha$  and IL-6 was not correlated with each other in neither samples that were studied.

Previously, Baulina *et al.*,<sup>[20]</sup> Suarez *et al.*,<sup>[21]</sup> and Mashima<sup>[22]</sup> showed that miR-155 can affect the NF-K $\beta$  pathway and SOCS1 gene which can cause an increase in the expression level of TNF- $\alpha$  and IL-6. It has been mentioned in previous studies that miR-155 and TNF- $\alpha$  have similar target genes so an increase in TNF- $\alpha$  can cause an increase in the expression level of miR-155<sup>[23]</sup> As well this increase can be explained by the fact that this cytokine can cause the increase in the level of miR-155 by affecting macrophages.<sup>[24]</sup> On the other hand, it is believed that miR-155 plays a vital role in regulating the IL-1 pathway in human



**Figure 4:** Relative expression level ratio of three genes according to Pfaffle test according to mean attachment loss in patients with chronic periodontitis in blood samples.

dendritic cells.<sup>[21,25]</sup> In our research, in accordance to study of Scapoli, a higher miR-155 expression level was directly related to higher expression level of TNF- $\alpha$  and IL-6.<sup>[26]</sup> Lavu *et al.* in 2015<sup>[27]</sup> demonstrated that an increase in miR-155 in blood samples will cause a decrease in the expression level of TNF- $\alpha$  and IL-6 which is not in accordance to results of the present study. Different sampling and study methods might be related to this inconsistency.

Previous studies on gingival samples have revealed a high expression level of miR-146a and miR-155 in patients with chronic periodontitis<sup>[9,18]</sup> Motedayyen *et al.*<sup>[2]</sup> believe that miR-146a has an anti-inflammatory effect which is responsible for a decrease in the expression level of TNF- $\alpha$  and IL-6. On the other hand, to keep balance between the production of cytokines, miR-155 will have a compensatory effect on the production of TNF- $\alpha$  and IL-6.

In the present study, the results showed that the expression level of TNF- $\alpha$  and IL-6 was higher in tissue samples compared to blood samples which are in accordance to study of Politano *et al.*<sup>[28]</sup> who showed that the amount of these two cytokines is higher in tissue samples and they believe that this might be because of the lower half-life time of these molecules in blood.

To date, no study has examined the expression level of miRNAs in serum and tissue samples in patients with chronic periodontitis, at one time, as was performed in the present study. In our research, it was shown that the expression level of miR-155 in blood and tissue samples has a meaningful correlation and this level was higher in blood samples. The higher level of this marker in blood might be explained by immunity against endogenous RNase activity.<sup>[19]</sup> This



molecule is secreted as an exosome with a two-layer membrane, which protects this molecule from degenerative enzymes. High levels of miR-155 in blood samples are important since this molecule can have different effects on biological systems of human body. According to the results of this research, it can be concluded that miR-155 can be an important circulating marker for chronic periodontitis and co-existing diseases such as atherosclerosis or diabetes. The results of our study showed that there is a positive correlation between the expression level of miR-155 and clinical parameters like pocket depth and attachment loss as well with inflammatory cytokines TNF- $\alpha$  and IL-6 [Figures 3 and 4].

### Strengths

Previous studies have evaluated the role of miRNAs in inflammatory and cancerous disorders and introduced it as a novel biomarker.<sup>[29-31]</sup> The present study was the very first research in which the presence of miR-155 in chronic periodontitis was evaluated in relation to important chronic periodontitis biomarkers such as TNF-a and IL-6.

Maintaining tissue and blood samples in the desired temperature and extracting miRNA from tissue samples were main limitations of the present study.

### CONCLUSION

miR-155 can be detected in chronic periodontitis and its level is higher in blood samples rather than gingival tissue sections. Level of MiR-155 was directly related to the level of TNF- $\alpha$  and IL-6. This study confirms the role of miR-155 as a circulating marker for early diagnosis of chronic periodontitis.

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### Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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