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Original Article

miR-205 and HMGB1 expressions in chronic periodontitis patients and their associations with the inflammatory factors

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Abstract: Objective: This paper sets out to investigate the miR-205 and HMGB1 expressions in chronic periodontitis (PO) patients and their associations with the inflammatory factors. Methods: From February 2016 to May 2018, 68 PO patients treated in our hospital were recruited for the study and placed in a patient group (PG), and 60 healthy volunteers were also recruited and placed in a healthy group (HG). Serum samples were collected from both groups for the identification of miR-205 using qPCR, as well as to determine the HMGB1 and inflammatory factor (IL-1β, IL-6, TNF-α) expression levels using ELISA. The correlations of the miR-205 and HMGB1 expression levels with the periodontal clinical indicators and the inflammatory factors were analyzed using a correlation analysis. Results: In comparison with the HG expression, the serum miR-205 expression was lower, and the HMGB1 was elevated in PG ($P < 0.05$). An ROC curve analysis showed that the areas under the curve (AUCs) of the serum miR-205 and HMGB1 expressions in diagnosing PO were 0.936 and 0.955 respectively. However, the serum miR-205 expression in PG increased while the HMGB1 expression decreased post treatment ($P < 0.05$). A correlation analysis revealed an inverse association between the serum miR-205 expression levels and the periodontal clinical indicators [bleeding index (BI), probing depth (PD), plaque index (PLI), and attachment loss (AL)], and the inflammatory factors [interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α)], but there was a positive association between the HMGB1 expression level and these parameters. Conclusions: miR-205 and HMGB1 are closely related to the progression of PO, and may be candidate biomarkers for the diagnosis and treatment of PO.

Keywords: miR-205, HMGB1, chronic periodontitis, inflammatory factors

Introduction

Periodontitis (PO) is a type of chronic inflammation triggered by bacteria invading the gums and periodontal tissues [1]. The main clinical presentations are gingival inflammation and bleeding, alveolar bone absorption, attachment loss (AL), and periodontal pocket formation, which can eventually lead to tooth loss and seriously affect patients' quality of life [2]. PO is a ubiquitous disease. According to the World Health Organization, 10-15% of the global population suffers from severe periodontitis [3]. It is not only considered as the primary cause of tooth loss in adults, it also increases the risk of multiple diseases such as cardiovascular disease, rheumatoid arthritis, adverse pregnancy outcomes, and cancer [4]. There is currently no valid method for treating the disease [5]. In view of this, it is paramount to better clarify the pathogenesis of periodontitis to find a better treatment.

miRs are highly conserved, endogenous short-chain non-coding RNAs, that are widely distributed in eukaryotic cells [6]. They can regulate physiological and pathological processes by blocking protein translation or inhibiting target gene expression by inducing mRNA degradation [7]. With our increasing understanding of miRs, they are not thought to play a key role in the development of a variety of inflammatory diseases. For example, miR-10a-5p is down-regulated in the synovia of rheumatoid arthritis

patients, and through the targeted regulation of TBX5, it induces inflammatory changes in human synovial sarcoma cells [8]. Myeloid-derived miR-223 is able to regulate intestinal inflammation by inhibiting NLRP3 inflammasomes [9]. And miR-495 is up-regulated in osteoarthritis and can promote the development of osteoarthritis by regulating AKT1 [10]. miR-205, an important member of the miR family, has drawn much attention because of its participation in the development of many diseases [11-13]. Previous studies have reported that miR-205 is down-regulated in the gingival tissues of periodontitis patients [14]. The high mobility group protein 1 (HMGB1) is a protein type widely distributed in eukaryotic cells to regulate gene expression and transcription, and it is highly expressed in manifold inflammatory diseases and is considered to be an essential regulator involved in inflammatory progression [15-17]. Moreover, HMGB1 has been found to be highly expressed in periodontitis and facilitates its progression [18].

In this paper, we examined the serum miR-205 and HMGB1 expressions in patients with periodontitis, and analyze their significance in disease diagnosis and evaluation.

Materials and methods

Study participants

From August 2017 to August 2019, 86 patients with chronic periodontitis treated in the Affiliated Hospital of North Sichuan Medical College, recruited for this study, and placed in the patient group (PG), and 60 healthy controls who concurrently underwent healthy check-ups were also recruited for this study and placed in the healthy group (HG). Inclusion criteria for the PG: patients who met the diagnostic criteria of periodontitis [19], patients with complete clinical data, and patients with more than 16 teeth remaining in their mouths. Exclusion criteria of both groups: patients with an organic dysfunction of the heart, liver, kidneys, or lungs, patients with autoimmune diseases, patients who were pregnant, lactating, or menstruating, patients who had undergone prior orthodontic treatment, patients who had taken antibiotics or immunomodulatory drugs within the previous three months, patients who had taken drugs and/or who had undergone surgical treatment for periodontitis within the previous three months, and patients with incomplete clinical data. This study was approved by the Medical Ethics Committee of the Affiliated Hospital of North Sichuan Medical College, and all the participants were informed and signed the informed consent to participate.

Quantification of the clinical indicators and the serum sample collection

Oral and total dental examinations were performed by our professional oral surgeons in our hospital, and bleeding index (BI), periodontal probing depth (PD), plaque index (PLI), and attachment loss (AL) levels were recorded. The average values of each of the above clinical indicators were taken and compared between the two groups.

The participants were required to fast and were prohibited from smoking for two hours before their blood was drawn. Then 5 ml venous blood was drawn from the median cubital vein and centrifuged (4°C, 1500 × g, 10 min) for the serum collection and subsequent storage in a freezer at -80°C for later analysis.

qRT-PCR quantification

Total RNA was extracted using TRIzol kits and its purity was tested. Then, cDNA reverse transcription was carried out following the TaqMan™ Reverse kit (Invitrogen, USA) protocol, followed by PCR amplification via a PCR amplification kit (Takara Bio, Japan). The amplification system: 10 μL SYBR qPCR Mix, 0.8 μL each of upstream and downstream primers, 2 μL cDNA, 0.4 μL 50 × ROX reference dye, and water in a final volume of 20 μL. The reaction conditions were pre-denaturation at 95°C for 60 s, denaturation at 95°C for 30 s, and annealing at 60°C for 40 s, cycling 40 times. U6 was utilized as an internal reference, and the primers were designed by Shanghai Genechem Co., Ltd. miR-205 upstream sequence: 5'-GGCGTGAGGCTGAGGCTA-3', downstream sequence: 5'-ATGGCTGAGCGAAATTGCGGAC-3'; U6 upstream sequence: 5'-CATCACCATCAGGAGAGTCG-3', downstream sequence: 5'-TGACGCTTGCCCACAGCCTT-3'. Data processing was performed by $2^{-\Delta\Delta ct}$.

ELISA quantification

The serum inflammatory factors, including interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), were quantified using ELISA.

Table 1. Comparison of the general information between the two series ([n (%)], x ± sd)

Groups	Healthy group (n=60)	Patient group (n=68)	χ^2/t	P
Gender			1.729	0.189
Male	42 (70.00)	40 (58.82)		
Female	18 (30.00)	28 (41.28)		
Age (years old)	57.26±7.15	59.11±8.63	1.310	0.193
Weight (KG)	62.24±6.12	63.56±7.22	1.108	0.270
Dietary preference			0.728	0.394
Light	29 (48.33)	38 (55.88)		
Greasy	31 (51.67)	30 (44.12)		
Residence			0.343	0.558
Urban	34 (56.67)	42 (61.76)		
City	26 (43.33)	26 (38.24)		
Do you have any exercise habits			2.008	0.157
Yes	26 (43.33)	38 (55.88)		
No	34 (56.67)	30 (44.12)		
History of smoking			0.212	0.645
Yes	41 (68.33)	49 (72.06)		
No	19 (31.67)	19 (27.94)		
History of drinking			0.694	0.405
Yes	30 (50.00)	29 (42.65)		
No	30 (50.00)	39 (57.35)		

ELISA kits specific for IL-1β, IL-6, and TNF-α were purchased from Wuhan BOSTER Biotechnology Co., Ltd. with catalog numbers EK0412, EK0412, and EK0525 respectively.

Statistical processing

SPSS 21.0 (IBM Corp, Armonk, NY, USA) and GraphPad Prism 7 were used for the statistical analysis and figure rendering respectively. The count data were compared using either chi-square tests or Fisher's exact tests. Independent sample t tests and paired t tests were used to carry the inter-group and intra-group comparisons, respectively. One-way analyses of variance were used for the comparisons among multiple groups of means, and Dunnett's tests were used for the pairwise comparisons. The diagnostic value of miR-205 and HMGB1 in the diagnosis of periodontitis was evaluated using receiver operating characteristics (ROC) curves [the higher the area under the curve (AUC) value, the better the diagnostic significance]. Pearson method was used to analyze the correlations between variables. A *p*-value < 0.05 was considered significant for all the tests.

Results

Comparison of the general data

Significant differences were absent in the general patient data such as gender, age, weight, diet preference, residence, exercise habit, smoking history, and drinking history in the two groups (P > 0.05) (**Table 1**).

A comparison of the serum miR-205 and HMGB1 expressions

As indicated by the qRT-PCR and ELISA assays, PG presented notably lower, and the serum HMGB1 was higher than the HG (P < 0.001). A Pearson correlation analysis revealed an inverse correlation between the miR-205 and HMGB1 levels in the serum of PO patients (P < 0.001) (**Figure 1**).

The diagnostic effect of the serum miR-205 and HMGB1 on periodontitis

The ROC curves revealed that the AUC of serum miR-205 in the diagnosis of periodontitis was 0.936, the sensitivity was 88.24%, and the specificity was 86.67%. The AUC, the sensitivity, and the specificity of the serum HMGB1 in diagnosing PO were 0.955, 89.71% and 95.00% respectively (**Table 2**; **Figure 2**).

Expression changes in the miR-205 and HMGB1 levels before and after the treatment

Comparing the expressions of miR-205 and HMGB1 before and after the periodontal basic treatment, it was found that miR-205 was increased while HMGB1 decreased post treatment (P < 0.001) (**Figure 3**).

The correlations of the miR-205 and HMGB1 expressions with the periodontal clinical indicators

The results of periodontal clinical indexes of the patients were PD (5.45±0.78) mm, AL

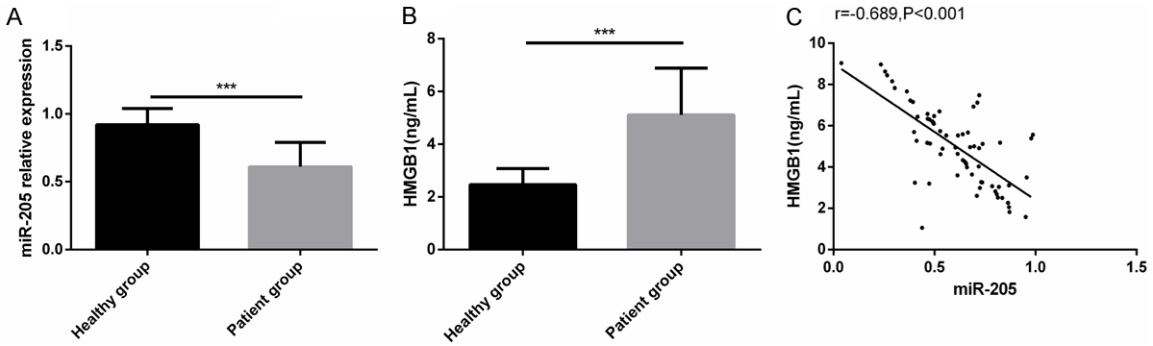

Figure 1. Comparison of the serum miR-205 and HMGB1 expressions in the two groups. A: Comparison of the serum miR-205 expressions in the two groups. B: Comparison of the serum HMGB1 expressions in the two groups. C: Correlation between the serum miR-205 and HMGB1 levels. Note: ***P < 0.001.

Table 2. The diagnostic value of the serum miR-205 and HMGB1 expression levels in periodontitis

Groups	AUC	95% CI	Standard error	Cut-off value	Sensitivity (%)	Specificity (%)
miR-205	0.936	0.896-0.975	0.020	0.811	88.24	86.67
HMGB1	0.955	0.916-0.995	0.020	3.364	89.71	95.00

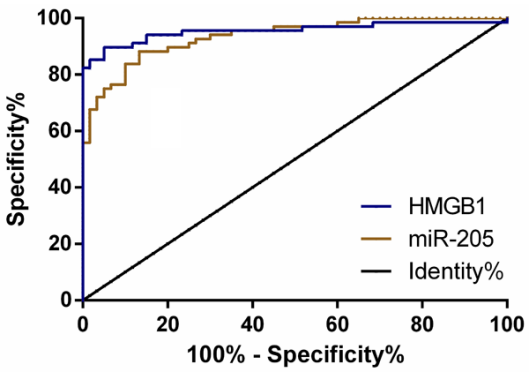

Figure 2. The ROC curves of the serum miR-205 and HMGB1 levels in the diagnosis of periodontitis. The AUC of serum miR-205 in the diagnosis of periodontitis was 0.936, the sensitivity was 88.24%, and the specificity was 86.67%. The AUC, sensitivity, and specificity of serum HMGB1 in the diagnosis of periodontitis were 0.955, 89.71% and 95.00%, respectively.

(4.59±0.68) mm, PLI (1.11±0.17), and GI (1.21±0.19). A Pearson correlation analysis showed that miR-205 is negatively related to PD, AL, PLI and GI, and HMGB1 is positively correlated with PD, AL, PLI, and GI (**Figure 4**).

Comparison of the serum IL-1β, IL-6, and TNF-α levels in the two groups

ELISA indicated that the serum IL-1β, IL-6, and TNF-α levels were higher in the PG than in the HG (P < 0.001) (**Figure 5**).

The correlation of the miR-205 and HMGB1 levels with the serum inflammatory factor levels

A Pearson correlation analysis showed that miR-205 has an inverse association with the IL-1β, IL-6, and TNF-α levels (P < 0.001), and that HMGB1 has a positive correlation with the above inflammatory factor levels (P < 0.001) (**Figure 6**).

Discussion

The investigation of the role of miRs in various diseases reveals that miRs are essential for the pathological development of periodontitis, which provides a new perspective and strategy for treating the disease [20, 21]. HMGB1 is a highly-conserved DNA binding protein that interferes with many biological processes such as DNA transcription, gene recombination, DNA repair, and nucleosome formation [22]. In PO, HMGB1 is abnormally elevated and can be released in the extracellular mediating inflammatory response [23]. In this paper, it was found that PO patients presented with notably lower serum miR-205 expressions and higher serum HMGB1 expressions than the controls, which agrees with the findings of previous studies [14, 24]. We also observed that serum miR-205 increased and HMGB1 decreased post treatment. Further, a negative association was identified between miR-205 and

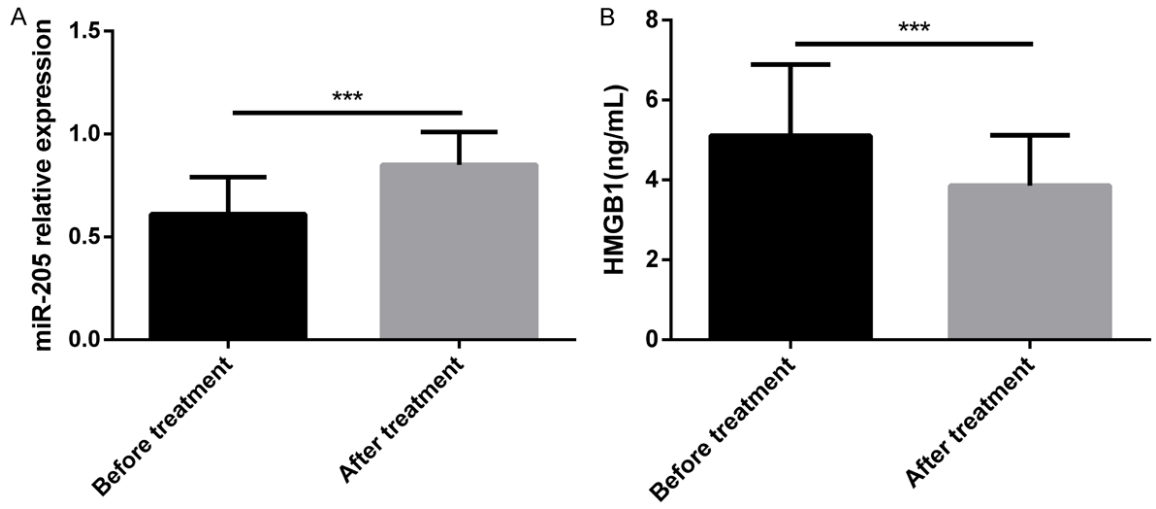

Figure 3. Changes in the serum miR-205 and HMGB1 expressions before and after the treatment. A: Changes in the serum miR-205 expressions before and after the treatment. B: Changes in the serum HMGB1 expressions before and after the treatment. Note: ***P < 0.001.

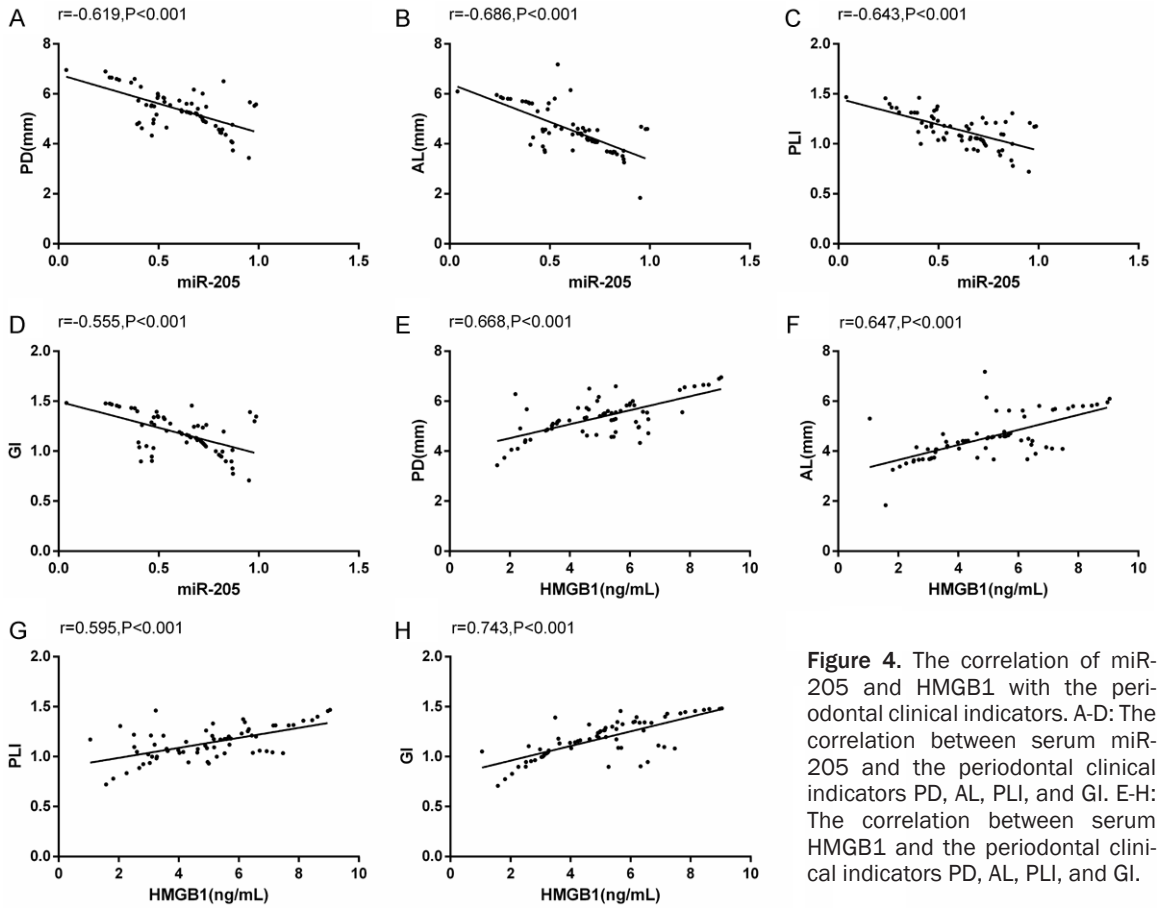

Figure 4. The correlation of miR-205 and HMGB1 with the periodontal clinical indicators. A-D: The correlation between serum miR-205 and the periodontal clinical indicators PD, AL, PLI, and GI. E-H: The correlation between serum HMGB1 and the periodontal clinical indicators PD, AL, PLI, and GI.

the periodontal clinical indicators PD, AL, PLI, and GI, while a positive correlation between HMGB1 and these periodontal clinical indicators was identified. These results suggest that miR-205 and HMGB1 are closely related to the occurrence of PO and may be potential targets

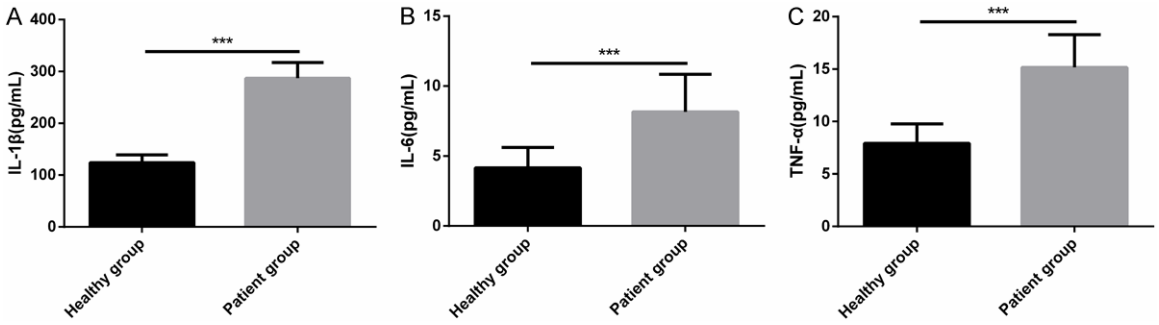

Figure 5. A comparison of the serum IL-1β, IL-6, and TNF-α levels in the two groups. A: A comparison of the serum IL-1β levels in the two groups. B: A comparison of the serum IL-6 levels in the two groups. C: A comparison of the serum TNF-α levels in the two groups. Note: *** P < 0.001.

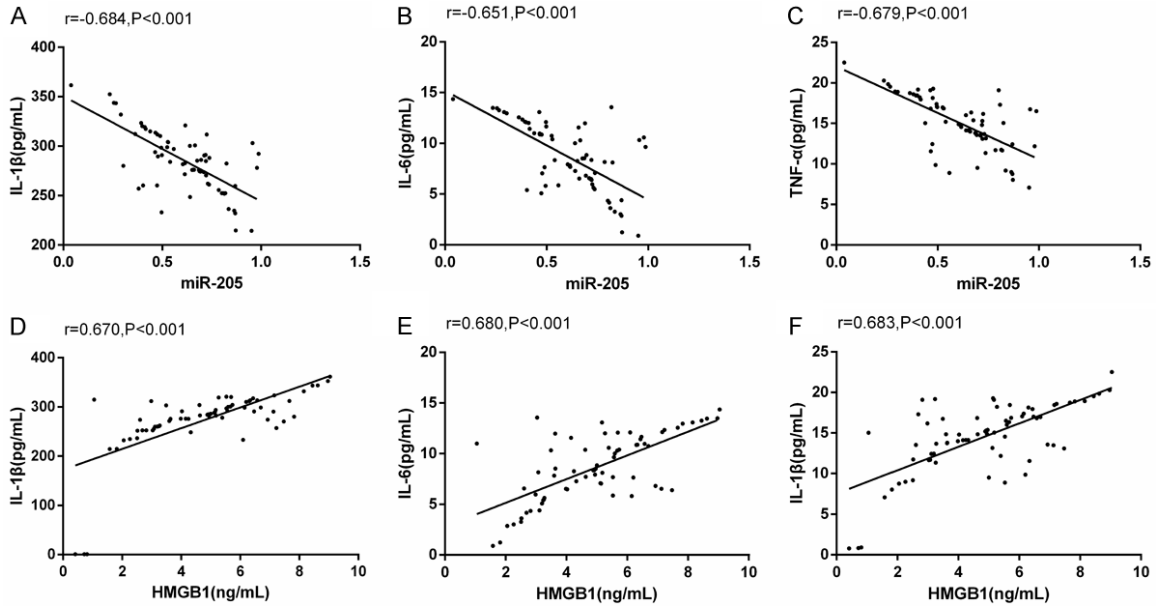

Figure 6. The correlation of miR-205 and HMGB1 with the serum inflammatory factors. A-C: The correlation between serum miR-205 and the inflammatory factors IL-1β, IL-6, and TNF-α in the serum. D-F: The correlation between the serum HMGB1 levels and the inflammatory factors IL-1β, IL-6, and TNF-α in the serum.

for its treatment. Past clinical experience suggests that prevention should be the priority for PO, and the early diagnosis and observation of PO progression can provide important information for PO treatment [25]. However, the early clinical diagnosis of periodontitis is not satisfactory, so finding efficient diagnostic methods is also warranted [26]. Previous studies have found that miR and HMGB1 are potential biomarkers for the diagnosis of many diseases [27, 28]. Subsequently, we analyzed the diagnostic effect of serum miR-205 and HMGB1 on periodontitis using ROC curves. The results demonstrated that the AUCs of serum miR-205 and HMGB1 in the diagnosis of PO were 0.936 and 0.955 respectively, suggesting that serum miR-205 and HMGB1 have the potential to serve as biomarkers for the diagnosis of PO.

The concentrations of the inflammatory factors such as IL-1β, IL-6, and TNF-α are reported to be significantly increased in periodontitis, a finding similar to the results of this study [29]. In the process of periodontitis, cytokines are closely related to inflammation, the immune response, and periodontal tissue destruction [30]. TNF-α is a common and crucial inflammatory factor in the development of PO, and it can promote the formation of osteoclasts, inhibit the transformation of periodontal liga-

ment fibroblasts into osteoblasts, and the activity of osteoblasts, resulting in the destruction of periodontal connective tissue and alveolar bone tissue, thus limiting the repair of periodontal tissue [30, 31]. IL-6 can facilitate the release of inflammatory mediators to aggravate the inflammatory reaction, inhibit the growth of periodontal ligament cells, and hinder the repair of periodontal ligaments, as well as induce the production of matrix metalloproteinase, ultimately leading to the degradation of bone matrix [32]. And IL-1β, produced mainly by monocytes, macrophages and neutrophils, is critical in inflammation and immunity [33]. To further understand the relationship between miR-205, HMGB1, and periodontitis, we analyzed the correlation between the serum miR-205, HMGB1 and inflammatory factor levels in PO patients. The results showed that miR-205 is negatively correlated with IL-1β, IL-6, and TNF-α, and HMGB1 is positively correlated with the above inflammatory factors. This suggests that miR-205 and HMGB1 may participate in PO progression by modulating the secretions of the cytokines such as IL-1β, IL-6, and TNF-α.

Finally, our Pearson correlation analysis revealed an inverse correlation between miR-205 and HMGB1. It is shown that miR-205 participates in the progression of sepsis, septicemia, and triple negative breast cancer through the targeted regulation of HMGB1 [13, 34, 35]. Therefore, miR-205 can also participate in the development of PO through the targeted regulation of HMGB1 expression. However, this study did not design a basic experiment for demonstrating this. So it is hoped that relevant experiments for verification will be carried out in subsequent studies.

To sum up, miR-205 and HMGB1 are closely related to the progression of periodontitis and may be candidate biomarkers for its diagnosis and treatment.

Disclosure of conflict of interest

None.

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