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Effect of combined periodontal-orthodontic treatment on NOD-like receptor protein 3 and high mobility group box-1 expressions in patients with periodontitis and its clinical significance

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Abstract

To investigate the effect of combined periodontal-orthodontic treatment on the gingival crevicular fluid (GCF) levels of high mobility group box-1 (HMGB1) and NOD-like receptor protein 3 (NLRP3) in chronic periodontitis.

A total of 60 patients with periodontitis who received combined periodontal-orthodontic treatment and 32 healthy individuals as normal controls were recruited in this study. Periodontal parameters were recorded. Enzyme-linked immunosorbent assay (ELISA) was used to examine GCF levels of HMGB1 and NLRP3.

The periodontal parameters and GCF levels of HMGB1 and NLRP3 in periodontitis patients were significantly higher before treatment, and observably decreased after 6 months of treatment as compared with the healthy group. However, significant positive correlations were observed between HMGB1, NLRP3, and periodontal parameters in chronic periodontitis patients.

Patients with chronic periodontitis showed higher levels of HMGB1 and NLRP3 in GCF.

Abbreviations: ANOVA = analysis of variance, BI = bleeding index, BOP = bleeding on probing, CAL = clinical attachment level, ELISA = enzyme-linked immunosorbent assay, GCF = gingival crevicular fluid, GI = gingival index, HMGB1 = high mobility group box-1, HRP = horse radish peroxidase, LPS = Lipopolysaccharide, NLRP3 = NOD-like receptor protein 3, PAGE = polyacrylamide gel electrophoresis, PBS = phosphate-buffered saline, PD = probing depth, PI = plaque index, SD = standard deviation.

Keywords: combined periodontal-orthodontic treatment, gingival crevicular fluid, high-mobility group box-1, NOD-like receptor protein 3, periodontitis

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The present study was approved by the ethic committee of Hospital of Nanjing university of science and technology. Written informed consent was obtained by all

participants.

All authors agreed the submission and the policy of the journal and copyright.

All data in this study can be obtained by proper request from the authors.

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Key Points

- The periodontal parameters were significantly elevated in periodontitis patients.
- GCF levels of HMGB1 and NLRP3 were decreased after orthodontic treatment.
- HMGB1 and NLRP3 levels were positively correlated with periodontal parameters.

1. Introduction

Periodontitis is a chronic oral disease characterize by periodontal inflammation and irreversible damage to periodontal tissues.[1] The diagnosis of periodontitis is mainly based on the routine examinations of visual inspection, probing diagnosis, and occlusal therapy.[2] Although its pathogenesis has not been completely illuminated, accumulating evidence has strongly implied that multiple factors such as bacterial infection, host immune mechanisms, genetic characteristics, and environmental factors are suggested to have potential influences on the production of cytokines in the gingival crevicular fluid (GCF).[3,4] To some extent, GCF factor inspection can also reflect the occurrence of periodontitis.[5]

High mobility group box-1 (HMGB1) is an inflammatory cytokine named for its high speed in migration in polyacrylamide gel electrophoresis (PAGE). A large quantity of evidence has

revealed that HMGB1 widely participates in cell replication, differentiation and maturation, DNA repair and recombination, steroid hormone regulation, and gene transcription regulation through non-specific binding to DNA.[6,7] Lipopolysaccharide (LPS) and other proinflammatory cytokines are reported to induce HMGB1 secretion from mononuclear macrophages and neutrophils. Furthermore, the released HMGB1 further stimulates the production of proinflammatory cytokines, forming a positive feedback loop in inflammatory diseases.[8,9] There is increasing evidence that HMGB1 is highly expressed in the gingival tissues and GCF of chronic periodontitis patients,[10] which is crucial for the initiation of periodontitis.[11]

Periodontitis is initiated by an imbalance of the host defense system and pathogenic microorganisms and metabolites, which is associated with inflammasomes activation.[12] NOD-like receptor protein 3 (NLRP3) is overexpressed inflammasome in the gingival tissues of patients with periodontitis.[13]

It is well documented that pathologic migration and tooth malpositions make prosthetic rehabilitation difficult. Combined periodontal-orthodontic treatment if properly used could improve tooth positions, and lead to additional attachment loss with plaque and gingival inflammation.[14] However, whether combined orthodontic-periodontic treatment influences the levels of some important inflammatory cytokines in periodontitis remains largely unclear. This study aimed to investigate the effect of combined periodontal-orthodontic treatment on the gingival crevicular fluid (GCF) levels of HMGB1 and NLRP3 in chronic periodontitis.

2. Methods

2.1. Subjects

The present study recruited a total of 92 participants consisting of periodontally healthy individuals (16 males and 16 females, aged 35 ± 12 years) and chronic periodontitis patients (36 males and 24 females, aged 48 ± 15 years) who underwent combined periodontal-orthodontic treatment between May 2015 and January 2018 in our hospital. The inclusion criteria for chronic periodontitis patients included:

1. patients diagnosed with mild to moderate periodontitis;
2. patients with bite anomalies and dental arch deformities.

The exclusion criteria for all participants were no history of medical and surgical diseases and bone metabolic diseases. Written informed consent was obtained from each subject and the study was approved by the Institutional Ethics Committee.

2.2. Orthodontic surgery

In addition to oral hygiene education, patients received periodontal basic therapy to eliminate periodontal inflammation 12 weeks before the start of orthodontic treatment. Orthodontic treatment was performed using straight-wire arch technique. In briefly, orthodontic brackets were bonded from the first molar to the molar on both the arches, then an 0.014-inch square nickel-titanium wires was inserted in these brackets. The corresponding periodontal clinical indicators were measured and recorded 1 month after orthodontic treatment. During the orthodontic treatment, periodontal conditions were reviewed regularly and periodontal maintenance treatment was carried out.

2.3. Clinical examinations

Clinical periodontal parameters of all subjects before and after operation were recorded including plaque index (PI) (0, no plaque; 1, plaque adhering to the free gingival margin; 2, visible plaque; 3, abundant plaque), bleeding index (BI) (0, no bleeding; 1, bleeding spots; 2, blood forms a confluent red line on mucosal margin; 3, heavy bleeding), probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) within 30 seconds.

2.4. GCF collection

Prior to GCF collection, the supra-gingival plaque was removed without touching marginal gingiva and the crevicular sites were dried with sterile dry cotton ball. Subsequently, GCF samples were collected after admission from periodontally affected sites of patients and from mesiobuccal sites of periodontally healthy subjects by inserting periopaper into the gingival sulcus and holding for 30 seconds. Samples contaminated with blood or saliva were discarded. Three paper strips from each group were placed into in Eppendorf tubes and stored dry at 80°C until analysis.

2.5. Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to determine the levels of HMGB1 and NLRP3 in GCF using commercial HMGB1 (Shino-Test Corporation, Kanagawa, Tokyo, Japan) and NLRP3 (Uscn Life Science Inc., Wuhan, Hubei, China) ELISA kits, according to the manufacture's recommendations. Prior to ELISA quantitation, the paper strips containing GCF were immersed in phosphate-buffered saline (PBS) and centrifugated at 1000 g for 15 minutes. Diluted standard or samples were added and incubated at room temperature for 2.5 hours. The wells were then washed 3 times with the wash buffer and coated with horse radish peroxidase (HRP)-labeled antibodies for 2 hours at room temperature. Substrate solution and stop solution was successively added into each well. The absorbance at 450 nm was read immediately with a microplate reader (Beckman Coulter, Fullerton, CA, USA).

2.6. Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). The measurement data were represented as mean ± standard deviation (SD) and detected by two-sided Student t test or One-way analysis of variance (ANOVA), while the enumeration data were expressed as integers or percentages and assessed using Chi-Squared test. Spearman rank correlation coefficient was used to analyze the relationship between clinical periodontal parameters and HMGB1 and NLRP3 levels. A value of $P < .05$ was considered to be statistically significant.

3. Results

3.1. Clinical characteristics

As shown in Table 1, healthy individuals and periodontitis patients were not statistically different in age and gender distribution (all, $P > .05$). The periodontal parameters (PI, BI, PD, CAL and BOP of sites) for collection of GCF in patients with chronic periodontitis were higher than those in periodontally

Table 1

Comparisons of baseline characteristics of study population.

Characteristics	Periodontally healthy subjects (n = 32)	Periodontitis (n = 60)	
		Before orthodontic treatment	After orthodontic treatment
Age (year)	35 ± 12	48 ± 15	
Gender (male/female)	4/4	9/6	
PI	0.5 ± 0.2	2.9 ± 0.6*	0.8 ± 0.6#
BI	0.10 ± 0.04	2.93 ± 0.62*	0.67 ± 0.13#
PD (mm)	2.3 ± 0.2	5.3 ± 0.4*	3.6 ± 0.6#
CAL (mm)	1.41 ± 0.24	6.00 ± 1.25*	3.63 ± 0.86#
Site with BOP (%)	0	70.1*	25.5#

BI = bleeding index, BOP = bleeding on probing, CAL = clinical attachment level, PD = probing depth, PI = plaque index.
* $P < .05$ compared with periodontally healthy paticipants.
# $P < .05$ compared with before orthodontic treatment.

Table 2

GCF levels of HMGB1 and NLRP3 proteins in the study population.

	Periodontally healthy subjects (n = 32)	Periodontitis (n = 60)	
		Before orthodontic treatment	After orthodontic treatment
HMGB1 (ng/ml)	3.66 ± 0.9	29.83 ± 4.73*	17.34 ± 3.94#
NLRP3 (pg/ml)	9.40 ± 1.32	31.28 ± 3.40*	16.13 ± 2.25#

* $P < .05$ compared with periodontally healthy paticipants.
# $P < .05$ compared with before orthodontic treatment.

healthy volunteers (all, $P < .05$). After 6 months of orthodontic treatment, these indexes were all significantly decreased in chronic periodontitis patients (all, $P < .05$).

3.2. HMGB1and NLRP3 concentration in GCF

GCF levels of HMGB1 and NLRP3 were shown in Table 2. The median levels of HMGB1 and NLRP3 were markedly higher in chronic periodontitis patients than in periodontally healthy subjects. HMGB1 and NLRP3 levels in patients were both decreased after 6 months of treatment (all, $P < .05$) but were higher than those in the healthy group.

3.3. Correlations between HMGB1 levels and clinical periodontal parameters

We further assessed the association between GCF levels of HMGB1 and clinical periodontal parameters in patients with chronic periodontitis. The correlation coefficient matrices indicated that significant positive correlations were existed between levels of HMGB1 and all periodontal parameters including PI, BI PD, and CAL (Table 3, all $P < .05$).

3.4. Correlations between NLRP3 levels and clinical periodontal parameters

Correlations between GCF levels of NLRP3 and clinical periodontal parameters of periodontitis patients were also examined. As presented in Table 4, the positive correlations were observed between NLRP3 concentration and PI, BI PD and CAL for collection of GCF (all, $P < .05$).

4. Discussion

Periodontitis is caused by the infection of dental plaque bacteria, affecting tooth-supporting tissues,[15] which is associated with a variety of systemic diseases, such as diabetes, cardiovascular diseases, and rheumatoid arthritis, etc.[16] Additionally, a possible association between periodontitis and the risk of various cancers, including pancreatic cancer was suggested by a recent study.[17] At present, the clinical diagnosis of periodontitis is based on measurements of PD, BI, gingival index (GI), PI, CAL and the radiographic pattern, which could objectively reflect the extent of periodontal tissue destruction. However, some researchers argue that these clinical indexes cannot accurately reflect the degree of inflammation of chronic periodontitis. GCF is

Table 3

Correlations between levels of HMGB1 and clinical periodontal parameters of periodontitis patients.

Variables	r	P
PI	0.602	.018
BI	0.587	.021
PD	0.799	<.001
CAL	0.732	.001

BI = bleeding index, CAL = clinical attachment level, PD = probing depth, PI = plaque index. r = correlation coefficient. * $P < .05$.

Table 4

Correlations between levels of NLRP3 and clinical periodontal parameters of periodontitis patients.

Variables	r	P
PI	0.519	.047
BI	0.599	.018
PD	0.819	<.001
CAL	0.666	.006

BI = bleeding index, CAL = clinical attachment level, PD = probing depth, PI = plaque index. r = correlation coefficient. * $P < .05$.

an inflammatory exudate composed of complements, antibodies, electrolytes, proteins, and enzymes infiltrated from the gingival connective tissues into gingival sulcus through the sulcular epithelium and junctional epithelium, playing an important role in the defense system of gingival tissues.[18,19] Therefore, in recent years, many scholars have attempted to seek a novel biomarker in GCF to evaluate the degree of periodontal inflammation.

The treatment of chronic periodontitis has always presented a challenge for clinicians. Although some patients with chronic periodontitis have a response to anti-inflammatory drug therapy, relapse represents the major cause of treatment failure. To the best of our knowledge, surgery treatment mainly included periodontic treatment that could effectively relieve periodontal symptoms, and orthodontic treatment that has inhibitory effect on pathologic tooth migration and bacterial plaques.[20,21] In this study, after 6 months of combined periodontal-orthodontic treatment, the performances of PD, PI, BI, CAL, and BOP in patients with chronic periodontitis were greatly decreased. Kramer et al[22] showed that combined orthodontic-periodontic treatment showed great clinical efficacy for periodontitis.

HMGB1, a non-histone DNA-binding protein secreted into the extracellular milieu in response to inflammatory stimuli, is identified as a late-acting mediator of inflammation via directly regulating chemokine, cytokine, metabolic, or neuroimmune activities.[23] It is noteworthy that HMGB1 is mainly located in gingival epithelial cells.[24] Our data showed that GCF levels of HMGB1 showed significant decrease after 6 months of combined treatment, which might be due to the relieving effect of the orthodontic treatment on the inflammatory response. In addition, the GCF levels of HMGB1 were associated with the periodontal parameters, which was consistent with the study suggested by Lin et al.[25]

NLRP3 is the well-studied NLR family member involved in regulating inflammation.[26] Xue et al[13] showed that NLRP3 was highly expressed in the periodontal epithelium layer of patients with chronic periodontitis. Moreover, Huang et al[27] indicated that the activation of NLRP3 in gingival tissues could promote tissue breakdown for patients with chronic periodontitis. Our study showed that GCF levels of NLRP3 were significant decreased after 6 months of combined treatment. In addition, the GCF levels of NLRP3 were positively associated with the periodontal parameters.

In summary, our study highlighted that combined periodontic-orthodontic treatment could effectively control the levels of inflammatory cytokines HMGB1 and NLRP3 in adult periodontitis patients and might be beneficial for periodontal status recovery.

Author contributions

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