Iran J Public Health, Vol. 50, No.10, Oct 2021, pp.1944-1952

Original Article

Changes in Matrix Metalloproteinase-8, Interleukin-6 and Tumor Necrosis Factor-A in Gingival Crevicular Fluid during Rapid Maxillary Expansion in Adolescent Patients

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(Received 15 Nov 2020; accepted 09 Feb 2021)

Abstract

Background: Rapid maxillary expansion (RME) is the standard treatment for correcting lateral maxillary defects commonly used in orthodontics. It is the most effective approach to increase maxillary width in clinical practice. At present, there are few studies on the level of molecular biology of periodontal tissue remodeling during RME. We aimed to investigate changes in matrix metalloproteinase (MMP)-8, interleukin (IL)-6 and tumor necrosis factor (TNF)-αin gingival crevicular fluid during RME.

Methods: Patients admitted to Department of Stomatology, the Third Affiliated Hospital of Qiqihar Medical University, Qiqihar, Heilongjiang Province, China between Dec 2016 and Dec 2018 were enrolled, and randomly divided into the observation group (76 cases) and control group (62 cases). Periodontal clinical indicators were recorded. Gingival crevicular fluid was collected and the periodontal clinical indicators were recorded. The levels of MMP-8, IL-6 and TNF-α were determined by ELISA, and the contents of the two groups were compared.

Results: The plaque index of the observation group was significantly higher than that of the baseline T0 ($P<0.05$) from T4, and the increase in the control group started from T5. The general clinical data of the two groups showed that the white blood cell count of the observation group was higher than that of the control group. The levels of MMP-8, IL-6 and TNF-α were the highest at T2, followed by T3, and gradually decreased at T4, and T5, and the differences were significant ($P<0.05$).

Conclusion: The changes in levels of MMP-8, IL-6 and TNF-α in adolescent patients during RME were related to the remodeling of periodontal tissue after RME.

Keywords: Matrix metalloproteinase-8; Interleukin-6; Tumor necrosis factor-α; Gingival crevicular fluid; Rapid maxillary expansion

Introduction

Rapid maxillary expansion (RME) was first proposed by Angell in the 1860s (1). It is the standard treatment for correcting lateral maxillary defects commonly used in orthodontics. It is the most effective approach to increase maxillary width in the clinic (2-5).

RME can quickly open the sulcus at the rapid growth stage (3). RME separates the sacral suture through a tooth dilator and expands the maxillary arch for therapeutic purposes. The healing of the sulcus in the RME process is influenced by many factors and is a dynamic, multifaceted physiological process involving a wide range of biological media (6-8).

Studies have found that mechanical stimulation during RME treatment can change periodontal tissue (7-9). During RME, the mechanical force generated by expansion of the bow causes the periodontal tissue to be pulled, resulting in an inflammatory reaction. Detection of inflammatory mediators in gingival crevicular fluid can reflect the process of periodontal inflammation. The composition of gingival crevicular fluid is mainly the breakdown products of microbes, epithelial tissues and intercellular fluids. Monitoring the changes in inflammatory factors during RME indirectly reflects the inflammation and repair process of gingival tissue. However, reports on the changes in matrix metal inflammatory substances in gingival crevicular fluid during RME in adolescent patients are rare.

In recent years, many researchers worldwide have been working on how to promote remodeling of periodontal tissues in the RME process. However, it has been found (8) that the levels of osteopontin (OPN), osteoprotegerin (OPG), interleukin (IL)-6 and tumor necrosis factor (TNF)-α in gingival crevicular fluid change significantly, which is related to the development of inflammation. Matrix metalloproteinase (MMP)-8 is involved in the remodeling of periodontal tissues. At present, there are few studies on the molecular biology of periodontal tissue remodeling during RME.

The aim of this study was to investigate the dynamic evolution of MMP-8, IL-6 and TNF-α in the process of RME, in order to provide a basis for clinical treatment.

Materials and Methods

Patient information

We enrolled patients aged 10–16 yr who were hospitalized or outpatients in the Department of Stomatology, the Third Affiliated Hospital of Qiqihar Medical University, Qiqihar, Heilongjiang Province, China between Dec 2016 and Dec 2018. Inclusion criteria were as follows: patients with stenosis of the maxillary arch and lateral developmental disorders; patients whose upper jaw teeth were moderately crowded, with a crowding degree of 0–4 mm; patients with no history of orthodontics; patients with healthy periodontal tissues and were informed of the study; patients or their families gave signed informed consent. Exclusion criteria were: patients with other serious systemic diseases; patients during pregnancy and lactation; patients with mental illness unable to complete the examination.

This study was reviewed and approved by the medical Ethics Committee of the Third Affiliated Hospital of Qiqihar Medical University, and all the patients signed the informed consent.

Patient grouping

Overall 138 patients were divided into two groups by random number table: 76 in the observation group and 62 in the control group. Observation group: continuous expansion for 1 wk; control group: only wearing the expansion device, with no force. Haas expanders were used and belt loops were made; the belt loop and the expander were joined together using a stainless steel wire and a base. In the observation group, the force was applied by an expander twice a day

for 1 wk; the bow was expanded by 0.5 mm/d, and the total expansion was 3.5–7.0 mm; and the maintenance period was 9 wk. Before the examination, the patients underwent a full mouth gingival cleansing and were guided for oral hygiene.

Sampling

Gingival crevicular fluid samples were taken at the first molar of the upper jaw at the following times: before RME (T0), 24 h after RME (T1), 1 wk after RME (T2), and maintenance for 1 wk after 1 wk RME (T3), maintenance for 4 wk (T4), maintenance for 7 wk (T5).

The disinfected filter paper strips were placed in the EP tubes and numbered. The first molars of the upper jaw were cleaned and dried, and the filter paper was placed in the direction of the tooth surface until resistance was encountered; the filter paper was removed after 30 s and put back into the EP tube for storage. PBS (150 mL) was added to the EP tubes, which were shaken for 1 h, and centrifuged at a low temperature for 10 min at high speed, and the supernatant was frozen in a refrigerator at −70 °C.

Detection of MMP-8, IL-6 and TNF-α

The levels of MMP-8, IL-6 and TNF-α were measured by ELISA (Shanghai Xin Yu Biotech Co., Ltd., China). The absorbance at 450 nm was measured.

Observation indicators

Periodontal status indicators were observed, including plaque index (PLI) and probing depth (PD).The score was counted according to the number of plaques and plaque thickness: no sterile plaque, 0 points; thin plaque on the tooth surface, 1 point; medium plaque, 2 points; lot of soft scale, 3 points. The periodontal probe was inserted into the base of the gingival sulcus to detect the distance from the gingival margin to the sulcus bottom; the values of the six sites on the cheek and lingual side (mesial, median, and distal sites) were recorded. The maximum value was taken as the PD value.

Statistical analysis

Statistical analysis was performed using SPSS (Chicago, IL, USA) version 22.0. The measurement data were expressed by mean ± standard deviation, and the *t* test was used for comparison between groups. The correlation between periodontal parameters and the levels of MMP-8, IL-6 and TNF-α was analyzed by Pearson analysis. α=0.05. $P<0.05$ indicated that the difference was significant.

Results

Comparison of general data

The general clinical data of the two groups were compared. The white blood cell count of the observation group was significantly higher than that of the control group ($P<0.001$). There was no significant difference between the two groups for age, sex, red blood cell count and platelet count (Table 1).

Table 1: Comparison of general clinical data between the two groups of patients

General data	*Observation group (n=76)*	*Control group (n=62)*	*$t/\chi^2/Z$ value*	*P*
Age (yr)	16.86±3.4	15.01±3.6	−0.765	0.451
Male (%)	41(53.9)	30(48.4)	0.427	0.241
White blood cells	11.39±2.1	8.65±4.3	−7.387	<0.001
Red blood cells	4.78±0.78	4.16±0.73	−0.242	0.567
Platelets	267.2±10.2	275.5±11.4	-0.796	0.435

Changes in periodontal parameters of each group

In the observation group, plaque increased significantly from T4, and the control group started from T5 (Fig. 1). PLI increased significantly ($P<0.05$).The PD values of the observation group at T1, T2, T3, T4 and T5 were significantly higher than those atT0 ($P<0.05$). There was no significant change in PD values at each time point in the control group (Fig. 2).

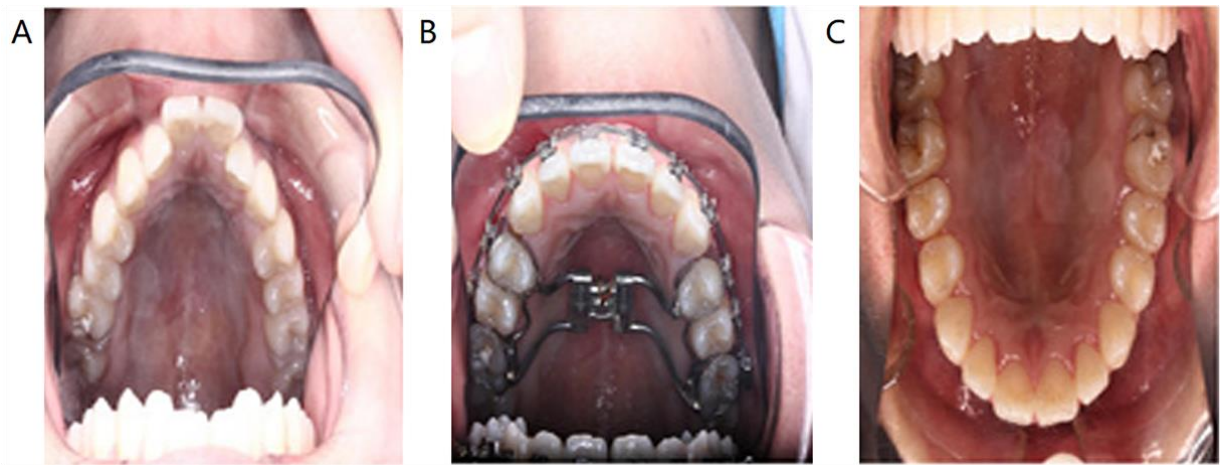

Fig. 1: Oral condition of patients in the observation group before, during and after RME. A: Oral condition before RME; B: oral condition at T4 during RME; C: oral condition after RME

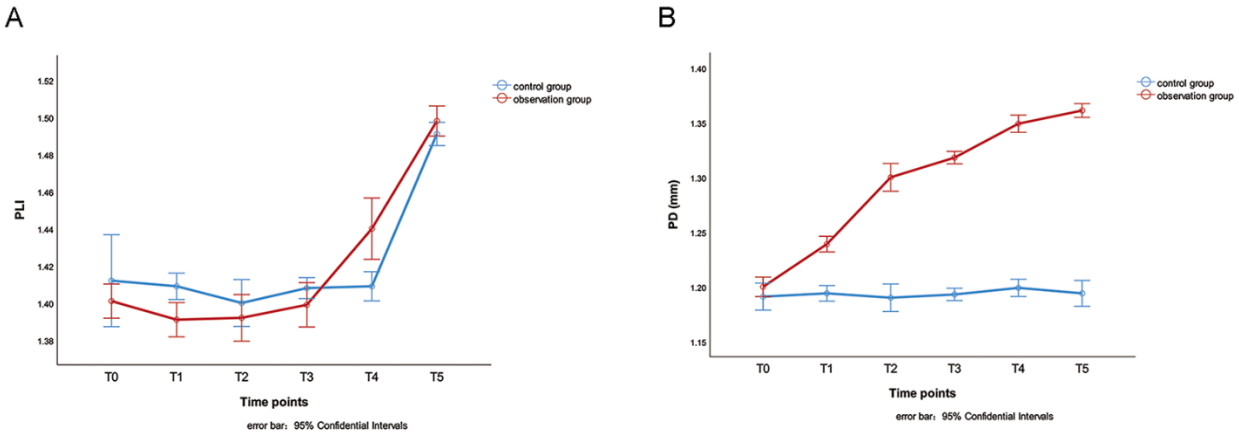

Fig. 2: PLI and PD values of different groups at the same time point.
A:PLI(plaque index); B: PD(probing depth)

Changes in MMP-8 levels in gingival crevicular fluid at different time points in the two groups

The MMP-8 level in the observation group increased from T1, reached a maximum at T2, and gradually decreased from T3 to T5, but was still significantly higher than at T0 ($P<0.05$). As for the control group, compared with T0, there was no significant change in the level of the MMP-8 from T1 to T5.There was no significant difference in the MMP-8 level in the gingival crevicular fluid between the observation and control groups at T0. MMP-8 level in the observation group was

significantly higher than in the control group at T2 to T5 (P<0.05) (Fig. 3).

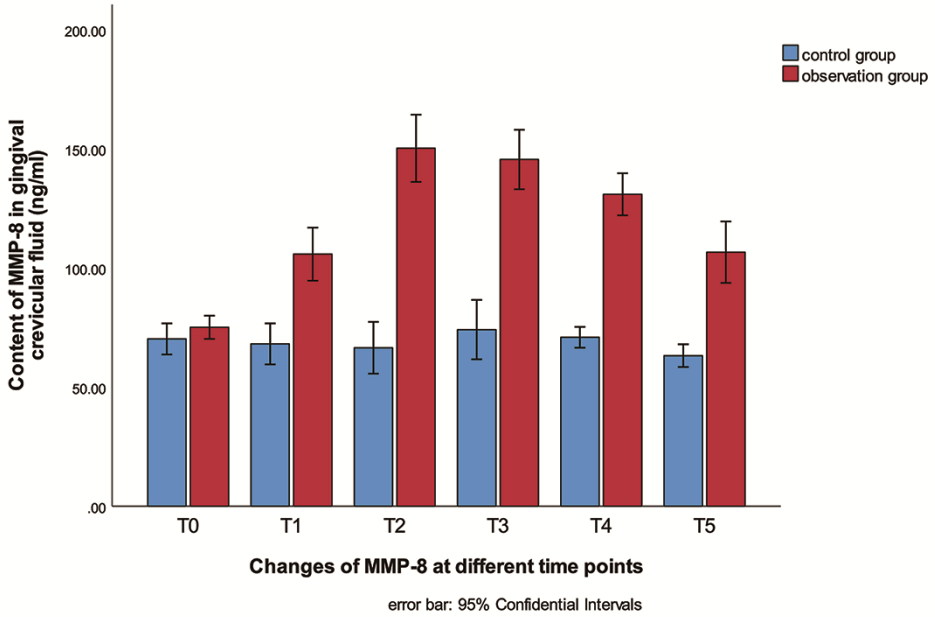

Fig. 3: Comparison of MMP-8 levels in gingival crevicular fluid at different time points in the two groups

Changes in IL-6 levels in gingival crevicular fluid at different time points in the two groups

The level of IL-6 in the observation group increased from T1, reached a maximum at T2, and gradually decreased from T3 to T5, but was still significantly higher than at T0 (P<0.05). There was no significant change in IL-6 level from T1 to T5 in the control group compared with T0. There was no significant difference in IL-6 level in the gingival crevicular fluid between the observation and control groups at T0. The IL-6 level in the observation group was significantly higher than in the control group at T2 to T5 (P< 0.05) (Fig. 4).

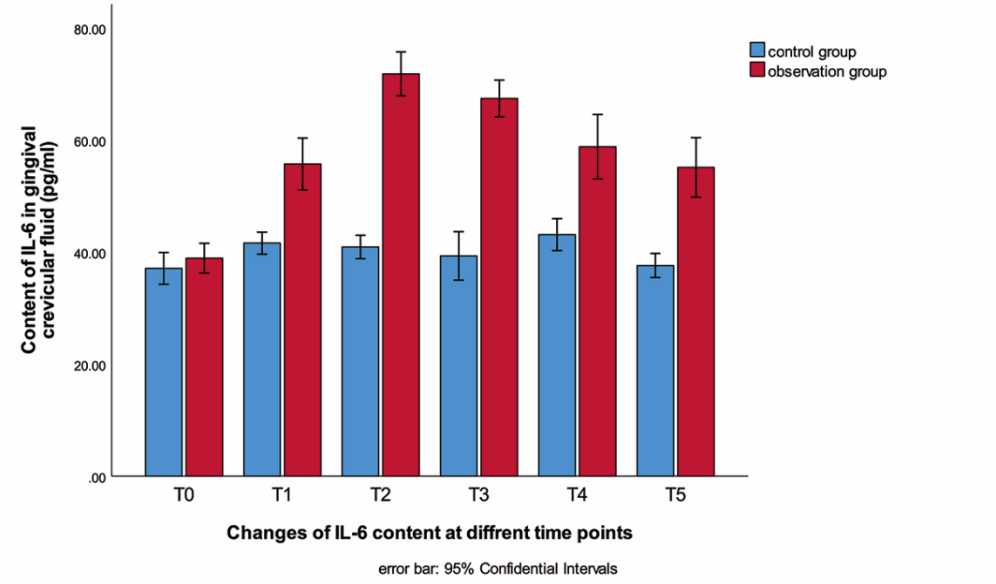

Fig. 4: Comparison of IL-6 levels in gingival crevicular fluid at different time points in two groups of patients

Changes of TNF-α levels in gingival crevicular fluid at different time points in the two groups

The level of TNF-α in the observation group increased from T1, reached a maximum at T2, and gradually decreased from T3 to T5, but was still significantly higher than at T0 (P<0.05).Compared with T0, the TNF-α levelsatT1 to T5 in the control group showed no significant change. There was no significant difference in the level of TNF-α between the two groups at T0, while the TNF-α levels in the observation group were significantly higher than in the control group at T2 to T5 (P<0.05) (Fig. 5).

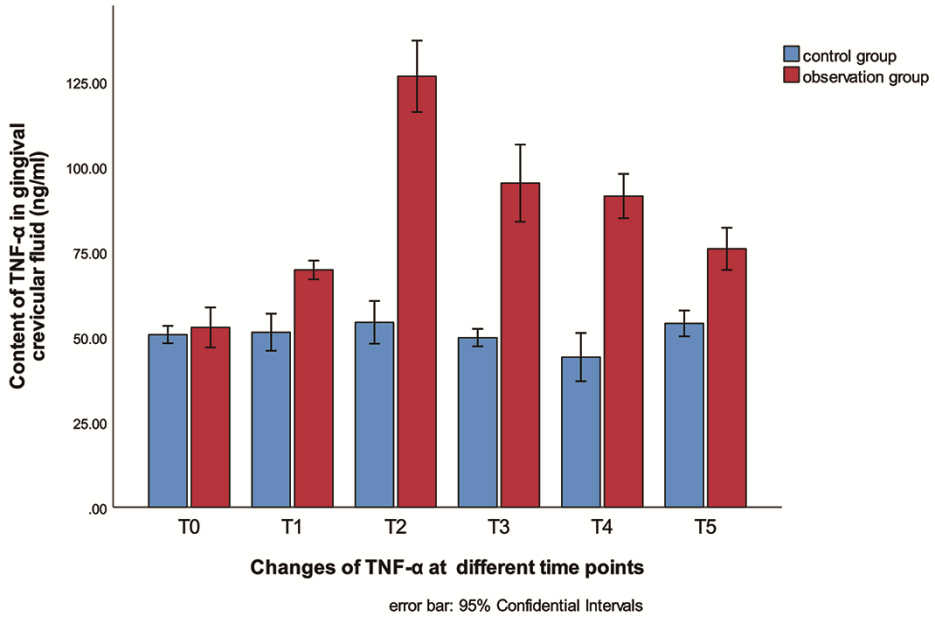

Fig. 5: Comparison of TNF-α levels in gingival crevicular fluid at different time points between the two groups

Correlation between periodontal clinical indicators and MMP-8, IL-6 and TNF-α levels in the observation group

Pearson correlation analysis showed that the periodontal clinical indexes such as PLI and PD values were significantly correlated with MMP-8, IL-6 and TNF-α levels, and the correlation coefficients were all > 0.6 (P<0.05) (Table 2).

Table 2: Correlation between periodontal clinical indicators and MMP-8, IL-6, TNF-α levels in the observation group

Indicators	*PLI*	*PD*
MMP-8	0.712/0.015	0.685/0.025
IL-6	0.602/0.021	0.786/0.013
TNF-α	0.793/0.004	0.657/0.023

PLI: plaque index; PD: probing depth

Discussion

The RME correction process stimulates the teeth and maxilla, causing changes in the expression

levels of some molecules in the gingival crevicular fluid. With the stabilization of periodontal tissue remodeling, the expression levels of various biological factors also tends to be stable. Therefore, through the detection of changes in the levels of certain biological factors in the gingival crevicular fluid, it is possible to indirectly determine the condition of periodontal remodeling. During orthodontic treatment, periodontal tissue is subjected to mechanical force, which changes the composition of the gingival crevicular fluid (10, 11). After orthodontics, interleukins and other substances in the gingival crevicular fluid increase, and inflammatory factors play an important role in the pathophysiological process after rapid expansion of the oral cavity (13, 14).

MMPs are proteases that degrade almost all components of the extracellular matrix (15). MMP expression is low when the body is disease free, and can be upregulated by inflammatory factors when inflammation or stress occurs. MMP-8 is produced by neutrophils. MMP-8 is involved in the degradation of dental tissues by actingon collagen components. It plays an important role in the pathophysiology of dental tissues after expansion (16). When external forces act on dental tissues, MMP-8 in gingival crevicular fluid is significantly increased (17). IL-6 is an important cytokine, which not only promotes the occurrence of inflammation, but also has an anti-inflammatory effect by promoting the proliferation and differentiation of B and T cells, and then plays a role in regulating human immunity (18-20). IL-6 is at the center of regulation of inflammation. It can stimulate white blood cells to produce CD1b/CD18, promote its binding to receptor cells, and then damage tissue (21), which is an indicator of the severity of inflammatory reactions (22). TNF-α is a small molecule protein secreted by macrophages and has a strong killing effect on tumor cells. In addition, it can increase vascular permeability in the inflammation site, promote leukocyte migration, and promote secretion of IL-6 and prostaglandin E2, etc. (23, 24). TNF-α can increase the proliferation of fibroblasts (25) and promote collagen production (26). TNF-α can promote the phagocytic ability of neutrophils (27,28) while promoting the inflammatory response, and enhance the activity of osteoclasts, leading to damage of connective tissue and affecting oral tissue repair. In summary, by detecting the levels of MMP-8, IL-6 and TNF-α in gingival crevicular fluid, the remodeling of periodontal tissue can be reflected.

In this study, we analyzed 76 patients in the observation group and 62 in the control group. The levels of MMP-8, IL-6 and TNF-α gradually increased after RME, and were highest at 1 wk, followed by maintenance for 1 wk. Their levels decreased gradually after maintenance for 4, 7 ~~and 9~~ wk. It is suggested that during RME, inflammatory substances are involved in the damage and subsequent periodontal tissue repair process, and that the inflammatory reaction is the cause of the increase inIL-6, TNF-α and MMP-8 in the gingival crevicular fluid.

This study analyzed the changes of periodontal clinical indicators in the RME process. The results showed that the PLI increased significantly from T4 in the observation group, and PLI increased significantly in the control group from T5. It is suggested that long-term wearing of the expander leads to accumulation of dental plaque, which increases the degree of gum inflammation. However, there was no significant change in the levels of MMP-8, IL-6 and TNF-α in the gingival crevicular fluid in the control group, suggesting that the expander does not affect the secretion of MMP-8, IL-6 and TNF-α.

This study mainly observed the changes in inflammatory substances in adolescent patients during RME, especially the changes in inflammatory substances at different time points, in order to provide a theoretical basis for clinical treatment. Necessary intervention at the appropriate time point may reduce the damage to periodontal tissue during RME.

Conclusion

Our study analyzed changes in periodontal clinical indicators and MMP-8, IL-6 and TNF-α levels in gingival crevicular fluid during RME and their

correlation. It is suggested that the changes inMMP-8, IL-6 and TNF-α levels in gingival crevicular fluid during RME are related to the remodeling of periodontal tissue after RME. The levels of IL-6, TNF-α and MMP-8 in gingival crevicular fluid can be used to judge the condition of periodontal tissue remodeling during RME. It has important guiding significance for the treatment of periodontal tissue after RME and helps to reduce clinical complications and side effects.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This study was funded by Project of Qiqihar Medical University (QMSI2020M-16).

Conflict of interest

The Authors declare that there is no conflict of interest.

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