

Original Research

Evaluation of IL-1 β and TNF- α expression and periodontitis under the influence of orthodontic appliances with minocycline

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Abstract: The current experiment was carried out to observe and analyze the effects of three orthodontic appliances combined with minocycline on periodontitis and inflammatory factors of gingival crevicular fluid. The patients included in this study were 180 patients with dental crowding treated in our hospital. They were divided into three groups, namely the invisible orthodontic appliance group (n=60), self-ligation orthodontic appliance group (n=60), traditional ligation bracket group (n=60), all of which were applied with minocycline therapy. The therapeutic effects of the three groups were observed. After three months of treatment, the periodontal index of each group showed an upward trend ($p < 0.05$). At six months to one year, the periodontal index of the self-ligation orthodontic appliance group and conventional ligation bracket group was higher than that of the invisible orthodontic appliance group, $p < 0.05$. After treatment, the expression of IL-1 β and TNF- α increased for the three groups, $p < 0.05$; the self-ligation orthodontic appliance group and the traditional ligation bracket group were the same as the invisible orthodontic appliance group in at 24 months, $p > 0.05$. In the early stage of orthodontic treatment, the invisible orthodontic appliance can keep the oral cavity in a clean and hygienic state. However, after 18 months of orthodontic treatment, all three orthodontic treatments did not have a significant impact on oral hygiene.

Key words: Minocycline; Invisible orthodontic appliance; Self-ligation orthodontic appliance; Traditional ligation bracket; Periodontitis; Inflammatory factor of gingival crevicular fluid; Impact.

Introduction

The important causes of gingivitis and periodontitis are dental plaque and toxin release, which bring an impact on patients' normal life quality to a different extent. Studies show that the development of dental plaque is closely related to environmental factors and individual factors, which are mainly reflected in oral hygiene, diet, saliva quality and fluoride exposure. Orthodontic appliance components (bracket, arch wire, and ligation wire) can produce a brushing dead corner (1-2), so that a large number of bacterial plaques may be accumulated, resulting in gingivitis and decalcification of hard tissues (Figure 1). In addition, some studies have shown that

during the assessment period of the impact of different orthodontic appliances on microbial flora and periodontal tissue, if the observation time is short, the changes of periodontal tissue inflammation will not be reflected (3-4). Therefore, this study analyzed the long-term effects of minocycline in combination with three kinds of orthodontic appliances (invisible orthodontic appliance, self-ligation orthodontic appliance and traditional ligation bracket) on periodontitis and gingival crevicular fluid inflammatory factors, which provides a basis for clinical selection of orthodontic appliance.

Materials and Methods

General data

The research objects were 180 patients who had been treated for dental crowding (Figure 2) in our hospital, from January 2015 to December 2018. Patients who met



Figure 1. Decalcification of dental hard tissue.



Figure 2. Dental crowding that investigated in this research.

the inclusion criteria were those aged above 18 years old, those who had no systemic antibiotics for nearly three months, those who had permanent teeth with normal overbite and overbite, those who had healthy oral mucosa without caries, and those who were expected to wear the appliance for more than 24 months. This study was approved by the ethics committee of our hospital, and patients signed informed consent. Patients who had ANB \geq 5, or had long centric over 2 mm, or had periodontal diseases when enrollment was excluded. The patients were randomly divided into three groups, namely, the invisible orthodontic appliance group, self-ligation orthodontic appliance group and traditional ligation bracket group, each containing 60 patients. The invisible orthodontic appliance group contained 30 males and 30 females, with an average age of (23.70 \pm 0.26) years old. The self-ligation orthodontic appliance group contained 32 males and 28 females, with an average age of (22.35 \pm 0.83) years old. 31 males and 29 females were included in the traditional ligation bracket group, with an average age of (23.62 \pm 0.75) years old. There was no significant difference in general data among the three groups ($p>0.05$).

Method

Reagents and instruments

Human IL-1 β ELISA (ThermoFisher) and human TNF- α ELISA kit (ThermoFisher) and the automatic enzyme marker Model680 (Bio-rad) and the ultra-low temperature refrigerator DW-HL528 produced by ZhongKeMeiLing Cryogenic Technology Co., Ltd.

Intervention method

The invisible orthodontic appliance (Invisalign), self-ligation orthodontic appliance (DAMON Q) and traditional ligation bracket (Victory Series) were adopted. Before wearing the appliance, the patients were asked to clean the plaque and dental stones carefully, and instructed to master the bass brushing method, brushing time, brushing frequency and application methods of mouthwash. Then the orthodontic treatment was carried out, and oral hygiene guidance and health supplements (toothbrush, toothpaste, interdental brush, dental floss, mouthwash) were provided in the treatment link. No additional periodontal treatment was performed during routine oral care, and the periodontal conditions were collected at different treatment times. One hour before sampling, fasting & drinking and brushing teeth were forbidden (5).

Observation indexes

Gingival index (GI) can be divided into four grades, namely 0, 1, 2 and 3. 0 refers to normal gingival, 1 refers to mild edema, color change, but no bleeding after probing, 2 refers to moderate inflammation with redness and edema, and probing bleeding, 3 refers to severe inflammation with significant edema and bleeding tendency.

Sulcus Bleeding Index (SBI) can be divided into the following grades. 0 represents gingival health, 1 represents no bleeding, 2 represents punctured bleeding only at the probing site, 3 represents bleeding spread along gingival margin, 4 represents bleeding overflow gingi-

val margin, and 5 represents gingival automatic bleeding tendency.

The evaluation standard of the calculus index (CI) can be described as follow. 0 represents there is no calculus above and below gingival. 1 represents the coverage area of supragingival calculus occupies less than one-third of the tooth surface. 2 represents the coverage area of supragingival calculus is between 1/3 and 2/3 of the tooth surface. 3 represents the coverage area of supragingival calculus occupies more than two-thirds of the tooth surface.

The evaluation standard of the Debris Index (CI) can be described as follow. 0 represents no soft scaling on the tooth surface. 1 represents the soft scaling covers less than one-third of the tooth surface. 2 represents the soft scaling covers 1/3 to 2/3 of the tooth surface. 3 represents the soft scaling covers more than 2/3 of the tooth surface.

The expressions of IL-1 β and TNF- α in the gingival crevicular fluid were evaluated. Cut filter paper strips into rectangles (10mm \times 2mm), and load them into 0.5mL Ep tube after weighing. Remove plaque calculus above the observation point, gargle with water for 10 minutes, prevent moisture, and blow-dry gingival groove. Insert filter paper into the middle, middle and distal gingival groove of the observation teeth with tweezers, and stop when there is resistance. Leave for half a minute and take it out, weigh it in the EP tube, obtain the weight of the gingival crevicular fluid, and then calculate the gingival crevicular fluid volume. Add 200 μ L PBS to Ep tube, shake for 1 hour, remove filter paper and freeze it in the refrigerator. Before the test, the samples were thawed at room temperature, and the concentrations of IL-1 β and TNF- α in the gingival crevicular fluid were measured strictly following the instructions of ELISA (6-8).

Statistical method

The statistical analysis software SPSS21.0 was adopted. Measurement data were expressed in the form of mean \pm average ($\bar{x} \pm s$), with a t-test for comparison of intergroup difference, while counting data were expressed as natural number (n) and percentage (%), with X^2 for comparison of intergroup difference. The intergroup difference was considered to be significant when $p < 0.05$.

Results

Comparison of the periodontal index at different time points

As shown in Table 1, the periodontal index of the three groups increased after three months of treatment, and the soft scale index of the invisible orthodontic appliance group was significantly lower than that of the other two groups ($p<0.05$). 6 to 12 months after treatment, the periodontal index of the self-ligation orthodontic appliance group and the traditional ligation bracket group increased ($p<0.05$). After 12 months, the periodontal index of the self-ligation orthodontic appliance group and the traditional ligation bracket group decreased significantly ($p<0.05$). In the 24th month, there was no difference in other periodontal indexes among three groups, $p>0.05$.

Table 1. Comparison of the periodontal index at different time points among three groups ($\bar{X} \pm s$).

Time	invisible orthodontic appliance group				invisible orthodontic appliance group				traditional ligation bracket group			
	GI	SBI	CI	DI	GI	SBI	CI	DI	GI	SBI	CI	DI
0 month	0.3 \pm 0.2	0.4 \pm 0.3	0.3 \pm 0.2	0.2 \pm 0.1	0.3 \pm 0.2	0.4 \pm 0.2	0.3 \pm 0.2	0.2 \pm 0.2	0.3 \pm 0.3	0.4 \pm 0.3	0.3 \pm 0.1	0.2 \pm 0.2
3 month	0.5 \pm 0.2	0.6 \pm 0.2	0.5 \pm 0.2	0.4 \pm 0.2	0.6 \pm 0.2	0.7 \pm 0.3	0.6 \pm 0.4	0.4 \pm 0.3	0.6 \pm 0.2	0.7 \pm 0.3	0.6 \pm 0.3	0.5 \pm 0.3
6 month	0.6 \pm 0.2	0.7 \pm 0.3	0.6 \pm 0.3	0.5 \pm 0.1	1.1 \pm 0.4	1.1 \pm 0.2	0.9 \pm 0.3	0.8 \pm 0.2	1.3 \pm 0.4	1.2 \pm 0.3	1.1 \pm 0.3	1.0 \pm 0.3
9 month	0.8 \pm 0.3	0.9 \pm 0.2	0.7 \pm 0.3	0.6 \pm 0.2	1.3 \pm 0.3	1.4 \pm 0.2	1.1 \pm 0.4	1.1 \pm 0.4	1.6 \pm 0.3	1.7 \pm 0.4	1.5 \pm 0.4	1.4 \pm 0.2
12 month	0.8 \pm 0.2	0.9 \pm 0.3	0.8 \pm 0.4	0.7 \pm 0.3	1.3 \pm 0.5	1.5 \pm 0.2	1.0 \pm 0.3	1.2 \pm 0.2	1.6 \pm 0.2	1.8 \pm 0.2	1.5 \pm 0.2	1.5 \pm 0.3
18 month	0.9 \pm 0.3	0.9 \pm 0.2	0.8 \pm 0.3	0.7 \pm 0.2	1.0 \pm 0.2	1.1 \pm 0.5	0.9 \pm 0.3	1.1 \pm 0.3	1.2 \pm 0.4	1.0 \pm 0.3	1.3 \pm 0.2	1.1 \pm 0.3
24 month	0.9 \pm 0.2	0.8 \pm 0.2	0.8 \pm 0.4	0.6 \pm 0.2	0.9 \pm 0.3	0.9 \pm 0.3	0.9 \pm 0.6	0.9 \pm 0.3	1.0 \pm 0.5	1.1 \pm 0.4	1.1 \pm 0.5	1.2 \pm 0.4

Table 2. Comparison of expressions of IL-1 β and TNF- α among three groups ($\bar{X} \pm s$).

Time	Invisible orthodontic appliance group		Invisible orthodontic appliance group		Traditional ligation bracket group	
	IL-1 β (mg/L)	TNF- α (mg/L)	IL-1 β (mg/L)	TNF- α (mg/L)	IL-1 β (mg/L)	TNF- α (mg/L)
0 month	15.3 \pm 3.2	0.3 \pm 0.9	14.9 \pm 0.3	0.3 \pm 0.3	15.4 \pm 4.3	0.3 \pm 0.7
3 month	42.7 \pm 5.4	0.7 \pm 0.2	64.3 \pm 12.1	0.8 \pm 0.2	58.0 \pm 13.2	1.1 \pm 0.3
6 month	63.2 \pm 9.0	1.1 \pm 0.5	104.3 \pm 23.2	1.3 \pm 0.6	146.5 \pm 30.4	2.2 \pm 0.9
9 month	91.2 \pm 4.6	1.5 \pm 3.2	155.8 \pm 31.2	1.8 \pm 0.4	243.6 \pm 42.1	3.3 \pm 0.8
12 month	96.7 \pm 10.2	1.5 \pm 0.2	136.7 \pm 24.6	1.7 \pm 0.7	218.9 \pm 32.0	3.1 \pm 0.2
18 month	95.7 \pm 9.8	1.5 \pm 0.8	108.9 \pm 26.5	1.6 \pm 0.2	122.3 \pm 20.1	2.4 \pm 0.2
24 month	93.2 \pm 11.3	1.4 \pm 0.3	96.7 \pm 12.8	1.5 \pm 0.9	106.5 \pm 13.6	1.6 \pm 0.9

Comparison of expressions of IL-1 β and TNF- α among three groups

As shown in Table 2 below, the expression of IL-1 β and TNF- α increased in the three groups after treatment, $p < 0.05$. After 24 months, the self-ligation orthodontic appliance group and the traditional bracket group were the same as the invisible orthodontic appliance group, $p > 0.05$.

Discussion

The orthodontic appliance is the first choice for clinical orthodontic treatment because of its mature technique and wide indications. However, using orthodontic appliances limit patients' ability to maintain oral hygiene and promote the accumulation of bacterial plaque, which may lead to gingivitis and even gingival recession (9-10). This study evaluated the long-term effects of three orthodontic appliances on periodontal tissue. The results showed that the periodontal index of patients using the invisible orthodontic appliance was lower than that using a fixed orthodontic appliance within nine months after treatment, indicating good oral hygiene. After one year of treatment, there was no significant difference in the effects of the three orthodontic appliances on periodontal health. Analysis suggests that there may be several reasons for the difference in short- and long-term periodontal health outcomes. First of all, during the two-year follow-up, many patients have received multiple hygiene instructions and developed good habits, which may play a key role in improving the periodontal index of patients with fixed orthodontic appliances, enabling them to catch up with patients with invisible orthodontic appliances without bracket (11-12). Secondly, patients gradually acquire more oral knowledge and realize the importance of periodontal health. After the doctor examines and informs them of gingival inflammation, patients may pay more attention to the method of brushing and the use of dental floss. Genetic research has also been done in relation to dental diseases (13), in which genome editing technology (14) can also be used.

In the inflammatory stage of periodontal tissue, IL-1 β directly participates in local immune response, which can increase the expression of metal matrix protease in human periodontal fibroblasts, making the periodontal tissue disintegrated. TNF- α cytokine involved in the destruction of periodontal inflammation plays a synergistic role with IL-1 β in the progression of periodontal inflammation. The detection of IL-1 β and TNF- α is helpful to determine the degree of periodontal inflammation and the accuracy is higher than that of subjective determination of periodontal index. This study shows that IL-1 β and TNF- α change with the development of periodontal inflammation, and the trend is the same as that of periodontal examination. It is also proved that the invisible orthodontic appliance is helpful to maintain oral hygiene in the early stage of orthodontic treatment, while the three kinds of orthodontic appliances have no significant difference in the influence on oral hygiene 18 months after orthodontic treatment.

In conclusion, this study presents a comparative ana-

lysis of the long-term impact of invisible orthodontic appliance, self-ligation orthodontic appliance, and traditional ligation bracket on oral health, which provides an important research basis for clinical selection of orthodontic appliances. According to the different influences of orthodontic appliances on oral health in different periods, doctors and patients should adopt a personalized periodontal health maintenance plan to effectively improve the orthodontic treatment level.

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