Effects of Kangfuxin solution on IL-1β, IL-6, IL-17 and TNF-α in gingival crevicular fluid in patients with fixed orthodontic gingivitis

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Abstract. Changes of interleukin (IL)-1β, IL-6, IL-17 and tumor necrosis factor-α (TNF-α) in gingival crevicular fluid in patients with fixed orthodontic gingivitis after treatment with Kangfuxin solution were analyzed to explore the clinical effect of Kangfuxin solution in patients with orthodontic gingivitis. A total of 78 patients diagnosed with fixed orthodontic gingivitis in Weifang People's Hospital from January 2015 to March 2017 were selected. Thirty-nine patients were treated with gingival cleansing as control group, and the other 39 patients were treated with gingival cleansing plus spraying and sublingual administration with Kangfuxin solution as treatment group. The general data of patients were collected, and the changes of IL-1β, IL-6, IL-17 and TNF-α in gingival crevicular fluid were measured, the bleeding index (BI), probing depth (PD), swelling and pain grades were recorded, and the clinical curative effects were compared between the two groups. The curative effect in treatment group was better than that in control group ($p<0.05$). After treatment, gingival BI and PD in both groups were lower than those before treatment. The curative effect in treatment group was better than that in control group ($p<0.05$). The levels of gingival pain and swelling after treatment in treatment group were mainly in grade I. The levels of gingival pain and swelling after treatment in control group were mainly in grade II and III ($p<0.05$). After treatment, the effective rate of control group was 76.92% and that of treatment group was 97.44% ($p<0.05$). It was found that the levels of IL-6 and TNF-α in gingival crevicular fluid were positively correlated with PD. The use of Kangfuxin solution in the treatment of patients with orthodontic gingivitis can effectively reduce the levels of IL-1β, IL-6, IL-17 and TNF-α in gingival crevicular fluid, and improve the periodontal conditions and the effective rate of treatment.

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Introduction

At present, more and more patients are clinically treated with fixed orthodontic treatment for dental malformation, and the effect is good. However, fixed orthodontic treatment also has its common complications (1), such as producing plaque to cause different degrees of irritation to the gingiva of patients, and causing gingivitis, which can be characterized by gingival swelling, pain or gingival irritating bleeding (2). If the treatment of gingivitis caused by fixed orthodontic treatment is not timely, it will not only affect the effect of treatment, but also affect the diet and quality of life of patients (3). Numerous studies have shown that a variety of inflammatory factors are involved in the production of fixed orthodontic gingivitis, thus effectively reducing the inflammatory level in gingivitis gingival crevicular fluid, which can effectively improve the condition, be helpful to fixed orthodontic effect and remove dental plaque. Reducing the level of inflammatory factors in gingival crevicular fluid is the main treatment for fixed orthodontic gingivitis (4,5).

Kangfuxin solution is a traditional Chinese medicine preparation, mainly consisting of ethanol extract from dried insect body of *Periplaneta americana*. It has the function of promoting blood circulation, nourishing *yin* and producing muscle, and has a good curative effect on ulcers, burns, and fistula. At present, it is increasingly used in the clinic (6). In patients with fixed orthodontic gingivitis, a polyol substance in Kangfuxin solution can promote increased isosynthesis of gingival epidermal cell type I isoelastic collagen, and generate new granulation tissues, so the local cells of gingival inflammation proliferate (7). The treatment of patients with fixed orthodontic gingivitis using Kangfuxin solution is gingivitis local spraying, followed by sublingual administration, which increases the direct contact time between the drug and gingivitis local inflammatory mucosa, accelerate the healing of inflammation, and improve the clinical effective rate (8).

Patients and methods

General data. A total of 78 patients diagnosed with fixed orthodontic gingivitis in the Department of Stomatology in Weifang People's Hospital (Weifang, China) from January, 2015 to March, 2017 were selected. The study was approved by the Ethics Committee of Weifang People's Hospital (Weifang, China). Signed informed consents were obtained from the patients or the guardians. Among them, 39 patients were treated with gingival cleansing as control group, and the other 39 patients were treated with gingival cleansing plus spraying and sublingual administration with Kangfuxin solution as treatment group. There were 41 males and 37 females, aged 13-28 years, with an average age of 16.07±1.86 years. The course of disease was 3-10 months, and the average course of disease was 6.23±0.91 months. All the included patients met the following diagnostic criteria: Gingival swelling, pain, congested mucosa, surface temperature rise, easily bleeding when stimulated, swelling and pain levels of grade III, gingival sulcus bleeding index (BI) >2, probing depth (PD) >4 mm, and all patients undergoing straight wire appliance. Exclusion criteria: Patients who had recently received antibiotic treatment or periodontal related treatment, patients with severe oral and periodontal diseases, severe heart, liver and kidney dysfunction, or poor eating and chewing habits, and patients who refused to sign the informed consent, those who quit midway or had incomplete clinical data.

Methods.

Therapeutic methods. All the included patients were treated with gingival cleaning routine treatment, that is, gingival cleaners were used to clean the plaque and periodontal stones, and gingival cleaning was performed inside the periodontal pocket. In treatment group, Kangfuxin solution was used on the basis of routine treatment. A 5 ml sterile disposable syringe was used to extract 5 ml Kangfuxin solution at a time and spray it on the local mucosa of gingivitis in patients in treatment group. The patients were told to take it in the mouth for 5 min and then swallow it twice a day. Relevant indexes of all patients were measured and recorded at 1 day before treatment and 7 days after treatment.

Inflammatory factor detection. At 1 day before treatment and 7 days after treatment, all the included patients gargled, and an air gun was used to dry the tooth surface. A filter paper strip was inserted into the gingival lateral sulcus of the patient, and it was taken out when there was a slight sense of resistance. The filter paper strip was observed, and if it had blood, the specimen was taken again. The specimen was stored at 4˚C and centrifuged at 3,000 x g, and the supernatant was collected. The levels of interleukin (IL)-1β, IL-6, IL-17 and tumor necrosis factor-α (TNF-α) in gingival crevicular fluid were determined by enzyme-linked immunosorbent assay (ELISA). The kit was provided by Shandong Science and Technology Co., Ltd (Shandong, China).

Periodontal condition measurement. A coarse blunt gingival probe was put into the lateral sulcus of the gingival, and BI and PD of the patient were calculated. The grades are as follows: 0 point, no bleeding, redness, or skin temperature rise after the probe touched the gingiva; 1, bleeding or redness, etc, after the probe touched the gingiva; 2, scattered point bleeding on the surface after the probe touched the gingiva; 3, scattered bleeding after the probe touched the gingiva; 4, larger amount of bleeding than that in gingival sulcus after the probe touched the gingiva; and 5 points, bleeding without the probe touching the gingiva.

Table I. Comparisons of general data between treatment and control group by Student's t-test.

General data	Treatment group (n=39)	Control group (n=39)	P-value
Age (years)	15.94±1.77	16.22±1.08	0.719
Sex (male/female)	20/19	21/18	0.908
Course of gingivitis (months)	5.99±0.87	6.32±1.01	0.779
BMI (kg/m^2)	21.73±4.62	20.64±3.91	0.813

Pain level. No obvious or slight pain, and normal life not affected was grade I. Moderate or paroxysmal pain was grade II. Intolerable pain and normal life affected was grade III.

Swelling level. No swelling or no periodontal pocket seen was grade I. Visible redness and swelling, but no periodontal pocket was grade II. Redness and swelling and periodontal pocket seen was grade III.

Therapeutic effect. Markedly effective: Gingival color and appearance are normal, PD<2 mm. Improved: Gingival color and appearance are improved significantly, 2 mm<PD<3.0 mm. Ineffective: No changes in gingival color and appearance, PD>3.0 mm.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 19.0 statistical software was used to process the data. Collection data were represented as mean ± standard deviation (SD). Student's t test was used for comparison of general data. ANOVA was used for comparison of multiple groups and the post hoc test was SNK test. Analysis of Pearson correlation between two factors was used. P<0.05 indicates that the difference was statistically significant.

Results

Comparison of general data before treatment between treatment and control group. There were no statistically significant differences in age, sex and the course of gingivitis between treatment and control group, and the data were comparable (p>0.05, Table I).

Comparison of inflammatory factors in gingival crevicular fluid before and after treatment between treatment and control group. There were no significant differences in the levels of IL-1β, IL-6, IL-17 and TNF-α before treatment between treatment and control group (p>0.05). After treatment, the levels of IL-1β, IL-6, IL-17 and TNF-α in both groups were lower than

Table II. Comparison of levels of inflammatory factors before and after treatment between treatment and control group by Student's t-test.

Groups	No.	Time	IL-1β (ng/l)	IL-17 (ng/ml)	IL-6 (ng/l)	TNF-α (pg/ml)
Control	39	Before treatment	14.23±1.76	3.26±0.71	16.34±2.23	13.29±4.18
		After treatment	10.97±1.78	1.87±0.33	10.41±1.16	9.45±1.44
Treatment	39	Before treatment	14.38±1.91	3.31±0.96	16.44±2.29	13.66±3.91
		After treatment	8.79±1.83	0.97±0.42	8.16±1.68	7.08±1.16

P<0.05, compared with that before treatment, and p<0.05, compared with that after treatment in control group. IL, interleukin; TNF-α, tumor necrosis factor-α.

Table III. Comparison of periodontal conditions before and after treatment between treatment and control group by Student's t-test.

Groups	No.	Time	BI	PD (mm)
Control	39	Before treatment	3.93±0.41	4.79±0.45
		After treatment	2.77±0.38	3.33±0.41
Treatment	39	Before treatment	3.89±0.37	4.81±0.49
		After treatment	1.54±0.49	2.36±0.37

P<0.05, compared with that before treatment, and p<0.05, compared with that after treatment in control group. BI, bleeding index; PD, probing depth.

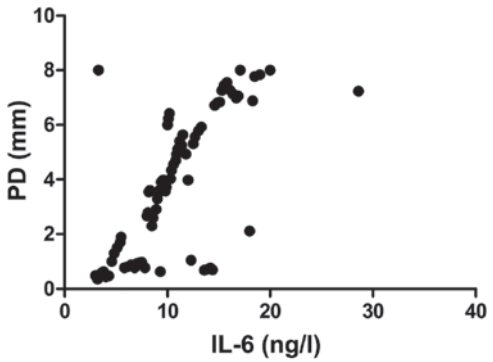

Figure 1. Correlation between IL-6 and PD. IL, interleukin; PD, probing depth.

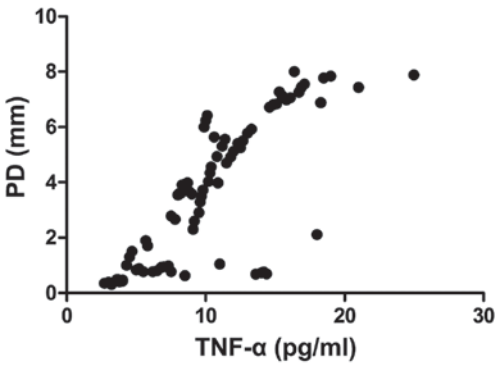

Figure 2. Correlation between TNF-α and PD. TNF-α, tumor necrosis factor-α; PD, probing depth.

those before treatment. Moreover, the curative effect of the treatment group was lower than that of the control group, and the difference was statistically significant (p<0.05, Table II).

Comparison of periodontal condition before and after treatment between the treatment and the control group. There were no significant differences in gingival BI and PD before treatment between the treatment and the control group. After treatment, gingival BI and PD in both groups were lower than those before treatment. The curative effect in the treatment group was better than that in the control group, and the difference was statistically significant (p<0.05, Table III).

Comparison of swelling and pain levels after treatment between the treatment and control the group. The levels of gingival pain and swelling in the treatment group were mainly in grade I, which were 89.74 and 92.31%, respectively. The levels of gingival pain and swelling after treatment in the control group were mainly in grade II and III, and the difference was statistically significant (p<0.05, Table IV).

Comparison of therapeutic effects between the treatment and control group. After treatment, the total effective rate of the control group was 76.92% and that of the treatment group was 97.44%. The difference was statistically significant (p<0.05, Table V).

Analysis of correlation of inflammatory factors with periodontal conditions. The levels of IL-6 (r=0.793, p<0.001) and TNF-α (r=0.667, p<0.001) in gingival crevicular fluid were positively correlated with PD, and the differences were statistically significant (Figs. 1 and 2).

Discussion

A membrane formed by plaque adheres to tooth surface, resulting in inflammation of gingiva, which is known as gingivitis. Gingivitis is more common in patients with fixed orthodontic teeth. During the formation of gingivitis in fixed orthodontic patients, many inflammatory factors in gingival crevicular fluid promote the occurrence and development of gingivitis (9). It has been reported that the levels of IL-1β, IL-6, IL-17 and TNF-α in gingival crevicular fluid are closely related

Table IV. Comparison of swelling and pain levels after treatment between treatment and control group by ANOVA.

		Pain level (n, %)			Swelling level (n, %)		
Groups	No.	Grade I	Grade II	Grade III	Grade I	Grade II	Grade III
Control	39	13 (33.33)	17 (43.59)	9 (23.08)	13 (33.33)	16 (41.03)	10 (25.64)
Treatment	39	35 (89.74)	3 (7.69)	1 (2.56)	36 (92.31)	2 (5.13)	1 (2.56)

P<0.05, the difference is statistically significant.

Table V. Comparison of therapeutic effects between treatment and control group by ANOVA.

Groups	No.	Markedly effective (n, %)	Improved (n, %)	Ineffective (n, %)	Effective (n, %)
Control	39	17 (43.59)	13 (33.33)	9 (23.08)	30 (76.92)
Treatment	39	34 (87.18)	4 (10.26)	1 (2.56)	38 (97.44)
P-value					0.001

to the occurrence of gingivitis in fixed orthodontic patients (10). Inflammatory factors contribute to the increase of synthesis and secretion of gingival proteinase, the decrease of collagen content and the weakening of cell proliferation, so the healing of the wound is slow (11). Numerous data indicate that in fixed orthodontic patients without gingivitis, the levels of inflammatory factors in gingival crevicular fluid are significantly decreased, and the higher the inflammatory level, the more severe the condition of gingivitis is (12). Fixed orthodontic patients with gingivitis generally manifest local gingival mucosal swelling, redness, increased skin temperature and bleeding, affecting the diet, and quality of life (13). However, if the treatment is not timely, it may develop into periodontitis, and bacteria may enter the body's blood circulation through the wound, leading to systemic inflammation, fever, and other organ infections (14,15). For gingivitis occurring in fixed orthodontic patients, gingival cleaning was used as the main treatment in the past. However, gingival cleaning is often unable to cure gingivitis, which can easily lead to recurrence and affect the quality of life of patients (16). If the drug can prolong the contact time with the local mucosal tissues of gingivitis, it is better for gingivitis, and Kangfuxin solution has this advantage (17). Kangfuxin solution in the treatment of gingivitis in fixed orthodontic patients has more advantages, such as improving the immune function of patients, increasing the activity of lysozyme in serum, and speeding up the clearance of necrotic cell tissues (18). Secondly, the local blood circulation is accelerated, the speed of capillary proliferation is accelerated, new granulation tissues are generated, and the healing of inflammation is accelerated (19).

It was found in this study that the levels of inflammatory factors in gingival crevicular fluid after treatment in the treatment and control groups were lower than those before treatment, and they were more obvious in treatment group than those in control group (p<0.05). Moreover, the periodontal conditions in treatment group were also improved significantly after treatment, and the overall effective rate was higher. Gingival crevicular fluid is the metabolite of epithelial cells, and cell fluid. The cytokine components in gingival crevicular fluid can represent the periodontal conditions. If the inflammatory level in gingival crevicular fluid is increased, the periodontal conditions are poor, and the condition of gingivitis is more serious (20). A positive correlation between inflammatory factors and periodontal conditions was found in this study (p<0.001), which was consistent with the above conclusion. Therefore, Kangfuxin solution effectively reduces the levels of inflammatory factors in gingival crevicular fluid, and improves the periodontal conditions at the same time, and it is more helpful in the treatment of fixed orthodontic patients complicated by gingivitis, thus improving the overall effective rate.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YL drafted this manuscript. YL and FM were responsible for the conception and design of the study. FM collected the patient data, and revised the manuscript critically for important intellectual content. LL and CS analyzed and interpreted the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Weifang People's Hospital (Weifang, China). Signed informed consents were obtained from the patients or the guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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