Relationships of inflammatory cytokines, oxidative stress markers and matrix metalloproteinases in gingival crevicular fluid with peri-implantitis.

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Abstract

This study aims to analyze the relationships of inflammatory cytokines, oxidative stress markers and metalloproteinases in gingival crevicular fluid (GCF) with peri-implantitis (PI). Forty patients who had received dental implantation were enrolled in this study. There were 52 implants in total, which were divided into PI group (42 implants) and health implant (HI) group (10 implants). Fifty-two healthy teeth with the same name were selected as the control group (healthy teeth, HT). The periodontal status was recorded. The GCF was collected and quantified. The levels of interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), hypersensitive C-reactive protein (hs-CRP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), matrix metalloproteinase (MMP)-13 and MMP-8 were detected using ELISA. Results showed that, the probing depth, sulcus bleeding index, GCF volume, and TNF- α , IL-6, hs-CRP, MMP-8 and MMP-13 levels in GCF in PI group were significantly higher than HI and HT groups, respectively (P<0.01 or P<0.05). The SOD and GSH-Px levels in PI group were significantly lower than HI and HT groups, respectively (P<0.05). Excepting hs-CRP, there was no significant difference of each index between HI and HT groups (P>0.05). In conclusion, TNF-α, IL-6, hs-CRP, SOD, GSH-Px MMP-8 and MMP-13 are involved in the occurrence of PI, and they may be used as reference indexes to evaluate the degree of PI. In addition, the clinical periodontal index SBI and PD are positively correlated with GCF volume, hs-CRP, MMP-8 and MMP-13.

Keywords: Peri-implantitis, Inflammatory cytokines, Oxidative stress, Metalloproteinases.

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Introduction

With the development and popularization of dental implant technology, more and more patients with tooth loss choose the implant denture to repair the tooth loss. However, the complications after denture implantation, including peripheral mucositis and peri-implantitis (PI), still universally exist [1]. PI is a chronic inflammation of the implant surrounding tissue after implantation or exerting functions. It is a disease that affects the combination of the implant and the surrounding tissue and leads to the gradual loss of soft and hard tissues [2]. PI is a major factor in the failure of denture implantation, and the incidence of PI is gradually increasing [3]. It is found that, PI is dominated by the bacteria, and it is related to the healing ability, occlusion, general condition of patient and so on. The etiology, pathology and clinical symptoms of PI are similar with those of the periodontitis, but the progress of PI is more rapid than that of periodontitis [4]. Previous studies show that, the expressions of inflammatory cytokines including tumor necrosis factor-a (TNF-a), IL-6, interleukin-6 (IL-6) and Creactive protein (CRP) in gingival crevicular fluid (GCF) are related to the health status of the tissues surrounding denture implant. It is suggested that the oxidative stress is involved in the development of chronic periodontitis [8]. In addition, matrix metalloproteinases (MMPs), such as MMP-8 and MMP-13, are also involved in periodontitis [9]. This study investigated the levels of inflammatory cytokines, hypersensitive C-reactive protein (hs-CRP), oxidative stress markers, and MMPs in GCF of PI patients, and explored the relationships of these indicators with PI. The objective was to provide a basis for early diagnosis and treatment of PI.

Materials and Methods

Subjects

Forty patients who had received dental implantation in our hospital from March 2011 to March 2015 were enrolled in this study. The dental implantation had been performed at least 1 year before. There were 24 males and 16 females. The age of patients was 24-58 years, with mean age of 38.2 ± 4.3 years. All patients met the following criteria: i) no systemic disease; ii) no smoking, good oral hygiene, no dental periphery disease; iii) not using antibiotic, immunosuppressive agent or nonsteroidal drug within at least 3 months; iv) no pregnancy for female patients; v) no occlusal trauma in implant; vi) good compliance to dental implantation; vii) the oral hygiene could be maintained after planting. There were 52 implants in total (ITI columnar two-section implant). According to the diagnose criteria, 52 implants were divided into PI group (probing depth (PD)>3 mm; bleeding on probing, with or without ecpyesis; gingival bleeding index (SBI)>2; 42 implants) and health implant (HI) group (PD \leq 3 mm; SBI \leq 2, mm; 10 implants). Fifty-two healthy teeth with the same name with the affected teeth in the patients were selected as the control group (healthy teeth, HT).

Recording of periodontal status

The periodontal probe was gently inserted in the gingival sulcus. The probe body was parallel to the long axis of tooth/ implant, and was close to root. The probing pressure was no more than 20 g. The PD values of four sites (mesio buccal, distal buccal, mesial lingual, distal lingual) of the implants and healthy teeth were measured. SBI of implants and the healthy teeth was recorded according to scoring standard as follows: 0 point, no bleeding at probing site; 1 point, only punctate bleeding at probing site; 2 points, linear bleeding in the gingival sulcus; 3 points, bleeding expanding along the gingival margin or overflowing the gingival margin.

Collection of GCF

The Whatman III filter paper was cut into 2 mm \times 20 mm strips, and then were placed it in a clean container. The tooth surface was wiped with dry sterile cotton, and was kept from wet. The large plaque on the tooth was removed. After gently blowing the gum, the filter paper strip was inserted into the gingival sulcus, until encountering the slight resistance. After staying for 1 min, the filter paper strip was taken out, and was placed in the Eppendorf tube, followed by immediately adding 300 µL PBS buffer. Each tube contained 4 filter paper strips of each sample. Finally the samples were kept at -70°C.

Quantification of GCF

The composition of GCF was similar with that of normal human serum, so the amount of GCF could be quantified according to the standard curve of normal human serum amount with the soaked area of filter paper [10].

Detection of inflammatory cytokines, oxidative stress markers and MMPs

The inflammatory factors and CRP were detected according to the reported methods [11-13]. Specimens were thawed at room temperature for 20 min. After centrifugation (2000 r/min, 4°C) for 10 min, the supernatant was obtained. The levels of IL-6, TNF- α , hs-CRP, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), MMP-13 and MMP-8 were detected using ELISA. The operation procedures were in accordance with the instruction of kits.

Statistical analysis

All statistical analysis was carried out using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Data were presented as mean \pm SD, and were compared using single-factor analysis of variance and SNK-q test. The correlation analysis was performed using Spearman's rank correlation test. P<0.05 was considered as statistically significant.

Table 1. Comparisons of PD, SBI and GCF volume among threegroups.

Group	roup n PD (mm)		SBI	GCF volume (µL)	
н	42	2.46 ± 0.38	0.51 ± 0.11	0.92 ± 0.19	
PI	10	4.33 ± 0.22^{a}	3.62 ± 0.32 ^a	2.02 ± 0.26^{a}	
HT	52	2.36 ± 0.16 ^b	0.47 ± 0.17 ^b	0.72 ± 0.23 ^b	

^aP<0.01 compared with HI group; ^bP<0.01 compared with PI group; PD: Probing Depth; SBI: Sulcus Bleeding Index; GCF: Gingival Crevicular Fluid; HI: Healthy Implants; PI: Peri-Implantitis; HT: Healthy Teeth.

Table 2. Comparisons of TNF-a, IL-6 and hs-CRP level in GCF among three groups.

Group	n	TNF-α (ng/ml)	IL-6 (ng/ml)	hs-CRP (ng/ml)
н	42	6.01 ± 2.33	0.61 ± 0.21	5.56 ± 2.38
PI	10	19.72 ± 4.53 ^a	4.77 ± 1.29 ^a	13.22 ± 5.62 ^a
HT	52	5.55 ± 1.92 ^b	0.44 ± 0.08^{b}	3.34 ± 0.50 ^{bc}

^aP<0.01 compared with HI group; ^bP<0.01 compared with PI group; ^cP<0.05 compared with HI group. TNF-α: Tumor Necrosis Factor-α; IL-6: Interleukin-6; hs-CRP: Hypersensitive C-Reactive Protein; GCF: Gingival Crevicular Fluid; HI: Healthy Implants; PI: Peri-Implantitis; HT: Healthy Teeth.

Results

Comparisons of PD, SBI and GCF among three groups

As shown in Table 1, the PD in HI, PI and HT groups was 2.46 \pm 0.38, 4.33 \pm 0.22 and 2.36 \pm 0.16 mm, respectively; the SBI in three groups was 0.51 \pm 0.11, 3.62 \pm 0.32 and 0.47 \pm 0.17, respectively; the GCF volume in three groups was 0.92 \pm 0.19, 2.02 \pm 0.26 and 0.72 \pm 0.23 µL, respectively. The PD, SBI and GCF volume in PI group were significantly higher than HI and HT groups, respectively (P<0.01). There was no significant difference in each index between HI and HT groups (P>0.05).

Comparisons of TNF- α , IL-6 and hs-CRP level in GCF among three groups

Table 2 showed that, the TNF- α level in GCF in HI, PI and HT groups was 6.01 ± 2.33, 19.72 ± 4.53 and 5.55 ± 1.92 ng/ml, respectively; the IL-6 level in three groups was 0.61 ± 0.21, 4.77 ± 1.29 and 0.44 ± 0.08 ng/ml, respectively; the hs-CRP level in three groups was 5.56 ± 2.38, 13.22 ± 5.62 and 3.34 ± 0.50 ng/ml, respectively. The TNF- α , IL-6 and hs-CRP levels in PI group were significantly higher than HI and HT groups, respectively (P<0.01). There was no significant difference in

TNF- α or IL-6 level between HI and HT groups (P>0.05). The hs-CRP level in HI group was significantly higher than that in HT group (P<0.01).

Table 3. Comparisons of SOD, GSH-Px and MDA level in GCFamong three groups.

Group	n	SOD (ng/ml)	GSH-Px (ng/ml)	MDA (ng/ml)
HI	42	223.67 ± 45.78	213.19 ± 31.23	6.79 ± 1.56
PI	10	202.34 ± 34.19 ^a	182.45 ± 27.01 ^a	6.82 ± 2.01
HT	52	226.38 ± 39.56 ^b	199.02 ± 30.33 ^b	6.45 ± 2.38

^aP<0.05 compared with HI group; ^bP<0.05 compared with PI group. SOD: Superoxide Dismutase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; GCF: Gingival Crevicular Fluid; HI: Healthy Implants; PI: Peri-Implantitis; HT: Healthy Teeth.

Table 4. Comparisons of MMP-8 and MMP-13 level in GCF among three groups.

Group	n	MMP-8 (mg/L)	MMP-13 (mg/L)
н	42	0.12 ± 0.03	11.32 ± 1.99
PI	10	3.85 ± 0.45 ^a	17.45 ± 2.28 ^a
HT	52	0.06 ± 0.01 ^b	10.12 ± 0.65 ^b

^aP<0.05 compared with HI group; ^bP<0.05 compared with PI group. MMP-8: Matrix Metalloproteinase-8; MMP-13: Matrix Metalloproteinase-13; GCF: Gingival Crevicular Fluid; HI: Healthy Implants; PI: Peri-Implantitis; HT: Healthy Teeth.

Comparisons of SOD, GSH-Px and MDA level in GCF among three groups

The SOD level in GCF in HI, PI and HT groups was 223.67 ± 45.78 , 202.34 ± 34.19 and 226.38 ± 39.5 ng/ml, respectively; the GSH-Px level in three groups was 213.19 ± 31.23 , 182.45 ± 27.01 and 199.02 ± 30.33 ng/ml, respectively; the MDA level in three groups was 6.79 ± 1.56 , 6.82 ± 2.01 and 6.45 ± 2.38 ng/ml, respectively. The SOD and GSH-Px levels in PI group were significantly lower than HI and HT groups, respectively (P<0.05). There was no significant difference in SOD or GSH-Px level between HI and HT groups (P>0.05), with no significant difference of MDA level among three groups (P>0.05) (Table 3).

Comparisons of MMP-8 and MMP-13 level in GCF among three groups

As shown in Table 4, the MMP-8 level in GCF in HI, PI and HT groups was 0.12 ± 0.03 , 3.85 ± 0.45 and 0.06 ± 0.01 mg/L, respectively; the MMP-13 level in three groups was 11.32 ± 1.99 , 17.45 ± 2.28 and 10.12 ± 0.65 mg/L, respectively. The MMP-8 and MMP-13 levels in PI group were significantly higher than HI and HT groups, respectively (P<0.05). There was no significant difference in each index between HI and HT groups (P>0.05).

Table 5. Spearman correlation coefficients of periodontal index withGCF volume, hs-CRP, MMP-8 and MMP-13.

GCF volume		hs-CRP		MMP-8		MMP-13	
r	Р	r	Ρ	r	Ρ	r	Р
0.891	<0.05	0.672	<0.05	0.762	<0.05	0.856	<0.05
0.572	<0.05	0.823	<0.05	0.823	<0.05	0.818	<0.05
	r 0.891 0.572	r P 0.891 <0.05 0.572 <0.05	r P r 0.891 <0.05 0.672 0.572 <0.05 0.823	r P r P 0.891 <0.05	r P r P r 0.891 <0.05	r P r P 0.891 <0.05	r P r P r P r 0.891 <0.05

GCF: Gingival Crevicular Fluid; hs-CRP: Hypersensitive C-Reactive Protein; MMP-8: Matrix Metalloproteinase-8; MMP-13: Matrix Metalloproteinase-13; PD: Probing Depth; SBI: Sulcus Bleeding Index.

Correlation analysis results

Spearman-rank correlation analysis showed that, the clinical periodontal index SBI and PD were positively correlated with GCF volume, hs-CRP, MMP-8 and MMP-13, respectively (P<0.05) (Table 5). Other indexes were not correlated.

Discussion

GCF of natural tooth is the serum fluid which infiltrates through the sulcular epithelium and junctional epithelium into the gingival sulcus. It contains a variety of factors and enzymes that are involved in the immune response and inflammatory reaction. The chemical composition analysis of GCF can reflect the inflammation of periodontal tissue, and has been widely used in the study of periodontal disease [14]. The GCF of plant is similar to that of the natural tooth. The analysis of chemical composition of plant GCF can reflect the health status of the implant surrounding tissues. The amount of GCF can reflect the health status of implant surrounding tissues in a certain extent [15]. Results of this study showed that, there was no significant difference of GCF volume between HI and HT groups (P>0.05). This is identical to the results of Prapulla et al. study [16]. In addition, the GCF volume in PI group was significantly higher than HI and HT groups, respectively (P<0.01). This indicates that, the GCF volume is related to the degree of inflammation in the surrounding tissues.

IL-6 is mainly secreted by monocytes-macrophages, endothelial cells and endothelial cells. It is a multifunctional cytokine. It exerts the functions mainly through autocrine or paracrine manner, and plays its physiological role in the regulation of hematopoietic and immune response [17]. TNF- α is mainly synthesized by mononuclear macrophages after stimulated by the endotoxin-lipopolysaccharide secreted by Gbacteria. Like IL-6, TNF-a also has many physiological functions, especially in inflammation and immune response [18]. CRP is a kind of acute-phase reactive protein that exists only in inflammatory disease of infection or non-infection. In acute inflammation, the serum level of CRP may rise by several hundred to thousands of times. The rising speed, amplitude and duration of CRP are positively correlated with the degree of tissue injury and infection. The concentration of CRP in blood decreases to normal after 24-48 h from cure of disease [19]. Results of this study showed that, the TNF- α , IL-6 and hs-CRP levels in PI group were significantly higher than HI and HT groups, respectively (P<0.01). There was no significant difference of TNF- α or IL-6 level between HI and HT groups (P>0.05). The hs-CRP level in HI group was

significantly higher than that in HT group (P<0.01). This indicates that, TNF- α , IL-6 and hs-CRP can be used as the indicators of PI.

SOD is a kind of antioxidant enzyme in vivo, which can catalyze the free radical disproportionation of superoxide anions in the body, and protect the cell from damage. It plays an important role in the antioxidant system [20]. GSH-Px is also an antioxidant enzyme which eliminates the hydrogen peroxide and lipid peroxide in the body. It can prevent the damage of oxygen free radicals to the body, thus playing an important role in the antioxidant system [21]. When the inflammation occurs in the periodontal tissue, excessive oxygen free radicals are produced. SOD, GSH-Px and other antioxidant enzymes are reduced due to the elimination of excessive oxygen free radicals. MDA is one of the decomposition products of lipid peroxidation. Its content can reflect the degree of lipid peroxidation in the body, which indirectly reflects the degree of cell injury [17]. Results of this study showed that, the SOD and GSH-Px levels in PI group were significantly lower than HI and HT groups, respectively (P<0.05), with no significant difference of MDA level among three groups (P>0.05). This indicates that, SOD and GSH-Px are also the clinical diagnostic indexes of PI.

MMPs mainly include 5 kinds of enzymes, such as collagenase, gelatinase, matrix metalloproteinase, etc. MMPs can destroy almost all extracellular matrix and basement membrane molecules whether in the pathological or physiological repair cases [22]. The collagenases are mainly composed of MMP-8 and MMP-13, and are closely related to periodontal tissue degradation. They can specifically act with type I, II and III collagen which compose the main components of periodontal tissues. In addition, they play a key role in the periodontal attachment loss, alveolar resorption and periodontal disease progression. Type I and type III collagen fibers are the most abundant in the gingival tissue, fibrous connective tissue and bone tissue around the implant. They constitute the main components of the implant surrounding tissues [23]. The periodontal research finds that, in the GCF of gingival tissues with mild inflammation or without inflammatory, the collagenase activity and level and amount of collagen cleavage products are lower than those of severe infection. The more serious the degree of periodontal tissue inflammation is, the higher the level and activity of MMP-8 and MMP-13 in GCF are [22]. In this study, the MMP-8 and MMP-13 levels in PI group were significantly higher than HI and HT groups, respectively (P<0.05). This indicates that, the MMP-8 and MMP-13 levels in GCF are closely correlated to the PI.

In conclusion, TNF- α , IL-6, hs-CRP, SOD, GSH-Px MMP-8 and MMP-13 are involved in the occurrence of PI, and they may be used as reference indexes to evaluate the degree of PI. In addition, the clinical periodontal index SBI and PD are positively correlated with GCF volume, hs-CRP, MMP-8 and MMP-13, respectively. These indexes should be detected for diagnosis and treatment of PI. This study still has some limitations. The sample size of this study is relatively small. Larger sample size will make the results more convincing. In our next studies, the sample size should be further increased for obtaining more satisfactory outcomes.

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