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

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Abstract
This study aimed to analyze the correlations of inflammatory cytokines, oxidative stress markers, and matrix metalloproteinases (MMPs) in gingival crevicular fluid (GCF) with peri-implantitis (PI). Forty patients receiving dental implantation were enrolled. There were 52 implants, which were divided into PI group (42 implants) and health implant (HI) group (10 implants). Fifty-two healthy teeth (HT) with the same names with affected teeth in the patients were selected as the control group. 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), MMP-13, and MMP-8 were detected using enzyme-linked immunosorbent assay (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 auxiliary indicators to evaluate the degree of PI. In addition, the clinical periodontal index probing depth and sulcus bleeding index are positively correlated with GCF volume, hs-CRP, MMP-8, and MMP-13.

Keywords
inflammatory cytokines, metalloproteinases, oxidative stress, peri-implantitis

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Introduction
Peri-implantitis (PI) is a chronic inflammation of the implant surrounding tissue after implantation or exerting functions. The clinical definition of PI is based on following criteria: (1) presence of peri-implant signs of inflammation, (2) radiographic evidence of bone loss following initial healing, and (3) increasing probing depth (PD) as compared with PD values collected after placement of the prosthetic reconstruction.1 PI is a disease which is dominated by the bacteria and related to the injury healing ability, occlusion of dentition, 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.2 Previous studies3,4 have
shown that the expressions of inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) in gingival crevicular fluid (GCF) are related to the health status of the implant surrounding tissues. It is suggested that the oxidative stress is involved in the development of chronic periodontitis. In addition, the matrix metalloproteinases (MMPs) are also involved in the periodontitis. This study investigated the changes 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. The implants were provided by Beijing Lianhe Denture Co., Ltd. (Beijing, China). 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: (1) no systemic disease; (2) no smoking, good oral hygiene; (3) not using antibiotic, immunosuppressive agent, or non-steroidal drug within at least 3 months; (4) no pregnancy for female patients; (5) no occlusal trauma in implant; (6) good compliance to dental implantation; and (7) the oral hygiene could be maintained after planting. There were 52 implants in total (ITI columnar two-section implant). According to the standard of clinical indexes, 52 implants were divided into PI group (PD > 3 mm; sulcus bleeding index (SBI) > 2; 42 implants) and health implant (HI) group (PD ≤ 3 mm; SBI ≤ 2, mm; 10 implants). Fifty-two healthy teeth (HT) with the same name with the affected teeth in the patients were selected as the control group. This study was approved by the ethics committee of University Hospital of Tsinghua University. Written informed consent was obtained from all participants.

Recording of periodontal status

Periodontal probe (570/1 type; MEDESY Medical Co., Italy) was gently inserted in the gingival sulcus. The probe body was parallel to the long axis of tooth/implant and was close to the root. The probing pressure was no more than 20 g. The PD values of four sites (mesio-buccal, distal-buccal, mesial-lingual, and distal-lingual) of the implants and HT were measured. SBI of implants and the HT 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

Whatman III filter paper (Whatman Co., Kent, UK) was cut into 2 mm × 20 mm strips, and then were placed in a clean container. The tooth surface was wiped with dry sterile cotton and was kept away from moisture. 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 (Eppendorf AG, Hamburg, Germany), followed by immediately adding 300 µL phosphate buffer saline (Sigma-Aldrich Corp., MO, USA). Each tube contained four filter paper strips of each sample. Finally, the samples were kept at −70°C.

Quantification of GCF

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 the filter paper.

Detection of inflammatory factors and CRP

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 enzyme-linked immunosorbent assay (ELISA). The operation procedures were in accordance with the instruction of kits (Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., Shanghai, China).
Table 1. Comparisons of PD, SBI, and GCF volume among three groups.

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

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-α, 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) |
|-------|----|---------------|--------------|----------------|
| HI    | 42 | 6.01 ± 2.33   | 0.61 ± 0.21  | 5.56 ± 2.38    |
| PI    | 10 | 19.72 ± 4.53a | 4.77 ± 1.29a | 13.22 ± 5.62a  |
| HT    | 52 | 5.55 ± 1.92b  | 0.44 ± 0.08b | 3.34 ± 0.50bc  |

TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; hs-CRP: high-sensitivity C-reactive protein; GCF: gingival crevicular fluid; HI: healthy implants; PI: peri-implantitis; HT: healthy teeth.

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 ± 0.38, 4.33 ± 0.22, and 2.36 ± 0.16 mm, respectively; the SBI in three groups was 0.51 ± 0.11, 3.62 ± 0.32, and 0.47 ± 0.17, respectively; the GCF volume in three groups was 0.92 ± 0.19, 2.02 ± 0.26, and 0.72 ± 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 of 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 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).

Statistical analysis

All statistical analyses were carried out using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The statistical power was 82.43%, which reached with the sample. Data were presented as mean ± 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.

Results

Comparisons of PD, SBI, and GCF among three groups

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 of 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

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 of each index between HI and HT groups.

Comparison of GCF among three groups

Spearman-rank correlation analysis showed that the PD was positively correlated with GCF volume,
hs-CRP, MMP-8, and MMP-13, respectively (PD with GCF volume: \( r = 0.891, P = 0.021 \); PD with hs-CRP: \( r = 0.672, P = 0.032 \); PD with MMP-8: \( r = 0.762, P = 0.019 \); PD with MMP-8: \( r = 0.856, P = 0.004 \)). In addition, the SBI was positively correlated with GCF volume, hs-CRP, MMP-8, and MMP-13, respectively (SBI with GCF volume: \( r = 0.572, P = 0.042 \); SBI with hs-CRP: \( r = 0.823, P = 0.005 \); SBI with MMP-8: \( r = 0.816, P = 0.008 \); SBI with MMP-8: \( r = 0.818, P = 0.011 \)). Other indexes were not correlated.

### Discussion

IL-6 is a multifunctional cytokine which plays a role in the regulation of hematopoietic and immune response.\(^7\) TNF-\(\alpha\) is mainly synthesized by mononuclear macrophages after inflammatory stimulation and has many physiological functions, especially in inflammation and immune response.\(^8\) CRP is a kind of acute-phase reactive protein that exists only in inflammatory disease of infection or non-infection.\(^9\) Results of this study showed that the TNF-\(\alpha\), IL-6, and hs-CRP levels in PI group were significantly higher than HI and HT groups (\( P < 0.05 \)), which indicates that SOD and GSH-Px are also the clinical diagnostic indexes of PI. MMP-8 and MMP-13 are the main kinds of MMPs and are closely related to the 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 reabsorption, and periodontal disease progression. 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.\(^6\) In this study, the MMP-8 and MMP-13 levels in PI group were significantly higher than HI and HT groups (\( P < 0.05 \)), which suggests that the MMP-8 and MMP-13 levels in GCF are closely correlated to the PI.

In conclusion, TNF-\(\alpha\), 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 auxiliary indicators 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. This study has provided a basis for early diagnosis and treatment of PI. This study still has some limitations. The sample size of this study is relatively small. In our next studies, the sample size should be further increased for obtaining more satisfactory outcomes.

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