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Original Article

# Comparison of the inflammatory states of serum and gingival crevicular fluid in periodontitis patients with or without type 2 diabetes mellitus

Xin-Ran Xu [a†], Jing-Ling Xu [a†], Lu He [a*], Xian-E Wang [a], Hong-Ye Lu [a,b], Huan-Xin Meng [a**]

[a] *Department of Periodontology, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Beijing, China*

[b] *Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Zhejiang Provincial Clinical Research Center for Oral Diseases, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Cancer Center of Zhejiang University, Zhejiang, China*

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**KEYWORDS**
Periodontitis;
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Interleukin 17;
Visfatin;
Receptor activator of nuclear factor-kappa B ligand;
Osteoprotegerin

**Abstract** *Background/purpose:*There is a two-way relationship between periodontitis and type 2 diabetes mellitus. This study aimed to compare the inflammatory states in serum and gingival crevicular fluid (GCF) in periodontitis patients with or without type 2 diabetes mellitus (T2DM) and healthy subjects.

*Materials and methods:* 20 subjects were systematic and periodontal healthy (H group), 40 subjects were with periodontitis (CP group), and other 40 were with periodontitis and type 2 diabetes mellitus (DC group). Fasting blood glucose (FBG) and HbA1c was tested. GCF and serum level of interleukin (IL) −17, visfatin, receptor activator of nuclear factor-kappa B (NF-κB) ligand (RANKL)/osteoprotegerin (OPG) ratio were measured.

*Results:* The GCF volume, total amount of IL-17, vastatin, RANKL/OPG ratio in GCF and their concentrations in serum were higher ($P < 0.05$) in CP and DC groups than in H group, which were also higher ($P < 0.05$) in DC group than in CP group except for visfatin in GCF and IL-17 in serum. At sample sites of PD $\leq$ 3 mm, GCF volume, IL-17, visfatin and RANKL/OPG ratio in DC and CP groups were higher ($P < 0.05$) than that in H group, which were also higher in DC

* Corresponding author. Department of Periodontology, Peking University School and Hospital of Stomatology, No. 22, Zhongguancun Nandajie, Haidian District Beijing, 100081, China.
** Corresponding author. Department of Periodontology, Peking University School and Hospital of Stomatology, No. 22, Zhongguancun Nandajie, Haidian District Beijing, 100081, China.
*E-mail addresses:* helupku@163.com (L. He), kqhxmeng@bjmu.edu.cn (H.-X. Meng).
† These authors contributed equally to this study.

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group than in CP group either with PD $\leq$ 3 mm or PD $>$ 3 mm. Inflammatory state in GCF was positively correlated to systemic inflammation, and both of them were positively correlated to FBG.

*Conclusion:* Moderate and severe periodontitis aggravated systemic inflammation. T2DM together with periodontitis resulted in more severe systemic inflammation. The positive correlation between the periodontal and systemic inflammation and their association with FBG indicated an inflammatory link between periodontitis and T2DM.



## Introduction

Chronic periodontitis (CP) is considered as an inflammatory disease caused by bacteria and affected by systemic factors.[1] It cannot only induce damage of periodontal soft tissue, cause bone loss, but also have impact on quality of life, and even relate to various systemic diseases.[1–3] Many studies have shown that there is a two-way relationship between periodontitis and type 2 diabetes mellitus (T2DM). Both clinical and animal studies show that T2DM can aggravate periodontal destruction, and increase the incidence of periodontitis.[4,5] On the other side, periodontitis may have an adverse effect on diabetes.[6] Nowadays, researchers generally agree that inflammation may serve as a bridge between the two diseases,[7] but the mechanism still need to be further studied.

IL-17, involved in many pathways, such as nuclear factor-kappa B (NF-κB) pathway and mitogen-activated protein kinase (MAPK) pathway, is strongly associated with the development of many diseases, including diabetes, periodontitis, obesity, inflammatory bowel disease (IBD), arthrosis and even cancers.[8] It has been reported that periodontal bone loss is mediated by IL-17 mainly from Th17, which leads to osteoclast activation and bone resorption by locally produced receptor activator of NF-κB ligand (RANKL).[9] In T2DM patients, higher serum level of IL-17 is found as well,[10] which may lead to enhanced expression of RANKL, increased osteoclastogenesis and high inflammation level in periodontal tissue, and then may result in more severe bone loss.

RANKL secreted by osteoclasts binds to its receptor and increases osteoclasts differentiation and mature osteoclasts. Osteoprotegerin (OPG) acts as a decoy receptor for RANKL and inhibits osteoclasts differentiation, then results in preventing osteoclastogenesis.[11] Thus, RANKL-OPG system is equivalently but oppositely regulated in the diseased state. Some studies found no significant difference in OPG[12] or both RANKL and OPG[13] in gingival crevicular fluid (GCF) under different periodontal inflammatory states, and another study showed higher RANKL and lower OPG in periodontitis patients.[14] While RANKL/OPG ratio changes were steady and identical for reflecting the periodontal condition in those studies. Therefore, monitoring RANKL and OPG together, which expressed as relative ratio of RANKL and OPG, may provide more adequate information on the state of periodontitis than RANKL or/and OPG examined individually.

Visfatin was defined as a pre-B-cell colony-enhancing factor by Fukuhara et al.[15] It is mainly secreted by adipocytes and also widely distributed in many types of cells. Its expression can be upregulated in variety of inflammatory diseases such as rheumatoid arthritis and IBD.[16] Therefore, the view that visfatin has proinflammatory effect in periodontitis has caught some researchers' attention.[17] Besides, visfatin can interfere with the insulin binding to its receptors, and stimulate glucose uptake in adipocytes.[15,18] Higher serum visfatin level in T2DM patients was observed.[19] But few studies mentioned its role on the relationship between T2DM and periodontitis via its expression in GCF and serum examined simultaneously.

With more and more attention paid to the relationship between T2DM and CP, it is of great significance to explore the difference and association of inflammatory state in periodontitis and diabetes patients from the systemic and local aspect simultaneously. Therefore, the aim of the present study was to compare the IL-17, RANKL/OPG ratio and visfatin level in serum and GCF in chronic periodontitis patients with or without T2DM, and systemic and periodontal health subjects as control, trying to deeply understand a link of inflammation between periodontitis and diabetes.

## Materials and methods

This study was approved by Ethical Committee of Peking University Health Science Center (Approval no. IRB00001052-08010). Each subject was given verbal and written instructions and signed an informed consent, fully understanding the study design and giving permission to use data obtained for research purpose.

### Subject selection

Subjects were selected from the population visited Department of Stomatology, Peking University Third Hospital, Beijing, China, during March 2011 to March 2012. Eighty chronic periodontitis patients were recruited, half with type 2 diabetes and half without systemic disease. Besides, twenty generally healthy (had healthy periodontium, with probing depth less than 3mm, no attachment loss, no gingivitis, no evidence of bone loss on radiographs, and no systemic disease) volunteers were recruited. All subjects had more than 16 teeth. The

diagnostic criteria of chronic periodontitis were based on the International Workshop for the Classification of Periodontal Diseases and Conditions in 1999, and classified as Stage II-IV, Grade B–C based on new classification in 2018. The patients with type 2 diabetes were diagnosed by the Department of Endocrinology in this hospital, according to the WHO criteria in 1999. The exclusion criteria are as followed: (1) other systemic diseases affect periodontal conditions, (2) severe complications of type 2 diabetes, such as diabetic nephropathy and retinopathy, (3) pregnancy or lactation, (4) history of antibiotic application and periodontal treatment within 6 months.

### Study procedures

A total of 100 subjects were included, 20 of whom were in health (H) group, 40 were in chronic periodontitis (CP) group and 40 were in chronic periodontitis with type 2 diabetes (DC) group. Fasting venous blood samples were collected and the fasting blood glucose (FBG) of all subjects in 3 groups were tested. The HbA1c level were tested in CP and DC group. Then GCF samples were collected before full mouth periodontal examination. After, the level of IL-17, visfatin and RANKL/OPG ratio in serum and GCF was measured and compared among three groups.

### Periodontal examination

Probing depth (PD) and attachment loss (AL) were measured by a Williams periodontal probe at 6 probing sites (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) per tooth in all subjects. Bleeding index (BI) and plaque index (PLI) were recorded at buccal and lingual side of every tooth. All clinical periodontal examination were performed by a periodontal specialist (Xu J).

### Gingival crevicular fluid sampling

2 teeth in different quadrants were selected based on clinical signs of inflammation and radiographic examination to sample GCF by intrasulcular method. After isolating the chosen tooth with cotton roll, supragingival plaque was removed softly by curettes and fluid on the tooth surface was dried by gentle airflow. A 3 MM CHR filter paper strip (Whatman, Maidstone, England), which was sterilized and weighed with an Eppendorf tube in advance, was gently placed into the pocket until resistance for 30 s. After that, the paper strip (Whatman) with the tube was weighed again, and the difference value was recorded and the volume of GCF was calculated. GCF sample from mesio-buccal site of each tooth was collected and tested separately. All samples were stored in Eppendorf tubes at −80 °C until tested.

### Blood sampling

The fasting venous blood sample were taken at early morning using non-anticoagulant tubes at room temperature. Serum was separated by centrifugation at 3500 rpm for 15 min after setting for 30 min at 4 °C. Then, the serum was immediately transferred to several Eppendorf tubes and stored at −80 °C until assayed.

### Measurement of cytokines in serum and gingival crevicular fluid

Both serum and GCF samples were setting at room temperature before tested. GCF in filter paper strip was eluted by centrifugation at 13000 rpm for 10 min with 100 μl PBS after 40 min vibration at 20 °C. IL-17 levels in serum and GCF were assayed using an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer. The concentration of IL-17, RANKL, OPG and visfatin of each sample in serum and GCF were determined based on standard curve, and the total amount of IL-17, visfatin in GCF and RANKL/OPG ratio in serum and GCF was calculated.

### Statistical analysis

The data was analyzed by software SPSS V. 26.0 (IBM, Armonk, NY, USA), and *P*-value less than 0.05 ($P < 0.05$) were considered statistically significant. Continuous variables were presented as mean ± SD, whereas categorical variable like gender was presented as number (%). For cross-sectional comparison, continuous variables that confirming to normal distribution were analyzed by a one-way ANOVA, followed by Least-Significant Difference (LSD) pairwise comparison, and the categorical variables were compared using a chi-square test. Partial correlation test adjusted for age, gender and body mass index (BMI) was conducted to determine the relationship between clinical periodontal parameters and biomarkers in GCF and in serum. Liner regression model was chosen to conduct univariate and multivariate analysis of the association of inflammatory state in periodontal tissue and circulation.

## Results

### The demographic and clinical characteristics of the subjects

The demographic data and clinical characteristics are summarized in Table 1. There were significant differences in age and BMI among 3 groups, while gender distribution and number of current smokers were similar. Obviously, FBG level and HbA1C level in DC group were highest among 3 groups ($P < 0.05$). FBG level in CP group was also higher than H group ($P < 0.05$), although both groups were at normal level.

PD, AL, BI and PLI of full mouth were significantly lower in H group compared to CP and DC group ($P < 0.05$). While periodontal status was similar between CP and DC group, except that mean value of BI in full mouth was significantly higher in CP group (2.71 ± 0.85 vs 2.20 ± 0.52, $P < 0.001$).

### GCF volume and cytokines level in serum and GCF

The GCF volume, cytokines level in GCF and serum in 3 groups are shown in Table 1. The GCF volume, the total

amount of IL-17 and RANKL/OPG ratio were significantly higher in DC group than in CP and H group, and higher in CP group compared to H group. The total amount of GCF-visfatin in H group was significantly lower compared to other two groups, while in DC group was higher than in CP group but without statistical significance. The concentration of serum IL-17 in H group was significantly higher than in DC group (194.15 ± 93.03 vs 104.74 ± 88.90, $P = 0.046$), and also higher than CP group but without statistical significance (194.15 ± 93.03 vs 137.96 ± 92.65, $P = 0.232$). The concentration of visfatin in GCF among three groups had no statistical significance. The concentration of IL-17, visfatin and RANKL/OPG ratio in serum was lower in H group than other 2 groups ($P < 0.001$). Furthermore, serum visfain and RANKL/OPG ratio in CP group were also significantly lower than in DC group ($P = 0.022$ and $P < 0.001$, respectively). While serum IL-17 was lower in CP group than DC group but without statistical significance.

## GCF volume and total amount of cytokines at sample sites with different PD

At sample sites with PD less than 3 mm (Fig. 1), Sample site BI was significantly lower in H group than CP and DC group ($P < 0.001$), and no significant difference between the latter two groups. The GCF volume was highest in DC group and lowest in H group, with statistical significance ($P < 0.001$). The total amount of IL-17 in GCF was lower in H group ($P < 0.001$), and no significant difference between CP and DC groups. And the total amount of visfatin and RANKL/OPG ratio was significantly lowest in H group, and highest in DC group.

At sample sites with PD more than 3 mm (Fig. 1), BI was significantly lower in DC group than in CP group. While GCF volume, total amount of IL-17, visfatin and RANKL/OPG ratio were all significantly higher in DC group.

## Correlation between clinical and biochemical parameters

Patrial correlation between clinical and biochemical parameters in GCF and serum was shown as heatmap in Fig. 2. After adjusting for age, gender and BMI, correlation could be observed between clinical parameters (PD and BI) and cytokines level in GCF and serum (including IL-17, visfatin and RANKL/OPG ratio). Besides, total amount of GCF -IL-17, -visfatin and -RANKL/OPG were closely correlated to their counterparts in serum ($P < 0.001$).

Univariate liner regression analysis (Table 2) in all subjects demonstrated that serum Visfatin ($\beta = 0.29$, $P = 0.003$), serum RANKL/OPG ratio ($\beta = 0.36$, $P < 0.001$), total amount of GCF- IL-17 ($\beta = 0.36$, $P < 0.001$), total amount of GCF Visfatin ($\beta = 0.47$, $P < 0.001$) and GCF-RANKL/OPG ($\beta = 0.51$, $P < 0.001$) were all associated with FBG. These five covariates entered the initial equation and adopting enter method to construct the model. Finally, only RANKL/OPG ($\beta = 0.40$, $P = 0.001$) in GCF was testified to be risk factors for FBG.

**Table 1** Demographic and clinical characteristics, and IL-17 level in serum and gingival crevicular fluid.

| | | H (n = 20) | CP (n = 40) | DC ( n = 40) | $P_1$ (H&CP) | $P_2$ (H&DC) | P3 (CP&DC) |
|---|---|---|---|---|---|---|---|
| Age | year | 33.55 ± 6.98 | 52.23 ± 9.65 | 57.23 ± 11.63 | <0.001 | <0.001 | 0.029 |
| Male | n (%) | 8 ( 40% ) | 18 ( 45% ) | 22 ( 55% ) | | 0.49 | |
| BMI | $Kg/m^2$ | 20.96 ± 2.95 | 23.37 ± 3.26 | 24.31 ± 3.39 | 0.008 | <0.001 | 0.20 |
| Current Smokers | n (%) | 0 (0%) | 5 (12.5%) | 7 (17.5%) | | 0.14 | |
| FBG | mmol/L | 4.89 ± 0.24 | 5.30 ± 0.49 | 7.80 ± 3.04 | 0.771 | 0.003 | <0.001 |
| HbA1C | % | | 5.40 ± 0.30 | 7.45 ± 1.72 | | | <0.001 |
| Full mouth | PD (mm) | 2.22 ± 0.54 | 3.67 ± 0.79 | 3.34 ± 0.67 | 0.001 | 0.015 | 0.065 |
| | AL (mm) | 0 | 3.45 ± 1.42 | 3.07 ± 1.67 | <0.001 | <0.001 | 0.132 |
| | BI | 0.63 ± 0.46 | 2.71 ± 0.85 | 2.20 ± 0.52 | <0.001 | <0.001 | <0.001 |
| | PLI | 0.98 ± 0.36 | 2.19 ± 0.53 | 2.32 ± 0.32 | <0.001 | <0.001 | 0.188 |
| Sample sites | PD (mm) | 2.30 ± 0.47 | 4.23 ± 1.74 | 4.23 ± 1.52 | <0.001 | <0.001 | 0.98 |
| | AL (mm) | 0 | 3.49 ± 2.78 | 3.56 ± 2.35 | <0.001 | <0.001 | 0.77 |
| | BI | 1.25 ± 0.44 | 2.65 ± 1.08 | 2.35 ± 0.73 | <0.001 | <0.001 | 0.17 |
| GCF | Volume (ul) | 0.35 ± 0.09 | 0.94 ± 0.67 | 1.56 ± 1.21 | <0.001 | <0.001 | 0.005 |
| | IL-17 (pg) | 63.61 ± 19.11 | 90.79 ± 27.51 | 96.95 ± 24.87 | <0.001 | <0.001 | 0.038 |
| | IL-17 (pg/ul) | 194.15 ± 93.03* | 137.96 ± 92.65* | 104.74 ± 88.90* | 0.232 | 0.046 | 0.14 |
| | Visfatin (ng) | 1.55 ± 0.66 | 3.40 ± 0.87 | 4.26 ± 1.12 | <0.001 | <0.001 | 0.166 |
| | Visfatin (pg/ul) | 4.71 ± 2.60 | 4.52 ± 1.80 | 4.72 ± 3.49 | 0.81 | 0.99 | 0.76 |
| | R/O | 12.54 ± 6.10* | 122.73 ± 20.61* | 221.41 ± 29.87* | <0.001 | <0.001 | <0.001 |
| Serum | IL-17 (pg/ul) | 0.11 ± 0.03 | 0.17 ± 0.05 | 0.18 ± 0.05 | <0.001 | <0.001 | 0.4 |
| | Visfatin (ng/ul) | 16.15 ± 5.09 | 33.87 ± 10.86 | 42.64 ± 11.71 | <0.001 | <0.001 | 0.022 |
| | R/O | 0.556 ± 0.13 | 1.26 ± 7.33 | 1.69 ± 0.32 | <0.001 | <0.001 | <0.001 |

Note: Data are the number (%) or mean ± SD. H, health group; CP, chronic periodontitis group; DC, chronic periodontitis with type 2 diabetes group; BMI, body mass index; FBG, fasting blood glucose; PD: probing depth; AL: attachment loss; BI: bleeding index; GCF, gingival crevicular fluid, IL-17, interleukin 17; R/O, RANKL/OPG. * : P < 0.001, compared to serum level in the same group.

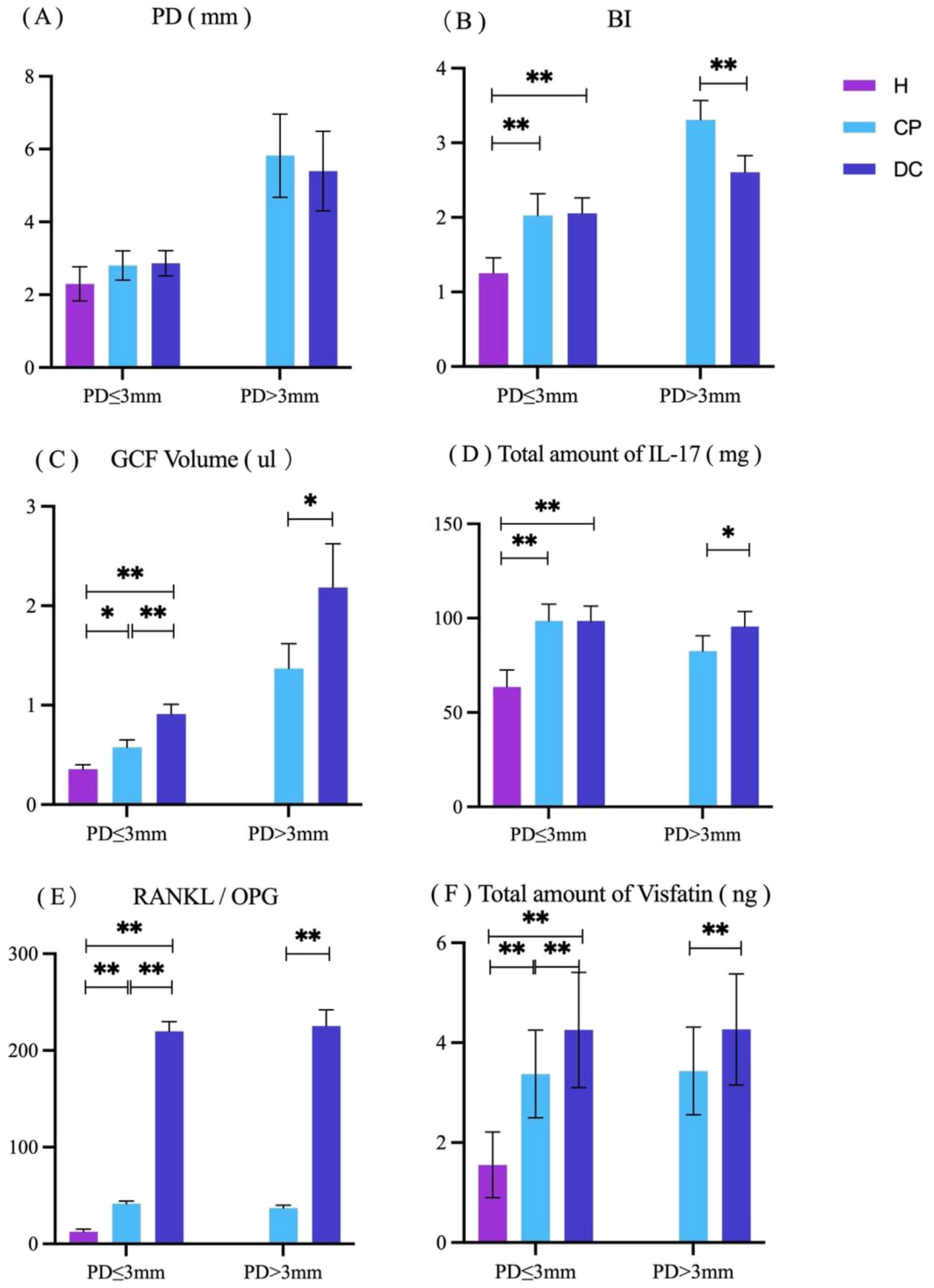


**Fig. 1** Probing depth (A), Bleeding index (B), gingival crevicular fluid volume (C), total amount of IL-17 (D), RANKL/OPG ratio (E) and total amount of visfatin (F) at sample sites with different probing depth.
Note: H, health group; CP, chronic periodontitis group; DC, chronic periodontitis with type 2 diabetes group;
PD $\leq$ 3 mm: n = 20 in H group, n = 41 in CP group, n = 37 in DC group; PD $>$ 3 mm: n = 39 in CP group, n = 43 in DC group.
PD, Probing depth; BI, bleeding index; IL-17, interleukin 17; R/O, RANKL/OPG.
*: $P < 0.05$ **: $P < 0.01$.

## Discussion

This study demonstrated periodontitis increase systemic inflammation. Periodontitis and diabetes together increase more severe systemic inflammation. Higher level of IL-17, visfatin and RANKL/OPG ratio of serum in periodontitis patients were observed when compared to healthy ones in the present study. Previous studies showed that systemic inflammation degree was much higher in periodontitis patients than in gingivitis or healthy ones.[20,21] It's worth pointing out that FBG in CP group was also significantly higher than H group, although both groups were within the normal range. This result was consistent with Shi et al.' s study,[22] which showed both chronic periodontitis and aggressive periodontitis had higher glucose level than healthy controls.

IL-17 can activate NF-κB pathway and then upregulate inflammatory cytokines genes expression, which are contributed to the insulin resistance and finally lead to development of T2DM.[23] Meanwhile, visfatin has insulin mimetic effect and influence insulin sensitivity, which acts as a pathogenic role in T2DM development.[18] Xu et al.[24] found that serum RANKL/OPG ratio was higher in patients with CP and poorly controlled T2DM than well controlled ones. Our study also showed a positive correlation between FBG and serum visfatin level and RANKL/OPG ratio. Thus, it's reasonable to presume that periodontitis may have influence on glycometabolism via inflammation pathways, and IL-17, visfatin, RANKL and OPG may be involved in this process.

With the in-depth understanding of T2DM till today, it has been considered as a chronic inflammatory disease. It was reported that chronic inflammation level in diabetic population was higher than in prediabetic and normal glucose tolerance ones, however, periodontal condition was not considered.[25] While in Sunandhakumari et al.' s study,[10] patients with diabetes had higher level of IL-17 in serum and more severe periodontal destruction than patients without diabetes. Pradeep et al.[26] reported that serum visfatin concentration in periodontitis patients with well controlled diabetes was higher than patients without diabetes, and both groups had similar BMI and periodontal destruction. While in our study, DC group had less periodontal inflammation (exhibiting as lower BI compared to CP group) but still represented higher systemic inflammation level with higher level of serum visfatin and RANKL/OPG ratio compared to CP group. These results suggested that periodontitis and diabetes could synergistically increase systemic inflammation. Periodontitis that persistently exist and without treatment can increase risk for incident of diabetes and its complications.[27]

In this study, total amount of IL-17, visfatin and RANKL/OPG ratio in GCF of periodontitis patients was higher than healthy ones, indicating these cytokines level may reflect the periodontal inflammatory condition. The positive correlation between PD or BI with IL-17, visfatin or RANKL/OPG ratio in GCF further underlined their roles in periodontitis development. While diabetes can exacerbate inflammatory state in periodontal tissue was further proved in this study. Our study showed DC group had higher GCF volume, GCF-IL-17 amount and RANKL/OPG ratio compared to CP group with similar clinical periodontal status. Previous study also showed that in the case of similar periodontal status periodontitis patients with diabetes had higher level of GCF-IL-17, visfatin and RANKL/OPG ratio, which was more severe in poorly-controlled T2DM.[26,28] There's also an interesting finding that FBG was associated with serum visfatin, serum RANKL/OPG, GCF total amount of IL-17, GCF total amount of visfatin and GCF RANKL/OPG by univariate analysis,

**Table 2** Univariate and multivariate liner regression analysis of association between fasting blood glucose and inflammatory parameters in periodontal and circulation.

| Parameters | Univariate analysis | | | Mulitivariate analysis | | |
|---|---|---|---|---|---|---|
| | β | t | *P* | β | t | P |
| age | 0.13 | 1.28 | 0.20 | | | |
| gender | 0.08 | 0.85 | 0.40 | | | |
| BMI | 0.15 | 1.49 | 0.14 | | | |
| PD | 0.16 | 1.55 | 0.12 | | | |
| BI | 0.09 | 0.89 | 0.38 | | | |
| S-IL-17 | 0.16 | 1.58 | 0.12 | | | |
| S-Visfatin | 0.29 | 3.02 | 0.003 | −0.061 | −0.576 | 0.566 |
| S-R/O | 0.36 | 3.86 | <0.001 | −0.006 | −0.048 | 0.962 |
| G-IL-17 | 0.36 | 3.83 | <0.001 | 0.1 | 0.853 | 0.396 |
| G-Visfatin | 0.47 | 5.24 | <0.001 | 0.235 | 1.7 | 0.092 |
| G-R/O | 0.51 | 5.82 | <0.001 | 0.397 | 3.281 | 0.001 |

Note: BMI, body mass index; PD: probing depth; BI: bleeding index; S-IL-17, concentration of IL-17 in serum; S-visfatin, concentration of visfatin in serum; S-R/O, RANKL/OPG ratio in serum; G-IL-17, total amount of IL-17 in gingival crevicular fluid; G-visfatin, total amount of visfatin in gingival crevicular fluid; G-R/O, RANKL/OPG ratio in gingival crevicular fluid.

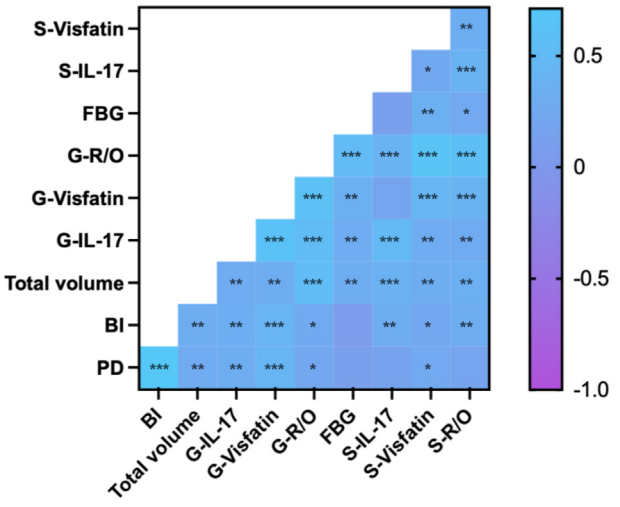


**Fig. 2** Heatmap of partial correlation between clinical periodontal parameters, fasting blood glucose total amount of cytokines in gingival crevicular fluid and concentration of cytokines in serum.
Note: PD: probing depth; BI: bleeding index; G-IL-17, total amount of IL-17 in gingival crevicular fluid; G-visfatin, total amount of visfatin in gingival crevicular fluid; G-R/O, RANKL/OPG ratio in gingival crevicular fluid; FBG, fasting blood glucose, S-IL-17, concentration of IL-17 in serum; S-visfatin, concentration of visfatin in serum; S-R/O, RANKL/OPG ratio in serum;
*: $P < 0.05$ **: $P < 0.01$ ***: $P < 0.001$.

while only RANKL/OPG in GCF was testified to be risk factors for FBG in multivariate analysis. Previous study reported that glucose level in GCF was higher at periodontitis sites of systemically healthy subjects.[29] Glycated albumin was also identified in GCF, which was higher in diabetes patients and positively correlated with blood glycated albumin and HbA1c level.[30] Elevated glucose level in GCF might support the growth of anaerobic pathogens and also affect the physiological functioning of periodontal ligament cells.[31] These results indicated that diabetes also aggravated inflammation in periodontal tissue, and the worse the glycemic control, the more severe the inflammatory state of periodontal tissue was.

Periodontitis is a bacteria-induced inflammatory disease, and sub-gingival microorganism plays an important role in the process. Patients with moderate and severe periodontitis have more complicated and pathogenic subgingival and saliva microbiota,[32] thus they are at higher risk of pathogenic bacteria colonization into shallow pockets than healthy people. With the stimulation of bacterial plaque, dendritic cells (DCs) in periodontal tissue recognize the pathogen like *Porphyromonas gingivalis* (*Pg*) and present the antigens to T cells and promote the differentiation into Th17 cells, which can release IL-17. Erkan Ozcan et al.[33] reported that *Pg* might increase visfatin secretion by inflammatory cytokines, though the mechanism was unknown. Yao et al.[34] found that visfatin was upregulated in inflamed gingiva. They also cultured hPDLCs with *Pg* LPS and found that the mRNA expression of visfatin presented a profile of time-dependent upregulation and significantly raised compared to untreated control group. It gave us a hint that visfatin in GCF might have other origination in periodontal tissue rather than adipocytes. Our study also showed a much higher concentration of visfatin in GCF than in serum. Nogueira et al.[35] demonstrated that *Filifactor alocis* could stimulate synthesis of visfatin from human macrophages via TLR2 and MAPK pathways. In our study, total amount of IL-17 and visfatin and RANKL/OPG ratio in GCF were positively correlated, and they were also correlated to periodontal destruction and inflammation, which suggested that these indicators did represent the inflammatory state of periodontal tissue to some extent, and might be even more sensitive than clinical periodontal parameters. This could explain why the sites of PD less than 4 mm in patients with periodontitis still had higher inflammatory state than in H group in the present study, showed as higher BI, more GCF volume and more total amount of GCF-IL17, visfatin, and higher RANKL/OPG ratio.

To the best of our knowledge, our study firstly showed diabetes exacerbated inflammatory state in shallow pockets of periodontitis patients. And this exacerbation was further amplified in deeper pockets. GCF volume, total amount of GCF-visfatin and RANKL/OPG ratio in sites of PD less than 4 mm was still higher in DC group than CP group, though both BI and amount of GCF-IL-17 in the two groups were similar. DC group also showed more severe inflammation in pockets of PD more than 3 mm when compared to CP group. Even with lower BI in full mouth, DC group still had higher GCF volume and amount of IL-17, visfatin, RANKL/OPG ratio in GCF. These results further proved that diabetes, as a systemic disease, could increase periodontal inflammation in periodontitis patients. Diabetes can shape the periodontal microbiome: the relative abundance of health-compatible species decreases and species belonging to disease-associated genera increases.[1] Animal study shows that DM increase the expression of some inflammatory cytokines in gingiva and also make oral microbiota more pathogenic, which can be reduced by IL-17 antibody treatment.[36] Other studies show that periodontitis patients with diabetes have higher level of visfatin in GCF,[26] which decreases after non-surgical periodontal treatment.[37] Therefore, diabetes is associated with more severe periodontal inflammation, and dysbiosis of sub-gingival microbiome aggravated by diabetes may be one of the correlated factors.

Therefore, IL-17, visfatin and RANKL/OPG ratio are supposed to be important proinflammatory indicators during the development of periodontitis with or without diabetes. While when periodontitis combined with diabetes mellitus, it is easier to develop into even worse periodontal status and make shallow pockets develop into deep pockets if not maintained regularly. Thus, glycemic control is supposed to be a crucial part in periodontal treatment for patients with periodontitis and diabetes both. Besides, this study was a cross-section design, thus, interpretation of dynamic changes in these cytokines were not provided. Further studies need longitudinal design to gain insight into the relationship between periodontitis and diabetes.

This study compared the level of IL-17, visfatin, RANKL/OPG ratio in GCF and serum among subjects suffered from periodontitis with or without diabetes, as well as general health. Their associations with periodontal parameters and glucose level were also discussed. It was demonstrated that moderate and severe periodontitis increased systemic inflammation, which could be aggravated by T2DM together with periodontitis, resulting in more severe systemic inflammation. T2DM could worsen periodontal inflammation, either. The positive correlation between the local and systemic inflammation and their association with FBG indicated an inflammatory link in the relationship between periodontitis and T2DM.

The limitation of the present study is lack of a group of diabetes patients with healthy periodontal tissue, which would make it easier to explain the severe inflammatory state that diabetes led to, either in GCF or serum. Besides, if the dynamic changes in inflammatory indicators after periodontal treatment and their differences between groups could be observed, it would be more convincing to discuss the inflammation-related association of periodontal and diabetes. Therefore, much work remains to be done to explore in depth the bidirectional link between periodontitis and diabetes.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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