Effect of Periodontal Pathogens on Total Bone Volume Fraction: A Phenotypic Study*

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Summary: Studies have shown that periodontal pathogens can enter the bloodstream, causing a series of reactions that can lead to a variety of systemic diseases. Epidemiological investigations also found a tight correlation between periodontitis (PD) and osteoporosis. This study aimed to further explore the effect of periodontal pathogens on bone volume fraction like bone tissue and mass, and explain the relationship between PD and osteoporosis. Sprague Dawley rats (female, 16 weeks old) were divided into the wild-type (WT) control group (n=9) and PD group (n=9). After eight weeks, periodontal tissues and ligatures, the fourth lumbar vertebra, the femur, the tibia, and blood were extracted and analyzed by micro-computed tomography (micro-CT), hematoxylin and eosin (H&E) staining, tartrate-resistant acid phosphatase (TRAP) staining, polymerase chain reaction (PCR), and enzyme-linked immunoassay (ELISA), respectively. We found that the bone mass of the lumbar vertebra, femur, and tibia was decreased in the PD group. The number of osteoclasts was higher in bone tissue in the PD group than in the WT group (P<0.05). The levels of inflammatory mediators and type I collagen C-terminal peptide (CTX-1) were higher in the PD group than in the WT group (P<0.05), although no significant difference in bone glutamic acid protein (BGP) levels was observed (P>0.05). In addition, we detected several periodontal pathogens, such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum*, in blood samples from rats in the PD group. These findings suggest that periodontal pathogens can enter the blood circulation from periodontal tissue, promote a systemic inflammation response, and subsequently reduce systemic bone density.
Key words: periodontitis; periodontal pathogens; bone mineral density; inflammation; systemic disease

Periodontitis (PD) is a chronic destructive disease of periodontal support tissue and it leads to the inflammation of gums, absorption of alveolar bone, loosening and shedding of the teeth. According to an epidemiological survey among people aged 35–44 years in 2005 in China, the national periodontal health rate was only 14.2%, the detection rate of bleeding on probing (BOP) (+) was 77.3%, and the detection rate of deep periodontal pocket was 40.9%. However, in 2015, the BOP (+) detection rate was increased to 87.4%[1], indicating that PD has become more common. It is well known that PD is primarily caused by periodontal pathogens, such as *Porphyromonas gingivalis* (Pg), *Fusobacterium nucleatum* (Fn), *Actinobacillus actinomycetemcomitans* (Aa), *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf), and *Treponema denticola* (Td)[2]. These pathogens can release antigenic factors, such as lipopolysaccharide (LPS) and fimbriae, activate antigen-presenting cells, promote cytokine production and secretion, and induce irreversible damage to periodontal tissues[3]. Many studies have shown an association of PD with systemic diseases, such as cardiovascular diseases, diabetes, and rheumatoid arthritis (RA)[4].

Osteoporosis is a multifactorial disease, characterized by a decrease in bone mass and progressive deterioration of bone microstructure that can lead to a gradual increase in bone fragility, and even bone fracture. As the population ages, the incidence of osteoporosis increases and this condition ranks

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third among chronic diseases[5]. Epidemiological studies have shown that the average incidence rate of osteoporosis in China was 13%, with 8.5% in males and 15.7% in females[6, 7].

PD and osteoporosis share some risk factors, such as smoking, alcohol consumption, age, genetics, and systemic inflammation. Both diseases can lead to the loss of bone tissue. Furthermore, studies have shown a strong correlation between PD and osteoporosis[8, 9]. A cross-sectional study of 400 women in Jordan suggested that whole-body bone density was significantly associated with severe alveolar ridge height loss[10]. Given the involvement of PD in many systemic diseases, it seems that PD may participate in the progression of osteoporosis. However, strong evidence for this hypothesis is still lacking. In this study, we demonstrated that PD decreased bone mass by promoting a systemic inflammatory response. Our findings provide new insight into the effect of PD on osteoporosis.

1 MATERIALS AND METHODS

1.1 Animals

This study was approved by the Animal Ethics Review Committee of Tongji Medical College, Huazhong University of Science and Technology (China). Sixteen-week-old female Sprague Dawley rats (Beijing Vital River Laboratory Animal Technology Company, China) were randomly divided into the wild-type (WT) control group (n=9) and PD group (n=9). All the rats were anesthetized with 10% chloral hydrate in a dose of 0.4 mL/100 g. After anesthesia, rats in the PD group were fixed on the operating table. The upper and lower jaws were fixed with rubber bands. First, the upper jaw teeth were exposed, and the first and second molars were subjected to periodontal ligation using a 3-0 silk thread. The thread was inserted as deep as possible into the bottom of the groove, and reinforced with a 0.2 mm orthodontic ligation wire. After the maxillary teeth were ligated, the rats were fixed on the operating table in the prone position, the mandibular molars were exposed, and the first and second molars of the mandible were ligated in the same manner. The ligation silk was replaced after two, four, and six weeks. Eight weeks later, the rats were sacrificed and the fourth vertebra, the femur, the tibia, periodontal tissues, and blood were harvested for further analysis.

1.2 Bacterial Detection in Periodontal Ligation Silk and Blood

Total DNA from periodontal ligation silk and blood was extracted using a DNA extraction kit (Tiangen, China) following the manufacturer's instructions. Amplification of the bacterial 16S ribosomal RNA gene was carried out with specific bacterial primers. The 16S rDNA primer sequences and the annealing temperature are listed in table 1. Polymerase chain reaction (PCR) was performed as described previously[11]. The PCR products were purified by 1.5% agarose gel electrophoresis.

1.3 Enzyme-linked Immunoassay Measurements of Inflammatory Factors and Bone Markers

Blood was centrifuged at 3500 r/min for 20 min, after which the serum was collected and stored at −80°C. The serum was equilibrated at room temperature. Serum levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), bone glutamic acid protein (BGP), and type I collagen C-terminal peptide (CTX-1) were analyzed with an enzyme-linked immunoassay (ELISA) kit (NeoBioscience, China) in accordance with the manufacturer's instructions. The concentration of each protein was calculated by measuring the absorbance (A_{450}) and comparing it with a standard curve.

1.4 Micro-computed Tomography for Bone Analysis

After the rats were sacrificed, fresh specimens from the jaw, femur, tibia, and fourth lumbar vertebra were isolated and scanned by micro-computed tomography (micro-CT). Micro-CT scanning was performed by the same person using the same machine (Skyscan 1276) under the same conditions (filter Cu+Al, 120 μA, scanning thickness 9 μm). After scanning, the micro-CT results were analyzed with the indicated software.

1.5 Bone Histopathology

After the rats were sacrificed, the upper and lower jaws, fourth lumbar vertebra, femur, and tibia were

Table 1 PCR primer sequence

Bacteria	Upstream primer (f)	Downstream primer (r)	Product size (bp)	Annealing temperature (°C)
Pg	AGGCAGCTTGCCATACTGCG	ACTGTTAGCAACTACCGATGT	404	62
Aa	AAACCCATCTCTGAGTTCTTCTTC	ATGCCAACTTGACGTTAAAT	557	56
Fn	AGAGTTTGATCCTGGCTCAG	GTCATCGTGCACACAGAATTGCTG	360	56
Pi	TTTGTTGGGGAGTAAAGCGGG	TCAACATCTCTGTATCCTGCGT	575	56
Tf	GCGTATGTAACCTGCCCGCA	TGCTTCAGTGTCAGTTATACCT	641	56
Cr	TTTCGGAGCGTAAACTCCTTTTC	TTTCTGCAAGCAGACACTCTT	598	56
Sm	GGCACCACAACATTGGGAAGCTCAGTT	GGAATGGCCGCTAAGTCAACAGGAT	433	51
Td	TAATACCGAATGTGCTCATTTACAT	TCAAAGAAGCATTCCCTCTTCTTCTTA	311	51
Pn	ATGAAACAAAGGTTTTCCGGTAAG	CCCACGTCTATGTGGGCTGCGA	804	56

Pg: *Porphyromonas gingivalis*; Aa: *Actinobacillus actinomycetemcomitans*; Fn: *Fusobacterium nucleatum*; Pi: *Prevotella intermedia*; Tf: *Tannerella forsythia*; Cr: *Campylobacter rectus*; Sm: *Streptococcus mutans*; Td: *Treponema denticola*; Pn: *Prevotella nigrescens*

isolated and fixed in 4% paraformaldehyde at room temperature for three days. Next, the samples were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) for two months, embedded in paraffin, and cut into 5 μm thick sections for hematoxylin-eosin (H&E) and tartrate-resistant acid phosphatase (TRAP) staining.

1.6 Statistical Analysis

Statistical analysis and mapping of the data were performed using SPSS 20.0 and GraphPad Prism 7.0 software. An unpaired two-tailed Student's *t*-test was used to compare the two groups. Results are presented as mean±standard deviation. $P<0.05$ was considered statistically significant.

2 RESULTS

2.1 PD Model Establishment

To establish the PD model, we performed periodontal ligation using 3-0 silk for eight weeks. The jaw specimens were separated carefully and scanned by micro-CT to analyze the alveolar bone absorption. The jaw bone sections were stained with H&E for morphological analysis and with TRAP for osteoclast observation. Micro-CT analysis showed that the resorption of alveolar bone was significantly faster in PD rats than in WT rats (fig. 1A). The staining results revealed that the apical damage in PD rats was more severe than in WT rats (fig. 1B), and that the number of osteoclasts was significantly greater in PD rats (fig. 1C). These data indicate that the PD model was successfully established.

2.2 Changes of Bone Mass in PD Rats

Specimens from the fourth lumbar vertebra, femur, and tibia were scanned by micro-CT. The bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular bone (Tb.N), and trabecular separation (Tb.Sp) values were calculated. In the fourth lumbar vertebra, BV/TV was significantly lower and Tb.Sp was significantly higher in PD rats ($P<0.05$); no significant difference in Tb.Th or Tb.N was observed between the groups (fig. 2A). In the femur, BV/TV and Tb.N were

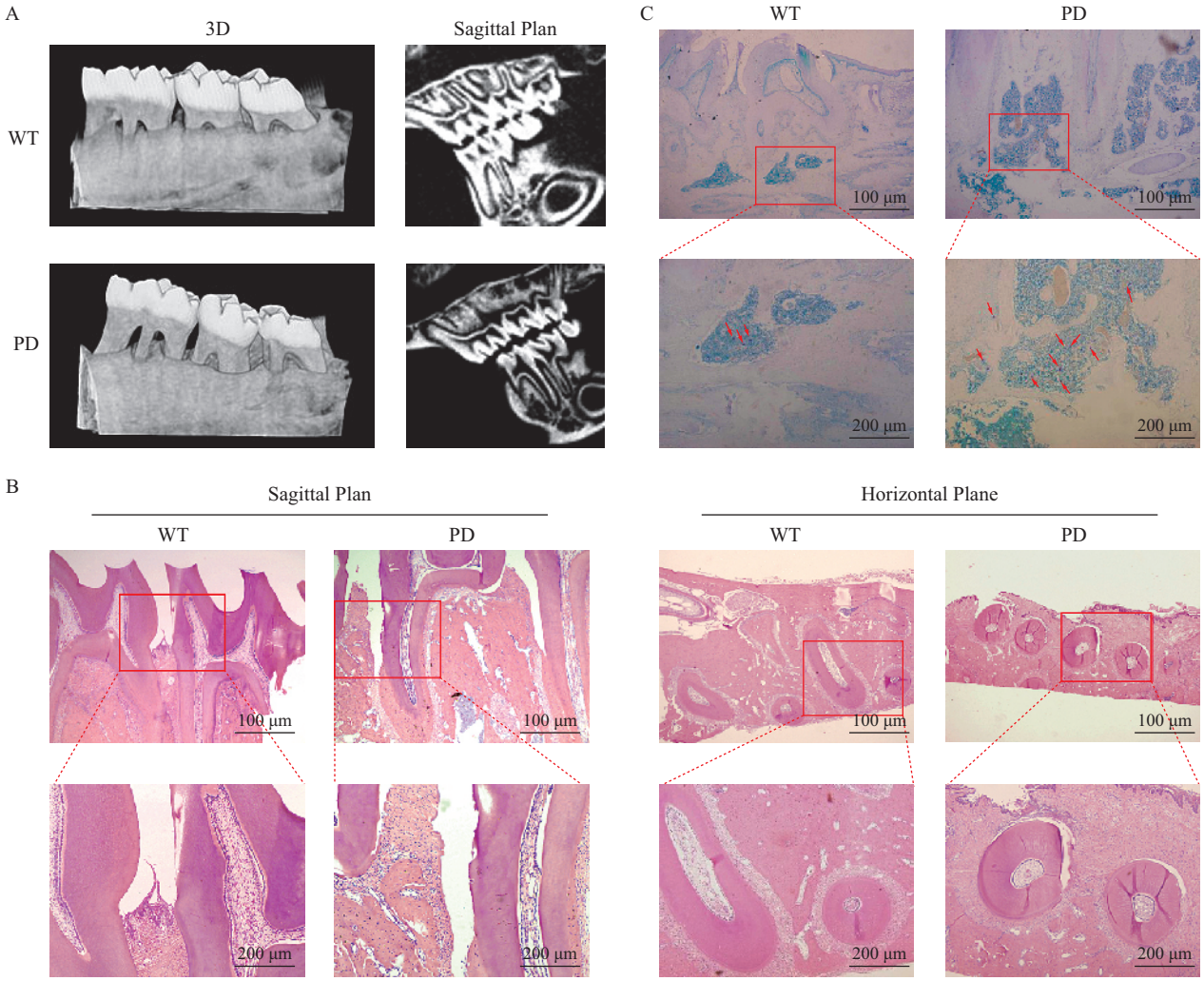

Fig. 1 Successful establishment of PD model in rats
Ligated periodontium and teeth from wild-type (WT) and periodontitis (PD) rats were analyzed by micro-CT (A), hematoxylin and eosin staining (B), and tartrate-resistant acid phosphatase staining (C). Red arrows in fig. 1C indicate osteoclasts.

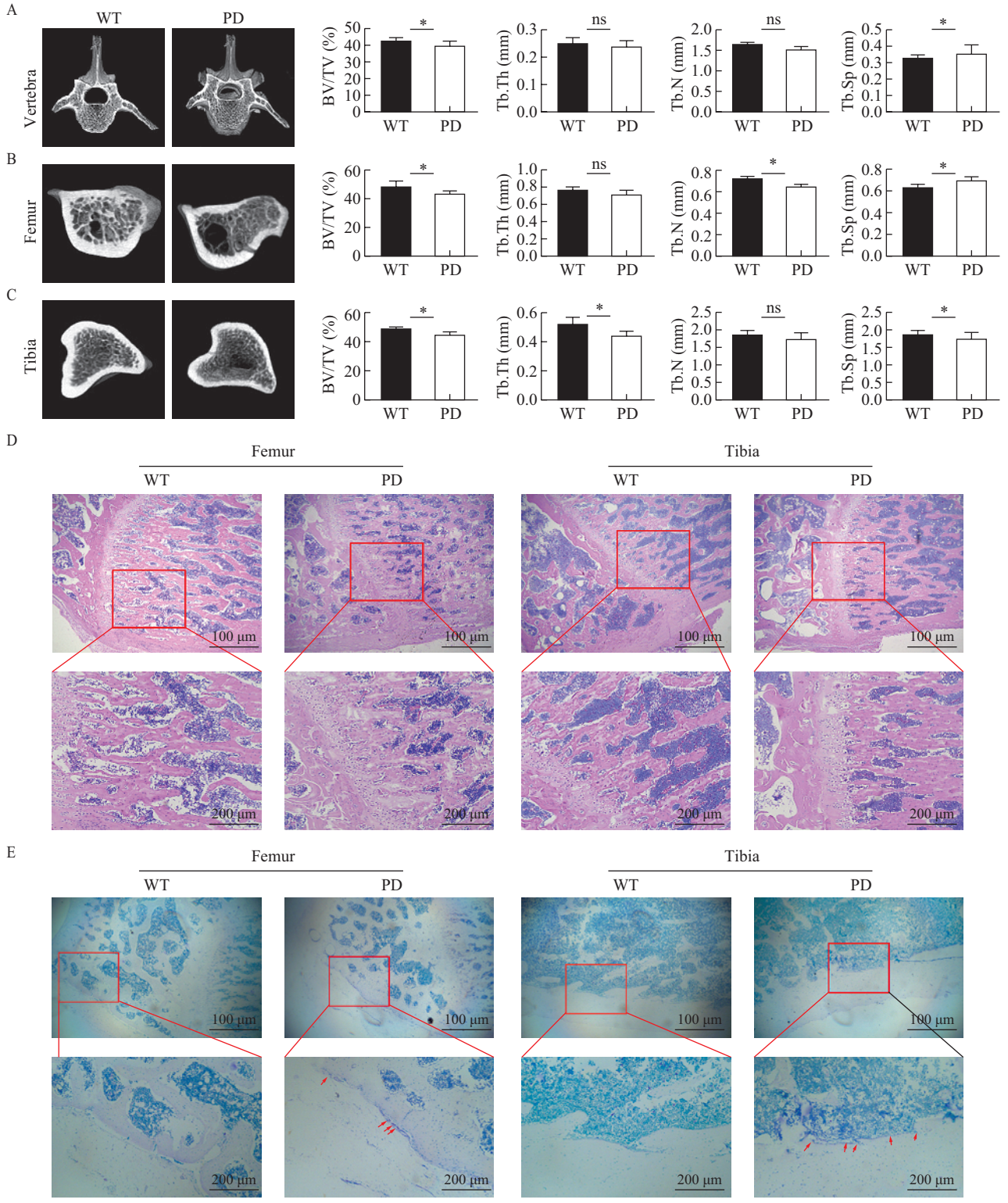

Fig. 2 Changes of bone mass in PD rats

Vertebra, femur, and tibia samples were taken from the rats during the eighth week after periodontal ligature. The fourth vertebra (A), femur (B), and tibia (C) in the WT and PD groups were analyzed by micro-CT, with quantification analysis shown on the right. Hematoxylin and eosin staining (D) and tartrate-resistant acid phosphatase staining (E) of the femur and tibia are also shown. Lower panel shows the magnification of the images shown by red rectangles. Red arrows in fig. 2E indicate osteoclasts.

significantly lower and Tb.Sp was significantly higher in PD rats than in WT rats (P<0.05) (fig. 2B). In the tibia, BV/TV and Tb.Th were significantly decreased and Tb.Sp was significantly increased in PD rats as compared with those in WT rats (P<0.05) (fig. 2C). Accordingly, in the transverse sections of the femur

and tibia, we observed enlarged bone marrow cavities and thinner cortical bone in PD rats (fig. 2D). The number of osteoclasts within the femur and tibia was significantly higher in PD rats than in WT rats (fig. 2E). Collectively, these results suggest that PD has a harmful effect on bone mass.

2.3 Food Intake and Body Weight

Considering the fact that other factors may induce differences in total bone mass, we monitored the food intake and body weight of rats in both groups. We found that no significant difference in food intake or body weight was observed (fig. 3), suggesting that the decrease in bone mass was not caused by food intake and body weight.

2.4 Changes in Inflammatory Factors and Bone Markers in the Serum

The activation of TNF-α and IL-6 can reduce total bone mass[12]. Bone markers in serum, such as BGP and CTX-1, can effectively reflect changes in bone mass[13]. ELISA showed that serum TNF-α and IL-6 levels were higher in PD rats, especially after eight weeks (fig. 4A and 4B). In addition, serum CTX-1 levels were significantly higher in PD rats throughout the experiment, while BGP levels were not significantly different (fig. 4C and 4D).

2.5 Periodontal Pathogens in Blood

Periodontal pathogenic bacteria can enter the bloodstream and cause bacteremia, which activates the production of systemic cytokines and affects other parts of the body. To analyze the periodontal pathogens in the blood circulation, we analyzed the DNA of several periodontal pathogens from periodontal ligatures and blood samples. Pg, Aa, and Fn were found in the samples from the periodontal ligatures, blood and subgingival plaque, suggesting that these periodontal pathogens may be the reason behind the elevated inflammatory response (fig. 5).

3 DISCUSSION

The prevalence of osteoporosis and periodontal disease is increasing globally[4]. In 2015, an epidemiological survey in Taiwan showed that after controlling for age, gender, income, and geographic region, there was a significant association between PD and osteoporosis in women[14]. Kobayashi *et al* found that compared with rats with normal bone density, rats with osteoporosis and osteopenia showed more loss of alveolar bone height and bone mass[15]. A study that evaluated osteoporosis of the jaw by two-photon absorption measurements found that osteopenia in patients with osteoporosis was directly related to the reduction of mandibular bone density[16]. The alveolar bone height in menopausal women with osteoporosis and PD decreased faster than in women without osteoporosis, and the rate of alveolar bone loss was significantly lower after taking bisphosphonates. Estrogen deficiency was associated with osteoporosis in women, and was also associated with an increased frequency of alveolar height reduction in an entire

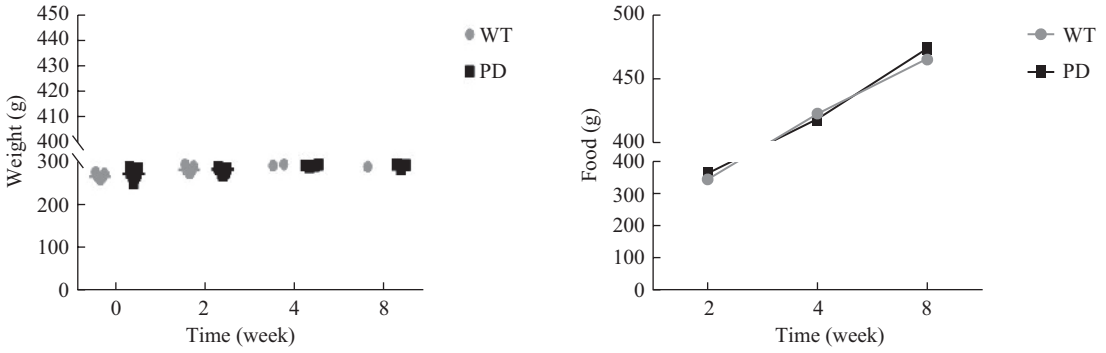

Fig. 3 The weight and food intake of rats

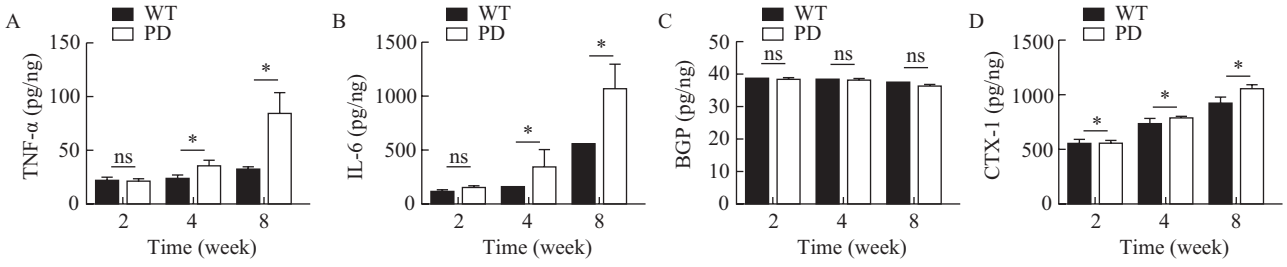

Fig. 4 The serum levels of TNF-α, IL-6, BGP, and CXT-1 in WT and PD rats
ELISA was used to determine the concentration of TNF-α (A), IL-6 (B), BGP (C), and CXT-1 (D) in serum from WT and PD rats at the indicated time points. $^{*}P<0.05$

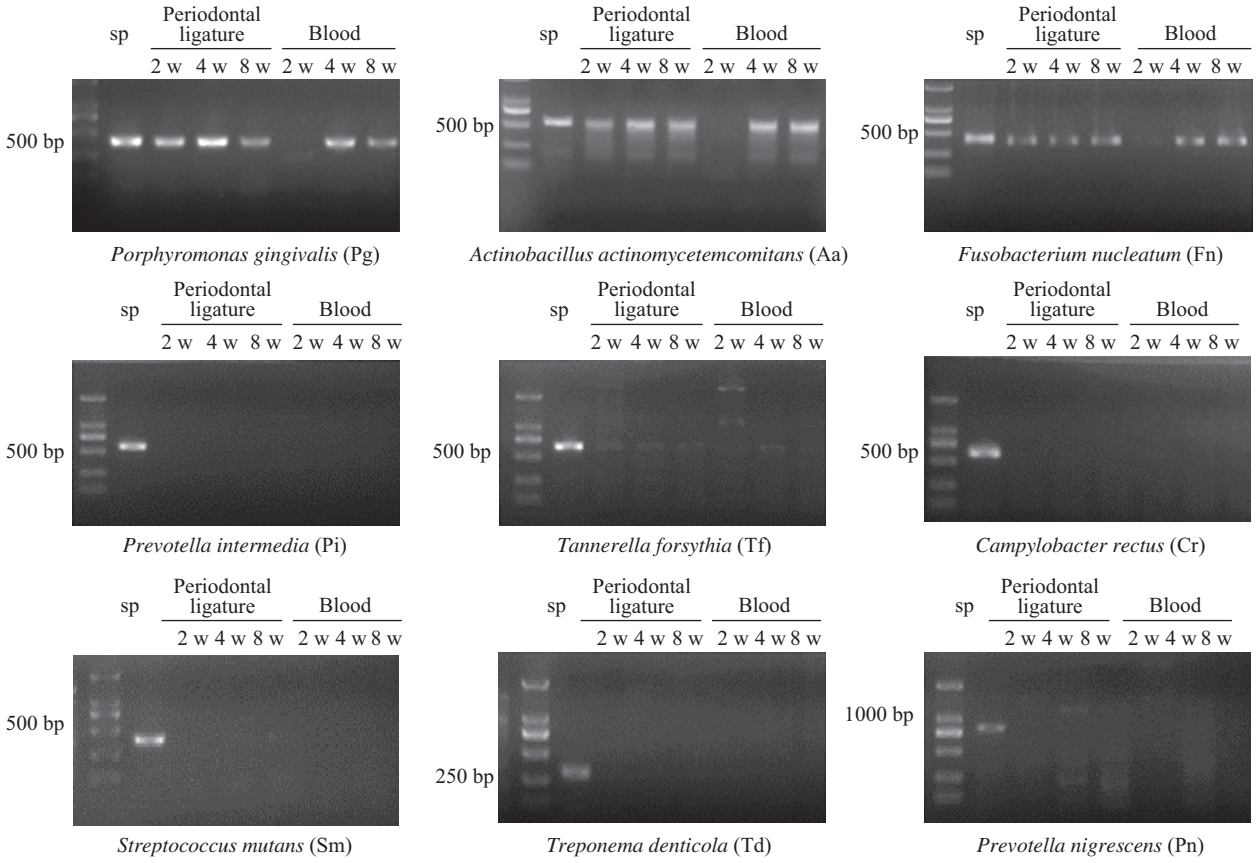

Fig. 5 Periodontal pathogen detection in periodontal ligatures and blood
16S rDNA detection of Pg, Aa, Fn, Pi, Tf, Cr, Sm, Td, and Pn by PCR in periodontal ligation silk and blood from PD rats.
sp: subgingival plague

study population[15]. Researchers found that rats with osteoporosis caused by reduced glucocorticoid levels had a greater risk of alveolar bone height loss than control rats[17]. These findings indicate that osteoporosis, osteopenia, and estrogen deficiency are risk factors for alveolar bone density loss in postmenopausal women. However, whether the development of PD can accelerate osteoporosis is still unknown.

Korean researchers conducted an epidemiological survey on 13 464 participants (8884 males and 4580 females) in 2017, and indicated that after controlling for all variables, the risk factors for osteoporosis were age, Charlson comorbidity index, and PD[18]. Some studies have also investigated osteoporosis in animals, and found that once an animal has PD, its osteoporosis becomes worse than that of normal control animals. These experiments suggested that PD may contribute to the development of osteoporosis. To explore the effect of PD on osteoporosis, we used 16-week-old rats to establish a PD model. To obtain accurate experimental results, we controlled for food intake and body weight. Rats in the PD group already had PD after two weeks. We found that PD had a negative effect on bone mass and it might be a risk factor for osteoporosis.

As it is secreted by osteoblasts and its levels reflect osteoblast activity, BGP is a specific marker for bone formation. CTX-1 plays an important role in osteoclast-mediated bone resorption and degradation. A large number of studies have shown that serum BGP and CTX-1 levels in patients with osteoporosis are significantly higher than in controls; because of this, BGP and CTX-1 are widely used to detect osteoporosis[19]. In the present study, we collected serum from rats, and found that the serum CTX-1 levels in PD rats were higher than in WT rats. However, no significant difference in BGP levels was noted. We speculate that this was due to the relatively old rats used in our experiments, as their bone forming ability was relatively low.

IL-6 and TNF-α are pleiotropic cytokines that play important roles in regulating endocrine and metabolic processes, such as inflammation and immune response[20]. Melton found that IL-6 levels in osteoporosis patients with estrogen deficiency were significantly increased. IL-6 can activate osteoclasts and accelerate bone resorption[12]. TNF-α is another major pro-inflammatory cytokine. TNF-α is involved in bone remodeling, and can exhibit synergy with other cytokines, such as IL-6, by activating the protein kinase R-like endoplasmic reticulum kinase pathway[21], the NF-κB pathway, and the Wnt pathway[22, 23], thereby increasing the expression of osteoclast markers,

increasing the production of osteoblasts, accelerating apoptosis, and inhibiting osteogenic transformation of bone marrow mesenchymal stem cells[13]. Inflammation factors such as IL-6 and TNF-α activate the NF-κB pathway, which increases the expression of CTX-124. In our study, we detected increased levels of IL-6 and TNF-α in the PD group and found that serum TNF-α and IL-6 levels were significantly higher in PD rats than in WT rats after eight weeks, which were consistent with results found by previous studies[24], and it was suggested that the inflammation response may be responsible for the loss of bone mass in PD[25].

Plaque is one of the most important factors in the development of PD, as it is a "hotbed" for the growth of bacteria such as Pg and Aa, which not only have the ability to resist host defense mechanisms, but also produce powerful virulence factors, release proteases, and cause damage to tissues[26–28]. Periodontal pathogens secrete LPS and other virulence and cytostatic factors, destroy alveolar bone, and prevent periodontal defense resistance[26, 29]. The virulence factors, pathogenic enzymes, antigenic components, and their metabolites secreted by periodontal pathogens can cause an immune response in periodontal tissue as well as a systemic immune response[30]. Large amounts of IL-6 and TNF-α can be detected in the gingival crevicular fluid and blood of PD patients[24], which may occur because pathogenic bacteria can activate the expression of a variety of cytokines, such as IL-1, TNF-α and IL-6, and these cytokines then stimulate monocyte activation and regulate systemic reactions[31]. In the present study, Pg, Aa, and Fn were found from the blood of PD rats, suggesting that periodontal pathogens may contribute to the loss of bone mass. In experimental animal models, Gram-negative anaerobic bacteria (such as Pg) in the periodontium can induce protein citrullination by releasing specific deaminases that may stimulate the formation of anti-citrullinated protein antibodies in RA patients[32]. When periodontal pathogens enter the systemic circulatory system, they can promote the development of systemic diseases and aggravate the disease severity.

Studies have shown that bacteria have effects on bone quality. It has been reported that gut microbiota can affect the body's immune system and regulate calcium and phosphorus absorption by secreting estrogen, serotonin, and other substances, thereby inducing changes in the body's bone quality[33]. Sjogren also found that mice lacking intestinal flora had a higher bone density in the distal femur than normal mice, which may be because mice lacking gut flora have fewer osteoclasts, and the osteoblast numbers remain unchanged[34]. Moreover, the expression levels of IL-6 and TNF-α in mice lacking intestinal flora were lower than in normal mice[3]. Because periodontal pathogens have some commonality with gut microbiota, they may also affect bone mass.

In summary, our study demonstrated that PD can promote the development of osteoporosis and periodontal pathogens can cause systemic bone changes, primarily due to activating a systemic immune response and increasing inflammation. Periodontal pathogens may activate the expression of various proteases, collagenases, and cytokines, as well as some pathological pathways, resulting in elevated CTX-1 levels and changing the total bone mass of the whole body. However, the specific mechanisms underlying the systemic bone changes caused by the pathogens remain to be elucidated.

Conflict of Interest Statement

The authors declare no competing financial interests.

REFERENCES

1 Cao CF. Prevalences of periodontal diseases and dental caries in China: re-analysis of the data from the Third National Epidemiological Survey on Oral Health. Zhonghua Kou Qiang Yi Xue Za Zhi (Chinese), 2013,48(5):257-259

2 Roberts AP, Mullany P. Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance. Expert Rev Anti Infect Ther, 2010,8(12):1441-1450

3 Darveau R. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol, 2010,8(7):481-490

4 Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim), 2017,11(2):72-80

5 Joseph ML. Adverse outcomes of osteoporotic fractures in the general population. J Bone Miner Res, 2003,18(6):1139-1141

6 Xue J. Epidemiological survey of osteoporosis. New Med Sci Osteop (Chinese), 2007,38(1):7-8

7 Ma ZJ, Wang J. Epidemiology of Osteoporosis. Internat J Osteol (Chinese), 2007,28(5):315-316

8 Dodd DZ, Rowe DJ. The relationship between postmenopausal osteoporosis and periodontal disease. J Dent Hyg, 2013,87(6):336-344

9 Juluri R, Prashanth E, Gopalakrishnan D, *et al*. Association of Postmenopausal Osteoporosis and Periodontal Disease: A Double-Blind Case-Control Study. J Int Oral Health, 2015,7(9):119-123

10 Habashneh R, Alchalabi H, Khader YS, *et al*. Association between periodontal disease and osteoporosis in postmenopausal women in Jordan. J Periodontol, 2010, 81(11):1613-1621

11 Xie M, Tang Q, Nie J, *et al*. BMAL1-Downregulation Aggravates Porphyromonas Gingivalis-Induced Atherosclerosis by Encouraging Oxidative Stress. Circ Res, 2020,126(6):e15-e29

12 L Joseph Melton 3rd. Adverse outcomes of osteoporotic fractures in the general population. J Bone Miner Res, 2003,18(6):1139-1141

13 Liu Y, Wang L, Kikuiri T, *et al*. Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-gamma and TNF-alpha. Nat Med,

2011,17(12):1594-1601
14 Lin TH, Lung CC, Su HP, *et al*. Association between periodontal disease and osteoporosis by gender: a nationwide population-based cohort study. Medicine, 2015,94(7):e553
15 Kobayashi M, Matsumoto C, Hirata M, *et al*. The correlation between postmenopausal osteoporosis and inflammatory periodontitis regarding bone loss in experimental models. Exp Anim, 2012,61(2):183-187
16 Maria B, Martina E, Josh E, *et al*. Periodontal Disease and Osteoporosis-Shared Risk Factors and Potentiation of Pathogenic Mechanisms. Current Oral Health Reports, 2018,5(7):1-7
17 Sousa LH, Linhares EV, Alexandre JT, *et al*. Effects of Atorvastatin on Periodontitis of Rats Subjected to Glucocorticoid-Induced Osteoporosis. J Periodontol, 2016, 87(10):1206-1216
18 Choi JK, Kim YT, Kweon HI, *et al*. Effect of periodontitis on the development of osteoporosis: results from a nationwide population-based cohort study (2003-2013). BMC Womens Health, 2017,17(1):77
19 Mohammed N, Kisma M, Turki M, *et al*. Serum Osteocalcin and Serum Osteopontin Levels in Osteoporotic Postmenopausal Women with and without Vertebral Fractures. J Fac Med Baghdad, 2015,5757(33): 257-262
20 Carlene P, Mariani Mélissa K, Yawen Y, *et al*. Interleukin (IL)-6 Inhibits IL-27- and IL-30-Mediated Inflammatory Responses in Human Monocytes. Front Immunol, 2018,9:256
21 Verena M, Penninger J. RANK and RANKL of Bones, T Cells, and the Mammary Glands. Osteoimmunology, 2016:121-142
22 Wang L, Shi X, Zhao R, *et al*. Calcitonin-gene-related peptide stimulates stromal cell osteogenic differentiation and inhibits RANKL induced NF-kappaB activation, osteoclastogenesis and bone resorption. Bone, 2010,46(5):1369-1379
23 Maeda K, Takahashi N, Kobayashi Y. Roles of Wnt signals in bone resorption during physiological and pathological states. J Mol Med (Berl), 2013,91(1):15-23
24 Trindade SC, Olczak T, Gomes-Filho IS, *et al*. Porphyromonas gingivalis HmuY-induced production of interleukin-6 and IL-6 polymorphism in chronic periodontitis. J Periodontol, 2013,84(5):650-655
25 Song J, Jing Z, Hu W, *et al*. alpha-Linolenic Acid Inhibits Receptor Activator of NF-kappaB Ligand Induced (RANKL-Induced) Osteoclastogenesis and Prevents Inflammatory Bone Loss via Downregulation of Nuclear Factor-KappaB-Inducible Nitric Oxide Synthases (NF-kappaB-iNOS) Signaling Pathways. Med Sci Monit, 2017,23:5056-5069
26 Hienz SA, Sweta P, Saso I. Mechanisms of Bone Resorption in Periodontitis. J Immunol Res, 2015,2015:54-61
27 Larry J, Cássio L, Christina M, *et al*. Oral infection of mice with Fusobacterium nucleatum results in macrophage recruitment to the dental pulp and bone resorption. Biomed J, 2018,41(3):184-193
28 Murakami S, Mealey BL, Mariotti A, *et al*. Dental plaque–induced gingival conditions. J Periodontol, 2018,89(Suppl 1):S17-S27
29 Amar S, Engelke M. Periodontal innate immune mechanisms relevant to atherosclerosis. Mol Oral Microbiol, 2015,30(3):171-185
30 Nora S, Loreto A, Denisse B, *et al*. Host response mechanisms in periodontal diseases. J Appl Oral Sci, 2015,23(3):329-355
31 Gustav VN, Anna-Mart E. Inflammation-induced metabolic derangements or adaptation: An immunometabolic perspective. Cytokine Growth Factor Rev, 2018, 43:47-53
32 Ziebolz D, Pabel SO, Lange K, *et al*. Clinical periodontal and microbiologic parameters in patients with rheumatoid arthritis. J Periodontol, 2011, 82(10):1424-1432
33 Shiheido Y, Maejima Y, Suzuki JI, *et al*. Porphyromonas gingivalis, a periodontal pathogen, enhances myocardial vulnerability, thereby promoting post-infarct cardiac rupture. J Mol Cell Cardiol, 2016,99:123-137
34 Sjogren K, Engdahl C, Henning P, *et al*. The gut microbiota regulates bone mass in mice. J Bone Miner Res, 2012,27(6):1357-1367

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