

The influence of diabetes mellitus on periodontal tissues: a pilot study

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Purpose: The purpose of this study was to preliminarily evaluate the influence of diabetes mellitus (DM) on periodontal tissue without establishment of periodontitis.

Methods: Seven-week-old db/db mice were used for the diabetic experimental group and systematically healthy mice of the same age were used as controls. After 1 week of acclimatization, the animals were sacrificed for hard and soft tissue evaluation. The pattern of bone destruction was evaluated by stereomicroscope evaluation with alizarin red staining and radiographic evaluation by microscopic computerized tomography images. Histological evaluation was performed with hematoxylin and eosin stain for evaluation of soft tissue changes.

Results: In both stereomicroscope evaluation and radiograph image analysis, aggressive form of bone destruction was observed in diabetic animals when compared to the systematically healthy controls. In histological evaluation, apical migration of junctional epithelium with slight inflammatory cell infiltration was observed with disarrangement of connective tissue fibers.

Conclusions: Within the limits of this study, diabetic animals presented distortion in periodontal attachment and an aggressive bone loss pattern when compared to the healthy controls, suggesting that DM has an independent effect on periodontal tissue destruction irrespective of the presence or absence of periodontal disease.

Keywords: Diabetes mellitus, Inflammation, Periodontal disease.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder manifested by abnormally high levels of glucose. The hyperglycemic state developed from either a deficiency in insulin secretion or an impaired cellular resistance to the action of insulin is associated with a number of complications, leading to retinopathy, neuropathy, nephropathy, angiopathy, atherosclerosis, periodontitis, and impaired wound healing [1,2]. The in-

fluence of DM on the oral cavity has been well researched, and studies have reported diabetes as a risk factor for gingivitis and periodontitis [3,4]. Several epidemiological studies have shown that diabetes increases the risk of alveolar bone loss and attachment loss when compared to non-diabetic individuals [2,5] and presented a positive correlation between degree of glycemic control and disease prevalence and progression [6]. The mechanisms by which hyperglycemia can induce periodontal destruction are not yet fully understood,

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but is thought to have an association with altered immune function, advanced glycation end products (AGEs) and changes in collagen [7-9].

Alteration in immune function takes place in diabetes. Exaggeration in innate immune response and impaired polymorphonuclear leukocyte function may facilitate bacterial persistence in the tissues and accumulation of AGEs, which results from chronic hyperglycemia and increases secretion of pro-inflammatory cytokines such as tumor necrosis factor- α , interleukin-1 and prostaglandin E-2. Reduction in collagen synthesis and an increase in collagenase activity will adversely influence collagen metabolism. Taken collectively, these mechanisms cause periodontal tissue breakdown and compromised wound healing.

With an increase in diabetic patients today, a more proper understanding on its pathophysiology is a key for an appropriate treatment approach. Therefore, the aim of the present study was to preliminarily characterize the morphological alterations in periodontal tissues of diabetic mice through his-

tology and radiography.

MATERIALS AND METHODS

Animals

Seven-week-old db/db mice (*C57BLKS/J-Leprd/Leprd* mice) were used as the diabetic model and systematically healthy mice of the same age were used as controls (Orient Bio Inc., Seoul, Korea). The animals were housed in cages and were fed with a standard laboratory diet and tap water. The animals were divided into test (diabetic mice) and control (non-diabetic mice) groups with 8 animals in each group. The animal experiment was conducted in accordance with the guidelines approved by the Animal Ethics Committee of the Yonsei University College of Dentistry, Seoul, Korea.

Stereomicroscope evaluation

Mandibular jaws were dissected and removed after sacrifice. Soft tissues were removed by use of a scalpel blade and



Figure 1. Stereomicroscope images of buccal and lingual aspects of control (A, B) and diabetic (C, D) animals. Note the ruffled and nibbled bone surface at the alveolar crest area (arrow) in the diabetic animals, whereas a relatively smoother surface can be observed in the control animals (Alizarin-red stain, $\times 400$).

were fixed in 10% formalin. The specimens were dehydrated by a serial increase in the concentration of ethanol followed by immersion in 1% potassium hydroxide for 2 days. Alizarin red staining was performed and the specimens were stored in glycerin. Visual examination of the specimens was performed by using a stereomicroscope coupled with a digital camera (Leica MZ FLIII, Leica, Wetzlar, Germany).

Radiographic evaluation

Three-dimensional analysis of the alveolar bone loss was assessed by using microscopic computerized tomography (micro-CT, Skyscan 1076[®], Skyscan, Aartselaar, Belgium). The specimens were scanned at a resolution of 35 μm (100 kV, 100 μA) and the data was processed in digital imaging and communications in medicine (DICOM) format for 3-dimensional image reconstruction (OnDemand 3D[®], Cybermed, Seoul, Korea). Macroscopic evaluation of bone loss around the teeth was performed.

Histological evaluation

Mandibular jaws were fixed in 10% formalin for 10 days and were then decalcified in 5% nitric acid for 14 days. The specimens were embedded in paraffin wax and were serially sectioned in buccal-lingual direction with a thickness of 5 μm . The specimens were stained with hematoxylin and eosin (H&E) stain and were evaluated under a light microscope (Leica DM-LB, Leica, Wetzlar, Germany).

RESULTS

Morphological and macroscopic observation

Both diabetic and non-diabetic mice presented alveolar bone loss when observed from the cemento-enamel junction level. The pattern of bone loss in the diabetic group presented a rougher and more irregular destruction pattern at the crestal area than in the non-diabetic control animals (Fig. 1).

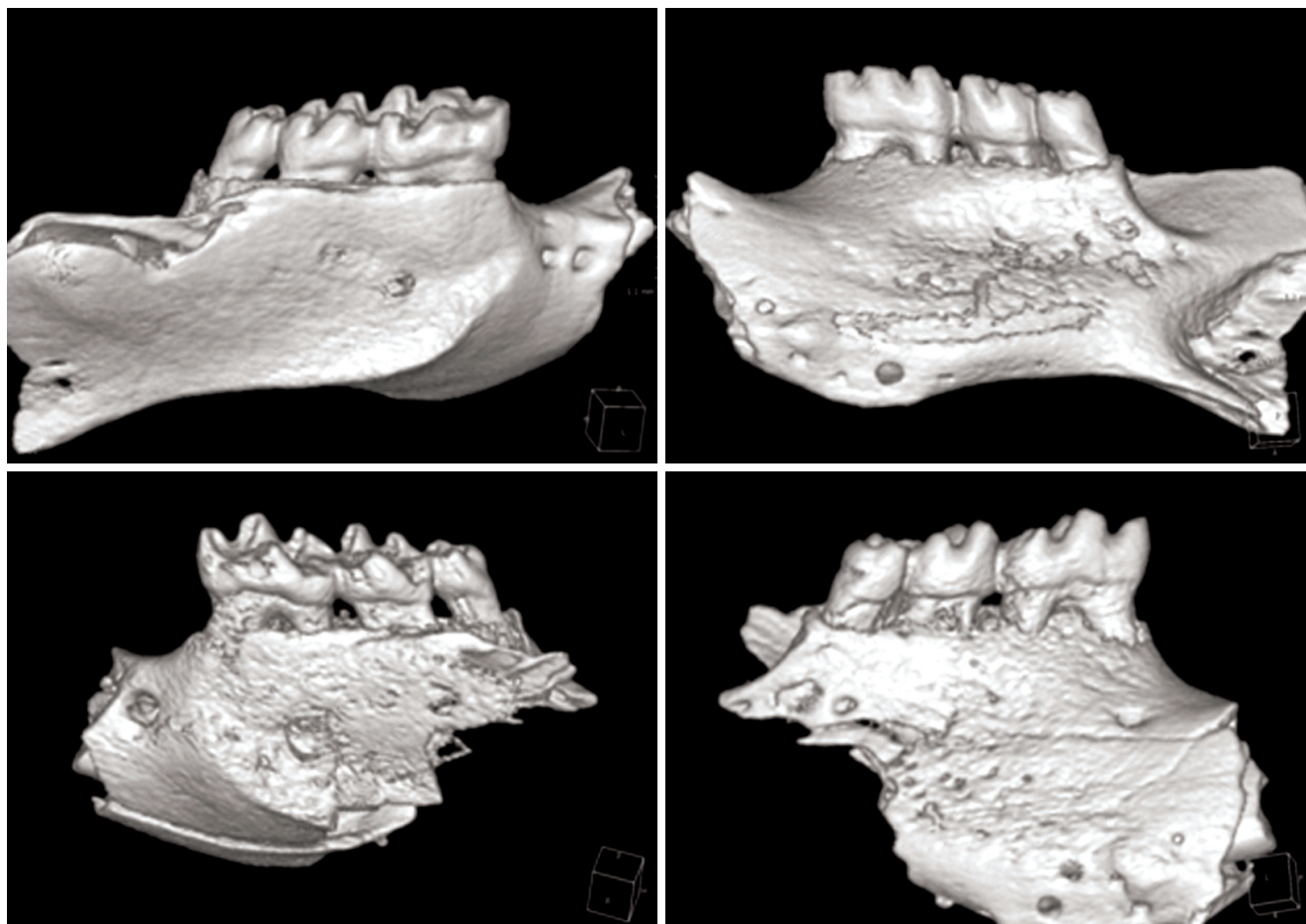


Figure 2. Three-dimensional reconstructed micro-computerized tomography images of buccal (left) and lingual (right) aspects of the control (upper row) and diabetic (lower row) animals. Alveolar bone loss was observed from the cemento-enamel junction level of the tooth in both groups with slightly more loss in the diabetic animals. The margin of the crestal bone and the overall surface of the alveolar bone presented a ruffled and nibbled appearance when compared to the controls.

Radiographic evaluation of alveolar bone loss

The radiographic results confirmed the results of the stereomicroscope evaluation. A clearer image of the bone destruction pattern was observed by radiography than by stereomicroscope. The level of bone loss generally seemed to be similar in both but with slightly greater loss in diabetic animals. The pattern of bone destruction was more aggressive with rougher ruffled and nibbled surface in diabetic animals than in non-diabetic controls (Fig. 2).

Histological observation

The overall gingival epithelium of both diabetic and non-diabetic control animals generally had a similar appearance, except that in some specimens, a slightly thicker keratin layer

(stratum corneum) was observed in the diabetic animals (Figs. 3 and 4). Apical migration of junctional epithelium was also observed with slight inflammatory cell infiltration in the connective tissue layer of diabetic animals (Fig. 5). In addition, there was distortion of the connective tissue fibers above the alveolar crest with loss of fiber arrangements, whereas relatively intact connective tissue fiber attachments were observed in the controls. In the alveolar bone region, there were no specific pathological changes and presence of osteoclastic cells was rare in both groups (Fig. 6).

DISCUSSION

Prevention of periodontal disease in diabetic patients is

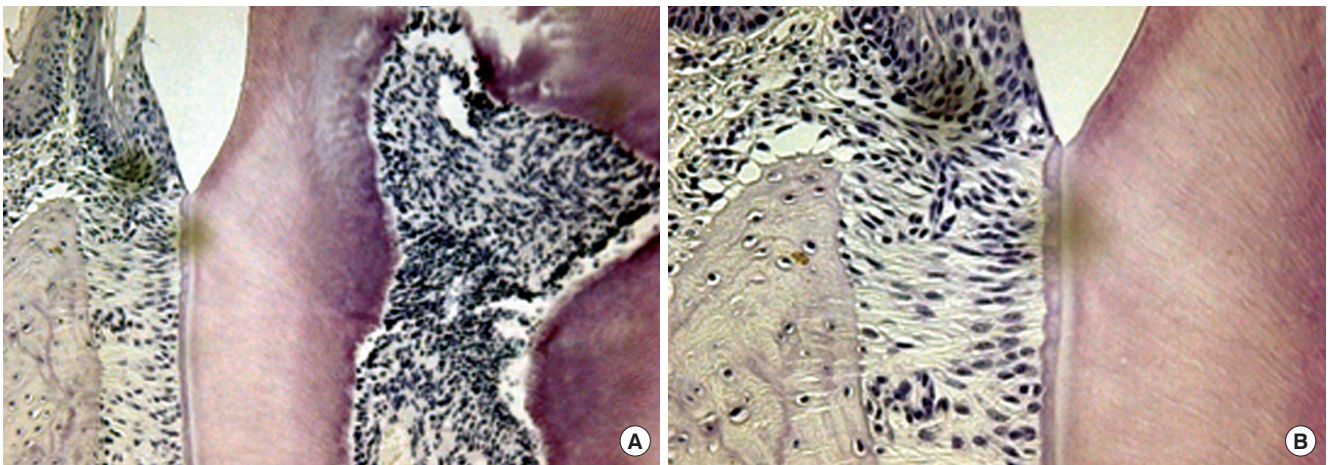


Figure 3. The overall histological image of the control animal. Junctional epithelium was located at the cemento-enamel junction level. Parallel and well organized fiber attachments were observed below the epithelium with minimal inflammatory cell infiltration (A) H&E stain, $\times 200$, (B) H&E stain, $\times 400$.

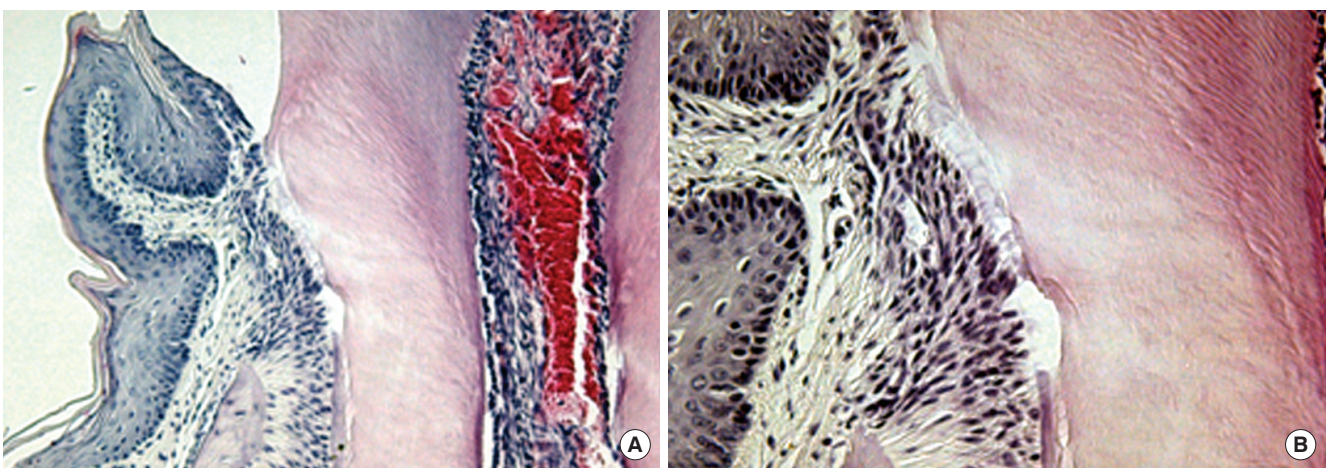


Figure 4. Higher magnification images of the oral epithelium of control (A) and diabetic (B) animals. The epithelium consisted of four definite layers with a thicker keratin layer in the diabetic group. The height of the dermal papilla was generally similar and there was no specific difference between the control and diabetic animals (H&E stain, $\times 400$).

fundamentally important due to its potential adverse effect on glycemic control and promotion of diabetic complications. The dynamics of periodontal tissue destruction in periodontal disease of diabetic patients is complex and has been not clearly elucidated [10]. Understanding its causative pathways plays a crucial role in determining an effective treatment approach. The purpose of the present study was to evaluate the influence of diabetes on morphological alterations to periodontal tissues.

The diabetic animals used in the present study were db/db mice. The db/db mouse is a useful model for diabetes [11]. The db/db mouse has a point mutation in the gene for leptin receptor which is responsible for leptin hormone (adipocyte-specific hormone) regulation. Leptin controls food intake

and energy expenditure by acting on receptors, and mice with leptin receptor deficiency have an increase in appetite causing obesity. The db/db mice are reported to develop significant obesity within 6 weeks of age showing hyperglycemia and hyperinsulinemia at fasting [11]. For these reasons, in the present study, 7-week-old db/db mice were used as diabetic animals.

Periodontal disease is characterized by gingival inflammation, periodontal tissue destruction, and alveolar bone loss [12]. One of the first changes in the periodontium with periodontitis is apical migration of the junctional epithelium [13]. This structural change is accompanied by neutrophil migration from the adjacent connective tissue and crevicular exudate flow to the epithelium. The histological findings of the

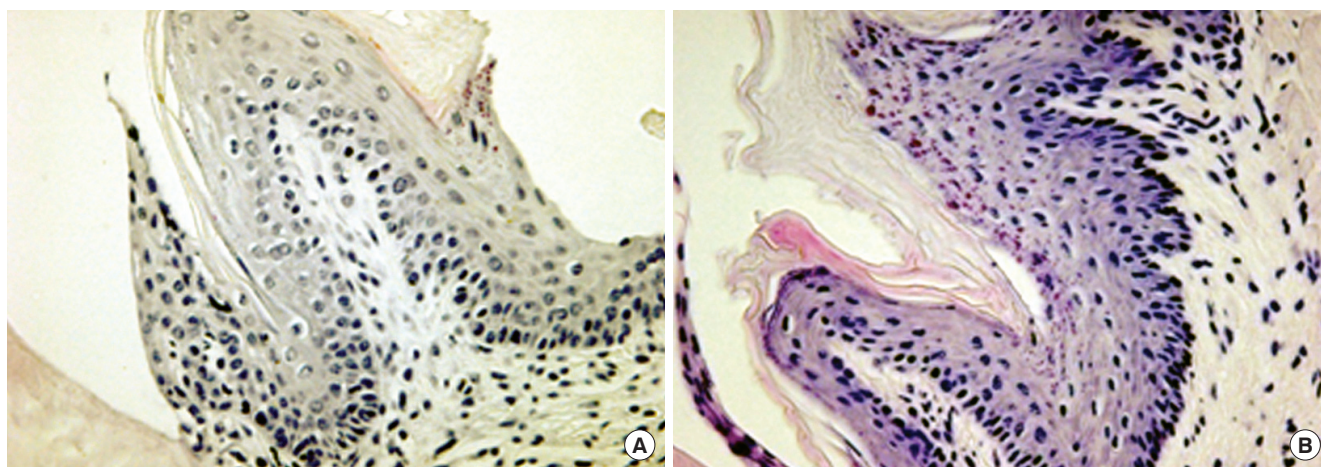


Figure 5. The overall histological image of the diabetic animal. Apical migration of junctional epithelium below the cemento-enamel junction was observed with distortion of fiber attachments. Slight inflammatory cell infiltration was observed in the connective tissue (A) H&E stain, $\times 200$, (B) H&E stain, $\times 400$.

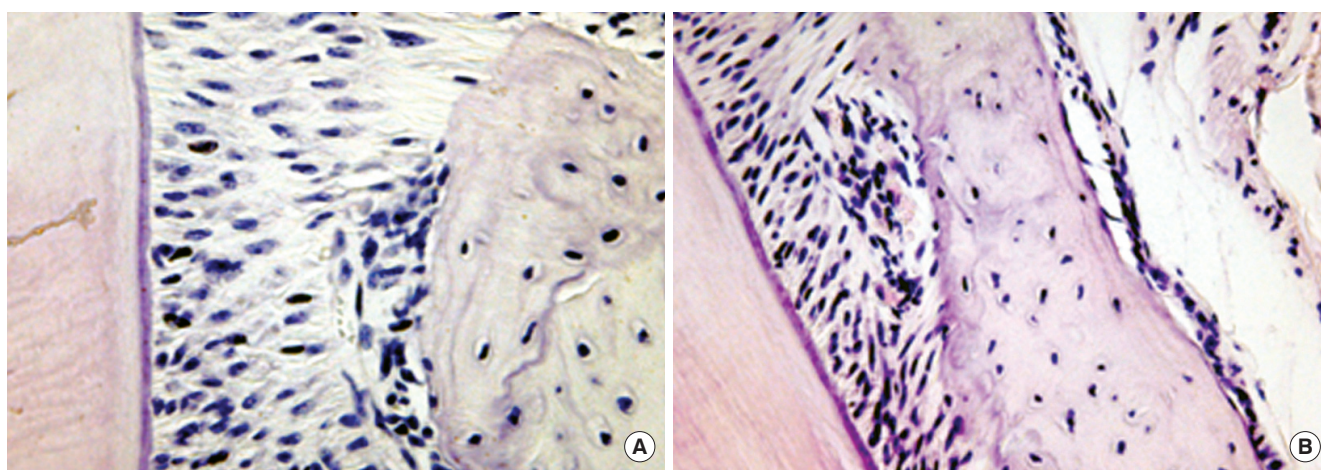


Figure 6. Higher magnification images of alveolar bone region of control (A) and diabetic (B) animals. Parallel fiber attachments between cementum and alveolar bone were observed. There was no specific pathological change in either control or diabetic animals (H&E stain, $\times 400$).

present study showed apical migration of the junctional epithelium with inflamed adjacent connective tissue in the diabetic mice, representing a breakage in the first defense barrier of the periodontium. In addition, there was distortion in the connective tissue fiber arrangements when compared to the healthy controls (Figs. 3 and 5)

Alterations in the gingival epithelium and connective tissue with diabetes have been reported by several authors. Atrophy and pleomorphism of the gingival epithelium, with decreased cellular organelles and increased intercellular spaces, have been seen in diabetic individuals, and according to Silva et al. [14], diabetic animals presented a thicker keratinized layer and reduction in the height of the dermal papilla. The increase in mitotic activity of the basal layer may have contributed to compromised differentiation of the epithelial cells resulting in thickening of the keratinized layer, and flattening of the dermal papilla may be due to a direct response to inflammation or an adaptive remodeling of the weakened connective tissue. Moreover, reduction in soluble collagen and distortion of reticular fibers were also reported in diabetic animals. However, these findings were not all observed in the histological results of the present study, and this may be due to the difference in the severity of diabetes and existence of periodontitis.

The pattern of alveolar bone loss was investigated by evaluation under stereoscope with alizarin red staining and radiographic evaluation of micro-CT images. The level of alveolar bone loss was similar in both diabetic and non-diabetic animals. However, the pattern of bone destruction presented in a more aggressive form in the diabetic animals (Figs. 1 and 2). Previous studies have reported that mineralized tissues were also affected by diabetes, and alveolar bone loss was greater in diabetic patients when compared with that in non-diabetic subjects [15]. Research on diabetes has shown a decrease in gene expression of osteoblast differentiation, a decrease in growth factor, and diminished extracellular matrix production, which compromises bone formation [16-18]. In addition, accumulation of AGEs impaired bone healing [19] by promoting apoptosis of matrix producing cells. Moreover, recent studies provide evidence of the important role of cell apoptosis in diabetic complications and evidence that diabetes is associated with the production of pro-apoptotic factors such as reactive oxygen species, tumor necrosis factor, and AGEs [9,20].

The pathogenesis of periodontitis starts from bacterial invasion into the periodontal tissues and there has been no study that has induced periodontitis in diabetic individuals without local factors. Thus, diabetes was reported to be a predisposing factor or modifying factor that can accelerate periodontal breakdown initiated by microorganisms [21]. The

model used in the present study was not induced to have periodontitis. However, the results showed a pathologic change in periodontal tissues in diabetic animals without an infective source. This shows that DM may have an independent influence on periodontal tissues.

In a recent rat experimental study by Sakallioğlu et al. [22], the authors reported that the level of monocyte chemoattractant protein-1 (MCP-1), which is considered to be the major signal for chemotaxis of mononuclear leukocytes, was similarly increased in gingival tissues of diabetes without periodontitis when compared to non-diabetes with periodontitis. Monocytes play an important role in periodontal tissue breakdown and patients with periodontitis have shown enhanced MCP-1 expression in the periodontal tissues [23,24]. In addition, increase in MCP-1 level has been also reported in diabetic patients when compared to systemically healthy patients [25,26]. The authors concluded that DM may lead to an increase in MCP-1 production in periodontal tissues as much as in periodontitis and that MCP-1 may be an important factor in the role of periodontal tissue destruction in those with DM.

Although it cannot be directly concluded from the results of our study, there is a possibility of DM (without periodontitis) having an influence on periodontal tissue destruction. The histological and radiographic results showed a difference between the healthy control animals and diabetic animals. Consequently, we can speculate that DM itself may cause pathological changes in periodontal tissues. In order to be more certain of this hypothesis, further experiments with a larger sample size and comparison with periodontitis-induced animals with or without diabetes should be performed including immunohistochemical analysis of related cytokines and chemokines. Moreover, the blood glucose level of the diabetic animals should be further investigated to correlate glycemic level and periodontal tissue destruction.

In conclusion, within the results of this preliminary study, the periodontium of mice with DM showed destruction in periodontal tissue fiber arrangement with a rougher and more nibbled bone surface compared to systematically healthy periodontium.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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