

## The evaluation of mandibular bone density in chronic periodontitis models

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### ABSTRACT

**Background:** Bone density, an important factor in functional bone quality, can affect the success of implant osteointegration or orthodontic treatment. A number of studies report that chronic periodontitis constitutes one risk factor of osteoporosis characterized by low bone mineral density and that the mandible is susceptible to osteoporosis. **Purpose:** The purpose of this study was to evaluate mandibular bone density in animal subjects suffering from chronic periodontitis. **Methods:** 40 male Wistar rats were divided into four chronic periodontitis groups and four control groups (each group n=5). As chronic periodontitis models, the subjects were injected with  $2 \times 10^9$  CFU/ml of *Porphyromonas gingivalis* in the sulcular gingiva, whereas control group members were injected with normal saline. After 2, 3, 4 and 6-week injection periods, the subjects were sacrificed and radiographic examination of the mandibular bone subsequently performed. Mandibular bone density was evaluated by histometric analysis. **Results:** The mandibular bone density in members of the chronic periodontitis group was significantly lower than those of the control group ( $p < 0.05$ ). The reduced mandibular bone density in the chronic periodontitis group was in line with the protracted bouts of periodontitis. **Conclusion:** Reduced mandibular bone density was found in the chronic periodontitis model. The longer the duration of a bout of chronic periodontitis, the greater the reduction in mandibular bone density.

**Keywords:** chronic periodontitis; mandibular bone density; *Porphyromonas gingivalis*

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### INTRODUCTION

Bone density is an important factor determining functional bone quality, with lower mandibular bone density impairing both implant osteointegration and orthodontic treatment. During dental implant therapy, bone quality should be evaluated because of its importance as a predictor of implant osseointegration. There are many factors that influence successful implant osseointegration, including bone mineral density. Poor bone quality, characterised by a thin cortex, sparse trabeculae or altered trabecular architecture, eventually results in poor implant stabilization and longer osseointegration.<sup>1</sup>

Osteoporosis, a form of bone disturbance characterised by low bone mineral density, constitutes a condition negatively affecting bone quality. The jawbones, particularly the mandible, can be affected by osteoporosis which decreases mandibular bone density.<sup>2</sup> In addition to bone mineral density, trabecular bone structure can be evaluated as a means of determining bone quality. A dense mandibular alveolar trabecular pattern in a dentate subject represents a reliable indicator of normal bone mineral density, while a sparse trabecular pattern confirms low bone mineral density.<sup>3</sup>

Chronic periodontitis has been reported as one of the risk factors relating to osteoporosis since the increasing severity of periodontitis exacerbates susceptibility to the condition.<sup>4</sup>

Chronic periodontitis constitutes a severe infectious disease resulting in inflamed supporting tissues of the teeth, progressive attachment loss and bone loss.<sup>5</sup> Considerable research has been conducted into the relationship between periodontitis and osteoporosis. However, to date, the results remain inconclusive. Several studies found a positive correlation between chronic periodontitis and osteoporosis, while others did not. The investigation conducted by Lafzi *et al.*<sup>6</sup> indicated that the bone mineral density of patients suffering from chronic periodontitis is lower than those afflicted with gingivitis. The mandibular bone density found in individuals with chronic periodontitis was significantly lower than that of healthy individuals.<sup>7</sup> However, another study reported that no correlation existed between chronic periodontitis and osteoporosis.<sup>8,9</sup> Therefore, understanding the nature of mandibular bone density in cases of chronic periodontitis remains a crucial research topic. Consequently, the aim of this study was to evaluate the mandibular bone density in a subject suffering from chronic periodontitis that had been induced by a local injection of *Porphyromonas gingivalis* (*P. gingivalis*).

## MATERIALS AND METHODS

This study constituted a true experiment incorporating a completely randomized design. Its experimental procedures were all performed under a protocol approved by Ethical Clearance Certificate Number 017/HRECC.FODM /II/ 2017, issued by the Ethical Committee of the Faculty of Dental Medicine at Universitas Airlangga. The protocol to develop a model of chronic periodontitis was based on previous study with minimal modification.<sup>10</sup> The bacterial suspension was prepared by means of the following procedure. *P. gingivalis* (ATCC 33277, MediMark, France) was cultured at 37°C on trypton soya agar (TSA) plates with 10% sheep blood, supplemented with 0.4 µl/ml vitamin K and hemin in an oxygen-free atmosphere (80% nitrogen, 10% carbon dioxide and 10% hydrogen). After 14 days of growth on TSA, certain *P. gingivalis* colonies were selected and a solution of 2x10<sup>9</sup> CFU/ml in 100 µl of 0.9% sodium chloride subsequently prepared for immediate use. Gram's method was applied to confirm the purity of the colonies.

Chronic periodontitis was induced in Wistar-strain *Rattus norvegicus* subjects by injection of *P. gingivalis* into the gingiva. 3-4 month-old male subjects ( $n = 40$ ), each 200-220 grams in weight, were randomly divided into four periodontitis groups and four control groups, each containing five members. The periodontitis group subjects were injected three times a week at 2-day intervals with 0.05 ml live *P. gingivalis* at a concentration of 210<sup>9</sup> CFU/ml into the buccal and lingual subgingival sulcus area between the first and second left mandibular molar. The injection involved the use of a tuberculin disposable syringe (OneMed, Indonesia) with a 30 gauge needle (BD PrecisionGlide™ Needle, USA). Control group members were injected with normal saline in accordance with

the periodontitis group protocol. For the duration of the experiment, the subjects were housed in plastic cages, fed a standard laboratory diet and provided with water ad libitum. The periodontitis and control group subjects were sacrificed during the 2<sup>nd</sup>., 3<sup>rd</sup>., 4<sup>th</sup>. and 6<sup>th</sup>. weeks following injection. The mandibular specimens were harvested and fixed in normal buffer formalin.

Radiographic examination was performed to evaluate the alveolar bone resorption as a clinical symptom of chronic periodontitis. The sample was confirmed as a case of chronic periodontitis if alveolar bone resorption was present in the interproximal area between the first and second molar. Furthermore, the mandibular specimens were decalcified in 10% ethylenediamine-tetra-acetic acid (EDTA) at pH 7, which was replaced twice a week during a period of 6-8 weeks until the process was completed. The decalcified specimens were subsequently submitted to routine histologic processing and embedded in paraffin. In order to facilitate histologic descriptive analysis, each sample was sliced into 5 mm continuous sections in the mesial-distal direction and prepared for haematoxylin and eosin (H&E) staining.

Histologic analysis was performed on mandibular bone from the mesial section of the first molar mesial root and the distal section of the second molar distal root. The mandibular bone density was considered to be the percentage of trabecular bone volume calculated by dividing the trabecular bone area by the total bone area, including the trabecular bone and bone marrow. All examination procedures involved the use of a Nikon H600L light microscope at 100x magnification fully equipped with a DS Fi2 300 megapixel digital camera and calibrated image processor using Nikkon Image System software. Two examiners unsighted as to the allocation of samples and subjects to specific groups performed this analysis with the average of two sets of results being calculated as the data of each sample.

Data was presented in the form of a mean  $\pm$  SD. The differences between the mandibular bone density of groups were analysed using one-way ANOVA followed by an LSD post hoc test. The data was considered statistically significant when the p value was less than 0.05.

## RESULTS

The result of radiographic examination showed there to be alveolar bone resorption in the interproximal area between the first and second molar in all periodontitis group members which was confirmed as a chronic periodontitis model (Figure 1). The alveolar bone crest resorption was found in the 2-week periodontitis group and the alveolar bone resorption was greater in line with the injection period.

The mandibular bone density of all control groups was not significantly different. The average mandibular bone density of the periodontitis groups was lower than that of

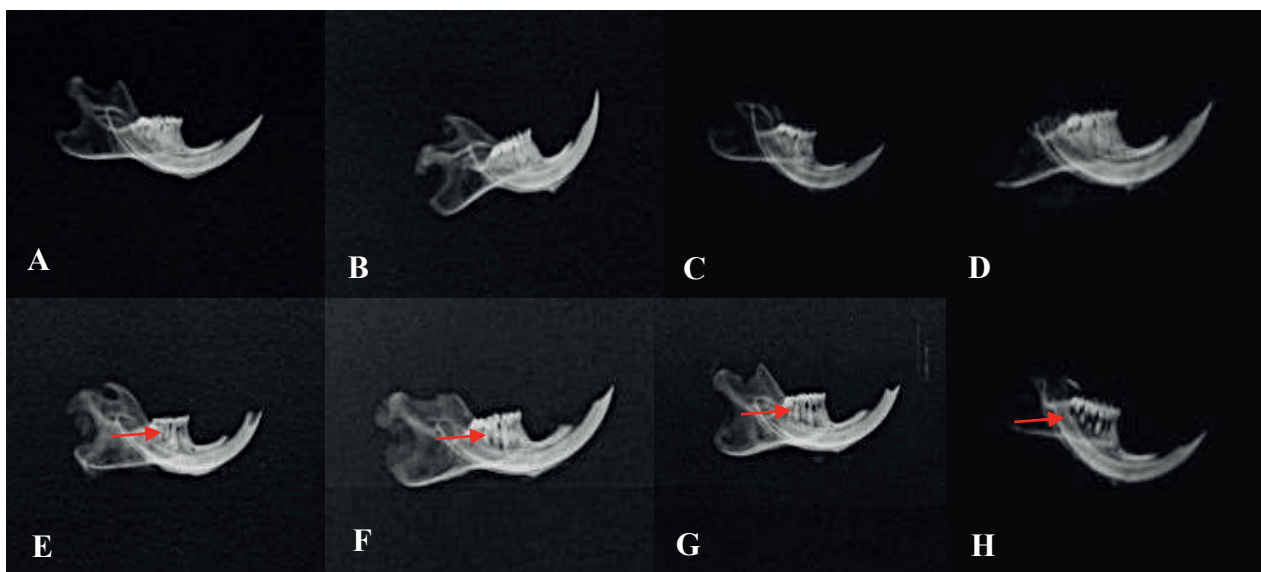
the control groups (Table 1), but the difference between the 2-week periodontitis group and control group was not significant. The mandibular bone density between the 3, 4 and 6-week periodontitis and control groups was significantly different ( $p < 0.05$ ). The mandibular bone density in the periodontitis groups was more reduced than that in the control groups. The greatest reduction occurred in the 6-week periodontitis group (Table 1). The reduced mandibular bone density coincided with the injection periods (Figure 2). The longer the period during which injections were administered, the lower the mandibular bone density. The mandibular bone density of the 4 and 6-week periodontitis groups was significantly lower than that of the 2-week group ( $p < 0.05$ ). A significant difference existed between the respective mandibular bone density of the 3 and 6-week periodontitis groups ( $p < 0.05$ ). However, no significant differences were observed between the 3 and 4-week periodontitis groups or between the 4 and 6-week periodontitis groups.

The histological feature of the mandibular bone density in the periodontitis and control groups is presented in Figure 3. The histological feature of the control groups was a dense and compact trabecular bone, whereas the bone marrow was narrow. This result was similar to the histological feature of the 2-week periodontitis group. In the 3, 4 and 6-week periodontitis groups, the bone marrow was wider and greater in quantity, while in the trabecular bone it was narrow. This sparse trabecular bone in the periodontitis groups is considered to have a lower mandibular bone density than that of the control groups.

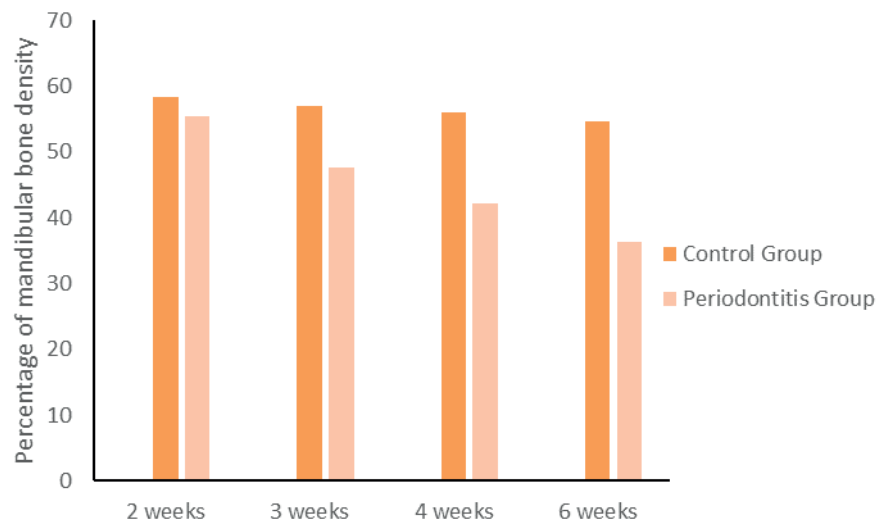
## DISCUSSION

Bone density is an important factor influencing functional bone quality. Reduced mandibular bone density will impair implant osteointegration or orthodontic treatment. Certain diseases are known to reduce bone density, including osteoporosis which can affect the mandibular bone. An association exists between osteoporosis and periodontitis<sup>4,6,7</sup> and it is assumed that chronic periodontitis not only causes reduced alveolar bone height, but also reduced bone density. Therefore, in this study, the mandibular bone density of a subject which had suffered from chronic periodontitis for a protracted period was evaluated.

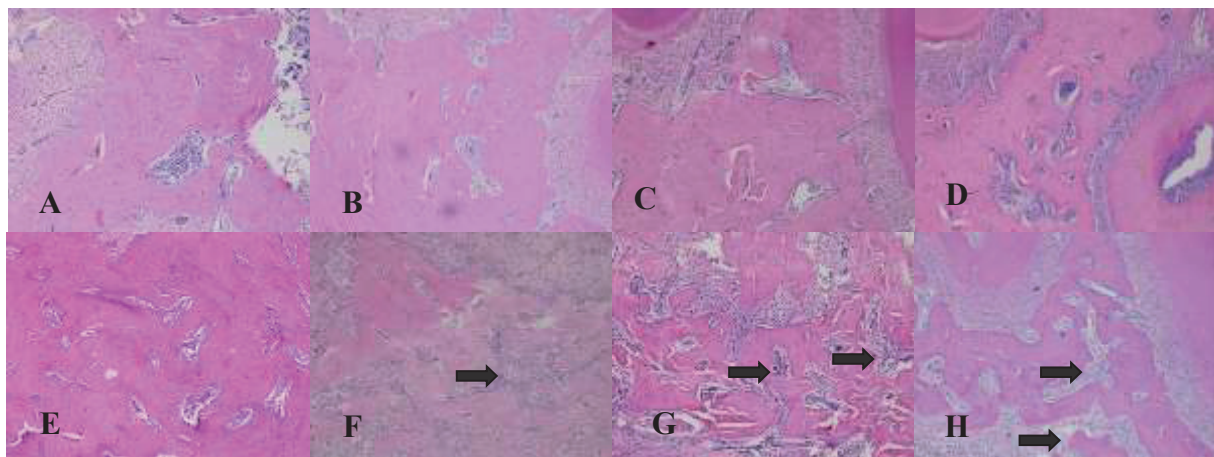
Animal subjects have long been used to investigate the pathogenesis of periodontitis, because studies involving humans are limited due to the difficulty of controlling the pathogenesis of periodontal disease.<sup>11</sup> Rats were employed as research subjects because the periodontal anatomy of their molar region shares several similarities with that of humans.<sup>12</sup> An study of periodontal disease in animals plays an important role in facilitating the study of defined aspects of periodontitis, including: pathogenesis, the development and progress of disease.<sup>11</sup> There are certain methods of initiating experimental periodontitis, including: ligature, injection of a bacterial or pathogenic component, and oral gavage.<sup>12</sup> In this study, a model of chronic periodontitis as occurs naturally in humans was induced by localized injection of live *P. gingivalis*. *P. gingivalis* has been identified as one major periodontal pathogen of chronic periodontitis.<sup>13</sup> About 40–100% of adults afflicted by



**Figure 1.** Representative radiographic images of the mandibular bone in the chronic periodontitis model. A greater degree of alveolar bone resorption occurs in the periodontitis groups compared to the control groups. The 2-week periodontitis group presented resorption in the alveolar bone crest (red arrow) (E), a greater degree of resorption occurring in the 3-week periodontitis group (F), increased resorption at 4 weeks (G) and the highest level in the 6-week periodontitis group (H). The control group at 2, 3, 4 and 6 weeks is shown in images A,B,C and D respectively.



**Figure 2.** The mandibular bone density of the chronic periodontitis model at various injection times.



**Figure 3.** A histological section of mandibular bone in the periodontitis and control groups (x100). The trabecular bone of the control group after 2 weeks (A), 3 weeks (B), 4 weeks (C) and 6 weeks (D) showed significant density. The 2-week periodontitis group demonstrated the same pattern as the 2-week control group (A). The sparse trabecular bone with wider bone marrow (black arrow) was present in periodontitis groups at 3 weeks (F), 4 weeks (G) and 6 weeks (H).

**Table 1.** The percentage of mandibular bone density in a chronic periodontitis model

	Group	n	Average ( $\pm$ SD)	p
2 week	Control	5	58.43 ( $\pm$ 9.18) <sup>d</sup>	0.000*
	Periodontitis	5	55.41 ( $\pm$ 9.84) <sup>cd</sup>	
3 week	Control	5	57.06 ( $\pm$ 3.17) <sup>d</sup>	
	Periodontitis	5	47.64 ( $\pm$ 4.37) <sup>bc</sup>	
4 week	Control	5	55.97( $\pm$ 5.55) <sup>d</sup>	
	Periodontitis	5	42.22 ( $\pm$ 6.66) <sup>ab</sup>	
6 week	Control	5	54.60 ( $\pm$ 5.03) <sup>cd</sup>	
	Periodontitis	5	36.25 ( $\pm$ 4.37) <sup>a</sup>	

\*significant at  $\alpha=0.05$  <sup>a,b,c,d</sup> the same superscript showed no difference between groups



periodontitis have been infected by these opportunistic bacteria.<sup>14</sup> This research confirmed a reduction in alveolar bone height of the periodontitis groups compared with that of the control group following injection of live *P. gingivalis* (Figure 1). Alveolar bone crest resorption was detected in the 2-week periodontitis groups and increased over time. The results reported here were similar to those of the research conducted by Han *et al.*<sup>15</sup> and Zhang *et al.*<sup>16</sup> indicating that *P. gingivalis* infection results in increased bone resorption. Numerous reviews explain the bone resorption mechanism in periodontitis. In short, the lipopolysaccharide (LPS) and other bacterial toxins can stimulate the immune cells and osteoblast to release proinflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ , prostaglandin E2, komponen MMPs and to increase the RANKL/OPG ratio that regulates the proliferation, differentiation and activation of osteoclast resulting in bone resorption.<sup>17</sup> *P. gingivalis* LPS, lipids and metabolic products have been reported as possessing the ability to modulate RANKL and/or OPG expression in osteoblasts to stimulate osteoclastogenesis.<sup>15</sup>

In many reports, alveolar bone loss can be detected as early as two weeks after the final oral bacterial infection or three weeks after initiating infection,<sup>18</sup> although the study conducted by de Molon *et al.*<sup>11</sup> found that oral infection through *P. gingivalis* was ineffective at inducing alveolar bone resorption compared with the ligature model. The different strains, concentration of *P.gingivalis* used in the study and the length of the experimental period may explain the variability of the research results. In this study, subjects were injected with 100 $\mu$ l live-*P.gingivalis* at a concentration of  $2 \times 10^9$  CFU/ml a total of eighteen times, while in the research conducted by Molon *et al.*<sup>11</sup> subjects were infected five times with  $1 \times 10^9$  CFU/ml.

This investigation demonstrated that chronic periodontitis resulted in both reduced alveolar bone height and bone density, while a reduction in mandibular bone density was observed in the periodontitis group (see Table 1). The sparse trabecular bone was observed in the 3-week, 4-week and 6-week periodontitis groups (Figure 3) and indicated lower mandibular bone density.<sup>3</sup> The lowest bone density was that of the 6-week periodontitis group. This reduced mandibular bone density increased progressively during the period of the experiment (Figure 2). This study indicated that chronic periodontitis not only reduces alveolar height, but also bone density, thereby paralleling the findings of a clinical study completed by Tonguc *et al.*<sup>7</sup> that the mandibular bone mineral density of subjects suffering from periodontitis was significantly lower than that of the periodontally healthy subjects. A study of animal subjects conducted by Zhang *et al.*<sup>16</sup> demonstrated significantly decreased residual alveolar bone volume and mineral density in *P. gingivalis*-infected animals compared with sham infected controls. Similar to the alveolar bone resorption which appeared in radiographic images, the reduction in mandibular bone density among periodontitis

group members was found to be greater in more prolonged periods. The 6-week periodontitis group presented the largest reduction in mandibular bone density. However, although reduced mandibular bone density was detected in the 2-week periodontitis group, a significant difference was found early in the 3-week periodontitis group. From this result, it can be assumed that in chronic periodontitis, alveolar crest resorption occurs earlier than reduced bone density. Nevertheless, further study is required to confirm the validity of this assumption.

The mechanism of reduced mandibular bone density in chronic periodontitis has yet to be clearly explained, although there is theoretical uncoupling between bone destruction and remodelling. Increased osteoclastic destruction, decreased osteoblastic formation, or a combination of the two will reduce bone density.<sup>19</sup> In addition to *P. gingivalis* possessing the ability to stimulate osteoclastogenesis, the components of *P. gingivalis* can inhibit the differentiation and osteogenesis of osteoblasts, thereby impeding alveolar bone formation.<sup>20</sup> Several potential questions emerge from the results of this study, for example, whether the mechanism of reduced bone density is the same as that of alveolar resorption, which bone cells play a dominant role in that mechanism and whether a specific cytokine determining the timing of reduced bone density exists.

This study successfully showed that chronic periodontitis reduced mandibular bone density which was also found to occur during orthodontic tooth movement. However, in the latter case, the reduced bone density recovered to its pre-orthodontic treatment level.<sup>21</sup> It was interesting to investigate whether the reduced mandibular bone density caused by chronic periodontitis can be repaired following periodontal treatment, or whether it will be continue even if bacterial infection has been cured. However, based on result of this study, mandibular bone density examination is recommended for chronic periodontitis patients, especially those due to receive a dental implant or planning orthodontic treatment.

The role of mandibular bone density in chronic periodontitis remains a critical topic for investigation. Further studies should explore the mechanism that promotes a reduction in mandibular bone density as a result of chronic periodontitis, particularly in order to discover the key molecule or cytokine in the pathogenesis of reduced mandibular bone density. Such investigation would support the prevention and treatment of reduced mandibular bone density caused by chronic periodontitis.

In conclusion, reduced mandibular bone density was found to have occurred in a chronic periodontitis model which suggests that the condition constitutes a risk factor in osteoporosis. The longer the periods of chronic periodontitis, the greater the reduction in mandibular bone density.

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