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Scaling and root planning effects on alveolar bone density and amount of *Porphyromonas gingivalis* and *Treponema denticola*

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Abstract. *Porphyromonas gingivalis* (*Pg*) and *Treponema denticola* (*Td*) are the main etiology of alveolar bone loss. The elimination of these bacteria with scaling and root planing can promote bone healing and initiate an increase in radiographic bone density. The aim of this research to analyze radiographic bone density and the amount of *Pg* and *Td* before and after scaling and root planing. A total of 40 subjects gave informed consent, and underwent clinical examination, radiographic examination for bone density, and laboratory examination for the load of *Pg* and *Td* using RT-PCR. The results showed there is differences between radiographic bone density and the amount of *Pg* and *Td* before and after scaling and root planing, indicating an inverse association between the amount of *Pg* and *Td* and the bone radiographic density. In conclusions, Scaling and root planing decrease the amount of *Pg* and *Td* and increase the radiographic bone density.

1. Introduction

Worldwide, 50% of the adult population suffers from periodontal disease [1,2]. According to the data from the Health Department of the Republic of Indonesia in 1999 the prevalence of periodontal disease, measured by the presence of dental calculus, is 42.8%. According to Magdarina’ study conducted in nine provinces in Indonesia, the prevalence of periodontal disease in the productive age group is 88.67% [3].

The most dominant periodontal disease is chronic periodontitis. In 2002, a survey by the Periodontia Clinic Dental and Oral Hospital, Faculty of Dentistry, Universitas Indonesia, analyzed the distribution of periodontal disease and found periodontitis occupies the most prominent position at 89% [4]. The symptoms of chronic periodontitis include the inflammation of the periodontal tissues caused by plaque bacterial infection, causing loss of attachment, bone destruction, and tooth mobility [5-7].

Living microorganisms in the biofilm, manifesting as both calcified and uncalcified sub- and supragingival plaque, cause periodontitis. Three bacteria—*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*—are associated with the occurrence of periodontitis, and are all anaerobic, red complex bacteria [8]. In a clinical trial the prospective quantity of *Porphyromonas gingivalis* (*Pg*) and *Treponema denticola* (*Td*) quantified with reverse transcription-polymerase chain...
reaction (RT-PCR) could predict the degree of attachment loss three months into the future; consequently, \textit{Pg} and \textit{Td} are claimed as pathogenic bacteria of great importance in the etiology of chronic periodontitis [8].

In evaluating the healing of periodontal diseases, clinical, and radiographic assessment are necessary—clinically, including the evaluation of the signs of healing of the soft tissues, and radiographically, including the visualization of the hard tissue healing responses [9]. A change in bone quality precedes every change in bone quantity; thus, the evaluation of the alveolar bone quality after periodontal treatment requires high quality radiographics [10]. Bitewing and periapical techniques are the appropriate radiographic techniques for periodontal cases, and will provide detailed radiographic features that are useful in evaluating the interdental alveolar bone [11]. However, because of its large distortion the use of panoramic projection for periodontal cases is not recommended [11,12].

Radiographical assessment of alveolar bone density has a long history of numerous studies and plays a vital role both pre- and post-treatment [13]. The timing of the radiographical measurement of the alveolar bone density in evaluating periodontal treatment is critical, and must take into account the time needed for bone remodeling. Bone mineralization must reach 40% before the density change in radiographical images can be the visualized [14]. Ellis et al. state bone healing requires four to six months [15]. Eugene states six to eight months is required to achieve mature bone [16]. Schropp et al., in a study on post-extraction bone healing, finds the highest rate of bone formation to be during the first three months. Although in the last six months, the bone formation rate is low, bone formation continues for up to twelve months [17].

The removal of the disease’s etiology and the prevention of its return are the main treatments for periodontitis. This includes cleaning supra- and subgingival plaque, both chemically and mechanically, repairing the host’s response toward the bacteria, and motivating the patient to routinely and adequately clean the agent causing the periodontal disease at home [18].

Scaling and root planning (SRP), the gold standard of chronic periodontitis treatment, has been proven to aid in healing clinically, but radiographic and microbiological evaluation of the increase in bone density, an early sign of bone healing, and also quantitative differences in \textit{Pg} and \textit{Td}—the main pathogen of bone destruction, are lacking.

2. Methods

This study was a clinical experiment. The subjects were localized periodontitis patients stage 2 grade A at Teaching Dental Hospital, Faculty of Dentistry Universitas Indonesia, between January 2013 and June 2013. The inclusion criteria were patients suffering from chronic periodontitis, with one proximal surface of the posterior teeth having a 4 mm–6 mm periodontal pocket, a loss of attachment ≥4 mm, and bleeding on probing, aged between 30 years and 55 years, who had not consumed antibiotics in the last three months nor received periodontal treatment in the last six months, who were not medically compromised through anamnesis, and who could be radiographically examined. Patients suffering from diabetes mellitus, overhanging proximal restoration, proximal and cervical caries, smokers, those suffering from malocclusion or malpositioned teeth, and those consuming drugs that influence the bone, such as osteoporosis medication, cancer medication, and so on, were excluded.

The subjects, localized periodontitis patients between January 2013 and June 2013, met all the inclusion criteria. The sample total was 40 teeth. SRP was performed and the pocket depth of samples, BOP, and loss of attachment were assessed. These measurements were counted before the treatment (day-0).

On the day of assessment, the subjects assessed were accepted only if they had two teeth of which one surface had a 4 mm–6 mm deep pocket (the deepest pocket was chosen) and a ≥ 4 mm loss of attachment. Before therapy ensued, subgingival plaque was collected using an excavator and stored it in a tube containing PBS. Then, the BOP, pocket depth, and the loss of attachment were recorded. Thereafter, SRP was performed on the patient (full mouth instrumentation). Radiographic assessment was performed after SRP and a bite registration was made using Futar D.
Plaque control and BOP assessment were performed on the same region as day-0 on the seventh day after SRP. On day 14 and day 21, the same therapy was given and the same assessments were performed. On the second month, the same therapy and assessment was conducted in addition to measure the pocket depth and the loss of attachment. On the third month, periapical radiographic assessment was performed using bite registration that was prepared before. On the sixth month the procedure was repeated.

Univariate data analysis was performed to evaluate the subject distribution and research variables, and bivariate analysis to quantitatively evaluate the decrease in \( P_g \) and \( T_d \) bacteria and the increase in the alveolar bone density. The relationship between \( P_g \) and \( T_d \) decrease and the alveolar bone density increase was also analyzed.

To evaluate the relationship between the independent variables and the dependent variables, the repeated ANOVA test with a 95% confidence level was conducted. Shapiro-Wilk normality test was performed before conducting the repeated ANOVA test. Where the test result was not normal, Friedman test followed by the Wilcoxon post hoc test were performed.

3. Results
The primary data were collected by measuring pocket depth, by radiograph assessment, and by RT-PCR. Initially, 43 subjects that met the inclusion criteria participated in this study, but during the study three subjects left the study due to taking antibiotics or relocating. The data distribution can be seen in Table 1.

| Variable       | Mean ± SD | Min-Max |
|----------------|-----------|---------|
| Age (year)     | 44.23 ± 6.99 | 33–55   |
| Weight (kg)    | 63.4 ± 10.61 | 44–90   |
| Height (cm)    | 158.35 ± 5.31 | 148–170 |

According to the Shapiro-Wilk normality test performed using (subjects < 50 people) the distribution was not normal; therefore, we used the Friedman test for the bivariate analysis, followed by the Wilcoxon post hoc analysis.

| Variable    | N   | Mean ± SD | P value |
|-------------|-----|-----------|---------|
| \( T_d \) count |     |           |         |
| initial     | 40  | 10.78 ± 2.89 | 0.000*  |
| third month | 40  | 6.01 ± 3.92  |         |
| sixth month | 40  | 9.59 ± 1.95  |         |
| \( P_g \) count |     |           |         |
| initial     | 40  | 5.64 ± 1.65  | 0.000*  |
| third month | 40  | 3.65 ± 1.18  |         |
| sixth month | 40  | 4.56 ± 1.73  |         |
| Bone density |     |           |         |
| initial     | 40  | 70.43 ± 16.25 | 0.000*  |
| third month | 40  | 72.63 ± 15.17 |         |
| sixth month | 40  | 79.02 ± 19.37 |         |

*Friedman test, \( p < 0.05 \).
Table 2 shows the mean $Pg$ and $Td$ counts had decreased by the third month compared with the initial count before SRP was performed, but had increased by the sixth month compared to the third month. Mean bone density had increased by both the third and the sixth month. The Friedman test showed at least two measurements in each variable with a significant difference. To analyze which measurements were significantly different, we used the Wilcoxon post hoc analysis (Table 3).

**Table 3. Post hoc analysis of $Td$ count, $Pg$ count, and alveolar bone density at the start of the study (initial), third month, and sixth month.**

| Variable                      | p value  |
|-------------------------------|----------|
| $Td$ count                    |          |
| initial and third month       | 0.000*   |
| initial and sixth month       | 0.007*   |
| third month and sixth month   | 0.000*   |
| $Pg$ count                    |          |
| initial and third month       | 0.000*   |
| initial and sixth month       | 0.013*   |
| third month and sixth month   | 0.002*   |
| Alveolar bone density         |          |
| initial and third month       | 0.000*   |
| initial and sixth month       | 0.000*   |
| third month and sixth month   | 0.000*   |

*Wilcoxon test, p < 0.05.

Post hoc analysis using the Wilcoxon test concluded there are significant differences in each measurement. Therefore, the minor hypothesis stating there is a significant increase in bone density after SRP is performed was accepted. This was also the case with a second minor hypothesis that there is a significant decrease in $Pg$ and $Td$ bacteria counts after SRP is performed.

The alveolar crest did not change between the start of the study, the third month, and the sixth month, with 35 subjects’ alveolar crests apparently missing and discontinuity of alveolar crests on five subjects’. Therefore, the hypothesis that the alveolar crest continuity changes after SRP was rejected.

**Table 4. The relationship between $Td$ and $Pg$ counts and bone density in the third and sixth month**

| Analysis                        | p value  |
|---------------------------------|----------|
| $Td$ count to bone density      |          |
| initial vs third month          | 0.000*   |
| initial vs sixth month          | 0.000*   |
| $Pg$ count to bone density      |          |
| initial vs third month          | 0.000*   |
| initial vs sixth month          | 0.000*   |

*Wilcoxon test, p < 0.05 = significant difference

Analysis using the Wilcoxon test showed a significant difference in the relationship between $Td$ and $Pg$ counts and alveolar bone density in the third and sixth months (Table 4). Therefore, the hypothesis that there is a significant relationship between $Pg$ and $Td$ counts, and alveolar bone density was accepted.
4. Discussion

Forty subjects aged between 30 years and 55 years participated in this study. The age group above 30 years was chosen because of the prevalence of localized chronic periodontitis mainly in this group, and to prevent bias with aggressive periodontitis cases which are usually seen in patients under 30 years old [19]. The age 55 was chosen as the age limit because patients above 55 years old are usually already entering menopause, and consequently undergoing a different approach to treatment because of potential periodontal tissue disturbance [20]. The subjects of this study (mean age 44.23 ± 6.99 years) were predominantly women, accounting for 77.5% of subjects.

Pocket depth was measured first to determine the position at which subgingival plaque would be extracted. Before measuring the periodontal pocket depth, we conducted a validity test for clinical examination by inter-examiner and intra-examiner calibration of measurement of the periodontal pocket using a periodontal probe. The examiners were periodontal specialist residents. The mean pocket depth was 4.8 mm with a 0.71 standard deviation. This is in accordance with one of the inclusion criteria, which is pocket depth of 4 mm–6 mm [6,7,21].

Van Steenberg states the main goal of periodontal treatment, both surgical and non-surgical, is to stop periodontitis activity, one route to this end being plaque control [7,22]. Baderstein et al. states SRP, both mechanical and sonic—combined with efficient plaque control in cooperative patients—is an effective main therapy in treating periodontal diseases that are slow progressing or do not progress [23].

Pg, Td, and Tf are important bacteria that contribute to chronic periodontitis, and are classified as red complex bacteria [24]. The elimination of these three bacteria will have a major impact on the healing of patients with chronic periodontitis.

This study found the Pg count before SRP was significantly different to the Pg counts in the third and sixth months after SRP. The mean count of Pg after SRP decreased after three months. These findings are in accordance with the findings by Baderstein et al. and Pawlowski et al., who stated that SRP is an effective main treatment [23,25]. Jervoe-Storm et al. who observed six periodontal pathogenic bacteria, also found SRP significantly decreased the bacterial count, but did not totally eliminate the bacteria [26]. The Pg count in the sixth month increased from that in the third month, although not to the same extent as the initial count. This is compatible with Merin’s directive that—considering microbiological factors—a patient with a history of periodontitis must routinely visit the dentist three times a month [27].

Td shares the same pattern with Pg. The amount of Td three and six months after SRP was significantly decreased relative to the amount before SRP. This result demonstrates.

The efficacy of SRP as a main treatment of chronic periodontitis and is in line with previous studies by Baderstein et al. [23], Pawlowski et al. [25], and Jervoe-Storm et al. [26] The load of Td after six months also increased, in accordance with statements from Merin and Johnson et al. who suggested periodic control every three months for patients with chronic periodontitis [27,28].

A change in bone quality precedes every change in the alveolar bone quantity. The alveolar bone density change is a change in quantity detectable using radiographic imaging; thus, it requires a high quality radiograph for evaluation of the quality of alveolar bone after periodontal treatment [10]. Periapical projection is recommended for the evaluation of periodontal treatment [11]. Hedstrom et al. state the lowest limit of normal bone density is 100; any value below this is considered pathological [29].

The projection used in this study is periapical, as recommended by Pharoah et al. Density evaluation before SRP [11], and three months and six months after SRP using the Friedman test followed by a Wilcoxon post hoc test showed a significant difference between the mean bone density before SRP (70.43 ± 16.25, which is below the normal limit set by Hedstrom et al.) and after SRP. These results show also bone density in chronic periodontitis differs from bone density in aggressive periodontitis. Iskandar, using the same measuring methods, found an increase in alveolar bone density with a mean of 132.59 ±5.9 in aggressive periodontitis cases [30]. In this study, the increase in bone density three months after SRP was 72.63 ± 15.17. This is in accordance with studies by Schropp et al.
who stated bone formation occurs from three to twelve months. The bone density increase in the sixth month after SRP was higher, with a mean of 79.02 ± 19.37. This accords also with the study by Schropp et al., who found the bone formation rate to be highest during the third to sixth months [17]. The study on aggressive periodontitis conducted by Iskandar et al. showed the mean value after healing to be 73.9 ± 6.7 [30]. (still below the normal limit by Hedstrom et al.) [29].

The alveolar crest is a cortical bone; its continuity is a parameter of chronic periodontitis healing [31]. We could not statistically quantify the alveolar crest continuity because no subjects showed any change in aveolar crest continuity. This is in line with Eugene et al., who stated cortical bone formation occurs during the last phases of bone formation. Furthermore, Eugene et al. stated bone formation occurs from six to eight months [16]. The last radiographic imaging in this study were taken six months after treatment.

\(Pg\) and \(Td\) are red complex bacteria, implying they are very influential toward the pathogenesis of chronic periodontitis [24]. Other studies state \(Pg\) and \(Td\) are bacteria that work synergistically to causes bone destruction. The load of these two bacteria are reported to be predictors of the degree of loss of attachment in the next three months [8]. The outermost protein membrane of \(Td\), called \(Td92\), can trigger the osteoclastogenesis process which then causes further bone destruction [32]. The trigger to alveolar bone healing is believed to be the elimination of these two bacteria.

The results of this study shows a decrease in \(Pg\) and \(Td\) load and an increase in alveolar bone density are significantly different, both at the third month after SRP and at the sixth month after SRP, to the measurements before SRP. Our findings are in accordance with studies conducted by Orth et al. Kesavalu et al. and Raj et al. who stated \(Pg\) and \(Td\) are the two main pathogens causing alveolar bone destruction [8,33,34].

5. Conclusion
This study was conducted on subjects with chronic periodontitis suffering from 4 mm–6 mm periodontal pockets. Both the \(Pg\) load and the \(Td\) load had decreased three months after SRP and increased by the sixth month. There was an increase in the alveolar bone density in the third and sixth months after SRP. There was no difference in the alveolar crest continuity before and after SRP. There is an inverse relationship between the \(Pg\) and \(Td\) loads and the alveolar bone density.

6. References
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