Microbial effects (Porphyromonas gingivalis, Tannerella forsythia) after scaling and root planing

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Abstract. Periodontitis patients with 4–6 mm deep pockets is generally treated surgically after the initial therapy. Scaling and root planing (SRP) can change the composition of bacterial pathogens. This study aimed to determine the microbiological effects (Porphyromonas gingivalis and Tannerella forsythia) of SRP for chronic periodontitis with 4–6 mm deep pockets. Forty subjects underwent SRP on the initial visit and on days 7, 14, and 21 and months 2, 3, and 6. At the initial, month-2, month-3, and month-6 visits, and subgingival plaque samples collected to identify the composition of the P. gingivalis and T. forsythia population using real-time PCR were examined before and after SRP. There was a decrease in pocket depth, gingival bleeding index, and P. gingivalis and T. forsythia populations (p < 0.05). SRP can improve microbiological conditions in the treatment of chronic periodontitis with 4- to 6-mm deep pockets.

1. Introduction

Periodontitis is an inflammation of the periodontal tissue caused by bacterial plaque infection, resulting in a loss of attachment, bone destruction, and tooth mobility [1-3]. Conventionally, periodontal therapy is recommended for changes to the oral environment and is less conducive to the development of bacterial plaque around the gingival tissues, especially the tissue attachment area [4]. Bacterial biofilm is disrupted and eliminated through various mechanical methods including oral hygiene techniques to obtain adequate oral hygiene levels, professional hygiene cleaning of the supragingival biofilms by a dentist in the subgingival root formation through scaling and root planing (SRP), inadequate filling correction, splinting, anti-infective periodontal therapy, and the removal of pockets or other surgical anatomical defects through curettage, gingivectomy, flap surgery without or accompanied by guided tissue regeneration, guided bone regeneration, mucogingival surgery, and other techniques [2,4-8].

Socransky and several other researchers have demonstrated the importance of the elimination of beneficial bacteria as a cause of periodontal disease [9]. Periodontitis begins with a shift in the normal
bacterial flora in the oral cavity to pathogens, especially around teeth where there are local factors that can aggravate the disorder [5]. Gram-negative microorganisms dominate the subgingival area and are organized as biofilms [5]. The number of microorganisms in the affected area is usually higher than that in healthy areas [10]. “Red” complex bacteria, Tannerella forsythia species, Porphyromonas gingivalis, and Treponema denticola, are detected in larger quantities in pockets ≥3 mm.[11] Red complex species are periodontal pathogens important for diagnosing periodontal abnormalities and are strongly associated with pocket depth and bleeding on probing [5,12,13]. The prevalence and number of T. forsythia and P. gingivalis (and T. denticola) increase with increasing pocket depth [12-14].

In many cases of periodontitis with a pocket depth of 4–6 mm, surgical treatment such as curettage or flap surgery is usually performed. However, in cases where the patient does not approve of surgery as well as for patients with poor oral hygiene, those with medical or mental compromise, elderly patients, or patients with functional impairment, a nonsurgical approach is more optimal [15].

Previous research has shown that SRP may alter or produce a shift in the bacterial composition associated with chronic periodontitis, back to a subgingival microbiota composition similar to that found in healthy periodontal tissue [16].

Based on the above points, this study aims to evaluate periodontal therapy in the form of SRP in cases of chronic periodontitis with a pocket depth of 4–6 mm over a period of 6 months. In this study, we will analyze the comparison between population of Gram-negative red complex pathogen bacteria, that is, P. gingivalis and T. Forsythia.

2. Methods
The design of this study was clinical, randomized, and longitudinal experimental, conducted from February 2013 until October 2013. The subjects of the study were localized periodontitis patients stage 2 grade A who visited Teaching Dental Hospital, Faculty of Dentistry, Universitas Indonesia. The participants consisted of 40 individuals who met the following inclusion criteria [17]: (1) chronic periodontitis with at least two teeth, one of the teeth having a pocket between 4 and 6 mm (deepest pockets were selected), loss of attachment more than or equal to 4 mm, and bleeding on probing, (2) age 30–55 years, (3) no periodontal treatment within the last 6 months, (4) no systemic disorders, and (4) nonsmokers. The exclusion criteria were [17] (1) proximal filling, (2) proximal and cervical caries, (3) teeth malposition, and (4) taking drugs that affect periodontal tissue healing.

Prior to treatment, subgingival plaque samples were taken from within the selected tooth pocket using a sterile excavator, and the plaque sample was placed in an epi tube containing phosphate buffer saline solution. Afterward, the degree of plaque, calculus, gingival bleeding index, and pocket depth were measured. This measurement was recorded on day 0 (initial), then on the subject of research SRP was performed on all elements of the teeth in the oral cavity (full-mouth instrumentation). On days 7 and 14, plaque control was applied and supragingival SRP was performed. On the second, third, and sixth months, the day-0 procedures were repeated. This study was conducted in accordance with the ethical policies from Dental Research Ethics Committee, Faculty of Dentistry, Universitas Indonesia.

To identify the changes in each variable between visits, a repeated ANOVA test with a 95% degree of confidence was performed if the distribution of the data was normal. If the distribution was not normal, the Friedman test was used. When the Friedman test showed a significant result, post hoc analysis was conducted to determine which groups were meaningful. The Bonferroni test was performed as post hoc analysis for repeated ANOVA test results, while the Wilcoxon test was used for Friedman test results. To identify the relationship between independent and dependent variables, a paired test with a 95% degree of confidence was performed if the distribution of data was normal. If the distribution was not normal, the Wilcoxon test was used.
3. Results
Primary data collection was conducted through questionnaires, with demographic data collection, signing of informed consent, periodontal examination, and subgingival plaque sampling. The number of subjects who met the inclusion and exclusion criteria was 40, comprising 31 women and 9 men. Table 1 shows distribution of mean, standard deviation, minimum, and maximum values for demographic data based on age, weight, and height.

Table 1. Distribution of mean, standard deviation, minimum, and maximum values for demographic data based on age, weight, and height

| Variable       | Mean ± SD   | Min–Max     |
|----------------|-------------|-------------|
| Age (years)    | 44.23 ± 7.00| 33–55       |
| Weight (kg)    | 63.40 ± 10.61| 44–90      |
| Height (cm)    | 158.35 ± 5.31| 148–170    |

Table 2. Distribution of mean, standard deviation, minimum, and maximum values for pocket depth, gingival bleeding index, and amount of *Porphyromonas gingivalis* and *Tannerella forsythia* in initial, second, third, and sixth months

| Variable                        | Mean ± SD   | Min–Max     |
|---------------------------------|-------------|-------------|
| Amount of *P. gingivalis* bacteria (CFU/mL) |             |             |
| Initial                         | 5.64 ± 1.65 | 2.89–8.84  |
| Second month                    | 3.85 ± 1.23 | 0–7.68     |
| Third month                     | 3.65 ± 1.18 | 0–5.67     |
| Sixth month                     | 4.56 ± 1.73 | 0–7.03     |
| Amount of *T. forsythia* bacteria (CFU/mL) |             |             |
| Initial                         | 8.11 ± 2.84 | 0–12.56    |
| Second month                    | 3.09 ± 2.55 | 0–7.51     |
| Third month                     | 2.73 ± 2.68 | 0–7.95     |
| Sixth month                     | 3.03 ± 2.85 | 0–7.58     |

Table 2 shows the clinical differences in the pocket depth measurement results, gingival bleeding index score, and the population of *P. gingivalis* and *T. forsythia* before and after SRP. Normality was evaluated using the Shapiro–Wilk test for pocket depth, gingival bleeding index, and the population of *P. gingivalis* bacteria, and the results showed that the population of *T. forsythia* bacteria at all measurement periods was not normal. Because the data distribution was not normal, the Friedman nonparametric test was conducted.

Table 3. Significance test for comparison between pocket depths, gingival bleeding index, amount of *Porphyromonas gingivalis*, and amount of *Tannerella forsythia* in initial, second, third, and sixth months

| Comparison variable                  | P value |
|-------------------------------------|---------|
| Amount of *P. gingivalis* population between visits | 0.000*  |
| Amount of *T. forsythia* between visits | 0.000*  |

*Friedman test, p < 0.05 significantly different

Table 3 shows a significant difference in the pocket depth, gingival bleeding index, and the population of *P. gingivalis* and *T. forsythia* for the initial, second, third, and sixth months, evaluated using the Friedman test, with p = 0.000 (p < 0.05). To confirm which measurements among the variables showed significant differences, a post hoc analysis was performed using the Wilcoxon test.
Table 4. Distribution of mean, standard deviation, minimum, maximum, increase, decrease, no-change, and significance test on the differences in pocket depth, gingival bleeding index, amount of Porphyromonas gingivalis, and amount of Tannerella forsythia in initial, second, third, and sixth months

| Variable | Mean ± SD | Min–Max | Decrease (N) | Increase (N) | No-change (N) | Total (N) | P value |
|----------|-----------|---------|-------------|-------------|--------------|-----------|---------|
| Porphyromonas gingivalis population changes (CFU/ml) | | | | | | | |
| Second month–initial | −1.79 ± 2.19 | −6.52–3.12 | 30 | 10 | 0 | 40 | 0.000* |
| Third month–initial | −2 ± 2.20 | −8.84–1.35 | 32 | 8 | 0 | 40 | 0.000* |
| Sixth month–initial | −1.08 ± 2.62 | −6.94–3.96 | 26 | 14 | 0 | 40 | 0.013* |
| Third month–second month | −0.20 ± 1.67 | −4.12–2.76 | 20 | 20 | 0 | 40 | 0.904 |
| Sixth month–second month | 0.71 ± 2.12 | −4.61–3.57 | 14 | 26 | 0 | 40 | 0.036* |
| Sixth month–third month | 0.91 ± 1.75 | −3.71–4.52 | 10 | 29 | 1 | 40 | 0.002* |
| Tannerella forsythia population changes (CFU/ml) | | | | | | | |
| Second month–initial | −5.02 ± 3.30 | −10.15–6.22 | 38 | 1 | 1 | 40 | 0.000* |
| Third month–initial | −5.37 ± 4.32 | −12.09–6.48 | 35 | 4 | 1 | 40 | 0.000* |
| Sixth month–initial | −5.07 ± 3.40 | −11.78–2.12 | 35 | 3 | 2 | 40 | 0.000* |
| Third month–second month | −0.35 ± 4.01 | −7.51–7.95 | 17 | 18 | 5 | 40 | 0.481 |
| Sixth month–second month | −0.05 ± 3.64 | −6.22–7.12 | 17 | 16 | 7 | 40 | 0.936 |
| Sixth month–third month | 0.30 ± 3.95 | −6.48–7.12 | 19 | 14 | 7 | 40 | 0.649 |

*Wilcoxon test, p < 0.05 hypothesis is accepted, p > 0.05 hypothesis is rejected.

The significance test results shown in Table 4 indicate that there was a significant difference in the pocket depth, gingival bleeding index, and both P. gingivalis and T. forsythia populations after SRP.

The results of the Shapiro–Wilk normality test (research subjects <50 people) showed an abnormal data distribution for all parameters evaluated. The bivariate analysis test used was the Wilcoxon nonparametric test.

4. Discussion

In this study, there was a significant decrease, and the number of P. gingivalis and T. forsythia (p < 0.05) in the second, third, and sixth months after SRP. This improvement continued until the third month, but there was a reversal in all microbiological parameters in the sixth month although a significant improvement remained compared with the initial data.

In this study, the observed pockets were only 4–6 mm deep and subjects were asked to return six times after the initial visit (day 0) on days 7, 14, and 21 and in months 2, 3, and 6. Microbiological data collection in the form of subgingival sampling for analysis via PCR, were performed on the initial visit before SRP as well as 2, 3, and 6 months thereafter. At the initial visit, supra and subgingival plaque and calculus removal were performed. On the next visit, only supragingival scaling was performed. During each visit, the patient was re-instructed on oral hygiene techniques to be performed at home.

The significant improvement in the parameters evaluated until the third month followed by a slight reversal in the sixth month is in accordance with research conducted by Haffajee et al., Carvalho et al. and Feres et al. [18–21]. In this study, 2 months after SRP, the total population of P. gingivalis and T. forsythia decreased significantly compared with the population before SRP. In the third month, the decrease of these two bacterial populations was insignificant compared with the second month. In contrast, there was an increase in the population of both bacterial populations in the sixth month, with P. gingivalis increasing significantly and T. forsythia increasing slightly compared with the second and third months. Nevertheless, the measurements after the second, third, and sixth months showed significant differences compared with the initial values. This is in accordance with the study by Haffajee et al. which showed that the prevalence of T. forsythia and P. gingivalis species was significantly reduced 3 months after SRP therapy but did not decrease further at 6 and 9 months after therapy [18].
The mean decrease in the *P. gingivalis* population in the second month after SRP was less than that of *T. forsythia*. However, between the third and sixth months, the mean increase in *P. gingivalis* was greater (Table 4). This suggests that *P. gingivalis* is more difficult to remove and is more likely to increase again compared with *T. forsythia*, similar to the results found in some previous studies. Research by Haffajee et al. categorizing pocket depths indicated that the mean percentage decrease in *P. gingivalis* after therapy was almost equal in all pockets, whereas the decrease in *T. forsythia* was most common in shallow and intermediate pocket depths, as was the case in this study [18]. Ximenez-Fyvie et al. and Haffajee et al. concluded that some periodontal pathogens such as the red complex can colonize the supragingival biofilm at high levels and proportions, and this contributes to the recolonization of the species in the newly treated subgingival region [11,22].

Another potential explanation for the less significant decrease in *P. gingivalis* compared with *T. forsythia* is that *P. gingivalis* has the ability to attach to other bacteria, epithelial cells, and fibrinogen and fibronectin connective tissue components [23]. This may explain why the *P. gingivalis* population decreased less than *T. forsythia*, despite the performance of SRP every visit.

Several other studies detected putative periodontal pathogens in healthy areas but in small numbers that were not clinically significant [24]. According to Riep et al. periodontal pathogens such as *P. gingivalis* and *T. forsythia* may be isolated from healthy control groups [25]. Tran and Rudney found that 55% of healthy areas contained *P. gingivalis*, 30% *A. Actinomycetemcomitans*, and 5% *B. forsythus* [26]. In this study, *P. gingivalis* species may have been found in healthy areas or those in the process of healing because the presence of complex interaction abilities between *P. gingivalis* and cross-talk between cells can produce a stable relationship in which organisms can survive in epithelial cells without causing great damage [24].

This study shows that SRP is indeed a gold standard for periodontal treatment because SRP alone can provide beneficial microbiological effects during periodontal healing. However, this effectiveness can only be sustained for the first 3 months. SRP was not performed in the next 3 months (until the sixth month), resulting in an increase in microbiological parameters. Nevertheless, these parameters remained significantly better than the initial data before SRP. This demonstrates that treatment every 3 months should be recommended for patients with chronic periodontitis. Maintenance care is a critical therapeutic phase, since long-term maintenance is associated with the frequency and quality of the phase [27].

Most patients with periodontitis require a long-term visitation schedule, with visits for professional cleaning every 3–4 months [27]. The idea of strict plaque control as part of the active phase of periodontal therapy was first introduced in 1970 and 1980 [21]. Researchers at that time (Nyman et al. Westfelt et al. Magnusson et al.) proposed that the removal of supragingival plaques every 2 weeks over a long period could provide clinically and microbiologically beneficial periodontal therapy results [28–31]. In this study, supragingival plaque removal was performed only once a week during three visits; hence it provided a less significant long-term effect in controlling supragingival plaque, which is the cause of periodontal disease (chronic periodontitis).

5. Conclusions
There was a decrease in the population of *P. gingivalis* bacteria, and population of *T. forsythia* bacteria in the third month after scaling and the initial root planing (SRP) treatment for periodontitis patients with pocket depths of 4–6 mm. These parameters increased after 6 months.

6. References
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