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ORIGINAL ARTICLE

Expression of Osteopontin mRNA During Periodontal Healing Following Scaling and Root Planing

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Abstract

Objective: To analyze osteopontin mRNA expression levels in subjects with periodontitis prior to (baseline) and 7, 14, and 28 days following scaling and root planing (SRP). **Material and Methods:** Gingival crevicular fluid was collected as clinical samples from four subjects with periodontitis (pocket depth, 4-5 mm) aged 35-54 years old as well as from three healthy subjects (controls). The osteopontin mRNA expression levels were measured by quantitative real-time polymerase chain reaction. Spearman's rank correlation between osteopontin levels in gingival crevicular fluid and the modified gingival index (MGI) was also performed. **Results:** The Wilcoxon signed-rank test showed no significant difference in osteopontin mRNA expression levels between baseline and 28 days following SRP (p=0.068). The Friedman test showed no significant difference in osteopontin mRNA expression levels between baseline and following SRP (7, 14, or 28 days) (p>0.05). Spearman's rank correlation showed no significant correlation between osteopontin mRNA expression levels and MGI (r=0.087; p=0.749). **Conclusion:** Following SRP of periodontal tissue, there was a decreasing trend in osteopontin mRNA expression; however, this finding was not statistically significant. Nevertheless, osteopontin can be used as a biomarker to monitor the healing process; however, further studies are required to clarify our results.

Keywords: Osteopontin; Periodontics; Root Planing; Dental Scaling.

Introduction

Periodontitis is defined as the chronic inflammation of the supportive periodontal tissue that progresses slowly or moderately fast [1,2]. Periodontitis can lead to permanent destruction of both soft and hard tissue, thereby leading to tooth loss [3]. Studies have shown that individuals aged 35-54 have a higher prevalence of periodontitis than those in other age groups [4,5].

Plaque bacteria are considered the main etiology of periodontitis; bacteria trigger the host immune-inflammatory response, which results in loss of periodontal attachment [6,7]. Inflammation of periodontal tissue can be caused by a numerous gram-negative anaerobic organisms, thereby inducing the production inflammatory cytokines and mediators, which can cause periodontal tissue destruction and bone resorption. Interleukin-1, prostaglandin E_2, tumor necrosis factor-α, and RANKL can initiate sequences of periodontal tissue destruction; if left without intervention, these molecules can induce increased expression of other inflammatory mediators and stimulate advanced damage.

Osteopontin (OPN) is a non-collagenous phosphoprotein found in bone extracellular matrix and functions as an inflammatory cytokine. OPN is produced by osteoclasts and is expressed at maturation and mineralization of osteoblasts during differentiation, also contributing to the resorptive stage [8,9]. Studies have shown that OPN plays an important role in the bone resorption process; however, its role in periodontal healing remains to be unclear [10,11]. OPN exists in Gingival Crevicular Fluid (GCF) and its levels correlate to increased pocket depth and progression of periodontal disease in the group of healthy control and periodontitis patients [8].

Moreover, previous research found that OPN levels were higher in advanced periodontal disease and comparatively low in healthy patients [12]. This study was held in three groups (patients who were periodontally healthy, had gingivitis, and periodontitis patients after scaling and root planing therapy and used ELISA (enzyme-linked immunosorbent assay) to estimate the OPN level. A systematic review reported that OPN was present during the early phase following implant placement and higher level of bone-biomarker was found on 7th, 15th, and 30th day (early stage of cellular response) and observed in recruitment of macrophages and lymphocytes [13].

Treatment for shallow periodontal pockets of 4-5 mm is still dilemmatic for clinicians [14], and this pocket depth is included in stage I and II periodontitis in the new classification of periodontal disease by the American Association of Periodontology [7,15]. A few studies have concluded that periodontal pockets shallower than 5 mm can be treated with non-surgical therapy; however, pockets deeper than 5 mm need surgery for a direct view [16]. One particular study concluded that the results of non-surgical treatment are not statistically different to those obtained by surgery with respect to mild-moderate periodontitis [14]. Scaling and root planing (SRP) is the gold standard in non-surgical periodontal treatment [17,18].

An increase in clinical attachment and decreased bleeding on probing (BOP) are considered as the main characteristics of periodontal healing [19]. However, the modified gingival index (MGI) was proposed as a non-invasive measurement to prevent interruption of pocket healing following SRP when probing the site. The MGI score is based on the clinical appearance of inflammation at the

site [4,20]. A few studies have observed an association between OPN levels and periodontal disease progression [8,12].

The present study was planned to use quantitative PCR (polymerase chain reaction) to detect OPN mRNA (level of gene expression) during the early stage of cellular response. In the present study, OPN mRNA expression levels in the GCF of subjects with periodontitis (pocket depth 4-5 mm; stage I and II periodontitis based the new classification of periodontal disease [7,15] were compared among baseline and 7, 14 [21,22] and 28 [23] days following SRP. Healthy subjects were evaluated as a control. The hypothesis of the present study was that there would be a statistical difference in OPN mRNA expression levels in the GCF of subjects with periodontitis between baseline and 28 days following SRP, and that there would be a correlation between these levels and the MGI that could be used as a clinical parameter of periodontal healing.

Material and Methods

Study Subjects

A total of seven patients aged 35-54 years old from the Clinic of Periodontology, Faculty of Dentistry, Universitas Indonesia Dental Hospital were divided into two groups: those with periodontitis (four subjects) and healthy controls (three subjects). Two calibrated operators recorded the preliminary periodontal status of the patients, which consisted of periodontal pocket depth and BOP score at baseline, and the modified gingival index (MGI) at baseline and following treatment.

Subjects with rapid progression of periodontitis, pregnancy, history of diabetes mellitus, smoking, or consumption of antibiotics within six months prior to examination were excluded from the present study. Each patient was subjected to a full periodontal status check by two calibrated operators. The diagnoses of periodontitis and healthy periodontal condition were confirmed based on the 2017 classification of periodontal diseases [15]. Teeth with 4-5 mm pocket depth were included in the present study.

Quantitative PCR (qPCR)

Gingival crevicular fluid (GCF) samples from up to three teeth with a 4-5 mm pocket depth were collected from each patient using sterile #30 absorbent paper points for 30 seconds [24], which were placed in 1.5 mL microfuge tubes with 300 µL Tris-EDTA buffer and stored in -80°C until use. Following collection of baseline samples, the SRP procedure was performed in all subjects. The GCF samples were collected three subsequent times (7, 14, and 28 days following SRP) from subjects with periodontitis, but only once for healthy control subjects.

The mRNA was extracted from the samples using GENEzol™ Reagent (Geneaid Biotech Ltd., New Taipei City, Taiwan), and cDNA was synthesized using ReverTra-Ace® (Toyobo Inc., Osaka, Japan). All procedures were performed according to the manufacturers' instructions.

qPCR was performed using the Applied Biosystems StepOne™ Real-Time PCR system and the SensiMix™ SYBR Hi-ROX Kit as a master mix. Primers for *OPN* were: forward, 5'–TTG CAG

CCT TCT CAG CCA A–3' and reverse, 5'–GGA GGC AAA AGC AAA TCA CTG–3' [25]. The pre-denaturation temperature was 95°C for 3 minutes, followed by 50 cycles of 95°C for 10 seconds, 60°C for 15 seconds, and 72°C for 10 seconds [26]. β-actin was used as the internal control: forward, 5' - TAA TGT CAC GCA CGA TTT CCC–3' and reverse, 5' - TCA CCG AGC GCG GCT–3' using the following protocol: 50°C for 2 minutes and 95°C for 10 minutes for the pre-denaturation temperatures, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute [27]. qPCR was performed in duplicate for each sample. Melting curve analysis was carried out after the end of the cycle. The expression levels were estimated using the $2^{-\Delta\Delta_{CT}}$ method [28,29], and the relative expression of *OPN* was normalized to β-actin.

Statistical Analysis

The difference between baseline and 28 days following SRP was assessed using Wilcoxon analysis, while differences among all study groups (baseline, 7, 14, and 28 days following SRP) were analyzed using the Friedman test. Spearman's correlation test was performed between OPN mRNA expression levels and the modified gingival index (MGI). All statistical analysis was performed with a 95% confidence interval (CI) using SPSS® version 23.

Ethical Aspects

All subjects agreed to participate in this research and signed a written informed consent. The study protocol was approved by the Ethical Committee of the Faculty of Dentistry, Universitas Indonesia (Protocol No. 090230218).

Results

The characteristics of the subjects are presented in Table 1. The mean age of the subjects with periodontitis was 47.25 years old, while that of the healthy subjects was 29.67 years old. Males accounted for 75% of the subjects with periodontitis and 66.67% of the healthy subjects.

Table 1. Distribution of patients with periodontitis and healthy subjects.

Variables	Periodontitis	Healthy
Age (Years)	47.25 (± 7.13)	29.67 (± 0.57)
Sex	N (%)	N (%)
Male	3 (75%)	2 (66.67%)
Female	1 (25%)	1 (33.33%)

The $2^{-\Delta\Delta_{CT}}$ method was used to analyze the relative OPN mRNA expression data between baseline and 28 days following treatment, and also among all groups (baseline, 7, 14, and 28 days following SRP). The Wilcoxon test comparing the relative OPN expression levels between baseline and 28 days following treatment showed no statistically significant difference (p=0.068). The Friedman test also showed no significant differences in the relative OPN expression levels among the four groups (p=0.308; Table 2). Spearman's correlation test between relative OPN expression levels and the modified gingival index (MGI) showed no correlation (r=0.087, p=0.749; Figure 1).

Table 2. Comparison of the relative OPN mRNA expression levels among subjects with periodontitis.

Fold Gene Expression of OPN	Median (Minimum-Maximum)	p-value
Baseline	2.482 (1.597–10.978)	0.308
7 Days After SRP	3.955 (0.056–8.122)	
14 Days After SRP	1.429 (0.058–3.331)	
28 Days After SRP	0.441 (0.009–0.792)	

Friedman test.

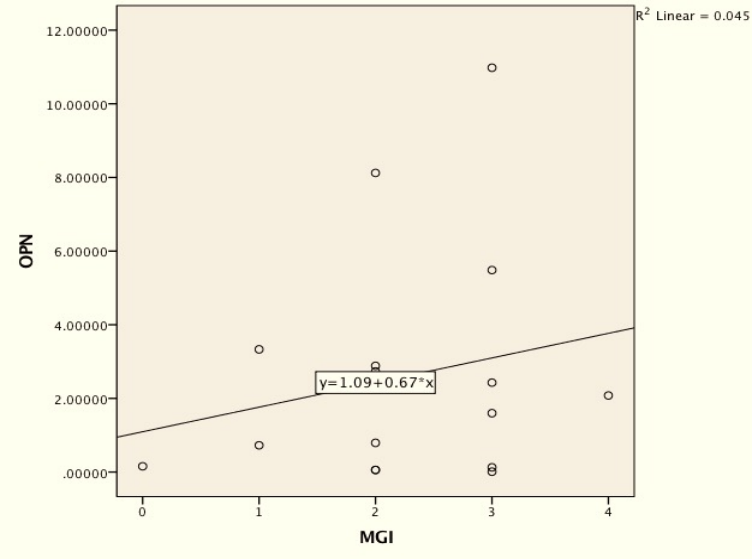

Figure 1. Correlation between relative OPN mRNA expression levels and the MGI. The scatter plot shows a very weak positive correlation, but there was no significant difference between relative OPN mRNA expression level and the MGI among baseline and 7, 14, and 28 days following treatment.

There was a decreasing trend in OPN mRNA expression levels from baseline to 7, 14, and 28 days following SRP as measured by q-PCR (Figure 2), but there were no statistically significant differences among any of the four groups. Spearman's correlation showed no correlation between relative OPN mRNA expression levels and MGI as a clinical parameter.

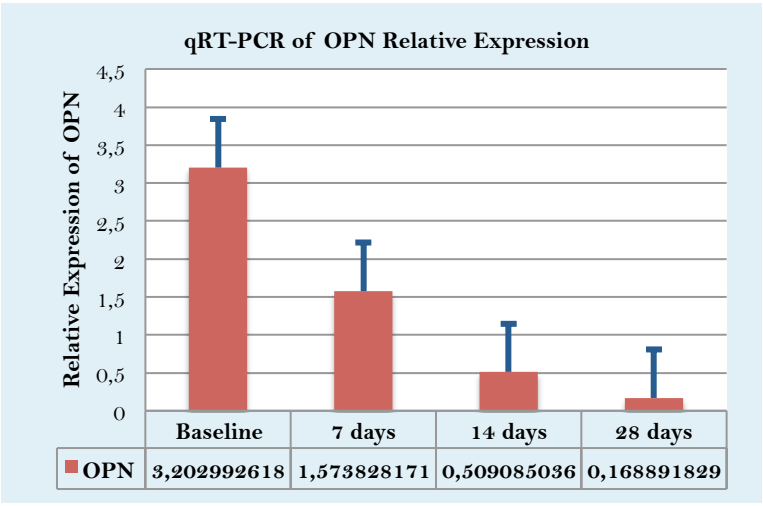

Figure 2. Comparison of relative OPN mRNA expression levels. The overall OPN mRNA expression in subjects with periodontitis relative to the healthy control subjects was 3.202 at baseline, and 1.573 on day 7, 0.509 on day 14, and 0.168 on day 28 following treatment. The relative expression of OPN was highest at baseline and gradually decreased 7, 14, and 28 days following treatment.

Discussion

The present study examined the relative osteopontin (OPN) mRNA expression levels in the GCF from teeth of subjects with periodontitis who presented with inflammation of the gingiva and a pocket depth of 4-5 mm prior to and following scaling and root planing (SRP) treatment.

As previously described, MGI was also used as one of the clinical parameters to confirm the effect of initial periodontal therapy (SRP) and resulted in a significant correlation of MGI and OPN on all groups (healthy, gingivitis, periodontitis) after SRP [12]. Although the present study did not succeed to give significant results, but the tendency of MGI and OPN level to correlate was reported.

Previous studies showed a significant difference between healthy and diseased subjects [8,12,30]. The present study also recognized higher OPN mRNA expression levels at baseline in subjects with periodontitis relative to healthy subjects, which is in accordance with previous studies.

Although no significant difference was found in the present study, the tendency of OPN to decrease after SRP indicated the healing process of the periodontium followed by decreasing number of osteoclasts which was reported on the previous study [13]. During the first month as the early stage of cellular response, OPN was found to tend to decrease based on the present study. This result might be considered as a sign of periodontal healing and discontinuance of bone destruction process of the disease that was marked on the first month after therapy [13].

It should be noted that previous studies have used ELISA to determine OPN protein concentration [8,12,30], while the present study used qPCR to detect OPN mRNA. Generally, qPCR has the disadvantage that mRNA expression levels do not always reflect actual protein expression levels; moreover, the highly sensitive system may not reach the detection threshold [31]. Previous studies have also been performed in a minimum of 45 subjects, while only seven subjects were included in the present study; the small number of subjects may have influenced the normality of the data and the outcome of the statistical analysis. The present study analyzed the OPN levels on day 7, 14, and 28 following SRP treatment, while other authors evaluated the OPN levels eight weeks following treatment [12,30]. The collection timing in the present study may have been another possible cause of the insignificant difference in the results.

Despite these limitations, the present study shows higher OPN mRNA expression levels relative to the healthy subjects, and the tendency to decrease gradually following SRP treatment (7, 14, 28 days) as the MGI decreases; however, neither the difference nor the correlation was statistically significant.

Conclusion

Osteopontin mRNA expression levels in the GCF of subjects with periodontitis were higher than those in healthy subjects. The initial periodontal therapy of SRP in subjects with periodontitis reduces OPN levels in the GCF as the MGI decreases; however, there was no statistical difference between baseline and any of the post-therapy groups. The decrease in OPN levels is in accordance with the decrease in MGI as a periodontal healing clinical parameter.

OPN mRNA expression levels can be used as a biomarker of periodontal healing or periodontitis progression that was confirmed on present study by the tendency of decreasing OPN level after SRP on early stage of cellular response that might lead to the decrease osteoclasts number and disease cessation. Further studies are required with a larger sample size and a longer observation period to determine OPN mRNA expression levels in periodontitis on every stage of bone remodeling, and to confirm the efficacy and success of initial periodontal therapy on periodontal healing.

Authors' Contributions: Each author made significant individual contributions to this manuscript. MM performed the experiment and wrote the manuscript, YS and RL designed the study and reviewed the manuscript, and BB performed the experiments and reviewed the manuscript.

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Conflict of Interest: The authors declare no conflicts of interest.

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