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Effects of initial periodontal therapy on interleukin-1β level in gingival crevicular fluid and clinical periodontal parameters

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Abstract: Inflammatory cytokines may have important roles in periodontitis. We assessed the effects of initial periodontal therapy on clinical periodontal parameters and interleukin-1β (IL-1β) level in gingival crevicular fluid (GCF) from chronic periodontitis (CP) patients. After initial screening, baseline periodontal parameters such as probing pocket depth (PPD) and bleeding on probing (BOP) were measured. GCF samples were collected from 13 shallow (≤3 mm) and deep (≥5 mm) PPD sites from 13 CP patients, and GCF volume and IL-1β concentration were determined at baseline (before scaling and root planning) and at 2 and 4 months after initial therapy. Baseline BOP rate, GCF volume, and IL-1β level were significantly higher at deep PPD sites than at shallow PPD sites. Significant improvements in PPD and BOP were observed at 2 and 4 months after periodontal initial therapy in deep PPD sites only. In contrast, GCF volume and IL-1β concentration were lower at 2 and 4 months after initial therapy at all sites. These results suggest that GCF volume and IL-1β level in samples reflect disease severity and that these variables are better than PPD and BOP as markers of gingival inflammation.
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Introduction

Periodontal diagnosis generally requires measurement of periodontal tissue destruction (e.g., probing pocket depth [PPD] and clinical attachment level [CAL]) and gingival inflammation (e.g., bleeding on probing [BOP] and gingival index [GI]). Although the techniques used are straightforward and noninvasive (1), these parameters are static and thus reflect disease history and not present disease activity (2). Therefore, it is necessary to develop diagnostic tests that can identify active periodontal sites, predict future disease progression, and assess response to periodontal treatment (3).

Periodontopathic bacteria increase the risk of periodontitis, and immune responses against bacterial products and subsequent secretion of proinflammatory cytokines are crucial in periodontal tissue destruction (4,5). Interleukin-1β (IL-1β) is an important mediator of inflammatory response and is involved in cell proliferation, differentiation, and apoptosis, and in the pathophysiology of periodontitis (6). Several studies reported a correlation between the inflammatory status of periodontal disease and IL-1β levels in gingival tissues and gingival crevicular fluid (GCF) (3,6,7). One report suggested that the role of IL-1 in osteoclastic bone resorption is in part mediated by induction of prostaglandin E_2 (PGE_2) release (8). In addition, IL-1 increased osteoclast formation and PGE_2 release in cultures of mouse bone marrow cells (9). Figueredo et al. reported that the IL-1β levels in GCF were induced in samples from periodontitis patients, independent of disease severity at the sampled

site. This suggests that IL-1β levels are representative of individual patients (10). Neither IL-1α nor IL-1β concentration significantly differed between shallow sulci and moderate/advanced sites in periodontitis patients (11). Individuals vary in periodontitis progression and treatment outcomes, and there is a strong correlation between periodontitis severity and IL-1 gene polymorphism (12). Therefore, we measured GCF volume and IL-1β concentrations in GCF sampled from shallow (≤3 mm) and deep (≥5 mm) PPD sites in 13 patients with chronic periodontitis (CP). The purpose of this study was to assess the effects of initial periodontal therapy on clinical periodontal parameters, GCF volume, and IL-1β level in a sample of Japanese CP patients.

Materials and Methods

Sampling

Thirteen nonsmoking CP patients (mean age, 57.6 ± 2.8 years) were included in this study. They received dental care at Nihon University Hospital School of Dentistry at Matsudo, Japan. This study was approved by the Ethics Board and Institutional Internal Review Board at Nihon University School of Dentistry at Matsudo (EC03-041, EC09-005). Written informed consent was obtained from each patient after all procedures had been explained.

Periodontal status was assessed by measuring probing pocket depth (PPD) and bleeding on probing (BOP). PPD was measured with a CP11 probe (Hu-Friedy, Chicago, IL, USA). CP was defined as the presence of at least two sites with a PPD ≥5 mm and attachment loss of >5 mm. All participants were in good general health and had no history of periodontal treatment or antibiotic therapy for at least 3 months before this study. Twenty-six GCF samples were collected from two periodontal PPD sites (≤3 mm and ≥5 mm) of the 13 CP patients at baseline, i.e., before scaling and root planning, and at 2 and 4 months after initial therapy. Before sampling, supragingival plaque was removed with sterile cotton pellets, and GCF was collected and measured with a Periotron 8000 device. Periopaper (Oraflow, New York, NY, USA) was then thrice inserted into the sample site and retained for 30 s at each insertion. GCF volume is expressed in microliters (μL). GCF samples were pooled in microcentrifuge tubes and stored at -80ºC until IL-1β measurement.

Enzyme-linked immunosorbent (ELISA) assay

IL-1β concentration was measured using a commercially available ELISA assay (R&D systems, Minneapolis, MN, USA), which is based on an antibody sandwich technique, as described previously (3). After the designated IL-1β samples were thawed on ice, plates precoated with a monoclonal antibody specific for human IL-1β were incubated with 200 μL of standard, control, or sample for 2 h. After washing, 200 μL of anti-IL-1β conjugate was added to each well for 1 h. After additional washing, 200 μL of substrate solution was added to each well for 20 min, after which 50 μL of stop solution was added to each well, with reading at 450 nm. The assay has a detection sensitivity of 1 pg/mL IL-1β.

Table 1 Characteristics of participants

First visit (Baseline) 13 chronic periodontitis patients	
Age (years)	57.6 ± 2.8
Male	6 (46%)
Female	7 (54%)
PPD (mm)	2.38 ± 0.14 (≤3 mm)
	5.38 ± 0.14* (≥5 mm)
BOP	3 (23%) (≤3 mm)
	12 (92%)* (≥5 mm)
GCF (μL)	1.51 ± 0.12 (≤3 mm)
	2.91 ± 0.12* (≥5 mm)
IL-1β (pg/site)	1.59 ± 0.11 (≤3 mm)
	5.48 ± 1.07** (≥5 mm)
IL-1β (pg/μL)	1.12 ± 0.10 (≤3 mm)
	2.00 ± 0.44*** (≥5 mm)

*$P < 0.001$, **$P < 0.005$, ***$P < 0.07$, mean ± SE
PPD: Probing pocket depth, BOP: bleeding on probing, GCF: gingival crevicular fluid

Statistical analysis

The chi-square for independence test, confirmed by Fisher's exact probability test, was used to determine whether BOP improved after initial periodontal treatment. Significant differences between baseline values for periodontal parameters and IL-1β level and values at 2 and 4 months after therapy were analyzed using one-way ANOVA.

Results

Table 1 shows the participants' age, sex, PPD, BOP, GCF volume, and IL-1β levels in GCF sampled from shallow (≤3 mm) and deep (≥5 mm) PPD sites at the first visit (baseline). The two baseline periodontal PPD sites were 2.38 ± 0.14 mm and 5.38 ± 0.14 mm. BOP was detected at 23% and 92% of sites, GCF volume was 1.51 ± 0.12 μL and 2.91 ± 0.12 μL, and IL-1β concentration was 1.12 ± 0.10 pg/μL (1.59 ± 0.11 pg/site) and 2.00 ± 0.44 pg/μL (5.48 ± 1.07 pg/site), respectively. Baseline BOP rate, GCF volume, and IL-1β level were significantly higher at deep PPD sites than at shallow PPD sites. The

Table 2 Baseline clinical characteristics of participants

Participant No.	Sex	Age	(≦3) PPD	BOP	FDI dental numbering	(≧5) PPD	BOP	FDI dental numbering
1	Male	54	2 mm	-	12	5 mm	+	27
2	Female	62	2 mm	-	11	6 mm	+	47
3	Male	39	2 mm	+	12	8 mm	+	21
4	Female	65	2 mm	-	21	5 mm	+	26
5	Male	51	3 mm	+	11	6 mm	+	25
6	Female	38	2 mm	-	21	6 mm	+	26
7	Male	63	2 mm	-	11	5 mm	+	25
8	Male	62	3 mm	+	13	6 mm	+	23
9	Male	73	2 mm	-	12	5 mm	+	26
10	Female	63	3 mm	-	23	5 mm	+	15
11	Female	58	2 mm	-	11	5 mm	+	16
12	Female	57	3 mm	-	11	5 mm	+	27
13	Female	64	2 mm	-	21	5 mm	-	16

Table 3 Clinical findings at baseline and 2 and 4 months after initial therapy

	Baseline	2 months after initial therapy	4 months after initial therapy
PPD (mm)	2.38 ± 0.14 (≤3 mm)	2.23 ± 0.12 (≤3 mm)	2.15 ± 0.10 (≤3 mm)
	5.38 ± 0.14 (≥5 mm)	4.62 ± 0.18* (≥5 mm)	4.00 ± 0.11* (≥5 mm)
BOP	3 (23%) (≤3 mm)	1 (8%) (≤3 mm)	0 (0%) (≤3 mm)
	12 (92%) (≥5 mm)	6 (46%)** (≥5 mm)	2 (15%)* (≥5 mm)

*$P < 0.001$, **$P < 0.005$, mean ± SE

Table 4 GCF volume at baseline and 2 and 4 months after initial therapy

	Baseline	2 months after initial therapy	4 months after initial therapy
GCF (μL)	1.51 ± 0.12 (≤3 mm)	1.16 ± 0.11** (≤3 mm)	1.00 ± 0.07** (≤3 mm)
	2.91 ± 0.12 (≥5 mm)	2.20 ± 0.17* (≥5 mm)	1.90 ± 0.11* (≥5 mm)

*$P < 0.001$, **$P < 0.005$, mean ± SE

Table 5 IL-1β concentration at baseline and 2 and 4 months after initial therapy

	Baseline	2 months after initial therapy	4 months after initial therapy
IL-1β (pg/site)	1.59 ± 0.11 (≤3 mm)	0.51 ± 0.03* (≤3 mm)	0.51 ± 0.03* (≤3 mm)
	5.48 ± 1.07 (≥5 mm)	1.15 ± 0.13* (≥5 mm)	0.96 ± 0.10* (≥5 mm)
IL-1β (pg/μL)	1.12 ± 0.10 (≤3 mm)	0.48 ± 0.05* (≤3 mm)	0.56 ± 0.05* (≤3 mm)
	2.00 ± 0.44 (≥5 mm)	0.51 ± 0.03* (≥5 mm)	0.51 ± 0.04* (≥5 mm)

*$P < 0.001$, mean ± SE

sex, age, PPD (≤3 mm and ≥5 mm), BOP, and tooth number (Federation Dentaire Internationale [FDI] dental numbering) of each patient are shown in Table 2.

Effects of initial periodontal therapy (toothbrushing instruction, scaling, and root planning) on clinical parameters (PPD and BOP) at shallow and deep PPD sites are shown in Table 3. As compared with baseline values, PPD and BOP were significantly improved at 2 and 4 months at deep PPD sites (Table 3), GCF volumes were significantly improved at shallow and deep PPD sites (Table 4), and IL-1β level (pg/μL and pg/site) was significantly lower at shallow and deep PPD sites (Table 5).

Discussion

We evaluated the effects of initial periodontal therapy on clinical periodontal parameters, GCF volume, and IL-1β concentration in GCF samples from Japanese CP patients. First, we examined baseline BOP rate, GCF volume, and IL-1β level in GCF sampled from shallow (≤3 mm) and deep (≥5 mm) PPD sites in 13 CP patients. Baseline BOP rate, GCF volume, and IL-1β concentra-

tion were significantly higher at deep PPD sites than at shallow PPD sites.

A previous study found that IL-1β concentration was significantly higher in GCF from patients with periodontitis than in those with gingivitis and healthy controls (6). In addition, levels of crevicular IL-1α and IL-1β closely correlated with periodontal disease severity (7). Another study found that the average amount of IL-1β collected from inflamed pockets was three times that from non-inflamed pockets and that IL-1β level declined after initial therapy (13). Initial therapy did not significantly reduce C-telopeptide pyridinoline cross-links and IL-1β level, despite the fact that IL-1β level was significantly higher at deep PPD sites than at shallow sites (3). Taken together, these findings indicate that IL-1β level is higher at inflammation sites than at healthy sites. However, other studies found no significant correlation among IL-1β level, GCF volume, and the clinical characteristics of the sample site (14,15). Previous studies reported that IL-1β concentration did not significantly differ between shallow and deep pockets in periodontitis patients (10,11). IL-1β concentration in GCF did not differ at baseline or after initial therapy between CP patients and healthy control patients, whereas total IL-1β level statistically differed among the three groups (16).

Inflammatory cytokines such as IL-1, IL-6, and IL-8 are critical in periodontal disease pathogenesis, and cytokine levels were elevated in sera from EBV-infected patients (17). We previously reported that Epstein-Barr virus (EBV) DNA was detected more frequently in deeper (≥5 mm) PPD sites of CP patients than in shallow (≤3 mm) PPD sites or in healthy controls (4). In addition, coexistence of EBV and *Porphyromonas gingivalis* was significantly more frequent in deep PPD sites of patients as compared with shallow PPD sites and healthy controls. Moreover, *in situ* hybridization of EBV-encoded small RNA (EBER) showed infiltration of B cells into inflamed gingival connective tissues of CP patients (4). These results suggest a correlation between EBV copy numbers in subgingival plaque and periodontitis severity (5). The envelope protein and genomic DNA of EBV can stimulate inflammatory cytokines in primary human monocytes (18,19). This may explain why IL-1β concentration was significantly higher at deep PPD sites than at shallow PPD sites in the present study. PPD and BOP had significantly improved at 2 and 4 months after initial periodontal therapy at deep PPD sites only. However, GCF volume and IL-1β level were significantly lower than baseline at 2 and 4 months after initial therapy at shallow and deep sites. Although our findings require confirmation in future studies, our results suggest that GCF volume and IL-1β level in samples depend on inflammation severity at the sample site and that these variables are better than PPD and BOP as markers of gingival inflammation.

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