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Effect of Periodontal Treatment on Reducing Chronic Inflammation in Systemically Healthy Patients With Periodontal Disease

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ABSTRACT

BACKGROUND: We determined the effects and an accurate marker of periodontal treatment on serum interleukin (IL)-6 and high-sensitivity C-reactive protein (HsCRP) levels in systemically healthy individuals with periodontal disease.

METHODS: This multicenter study included systemically healthy individuals with periodontal disease who received initial periodontal treatment and had no periodontal treatment history. Periodontal parameters, including periodontal inflamed surface area, masticatory efficiency, and periodontal disease classification; serum IL-6 and HsCRP levels; and serum immunoglobulin (Ig)G titers against periodontal pathogens were evaluated at baseline and after treatment. Subjects were classified as low or high responders (group) based on periodontal inflamed surface area changes.

RESULTS: There were 153 participants. Only periodontal inflamed surface area changes were markedly different between low and high responders. Periodontal treatment (time point) decreased both serum IL-6 and HsCRP levels. The interaction between group and time point was remarkable only for serum IL-6 levels. Changes in serum immunoglobulin (Ig)G titers against periodontal pathogens were not associated with IL-6 changes in high responders. We analyzed the indirect effect of serum anti-*Porphyromonas gingivalis* type 2 IgG titer changes using mediation analysis and found no significance. However, the direct effect of group (low or high responder) on IL-6 changes was considerable.

CONCLUSIONS: Periodontal treatment effectively decreased serum IL-6 levels, independent of periodontal pathogen infection, in systemically healthy individuals with periodontal disease.

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KEYWORDS: Chronic inflammation; HsCRP; Periodontal inflamed surface area; Periodontal pathogens; Serum IL-6; Systemically healthy patients with periodontal disease

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INTRODUCTION

Chronic serum interleukin (IL)-6 elevation is a risk factor for cancer, cardiovascular disease, psychiatric disease, and autoimmune disease, among others.[1-4] Obesity, ageing, hypertension, dyslipidemia, and sleep disorders cause chronic IL-6 elevation, even in healthy individuals.[5-7] IL-6 promotes C-reactive protein (CRP) production in the liver, and CRP is used as a marker of inflammation.[8-10] High-sensitivity CRP (HsCRP) is used as a reference value for low-grade chronic inflammatory diseases.[11] Both IL-6 and HsCRP have been widely used to evaluate the association of chronic inflammation with target diseases, including metabolic syndrome.[2,12-14]

Periodontal disease is an infectious chronic inflammatory disease caused by periodontal pathogens and is associated with systemic diseases such as diabetes mellitus, pneumonia, cardiovascular disease, rheumatoid arthritis, dementia, and COVID-19 severity.[15-19] To elucidate the role of periodontitis in systemic diseases, the periodontal disease prevalence and periodontal treatment effects in disease groups such as Alzheimer's disease patients or rheumatoid arthritis patients have been evaluated.[20,21] However, to elucidate the true role of periodontitis in systemic diseases, a study involving systemically healthy individuals with periodontal disease is necessary.

Few studies have reported the association between periodontal disease and chronic inflammation in systemically healthy individuals. Although periodontal disease treatment decreases serum IL-6 and HsCRP levels in healthy individuals, a relationship between the periodontal disease severity and IL-6 levels has not been observed,[22,23] probably due to inappropriate assessment of the overall inflammatory burden of periodontal disease. Herein, we used the periodontal inflamed surface area and masticatory efficiency to assess the inflammatory burden of periodontal disease. Periodontal inflamed surface area is a score that quantifies the inflamed surface area in the periodontal pocket; because it includes all remaining teeth, it can assess the burden of periodontitis in the oral cavity.[24] Several studies have reported the association of periodontal disease with systemic diseases using periodontal inflamed surface area;[25-27] however, no study evaluated periodontal disease in healthy individuals. Additionally, a study reported that the chewing efficiency score decreases in severe periodontitis, possibly indicating periodontal tissue inflammation.[28]

We aimed to identify the role of periodontal treatment in systemic inflammation in otherwise healthy individuals. Accordingly, we evaluated the effect of periodontal treatment on serum inflammatory markers in systemically healthy individuals with periodontal disease who were stratified into low and high responders based on periodontal inflamed surface area score changes. We also investigated the association between periodontal disease and systemic chronic inflammation using serum immunoglobulin (Ig)G titers against periodontal pathogens.

CLINICAL SIGNIFICANCE

- The interaction between periodontal treatment and reduced serum interleukin-6 levels in systemically healthy patients with periodontal disease is unclear.
- Periodontal inflamed surface area changes directly decreased serum interleukin-6 levels independent of the periodontal pathogen infection.
- The periodontal inflamed surface area is a novel and appropriate examination tool to understand systemic chronic inflammation in healthy patients with periodontal disease.

MATERIALS AND METHODS

Study Design

This multicenter study recruited systemically healthy individuals with periodontal disease who received initial periodontal treatment from 22 centers in Japan between 2019 and 2023. The study protocol was approved by the research ethics committees of the participating hospitals, and written informed consent was obtained from the subjects. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline.[29]

Participants

The inclusion criteria were as follows: individuals aged ≥20 years with a diagnosis of periodontitis (periodontal inflamed surface area>0), ≥20 remaining teeth, and no systematic periodontal treatment history. Pregnant women; individuals with severe metabolic disease, cardiovascular disease, or renal or hepatic dysfunction; individuals receiving antibiotics or anti-inflammatory drugs for autoimmune diseases; and individuals who received antibiotics within 3 months of initial presentation were excluded.

Laboratory Testing and Serum Antibody Measurement

Serum IL-6 and HsCRP levels were measured at SRL, Inc. (Tokyo, Japan). Serum antibody titers against periodontal pathogens were measured as previously reported.[30] Calibration was performed using pooled serum from 5 healthy individuals (periodontal inflamed surface area = 0). Using serial dilutions of the pooled control serum, the standard reaction was defined in enzyme-linked immunosorbent assay units, with 100 units corresponding to a 1:3200 dilution of the calibrator sample. Serum IgG titers against *Porphyromonas gingivalis* (*Pg*), fimbrie types 1, 2, 3, 4, 5; *Treponema denticola; Tannerella forsythia* (*Tf*); *Aggregatibacter actinomycetemcomitans* (*Aa*) Y4, 295293; *Eikenella corrodens; Fusobacterium nucleatum (Fn)* 25586, 10953; *Prevotella intermedia; Prevotella nigrescens*; and *Campylobacter rectus* were measured.

Periodontal Examination and Diagnosis

For periodontal examination, the tooth circumference was divided into 6 sections, and measurement using a periodontal probe (mm) was performed at one point in each section (a total of 6 points). The presence or absence of bleeding during probing was evaluated. The obtained results were applied to the formula developed by Nesse et al[24] for calculation of the periodontal inflamed surface area. The periodontal treatment effect was assessed by comparing the periodontal inflamed surface area prior to and after treatment. The difference in the periodontal inflamed surface area score was divided by the median, and the top and bottom groups were categorized into high responder and low responder groups, respectively. Masticatory performance was assessed using a test device (Gluco Sensor GS-II, GC Co., Ltd., Tokyo, Japan).[31] The participants were instructed to chew 2 g of gummy jelly for 20 seconds, and the amount of elutriated glucose obtained from a chewed gum sample was quantified using Gluco Sensor GS-II.[31] For periodontal disease diagnosis, the stage and grade were based on the new periodontal disease classification.[30]

Research Schedule

The first visit involved interviews; periodontal tissue examinations; chewing efficiency tests; blood sampling; and IL-6, HsCRP, and serum antibody titer measurements. First, basic periodontal treatment, including plaque control instruction with scaling and root planing, was performed by a periodontal specialist. Then, periodontal inflamed surface area, masticatory efficiency, IL-6, HsCRP, and serum antibody titers were measured.

Statistical Analysis

All statistical analyses were performed using JMP Pro software, ver.16 (SAS Institute Inc., Cary, NC, 1989-2023). IL-6, HsCRP, and serum antibody titers were natural log-transformed for normal or approximately normal distribution. Outliers with IL-6 values of 960 pg/mL and 1570 pg/mL for subjects with chronic periodontitis were treated as errors or false-positive results. Missing data for variables were handled using a missing data model in ordinary least squares analysis. The relationships between baseline IL-6 or HsCRP levels and periodontal inflamed surface area, masticatory efficiency, and periodontal disease classification were evaluated by ordinary least squares adjusted for sex, age, smoking, body mass index (BMI), uncontrolled hypertension, and dyslipidemia as covariates. A color map of multivariate correlations of infectious bacterial strains was created, and their relationship with the systemic infection degree was assessed by clustering. The χ^2 test or t test was used to compare variables between the low and high responder groups prior to and after treatment. The Wilcoxon test was used for non-normally distributed variables. Pre- and post-treatment differences in IL-6 and HsCRP were evaluated using linear mixed models. Within-subject (time points) and between-subject (groups) factors, as well as interactions between time points and groups, were evaluated. Age, smoking, and BMI, which are associated with the markers, were adjusted as fixed effects, while the subject ID and center were included as random effects. For high responders, we analyzed the association between the serum IL-6 decrease and changes in periodontal inflamed surface area or serum IgG titers using a mixed effects model for repeated measures with an unstructured covariance matrix. Fixed effects included age, smoking, BMI, and serum antibody titers against periodontal pathogens at baseline or periodontal inflamed surface area at baseline, while random effects included the subject ID and center. Mediation analysis was performed using a structural equation model, including the change in serum anti-*Pg* type 2 IgG titer as a mediator of the association between a change in periodontal inflamed surface area and the change in IL-6 levels. Assuming a model involving a decrease in periodontal inflamed surface area (X, group) → a decrease in serum anti-*Pg* IgG titer (M, anti-*Pg* type 2 IgG titer change) → a decrease in IL-6 (Y, IL-6 change), the total effect, the indirect effect with M as the mediator, and the direct effect (X → M, M → Y, and X → Y) were measured to test the influence of the mediator. To test the statistical significance of the mediation pattern, a bootstrapped mediation test was performed with 2000 re-samplings and a 95% confidence interval (CI). An α level of .05 was used for all tests.

RESULTS

Supplementary Table 1 (available online) shows the baseline characteristics and laboratory results for all participants (n = 153; female, 64.1%; age: mean, 57.0 years; standard deviation 12.0). The study flowchart is shown in Supplementary Figure 1 (available online). Supplementary Table 2 (available online) shows the associations between baseline IL-6 or HsCRP levels and other examinations. Periodontal inflamed surface area was significantly associated with serum IL-6 levels at baseline (β, 0.191, P = .021); no other parameter was associated with serum IL-6 or HsCRP levels. According to periodontal inflamed surface area changes (T1 [post-treatment] − T0 [baseline]), which indicated the periodontal treatment effects, we divided the subjects into low and high responders. Table 1 presents the characteristics of subjects in the low and high responder groups. Changes in periodontal inflamed surface area were significantly different (low responders: periodontal inflamed surface area change [range], 333.8 [−71.2-583.2]; high responders, 1120.9 [595.0- 3517.7]; $P \leq .001$). The other variables showed no significant differences.

The effect of group (low responders and high responders) and time point (prior to and after treatment) and their interaction (group × time point) were evaluated. Periodontal treatment (time point) decreased both serum IL-6 (B [95% CI], −0.12 [−0.05 to −0.19]; $P \leq .001$) and HsCRP (B [95% CI], −0.18 [−0.27 to −0.08]; $P < .001$) levels. However, a significant interaction was observed between group and time point for serum IL-6 levels (B [95% CI], −0.07 [−0.14 to

Table 1 Characteristics of Participants

	Low Responder (n = 72)	High Responder (n = 73)	*P* Value
Age, mean (SD)	58.5 (12.0)	54.7 (11.7)	.054
Female subjects (%)	46 (64)	49 (67)	.68
BMI, mean (SD)	18.3 (3.1)	17.9 (2.5)	.45
Smoker (%)	8 (11)	12 (16)	.35
Hypertension (%)	4 (< 0.1)	3 (< 0.1)	.68
Dyslipidemia (%)	1 (< 0.1)	0 (0)	.24
Number of teeth, mean (SD)	25.9 (2.8)	26.3 (2.5)	.36
Treatment period (day), median (range)	175.5 (54-488)	203 (50-690)	.29
PISA change (T1 − T0), median (range)	333.8 (−71.2-583.2)	1120.9 (595.0-3517.7)	< .001

BMI = body mass index; PISA = periodontal inflamed surface area; SD = standard deviation.
χ^2 tests or Student's *t* test was used to examine between-group differences.

−0.002]; *P* = .044), but not for serum HsCRP levels (B [95% CI], −0.02 [−0.11-0.08]; *P* = .75) (Table 2).

Supplementary Table 3 (available online) presents the serum IgG titers against periodontal pathogens at baseline. Titers with a median value of <100 were excluded from the analysis; eventually, titers of antibodies against *Pg* types 1, 2, 3, and 5; *Tf;* *Aa* Y4; *Aa* SUNY67; and *Fn* 10953 were analyzed. The color map of the periodontal pathogens demonstrated a strong association with serotype for each pathogen, and a strong correlation was identified between *Tf* and *Fn*, consistent with previous findings[32] (Supplementary Figure 2, available online). Changes in serum IgG titers were not associated with changes in IL-6 in high responders (*Pg*1: B [95% CI], −0.055 [−0.31-0.20]; *P* = .66; *Pg*2, −0.073 [−0.21-0.35]; *P* = .45; *Pg*3, −0.114 [−0.33-0.10]; *P* = .29; *Pg*5, −0.004 [−0.26-0.25]; *P* = .98; *Aa* Y4, −0.011 [−0.24-0.41]; *P* = .84; *Aa* SUNY67, −0.129 [−0.39-0.14]; *P* = .33; *Tf*, 0.171 [−0.14-0.18]; *P* = .77; *Fn*, 0.094 [−0.17-0.07]; *P* = .41), whereas changes in periodontal inflamed surface area were strongly associated with a serum IL-6 decrease (B [95% CI], 0.0005 [5.0 × 10-5-0.001]) (Table 3). This indicated that antibody titer changes did not directly affect serum IL-6 changes.

Therefore, we investigated whether antibody titer changes act as a mediator between periodontal inflamed surface area changes and serum IL-6 changes. We observed a significant relationship between periodontal inflamed surface area and the anti-*Pg* type 2 IgG titer (B [95% CI], 0.363 [0.082-0.645]; *P* = .012) (Supplementary Table 4, available online); periodontal inflamed surface area changes were significantly associated with anti-*Pg* type 2 antibody titer changes (B [95% CI], 0.0005 [0.0001-0.0009]) (Supplementary Table 5, available online). Accordingly, we analyzed the indirect effect of serum anti-*Pg* type 2 IgG titer changes using mediation analysis. This indirect effect (β, [95% CI], 0.017 [−0.010-0.137]; *P* = .29) was not significant, whereas the direct effect of group (low or high responder) on IL-6 changes was significant (β, [95% CI], 0.186 [0.03-0.341]; *P* = .019) (Figure).

DISCUSSION

To our knowledge, this is the first study to show a strong association between periodontal treatment effects (reflected by periodontal inflamed surface area changes)

Table 2 Changes in Serum IL-6 and HsCRP Levels After Periodontal Treatment in Low and High Responders

	Baseline Median (Range)	After Treatment Median (Range)		β (95% CI)*	*P* Value
IL-6, pg/mL					
Median (range)					
Low responders	1.65 (0.5-20.8)	1.3 (0.5-39.85)	Group	−0.05 (−0.15-0.04)	.29
High responders	2.8 (0.4-34.5)	1.3 (0.4-12.7)	Time	−0.12 (−0.19 to −0.05)	<.001
			Time × group	−0.07 (−0.14 to −0.002)	.044
HsCRP, μg/L					
Median, (range)					
Low responders	1960 (50-49,500)	1640 (50-19,400)	Group	−0.14 (−0.35-0.07)	.20
High responders	2060 (100-105,000)	1180 (50-39,200)	Time	−0.18 (−0.27 to −0.08)	<.001
			Time × group	−0.02 (−0.11-0.08)	.75

A mixed effects model was used.
CI = confidence interval; HsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin 6.
*Adjusted with age, body mass index, and smoker as fixed effects. Natural logarithmically transformed values of IL-6 and HsCRP were used for analysis.

Table 3 Post-Treatment Changes in Serum IgG Titers Against Periodontal Pathogens and Serum IL-6 Changes in High Responders

IL-6 change (T1 − T0)	B (95% CI)*	*P* Value
Anti-Pg type 1 IgG change	−0.055 (−0.31-0.20)	.66
Anti-Pg type 2 IgG change	0.073 (−0.21-0.35)	.45
Anti-Pg type 3 IgG change	−0.114 (−0.33-0.10)	.29
Anti-Pg type 5 IgG change	−0.004 (−0.26-0.25)	.98
Anti-Aa Y4 IgG change	−0.011 (−0.24-0.41)	.84
Anti-Aa SUNY67 IgG change	−0.129 (−0.39-0.14)	.33
Anti-Tf IgG change	0.171 (−0.14-0.18)	.77
Anti-Fn IgG change	0.094 (−0.17-0.07)	.41
PISA change	0.0005 (5.0×10^{-5}-0.001)	.030

A mixed effects model for repeated measures was used.

Aa = *Aggregatibacter actinomycetemcomitans*; CI = confidence interval; *Fn* = *Fusobacterium nucleatum*; IL-6 = interleukin 6; *Pg* = *Porphyromonas gingivalis*, fimbrie types 1, 2, 3, 4, 5; PISA = periodontal inflamed surface area; *Tf* = *Tannerella forsythia;*

*Adjusted with age, body mass index, smoker, and baseline serum IgG titers against periodontal pathogens or the baseline PISA as fixed effects.

and serum IL-6 changes in systemically healthy individuals with periodontal disease. This effect was direct and not influenced by the degree of infectivity of the periodontal pathogens.

Initially, we investigated periodontal disease features related to baseline serum IL-6 and HsCRP levels. Consistent with previous findings,[28] the masticatory efficiency score demonstrated a negative correlation with the periodontal disease severity (data not shown). Therefore, we considered that masticatory efficiency may be related to serum IL-6 and HsCRP levels. Only periodontal inflamed surface area could exhibit a strong relationship with IL-6. Only few studies have reported the effects of periodontitis in systemically healthy individuals, and our study confirmed that IL-6 was chronically elevated in these patients. IL-6 is associated with several diseases such as exacerbation of COVID-19,[33] rheumatoid arthritis,[34] and inflammatory bowel disease.[35] Chronic IL-6 elevation is strongly associated with survival in older individuals[36] and individuals with heart disease, including myocardial infarction.[2] Additionally, periodontitis-induced serum IL-6 is associated with brain inflammation and blood−brain barrier disruption in mice.[37] Considering these and the present findings, periodontal disease (elevated periodontal inflamed surface area score) may be an important confounding factor in the study of IL-6-related diseases, lifestyle diseases, and survival among older individuals.

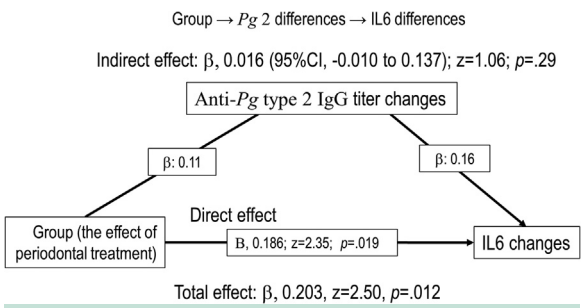

Figure Results of mediation analysis. Mediation analysis was performed to evaluate *Pg* type 2 infection as a mediator of the association between the effect of periodontal treatment (decrease in periodontal inflamed surface area) and decrease in serum IL-6 levels. Assuming a model involving a decrease in periodontal inflamed surface area (X, group) → a decrease in serum anti-*Pg* IgG titer (M, anti-*Pg* type 2 IgG titer change) → a decrease in IL-6 (Y, IL-6 change), the total effect, the indirect effect with M as the mediator, and the direct effect (X → M, M → Y, and X → Y) were measured to test the influence of the mediator. IgG = immunoglobulin G; IL-6 = interleukin 6; *Pg* = *Porphyromonas gingivalis*.

We investigated periodontal treatment effects on serum inflammatory markers in systemically healthy individuals with periodontitis who were classified as low or high responders in periodontal treatment (periodontal inflamed surface area score change). No differences in HsCRP levels due to group effects (periodontal inflamed surface area levels) were observed. Conversely, HsCRP decrease independent of the periodontal treatment effect indicates that the treatment itself may have caused some changes. The interaction between group and time point was remarkable only for serum IL-6 levels. Notably, previous reports verifying the periodontal treatment effect did not identify an interaction between the extent of the treatment effect and serum IL-6 levels.[22,23] Herein, by evaluating periodontal inflamed surface area, we demonstrated that the extent of the treatment effect can reflect serum IL-6 changes in systemically healthy individuals with periodontal disease.

Furthermore, we investigated the mechanisms by which periodontal treatment reduced serum IL-6 levels, with focus on periodontal pathogen titers. Infection was assessed by measuring serum IgG titers against periodontal pathogens, with exclusion of titers <100 (healthy periodontal tissue, periodontal inflamed surface area = 0) from the analysis. Multivariate correlation analysis demonstrated a strong correlation between bacterial serotypes, particularly *Fn* and *Tf*, indicating that serum IgG titers adequately reflect bacterial infection. Linear mixed effects model analysis for high responders demonstrated that, contrary to our expectations, changes in serum antibody titers against periodontal pathogens did not decrease IL-6 levels. To date, no relationship has been reported between periodontal pathogen infection and IL-6 levels; however, chronic inflammatory diseases such as inflammatory bowel disease and intestinal bacterial infection are strongly associated with serum IL-6 levels.[35] Therefore, changes in IgG titers against periodontal pathogens were expected to show a strong association with the decrease, although this was not observed in our study.

In this study, only *Pg* type 2 exhibited a strong association with periodontal inflamed surface area, and the anti-*Pg* type 2 IgG titer change after treatment was dependent on the periodontal inflamed surface area change. Based on these results, we examined whether the anti-*Pg* type 2 IgG titer change mediated the effect of group (low or high responder) on the serum IL-6 decrease. However, we observed a minimal effect that was most likely related to the direct effect of group on IL-6 reduction. Previous reports have shown increased antibody titers against *Pg* in periodontitis.[38,39] Furthermore, upon investigation of the relationship between systemic disease and *Pg*,[40-42] *Pg* infection was observed in many tissues. However, our results demonstrated that the *Pg* decrease due to periodontal treatment did not affect the IL-6 decrease in systemically healthy individuals with periodontal disease. Conversely, our results indicated that the serum IL-6 change was directly dependent on IL-6 production in periodontal tissues, rather than that due to systemic infection with periodontal pathogens.

Limitations

This study lacked a control group without periodontal treatment because of ethical considerations; periodontal treatment cannot be withheld after the diagnosis of periodontal disease. To address this limitation, we mimicked the median decrease in periodontal inflamed surface area in the non-treatment group and separately analyzed low and high responders. Further validation in a prospective cohort study can confirm the potential usefulness of periodontal inflamed surface area. Nevertheless, this study also had some strengths, including a large sample size of systemically healthy individuals with periodontal disease and the evaluation of serum IgG titers against various periodontal pathogens, which helped elucidate a clear association between periodontal disease and serum IL-6 levels in healthy individuals.

CONCLUSIONS

We demonstrated that periodontal treatment, the effect of which was assessed by the change in periodontal inflamed surface area, was strongly associated with the decrease in serum IL-6 levels, independent of infection by periodontal pathogens. Our results suggest that periodontal disease (increased periodontal inflamed surface area) causes chronic systemic inflammation with increased serum IL-6. Therefore, periodontal inflamed surface area may serve as a single marker to evaluate the periodontal treatment effects on the systemic inflammatory condition in healthy individuals with periodontal disease. Further prospective studies are required to determine the cut-off periodontal inflamed surface area value that influences baseline serum IL-6 levels, as well as the degree of change in periodontal inflamed surface area required for decreasing serum IL-6 levels.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amjmed.2023.11.001.

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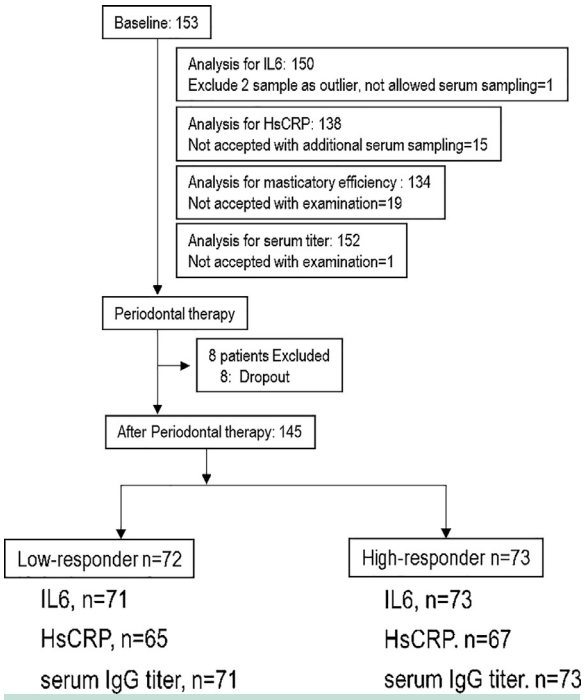
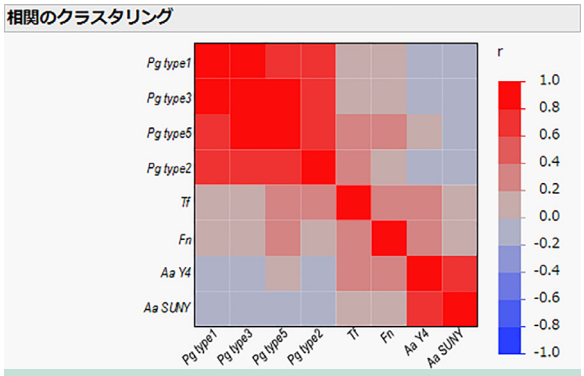


Supplementary Figure 2 Study flowchart.



Supplementary Figure 3 Color map for periodontal pathogens.

Supplementary Table 1 Baseline Characteristics, Periodontal Examination Data, and Serum Levels of Inflammatory Markers

Baseline Demographic and Characteristics		n
Female patients (%)	64.1	153
Age, years (Mean, SD)	57.01, 12.0	153
BMI (Mean, SD)	18.19, 2.8	153
Smoker	13.1	153
Hypertension	4.6	153
Dyslipidemia	0.007	153
Tooth number (Mean, SD)	26.07, 2.7	153
PISA, mm^2 (Median, range)	856, 26-4041.2	153
Masticatory efficiency, mg/dL (Median, range)	153, 30-357	133
HsCRP, μg/L (Median, range)	5740, 50-105000	138
IL-6, pg/mL (Median, range)	1.7, 0.4-34.5	150

BMI = body mass index; HsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; PISA = periodontal inflamed surface area; SD = standard deviation.

Supplementary Table 2 Coefficient Estimates for the Association Between Periodontal Examinations and Serum Inflammatory Markers Using Ordinary Least Squares Analysis

	B (95% CI)*	β	*t* Value	*P* Value
IL-6				
PISA	1.96×10^{-4} (3.0×10^{-5}–3.6×10^{-4})	0.191	2.33	.021
Masticatory efficiency	−0.0015 (−0.036-0.0007)	−0.119	−1.33	.19
Periodontal classification stage 3	−0.015 (−0.389-0.359)	−0.007	−0.08	.93
Periodontal classification stage 4	0.194 (−0.130-0.518)	0.099	1.18	.24
HsCRP				
PISA	2.15×10^{-5} (-3.3×10^{-5}–3.8×10^{-5})	0.011	0.12	.90
Masticatory efficiency	0.003 (−0.001-0.008)	0.137	1.47	.14
Periodontal classification stage 3	0.227 (−0.557-1.011)	0.052	0.57	.57
Periodontal classification stage 4	−0.312 (−0.989-0.365)	−0.080	−0.91	.36

CI = confidence interval; HsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; PISA = periodontal inflamed surface area.

*Adjusted with sex, age, body mass index, smoker, uncontrolled hypertension, and uncontrolled dyslipidemia.n = 150, PISA and periodontal classification for IL-6.n = 133, masticatory efficiency for IL-6 and HsCRP.n = 138, PISA and periodontal classification for HsCRP.

Supplementary Table 3 Serum IgG Titers Against Periodontal Pathogens at Baseline (n = 150)

	Antigen Titer Median	Range
Pg type I	5421.6	125-31,370
Pg type II	329.4	4.2-87,111.7
Pg type III	751.3	12.7-41,628.0
Pg type IV	49.1	4.1-674.6
Pg type V	220.8	3.0-12,332.9
Td	44.5	3.7-18,252.1
Tf	101.9	12.2-955.7
Aa Y4	162.3	17-10,682.1
Aa 29523	92.6	12-69,781.9
Aa SUNY67	176	11.6-620,389.8
Ec	58.7	1.1-447.4
Fn 25586	62.5	0.9-881.5
Fn 10953	133	5.7-78,043.2
Pi 25611	32.8	1.2-883.1
Pn 33563	45.3	1.6-688.3
Cr	55.7	3.1-1,270,638.8

Aa = Aggregatibacter actinomycetemcomitans; Cr = Campylobacter rectus; Ec = Eikenella corrodens; Fn = Fusobacterium nucleatum; Pg = Porphyromonas gingivalis; Pi = Prevotella intermedia; Pn = Prevotella nigrescens; Td = Treponema denticola; Tf = Tannerella forsythia.

IgG titer in periodontal healthy subject (PISA = 0), 100.

Supplementary Table 4 Coefficient Estimates for the Association Between PISA and Serum IgG Titers Against Periodontal Pathogens at Baseline Using Ordinary Least Squares Analysis (n = 150)

	B (95% CI)	*t* Value	*P* Value
Pg type1	0.194 (−0.105-0.493)	1.28	.20
Pg type2	0.363 (0.082-0.645)	2.55	.012
Pg type3	0.084 (−0.228-0.396)	0.53	.60
Pg type5	0.086 (−0.278-0.264)	0.94	.35
Tf	0.060 (−0.111-0.231)	0.7	.49
Aa Y4	−0.077 (−0.288-0.133)	−0.72	.47
Aa SUNY	−0.193 (−0.511-0.125)	−1.2	.23
Fn	0.012 (−0.233-0.256)	0.09	.93

Aa = Aggregatibacter actinomycetemcomitans; Fn = Fusobacterium nucleatum; Pg = Porphyromonas gingivalis; Tf = Tannerella forsythia.

Supplementary Table 5 Coefficient Estimates for the Association Between Changes in Serum Anti-*Pg* Type 2 IgG Titers and PISA Following Adjustment for Covariates, Using a Mixed Effects Model for Repeated Measures

	B (95% CI)	*F*	*P* Value
Age	−0.0080 (−0.028-0.012)	0.64	.43
Smoking [nonsmoking]	0.2700 (−0.069-0.609)	2.48	.12
BMI	−0.0286 (−0.111-0.054)	0.47	.49
Treatment term	0.0011 (−0.001-0.003)	1.65	.20
PISA differences	0.0005 (0.0001-0.0009)	6.64	.011

BMI = body mass index; CI = confidence interval; PISA = periodontal inflamed surface area.