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Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients

Kazuhisa Yamazaki[1], Tomoyuki Honda[2], Taro Oda[2], Kaoru Ueki-Maruyama[2], Takako Nakajima[2], Hiromasa Yoshie[2], Gregory J. Seymour[3]

[1]Periodontology and Immunology, Department of Oral Health and Welfare, Niigata University Faculty of Dentistry, Niigata, Japan, [2]Division of Periodontology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan and [3]Oral Biology and Pathology, School of Dentistry, The University of Queensland, Brisbane, Australia

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Background: Recent epidemiological studies have shown that individuals with periodontitis have a significantly increased risk of developing coronary heart disease. In addition to conventional risk factors, chronic infection and subsequent production of systemic inflammatory markers may be associated with this increased risk.

Objectives: The aim of the present study was to determine whether the presence of chronic periodontitis and subsequent periodontal treatment could influence the serum levels of C-reactive protein (CRP), interleukin-6 and tumor necrosis factor-α (TNF-α) in a Japanese population.

Methods: Sera were obtained from 24 patients with moderate to advanced periodontitis at the baseline examination and at reassessment after completion of treatment. As a control, sera were also obtained from 21 subjects without periodontitis. High-sensitivity CRP (hs-CRP) was measured using nephelometry with a latex particle-enhanced immunoassay and interleukin-6 and TNF-α were determined by sensitive enzyme-linked immunosorbent assay.

Results: The levels of hs-CRP and interleukin-6 in the sera of this Japanese population seemed to be much lower than those reported in other populations. TNF-α on the other hand, demonstrated similar levels between this Japanese and other populations. Periodontal status demonstrated a significant improvement in all patients following treatment. There was a trend toward higher hs-CRP levels in patients at baseline compared with control subjects. Hs-CRP level tended to decrease with improvement of the periodontal condition following treatment and approached that of control subjects, although this decline was not statistically significant. interleukin-6 and TNF-α levels did not change following periodontal treatment. Furthermore, there was no difference in the serum levels of these inflammatory cytokines between patients either at baseline or at reassessment and control subjects.

Dr Kazuhisa Yamazaki, Periodontology and Immunology, Department of Oral Health and Welfare, Niigata University Faculty of Dentistry, 5274 Gakkocho 2-ban-cho, Niigata 951–8514, Japan
Tel: +81 25 227 0744
Fax: +81 25 227 0744
e-mail: kaz@dent.niigata-u.ac.jp

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Conclusions: In this pilot study, we were unable to show that periodontal disease significantly affects the serum levels of systemic inflammatory markers. However, this does not necessarily mean that periodontitis does not contribute to the total burden of inflammation as there was a tendency for hs-CRP to decrease following successful periodontal treatment. Large-scale studies are clearly needed to determine the impact of periodontal disease on systemic inflammation.

The association between periodontal disease and systemic diseases has recently received much attention. Numerous cross-sectional studies have suggested that chronic periodontitis is a possible risk factor for atherosclerosis and subsequent cardiovascular disease (1–3). However, cross-sectional studies do not establish a cause and effect relationship, such that it may merely reflect confounding by common risk factors that are related to both periodontal disease and atherosclerosis, such as smoking, obesity and diabetes.

Increasing evidence suggests that atherosclerosis is a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation (4). As a consequence, markers of inflammation including acute phase proteins such as C-reactive protein (CRP) and serum amyloid A, proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1 and interleukin-6, and soluble forms of intercellular adhesion molecule 1 (sICAM-1) and vascular cell adhesion molecule 1 (sVCAM-1) have been used in terms of risk assessment for cardiovascular disease (5).

CRP is synthesized and secreted mainly by hepatocytes in response to cytokines such as interleukin-6 and has long been considered to be an inflammatory marker, with no specific role in the pathogenesis of atherosclerosis. interleukin-6 production is also stimulated by other cytokines such as TNF-α and interleukin-1. Recent evidence, however, has shown that CRP has a direct proinflammatory effect on human endothelial cells via up-regulation of ICAM-1, VCAM-1 and E-selectin (6). Thus, CRP and CRP-inducing cytokines may be involved indirectly as well as directly in the development of atherosclerosis. As with other infectious agents, periodontal pathogens have been considered to be possible candidate triggers of the inflammatory response in atherosclerosis. Furthermore, it has been proposed that patients with periodontitis may have elevated circulating levels of some of those inflammatory markers (7–10). Based on these, several researchers have attempted to clarify the association between periodontal status and systemic inflammatory markers with ischemic heart disease; however, available data are restricted to Caucasians and are sometimes inconsistent (11–19).

Because of the different genetic background and lifestyle, in particular diet, between Westerners and Japanese, the hypothesis that periodontal infection is a risk factor for atherosclerosis needs also to be verified in Japanese. In order to clarify further the contribution of periodontal disease to the systemic inflammatory response, the aim of the present study was to determine whether the presence of periodontitis and periodontal treatment could influence serum levels of CRP, interleukin-6 and TNF-α in a Japanese population.

Materials and methods

Patients

A total of 24 patients (15 females and 9 males) aged 20–59 (40.5 ± 11.1) with moderate to advanced periodontitis and who were non-smokers were included in order to exclude the confounding effects of smoking. The institutional review board of Niigata University Graduate School of Medical and Dental Sciences approved this study and the written informed consent was obtained from all the patients before inclusion in the study. Periodontal tissue destruction was assessed as described previously (20, 21). Clinical examination included plaque control record (22), probing depths, attachment levels and alveolar bone resorption. Probing depths and attachment levels were recorded at six sites around each tooth. Alveolar bone resorption was measured on the

Table 1. Clinical profiles of the study population

	Periodontitis ($N = 24$)		
	Baseline	Reassessment	Control ($N = 23$)
PD < 4 mm (% sites)	48.3 ± 25.5	87.6 ± 20.0*	99.4 ± 1.3
PD 4–6 mm (% sites)	36.5 ± 16.2	10.9 ± 15.6*	0.6 ± 1.3
PD > 6 mm (% sites)	14.1 ± 15.2	1.5 ± 2.8*	0.03 ± 0.1
CAL < 4 mm (% sites)	38.8 ± 24.9	57.8 ± 26.6*	98.5 ± 3.0
CAL 4–6 mm (% sites)	36.3 ± 16.2	29.2 ± 16.8	1.4 ± 2.9
CAL > 6 mm (% sites)	24.7 ± 22.6	13.0 ± 15.2*	0.03 ± 0.1
Mean bone loss (%)	42.2 ± 16.4	ND	ND
Number of teeth	25.6 ± 4.4	22.7 ± 5.7	27.7 ± 2.0

Data are expressed as mean ± SD.
PD, pocket depth; CAL, clinical attachment level; ND, not determined.
*Significantly different from baseline values at $p < 0.0001$ (Wilcoxon signed matched pairs test).

proximal surface of each tooth on a radiograph (23). All patients had no history of periodontal treatment and had not taken antibiotics within the 3 months prior to the baseline examination. The clinical profile of the study population is presented in Table 1. As a non-diseased control, sera were obtained from staff members of the Niigata University Dental Hospital consisting of 13 females and 10 males aged 29–56 (43.8 ± 6.6). None of these subjects had periodontal pockets, loss of attachment or alveolar bone resorption.

Treatment

Initial therapy consisted of mechanical plaque control, together with scaling and root planing under local anesthesia. The effect of the initial therapy was evaluated and surgical procedures applied for residual periodontal pockets. The number of sites receiving surgical procedures varied from patient to patient. After surgical procedures were completed, the patients were examined and followed monthly or every 3 months depending upon individual requirements. Antibiotics were usually prescribed for 4 days after periodontal surgery. Serum samples were taken at least 3 months after the completion of active therapy (scaling and root planing or periodontal surgery).

Measurement of CRP

Serum high-sensitivity C-reactive protein (hs-CRP) was measured by using nephelometry, a latex particle-enhanced immunoassay (NA Latex CRP kit, Dade Behring, Tokyo, Japan), on a commercial basis (SRL, Tokyo, Japan). The lower limit of the assay was 50 ng/ml. Undetectable CRP values were recorded as 25 ng/ml, halfway between 0 and the threshold of detection (24).

Measurement of serum interleukin-6 and TNF-α

Serum levels of interleukin-6 and TNF-α were determined by sensitive enzyme-linked immunosorbent assay (ELISA) using commercial kits (R & D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The lower limit of detection was 0.016 pg/ml for interleukin-6 and 0.06 pg/ml for TNF-α. The plates were read at a wavelength of 490 nm using an automated ELISA reader (Labsystems Oy, Helsinki, Finland) and analyzed using GENESIS LITE software (Labsystems).

Statistical analysis

Clinical and biochemical parameters at baseline and reassessment in the periodontitis patients were compared using Wilcoxon signed ranked matched pairs test. Clinical and biochemical parameters at baseline or at reassessment of periodontitis patients and those of control subjects were compared using Mann–Whitney *U*-tests. A value of $p < 0.05$ was considered to indicate a significant difference in statistical analyses.

Results

Clinical effect of periodontal treatment

The mean plaque score at the initial examination was 54.6 ± 22.5% and this declined to 15.9 ± 13.4% at reassessment. The condition of the periodontal tissues significantly improved following scaling and root planing and subsequent periodontal surgery. The percentage of sites showing probing depths > 6 mm or 4–6 mm significantly decreased at reassessment compared with that at baseline. In contrast, the percentage of sites showing probing depths < 4 mm significantly increased following treatment. The percentage of sites showing attachment level < 4 mm also increased at reassessment (Table 1).

Change of high sensitivity CRP, interleukin-6 and TNF-α levels in sera of periodontitis patients

As shown in Fig. 1, median serum CRP levels were 317.0 ng/ml and 261.5 ng/ml at baseline and reassessment, respectively. For interleukin-6, the median values at baseline and reassessment were 0.70 pg/ml and 0.69 pg/ml, respectively (Fig. 2). The median TNF-α level in the serum of periodontitis patients was 1.81 pg/ml at baseline and 1.59 pg/ml at reassessment (Fig. 3). There were no statistically significant differences at baseline and reassessment for all these markers. However, only hs-CRP level demonstrated a tendency to decrease following treatment ($p = 0.087$).

CRP, interleukin-6 and TNF-α levels in sera of control subjects

The median values of CRP, interleukin-6 and TNF-α in control subjects were 195.0 ng/ml, 0.56 pg/ml and

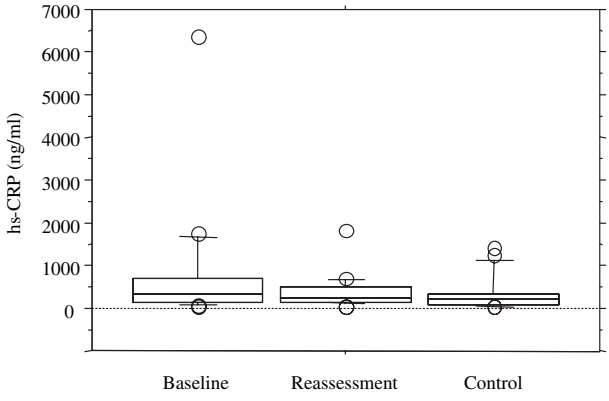

Fig. 1. Serum concentration of high sensitivity C-reactive protein (hs-CRP) of periodontitis patients at baseline and reassessment and control subjects. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as open circles. No significant difference was observed either between baseline and reassessment (Wilcoxon signed matched pairs test) or between periodontitis patients at baseline and control subjects (Mann–Whitney *U*-tests).

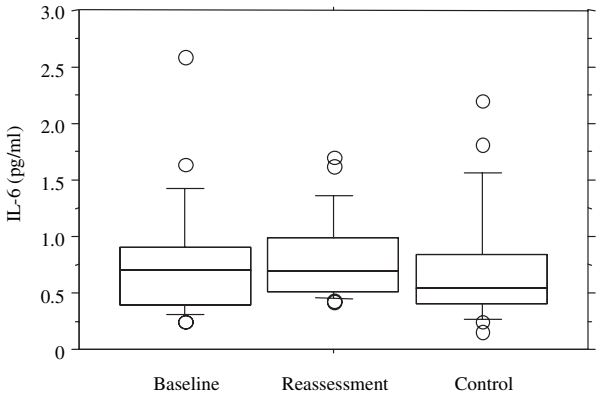

Fig. 2. Serum concentration of interleukin-6 (IL-6) of periodontitis patients at baseline and reassessment and control subjects. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as open circles. No significant difference was observed either between baseline and reassessment (Wilcoxon signed matched pairs test) or between periodontitis patients at baseline and control subjects (Mann–Whitney *U*-tests).

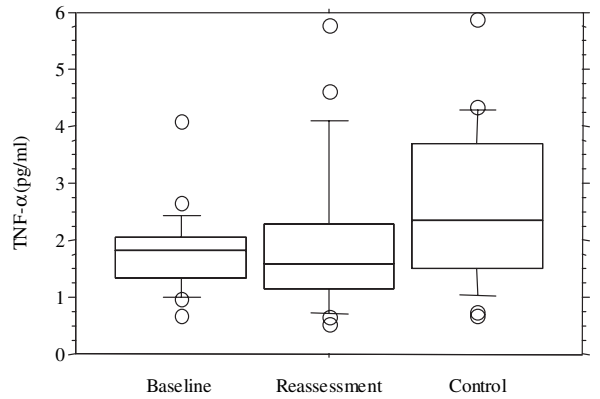

Fig. 3. Serum concentration of tumor necrosis factor-α (TNF-α) of periodontitis patients at baseline and reassessment and control subjects. Box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. Outside values are shown as open circles. No significant difference was observed either between baseline and reassessment (Wilcoxon signed matched pairs test) or between periodontitis patients at baseline and control subjects (Mann–Whitney *U*-tests).

2.35 pg/ml, respectively. All of these markers demonstrated no significant differences between control subjects and periodontitis patients at either baseline or reassessment (Figs 1–3). The difference in hs-CRP level between patients and control subjects demonstrated a trend towards higher levels in patients at baseline ($p = 0.056$). However, this difference declined following treatment ($p = 0.138$).

Discussion

In addition to the conventional risk factors for atherosclerosis, such as hypertension, hyperlipidemia and diabetes, the role of inflammatory mediators is intrinsic to the concept of atherosclerosis as a chronic inflammatory process (4). Therefore, serum markers of inflammation provide a possible insight into the pathophysiology of atherosclerosis and its complications. High-sensitivity CRP has received much attention and several studies support a strong link between baseline elevations of hs-CRP and future risk of coronary events (25, 26). Other proinflammatory cytokines such as interleukin-6 and TNF-α are also important in the inflammatory process. interleukin-6 provokes the augmented expression of CRP gene in the liver, and TNF-α can in turn stimulate the expression of interleukin-6. Prospective epidemiological studies have found an increased vascular risk in association with increased basal level of these cytokines (27–29). The stimuli that initiate and sustain the inflammatory process in atherosclerosis have not been fully identified. However, chronic infections with a range of organisms such as *Chlamydia pneumoniae* and cytomegalovirus have been postulated (30, 31). Although it has not been clarified as to how periodontal infection contributes to the development of atherosclerosis and subsequent life-threatening diseases, it is reasonable to assume that the periodontitis lesion is a potential source of inflammatory cytokines.

A number of reports have demonstrated elevated levels of inflammatory cytokines in gingival crevicular fluid from periodontitis-affected gingiva compared with non-diseased gingiva (32). These clearly suggest that infection with periodontopathic bacteria can induce a strong inflammatory response in the periodontium. However, it has not been fully elucidated as to how much the local inflammatory response in the periodontium can influence the systemic levels of inflammatory mediators. Clearly if periodontal infection does contribute significantly then it would follow that periodontal treatment could result in a reduction of systemic inflammation, hence decrease the risk for atherosclerosis. Several reports have demonstrated a statistically significant increase in serum CRP levels in periodontitis patients compared with non-diseased control subjects (11–13, 19). However, whether this increase in the serum CRP levels in periodontitis patients is in fact associated with periodontal infection has not been clarified. Only one report, by Noack *et al.* (13), showed that the extent of increase in CRP levels in periodontitis patients depends on the severity of the disease and that elevation of CRP was associated with the presence of periodontopathic bacteria.

In the present study we failed to demonstrate that inflammatory periodontal disease contributed to elevated serum TNF-α and interleukin-6, nor

were we able to demonstrate that periodontal treatment *per se* significantly improved these systemic inflammatory markers. However, it should be noted that serum hs-CRP levels of patients tended to be higher at baseline compared with that of control subjects ($p = 0.056$) and declined at reassessment, suggesting that periodontal treatment did lead to a reduction in systemic inflammation to some extent, albeit not statistically significant. This lack of statistical significance may reflect the various contributions played by periodontal disease to the total burden of inflammation in different patients and the relatively small numbers of patients. However, in the present study, patients with moderate as well as severe periodontitis were included and there was no relationship between severity of periodontitis at baseline and hs-CRP levels. These findings are consistent with those of Ide *et al.* who demonstrated that there were no statistically significant changes in the levels of interleukin-1β, interleukin-6, TNF-α and CRP in sera in spite of periodontal treatment and the subsequent improved plaque and bleeding scores and reduced probing depths (18). Mattila *et al.* also examined the effect of periodontal treatment on the serum levels of CRP and demonstrated that the median baseline CRP level of 1.05 mg/l decreased to 0.7 mg/l after periodontal treatment (17). However, as only six out of 30 patients showed higher levels of CRP than that of the 95th percentile limit of the Finnish population, they concluded that periodontitis seems to increase CRP only in some individuals (17). As the number of patients and controls in the present study is relatively small, the distribution of CRP levels in this study population needs to be compared with those in a large-scale study of the Japanese population, such as the study by Yamada *et al.* (24). In this large study (24), the distribution of serum CRP level was highly skewed to lower levels and ranged widely. The minimum value was less than 30 ng/ml and the maximum value was 68.2 μg/ml, with 25th, 50th and 75th percentile values of less than 30, 120 and 300 ng/ml, respectively. Yamada *et al.* nevertheless concluded that serum CRP levels correlated with atherosclerotic risk factors in the Japanese as well as in Westerners (24). However, the distribution of CRP in their study was quite different from that of previous studies, suggesting that Japanese have much lower CRP levels than populations of other developed countries. The levels of CRP seen in the present study are similar to but slightly higher than those of the above study.

Loos *et al.* showed that a higher frequency of plasma interleukin-6 levels occurred in localized periodontitis patients compared with controls and that plasma interleukin-6 levels were also higher in periodontitis patients (median 0.46 pg/ml) compared with controls (median 0.20 pg/ml) (12). However, it should be noted that the lower limit of detection of their assay was 0.4 pg/ml, which is higher than the median value of controls. Ide *et al.* also measured serum interleukin-6 levels before and after treatment and showed that there was no statistically significant change (18). The subjects in their study were divided into two groups that received either immediate periodontal treatment or periodontal treatment following a 3-month non-treatment phase. Our study group did not have a non-treatment phase and is therefore comparable to the immediate treatment group of Ide *et al.* in which median interleukin-6 levels at baseline and reassessment were 1.56 and 1.96 pg/ml, respectively. In the present study, the median interleukin-6 levels at baseline and reassessment in periodontitis patients were 0.70 pg/ml and 0.69 pg/ml, respectively, and 0.56 pg/ml in control subjects, which are again much lower than those in Westerners but comparable to other reports of the Japanese population (33). interleukin-6 has been demonstrated to increase the expression of adhesion molecules in human umbilical vein endothelial cells at 10 pg/ml (34). However, the biological effect of these low levels of interleukin-6 and the role of periodontal infection in the systemic levels of interleukin-6 remained to be clarified.

As with interleukin-6, median TNF-α levels in periodontitis patients were not different from those of the control subjects and the effect of treatment was obscure. TNF-α is also reported to up-regulate the adherence of lymphocytes to \endothelial cells at a concentration of 10 pg/ml (34). Although the level of TNF-α was variable among the subjects irrespective of presence or absence of periodontitis, none of the subjects showed high levels of TNF-α, the highest being 5.9 pg/ml. In contrast to CRP and interleukin-6, TNF-α levels were similar to those reported by Ide *et al.* (18).

Although there are limitations in the present study in which the number of subjects is relatively small and the data for conventional atherosclerotic risk factors are lacking, our data showed that serum hs-CRP level tended to be higher in untreated periodontitis patients compared with periodontally healthy subjects and demonstrated concomitant reduction of hs-CRP with successful periodontal treatment. However, the serum levels of TNF-α and interleukin-6 in periodontitis patients are not different from those in non-diseased control subjects and, even though successful, periodontal treatment had little effect on these markers. As the population-based CRP and interleukin-6 levels are different between Japanese and Westerners, simple comparison of the present study with others may be misleading. Nevertheless, as CRP and interleukin-6 are important atherosclerotic risk factors in Japanese, further large-scale and longitudinal studies are clearly needed to clarify the relationship between periodontal infection and atherosclerosis, especially in terms of systemic inflammatory markers.

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