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Original Research

The Reduction in Serum Inflammatory Markers by Periodontal Therapy

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ABSTRACT

Background: Periodontitis is associated with elevated inflammatory markers in otherwise healthy populations. The nature of this association has not been determined.

Aim: Our aim was to determine if periodontal therapy would result in decrease in serum markers of systemic inflammation.

Material and methods: Fifty systemically healthy subjects with severe generalized periodontitis participated in a prospective twelve-month blind intervention trial. Periodontal parameters and inflammatory markers (leukocytes-Le, C-reactive protein-CRP, and Fibrinogen-FIBR) were evaluated prior to 6, 12 and 24 months after delivery of standard non-surgical periodontal therapy.

Results: Six, twelve and 24 months after treatment, significant reductions in serum inflammatory markers were observed. Decreases in inflammatory markers were significant in subjects with above average clinical response to periodontal therapy after correction for possible confounders.

Conclusion: Periodontitis may add to the systemic inflammatory burden of affected individuals and periodontal therapy decrease in serum markers of systemic inflammation.

Key words: periodontitis, inflammation, inflammatory markers

INTRODUCTION

Chronic periodontitis (PD) is the most frequent form of the disease characterized by an inflammation of the tissues that support the teeth. Periodontitis results in destruction of the periodontal ligament and loss of the adjacent bone that support the teeth. [1] The clinical signs are seen in early middle age. It is a chronic, tissue-destructive inflammation. The more severe form of the disease is present in approximately 10-15% of an adult population, whereas 35% exhibit moderate signs of the disease. [2]

Recent studies have proven that periodontal disease can produce numerous changes in systemic health. Changes in the blood chemistry raise the inflammatory markers in the serum.[3] More recent evidence has indicated that patients with severe periodontitis have increased levels of inflammatory markers (C reactive protein-CRP, fibrinogen-FIBR, leukocytes-LE) when compared with unaffected control population.[4-6] In periodontitis patients, elevated serum inflammatory markers were associated with high levels of infection with periodontal pathogens.[7] These dates have received considerable attention indicating that periodontitis was the cause for the observed serum acute phase response, and that periodontitis play a role in the etiologic pathway of systemic inflammatory disease such as atherosclerosis and cardiovascular disease. Atherosclerosis is also very common and starts early in life, however, since disease progression is usually slow, clinical symptoms or hospitalization are rare before 40 years of age.[8]

Several studies over the last decade have suggested that development of coronary heart disease is related to various types of oral infections.[9,10]

Periodontal infections, in particular, have been suspected as a risk factor for cardiovascular disease[11],control of type II diabetes.[12] Experimental models have indicated that chronic infection with periodontal pathogens leads to thickening of the carotid intimae.[13] Several serological studies[14,15] support a direct relationship of pathogens, etiologically linked to periodontal disease with increased risk for subclinical, prevalent, and future cardiovascular diseases. Independently of the underlying mechanisms, systemic inflammation seems to be central for explaining the nature of the link between chronic infections and atherosclerosis.[16] The inflammatory markers are usually elicited by an inflammatory stimulus and mediated through a complex network of cytokines and have also a significant role as a predictor for future coronary events in healthy populations.[17]

The aim of this pilot intervention study was to assess whether individual response to periodontal treatment was associated with changes in serological markers of systemic inflammation in otherwise healthy individuals.

MATERIAL AND METHODS

The study was a prospective, pilot intervention study with 24-months follow-up (2009-2011). Participants were recruited from subjects referred to the Department of Periodontology of the Medical Faculty, University of Nis. Subjects presenting with generalized, severe periodontitis (pocket depths greater than 5 mm, and alveolar bone loss than 30%) were invited to participate in the study. This level of disease severity was chosen to increase probability of detection of a systemic burden from the local periodontal infection. The periodontitis group (a test group) was composed of 50 subjects, (27 men, 23 women; group mean age 46.5 years) who received periodontal treatment. The healthy, non-periodontal group (comparison group for inflammatory markers) comprised 25 subjects (7 men, 18 women; group mean age 41.5 years) none of whom had clinical signs of periodontal disease as measured by pocket depths over 5 mm or any clinical attachment loss.

Patients were excluded from the study if they had a known systemic disease (cardiovascular disease), presence of other infections, systemic antibiotic treatment in the preceding 6 months, and treatment with any medication known to affect the serum levels of inflammatory markers, and pregnant or lactating females. Smokers and patients with diabetes were also excluded. All patients gave informed consent and the study had been approved by the Ethics Committee of the Medical Faculty in Nis (No: 01-2800-5).

A baseline visit was conducted by a examiner who collected a complete medical and dental histories, socioeconomics, and health behavior, standard clinical periodontal parameters[18] and blood samples. Patients underwent a comprehensive periodontal examination including radiographs and the oral health status was verified by clinical examination.

Gingiva was evaluated, and the depth pocket was measured using a calibrated periodontal probe. Periodontal probing depth (PPD) is the distance between the gingival margin and the bottom of pocket measured for the four sides of each tooth. Mean pocket depth was ≥ 5 mm. Clinical attachment level (CAL) was computed like difference between depth pocket and cemento-enamel junction measured on six sites of each tooth. Mean attachment loss was ≥ 3mm. The dental plaque (PLI) was made visible by gently moving the tip of the probe along the gingival margin of the four sides of each tooth. Gingival inflammation was noted as bleeding on probing (BOP).

Patients underwent a standard phase of non-surgical periodontal treatment that was performed by a periodontist. All treatment consisted of oral hygiene instructions and subgingival scaling and root planning with the manual instruments. The therapeutic phase was completed within 2 months of the baseline visit. Periodontal parameters and serum markers of systemic inflammation were measured before and after periodontal therapy. Patients were re-examined at 6, 12 and 24 months after the completion of the treatment.

Venous blood samples were collected at different time points (baseline, 6, 12 and 24 months) and processed and analyzed in a standardized fashion. Concentration of biochemical inflammatory markers (CRP, fibrinogen, leukocytes) was measured using automated analyzers according to Biochemical Laboratory in Medical Clinic Center in Nis.

Statistical analysis

Changes in serum concentration of CRP, fibrinogen end leukocytes following periodontal therapy were used as the primary outcome variables. All data are expressed either as mean±std, or with frequencies and percents. Differences of mean values between groups were compared using Student' t-test. Chi Square test was used as nonparametric test. Differences between means obtained before and after periodontal treatment were proved for significance using ANOVA analysis the for paired samples. P values below 0.05 were considered to indicate significance.

RESULTS

Baseline demographic characteristics of the 50 patients (with periodontitis) and comparison group of 25 subjects (free of periodontitis) are given in Table 1 and 2

Table 1. Gender and age structure of examiners

Variables	**Periodontitis group (n=50)**	**Control group (n=25)**	**p-value**
Gender, N°, %			
male	27 (54 %)	11 (48 %)	t=8.715
female	23 (46%)	14 (52%)	p<0.05
Ages, mean±std	46.5±15.83	43.50±5.76	t=9.789 p<0.001

Examiners were 48.76±15.83 yrs of age, 54% were males and 46% women. In control group examiners were 42.80± 5.76 yrs of age, and 28% were males and 18% women.

Table 2. Characteristics of examiners with and without periodontitis

Variables	**Periodontitis group (n=50)**	**Control group (n=25)**	**p-value**
Education			
<12 yrs	32 (64%)	8 (32 %)	t=7.166
>12 yrs	18(36%)	17(68 %)	p<0.05
Social status, N°, %			
bad	33 (66%)	7 (32%)	t=7.166
good	17 (34%)	18 (68%)	p<0.05
Physical activity, N°, %			
Active	15 (30%)	6 (24%)	t=91.244
Moderate	35(70%)	19(76%)	p<0.05
BMI, kg/cm^2	19.0-33.5	17.8-35.9	t=15.784
mean±std	26.04±3.38	22.08±4.10	p<0.001

Footnote:
BMI – body mass index

Examiners were middle aged. Basic education and social status were lower in test group vs. control group. Patients in the test group exhibited a lower working capacity. Their average body mass index was 26.04±3.38 kg/m^2 (p< 0.001). During the 24-month study period, none of the examiners reported any change in diet, medication or any habits. The analysis of primary interest is the multiple logistic regressions since this is adjusted for age, gender variables. Significant association was between periodontitis and body mass index.

Table 3. Characteristics of clinical periodontal parameters before therapy

Variable	**Periodontitis group (n=50)**	**Control group (n=25)**	**p-value**
PLI	1.64±0.53	0.52±0.42	t=58.650
BOP	1.76±0.45	0.40±0.38	t=86.971 p<0.001
PPD, mm mean±std	4.68±1.11	1.90±0.55	t=94.454 p<0.001

Periodontal analysis showed the presence of dental plaque 1.64±0.53 vs. 0.52±0,42; bleeding on probing scores were 1.76±0.43 vs. 0.40±0.38. Periodontal pocket depth was in 66% cases <5 mm and in 34% = 5mm. Mean value pocket depth was 4.68±1.11mm and clinical attachment loss was 5.72±0.96mm.

Table 4. Clinical periodontal parameters 6, 12 and 24 months after therapy in the test group

Variable	Before therapy (0)	After 6 months	After 12 months	After 24 months	p-value
PLI	1.64±0.53	0.58±0.60	0.81±0.46	0.84±0.63	F=34.536 p<0.001
BOP	1.76±0.45	0.54±0.39	0.42±0.46	0.42±0.37	F=124.91 p<0.001
PPD, mm mean±std	4.68±1.11	3.66±0.69	3.13±0.57	2.97±0.53	F=51.472 p<0.001
CAL, mm mean±std	5.72±0.96	4.78±0.69	4.27±0.57	4.11±0.53	F=52.190 p<0.001

PLI – plaque index, **BOP** – bleeding on probing, **PPD** – periodontal pocket depth, **CAL** – clinical attachment loss

Therapy was highly effective (p<0.001) for the all analyzed clinical parameters. The clinical results showed that the mean values of sites with bleeding on probing, periodontal probing depth, and clinical attachment loss were significantly reduced in the test group 6 to 24 months after periodontal treatment. Levels of inflammatory markers at baseline and after treatment are summarized in tables 5 and 6.

Table 5. Markers of systemic inflammation before therapy

variable	Periodontitis group (n=50)	Control group (n=25)	p-value
CRP (mmol/l)	6.69±5.69	1.24±1.46	t=11.207 p<0.001
FIBR (g/l)	4.78±2.12	2.85±2.20	t=21.860 p<0.001
LE (10^9/l)	8.74±3.03	7.07±2.16	t=7.165 p<0.005

CRP – C-reactive protein, **FIBR** – fibrinogen, **LE** – leukocyte

Table 6. Values of inflammatory markers 6, 12 and 24 months after therapy in the test group

Variable	Before therapy (0)	After 6 months	After 12 months	After 24 months	p-value
CRP, (mmol/l)	6.69±5.69	5.07±3.65	4.94±4.21	4.25±2.63	F=3.030 p<0.05
FIBR, (g/l)	4.78±2.12	4.37±1.56	4.13±1.23	4.08±1.04	F=2.081 p>0.05
LE, (10^9/l)	8.74±3.03	7.41±1.62	7.22±1.75	6.86±0.93	F=8.583 p<0.001

CRP – C-reactive protein, **FIBR** – fibrinogen, **LE** – leukocyte

Among the tested inflammatory markers, CRP levels, fibrinogen concentration and leukocytes count were higher in the periodontitis group at baseline compared to the controls.

The baseline median CRP level was 6.69±5.69mmol/l, fibrinogen level was 4.78±2.12 g/l and leukocytes count 8.74±3.03 (Table 5). Statistically significant reduction in CRP levels, fibrinogen concentration and leukocytes count achieved after treatment. Median serum CRP level was decreased from baseline value of 6.69mmol/l to 4.25mmol/l. After treatment, the median serum fibrinogen was decreased to 4.08g/l. Leukocytes count was also decreased to 6.86x10^9/l after periodontal treatment. Significant correlations between CRP and both bleeding on probing and the periodontal pockets depth were also apparent.

A trend towards an association between periodontitis and increases in CRP and fibrinogen was also apparent (Table 5). Difference was statistically significant between baseline and 6, 12 and 24 months for all inflammatory markers, after periodontal therapy. Improvements in serum inflammatory markers were: the median change in CRP levels between baseline and 24 months was 2.44mmol/l, in fibrinogen concentration was 0.70g/l. Corresponding values for leukocytes were 1.88x10^9/l.

Further analysis with a repeated measure indicated that there was a significant association between the clinical outcomes of periodontal treatment and the serum inflammatory marker levels at different time points of treatment. This was significant after correction for other known covariates, such as age, gender and body mass index.

DISCUSSION

Periodontitis has been traditionally regarded as a chronic inflammatory oral infection and this oral disease may have profound effects on systemic health. In terms of the potential relationships between periodontitis and systemic disease, it is inflammatory markers that cause changes through mechanisms involving periodontitis. All hematological parameters were higher in the patients with periodontitis than patients in control group without periodontitis.

Our prospective study provides important evidence that near-complete elimination of periodontal infection by comprehensive local periodontal therapy was associated with a significant decrease in serum concentration of inflammatory markers in otherwise healthy individuals affected with severe, generalized periodontitis. This study showed an improvement in levels of serum inflammatory markers in patients who responded better to periodontal therapy.

The observed decreases in CRP were significantly

correlated with changes in fibrinogen and leukocytes. Significant decreases in CRP, fibrinogen and leukocytes were already observed 6 months after therapy, with trend decreases to 24 months.

These data indicate that periodontitis contributed to the systemic inflammatory responses in these patients, and that periodontal therapy is critical in the context of the design and implementation of a definitive trial.

Analysis of these data confirms previous observations that otherwise healthy subjects suffering from chronic periodontitis display a moderate increase in systemic inflammation.[19] Periodontitis results in higher systemic levels of CRP, fibrinogen and leukocytes. Results from a recent study suggest that destructive periodontal disease is associated with changes in serum components consistent with an acute-phase response.[20] In addition, patients with severe periodontitis had significantly higher CRP levels than patients with moderate periodontitis and both were significantly higher than the control patients.[21] In agreement with previous studies [22-24] analysis of recent data have indicated that CRP levels in the upper quartiles of normality are good predictors of future coronary events in healthy populations.[25] Moreover, systemic markers of inflammation, fibrinogen and leukocytes count are also predictors of present and future cardiovascular event and disease.[26]

A tendency towards an association between periodontitis and high levels of inflammatory markers (CRP, fibrinogen, leukocytes) is important, as inflammatory markers which are accepted like risk factors for atherosclerosis and also as predictors of present and future cardiovascular event and disease. Sustained elevations of serum inflammatory markers may have a serious negative impact on systemic health.

Our study showed significant correlation between inflammatory markers levels and the degree of gingival inflammation after therapy. Our observation that serum inflammatory markers are decreased by periodontal therapy is consistent with the finding of D'Aiuto et al [27], who measured serum inflammatory markers in 94 subjects before and after nonsurgical therapy for severe generalized periodontitis. Several evidences support the causative role of chronic infection and inflammatory responses in atherosclerosis. In the majority of studies, subjects with severe generalized periodontitis displayed increased odds ratios of cardiovascular events. Reports from prospective studies associated periodontitis with an increased risk for carotid atherosclerosis after correction for possible confounding factors. [28]

Periodontitis is an infection caused by Gram-negative bacteria that are organized in a biofilm in a subgingival location. Transient and recurrent bacteremia, which may be caused by periodontal infection, induce an intense local and systemic inflammatory response, leading to changes in the whole body. Periodontitis is believed to cause low, but long-lasting, systemic inflammation reaction, which in turn contributes to the development of atherosclerosis.[29] As such, its treatment requires, in the first instance, the removal of the biofilm by mechanical professional instrumentation. Following successful treatment, bacterial load is significantly reduced, and local inflammation significantly decreases, and there is a significant improvement of the clinical parameters (bleeding on probing, periodontal pocket depths, plaque index) (Tab. 4). In this study, periodontal treatment decreases the infection burden and periodontal inflammation as assessed by clinical parameters.

In agreement with previous observations, analysis of the data indicate that serum levels inflammatory markers were associated with a variety attributes as age, gender, education, social status, physical activity and body mass index of the examiners. Like a previous study [30] body weight was also significantly higher in patients with periodontitis. Traditional factors of cardiovascular risk, such as age, diabetes, lifestyle, tobacco use, obesity as well as novel risk factors, such as inflammation are presents and coexist in many periodontal patients. The

association between periodontitis and atherosclerosis may be because of common risk factors such as smoking, diabetes mellitus, aging, male gender and socioeconomic factors, but there is also good evidence of periodontitis being an independent risk factor of cardiovascular disease.[31] This finding most likely reflects different lifestyles of the subject group, as patients performed less well in the exercising test. These findings are in agreement with the hypothesis that the periodontal burden (infection and inflammatory responses) may play a role in systemic inflammatory disease such as atherosclerosis.

Short-term studies monitoring surrogate markers of cardiovascular disease, i.e. biochemical risk factors, have appeared to be very helpful in obtaining future evidence for periodontitis as an independent cardiovascular disease risk factor. In these studies, established risk factors of atherosclerosis, such as increased serum levels of CRP, fibrinogen and leukocytes were significantly decreased after (up to 24 months) local therapy of periodontitis. These finding are in accordance with studies of other authors.[32-34]

CONCLUSION

In summary, non-surgical periodontal therapy was effective in improving periodontal clinical data and in reducing the serum levels of CRP, fibrinogen and leukocytes in patients with severe periodontitis. Periodontitis seems to contribute to systemic inflammation. The potential significance of the reported findings relates to the magnitude of the observed significantly decreased plasma concentrations of the biochemical inflammatory cardiovascular disease risk markers after periodontal therapy, and the fact that periodontitis can be treated.

Our finding that periodontal therapy improves periodontal disease and decrease inflammatory markers is consistent with a possible causative role for periodontal disease in the pathogenesis of atherosclerosis, perhaps mediated through systemic inflammation.

A practical conclusion that can be drawn from the studies is that periodontal therapy may reduce other cardiovascular risk factors, and that has the systemic benefits. Patients with known risk factors should be examined for periodontitis. A conclusion could provide an easy and inexpensive benefit to both periodontal and cardiovascular health.

Therefore, treating inflammation may not only help manage periodontal diseases but may also help with the management of other chronic inflammatory conditions.

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