Original Article

The Effect of Nonsurgical Periodontal Treatment on Serum and Gingival crevicular fluid markers in patients with atherosclerosis

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Abstract

Background and Aims: The aim of this study is to compare patients with atherosclerosis and chronic periodontitis and patients who are systemically healthy and chronic periodontitis using alteration of adrenomedullin (ADM), chemokine (C-C motif) ligand 28 (CCL-28), white blood cell levels, platelet levels, high-density lipoprotein, low-density lipoprotein, high-sensitivity C-reactive protein, creatinine, and fibrinogen. **Materials and Methods:** Totally, 40 patients were involved in study; a test group of 20 patients with atherosclerosis-chronic periodontitis and a control group of 20 patients who were nonatherosclerosis-chronic periodontitis. Nonsurgical periodontal treatment was offered to all patients, in whom systemic markers of atherosclerosis were measured in serum; ADM and CCL-28 biomarkers were measured in gingival crevicular fluid. **Results:** Systemic markers of atherosclerosis, ADM, and CCL-28 levels have changed significantly in the test group compared to the control group after nonsurgical periodontal treatment. **Conclusions:** Treatment of local inflammation and reduction of systemic inflammatory markers are believed to lower the diagnostic criteria for atherosclerosis as well. It is possible to conclude that nonsurgical periodontal treatment of chronic periodontitis, which is a risk factor for atherosclerosis, has a positive effect on the atherosclerosis prognosis.

Keywords: *Adrenomedullin, atherosclerosis, chemokine (C-C motif) ligand-28, chronic periodontitis*

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Introduction

Antimicrobial peptides reach high levels in the event of infections and they are helpful in preventing the reproduction of many microorganisms.[1] Effects of antimicrobial peptides include increasing membrane permeability, affecting the cytoplasmic contents, inducing cell apoptosis, preventing the division, and inhibiting the functioning of cell.[2] Among these peptides, adrenomedullin (ADM) is known to act as a vasodilator hormone at certain concentrations in various tissues, particularly in blood. ADM is also reported to have functions on regulation of angiogenesis, regulation of damage related to stress and hypoxia, and extension of oxidative cell tolerance through its other effects.[3,4] Chemokine (C-C motif) ligand 28 (CCL-28) is pointed out as an antimicrobial peptide. CCL-28 has a role in angiogenesis. It also participates in guidance of cells in wound healing due to infection. CCL-28 has a homeostatic effect, also responsible for basal leukocyte migration and is produced structurally in certain tissue.[5]

Cardiovascular disease (CVD), correlation based on an underlying inflammation of chronic periodontitis.[6] For the purpose of identifying the CVD-associated risks; studies are conducted on biological mediators such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), and high-sensitivity C-reactive protein (hs-CRP).[7]

Atherosclerosis is the most common type of CVDs. Many systemic and/or local factors are engaged in atherosclerosis formation in addition to low level chronic inflammation, which was shown to play a role in progression of the disease.[8] Presence of microorganisms which was important for periodontal diseases was determined in the examinations of atheroma plaque. It

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is known that dental and periodontal infections may underpin atheroma plaque formation and development of CVD.[9] The connection between periodontal inflammation and CVD can be proven by circulation of pro-inflammatory mediators such as certain bacterial endotoxins and specific cytokines in blood stream, which cause the exposure of acute-phase reactants leading to increased inflammatory activity in atherosclerotic lesions.[10] The most advocated hypothesis to explain of the correlation between periodontal disease and CVD is systemic inflammatory process.[11]

In our study, based on the information which have investigated the correlation between periodontal diseases and CVD, the aim of the study was to compare patients with atherosclerosis and chronic periodontitis to patients who are systematically healthy and chronic periodontitis with regards to difference in periodontal clinical indices; alteration of ADM and CCL-28 markers as measured in gingival crevicular fluid (GCF); and change in levels of white blood cell (WBC) levels, platelet levels (PLT), LDL, HDL, creatinine, fibrinogen, and hs-CRP markers in serum samples.

Materials and Methods

Subjects

A total of 40 patients, between ages of 35 and 65 involving patients who applied to Department of Periodontology, Faculty of Dentistry of the Yüzüncü Yıl University (YYU) with oral and dental complaints, primarily periodontal complaints, were admitted to this study. Patients included in the study were divided into two groups. Once clinical periodontal examinations are completed, 20 patients among atherosclerosis patients who had chronic periodontitis were involved into the study as the test group. On the other hand, 20 patients who had been diagnosed to have chronic periodontitis, but were healthy in cardiovascular aspect as verified by necessary checks served as the control group.

We paid attention that patients who were involved in control group shall have no systemic disease, have not used any antibiotics or medications with an impact on the immune system, or have not received any periodontal and/or medical treatment within the last 6 months. We also cared that patients in the test group shall have no other systemic disease or condition except for CVD, have not used any antibiotics or medications with an impact on the immune system, or have not received any periodontal treatment within the last 6 months. In addition, following criteria were sought in both patient groups: Patients should be nonsmokers of cigarette or tobacco products (never used before), not in a pregnancy or breastfeeding term, without any obstacles for periodontal treatment in stable cardiovascular conditions. Materials and methods of our study were approved by the Non-drug Clinical Researches Ethics Committee of YYU, with ethics committee decision number YYU 05122013.00/06.

Criteria to make the diagnosis of chronic periodontitis

Criteria for the diagnosis of chronic periodontitis were regarded as the presence of a pathological periodontal pocket with a probing depth (PD) ≥5 mm. In addition, patients who were determined to have clinical attachment loss at >30% of their total existing dental areas and PD ≥5 mm along with bone destruction and therefore diagnosed with generalized chronic periodontitis were involved in the study.[12]

Criteria to make the diagnosis of atherosclerosis as a cardiovascular disease

After establishment of atherosclerosis diagnosis in 20 patients with CVD depending on the angiography and/or blood tests by Department of Cardiology of the YYU, these patients were involved in the study.

Clinical periodontal evaluations

Gingival index,[13] plaque index,[14] PD (mm), clinical attachment level (mm), and bleeding on probing (BOP) (±) data were collected from six aspects (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual, and mesiolingual) of all teeth, except for third molar teeth and recorded separately as pre- and post-treatment.

Obtaining gingival crevicular fluid samples

GCF samples were collected from vestibulo-proximal regions of different teeth with deepest pathological periodontal pockets (min PD = 5 mm and above). They were collected with the aid of paper strips (Periopaper Interstate Drug Exchange, Amityville, NY, USA) prepared specifically for this operation. Paper strips impregnated with GCF were measured in Periotron® device (Periotron 8000, Oraflow, NY, USA) following the standardization prior to each measurement. Once measurements were completed, paper strips were placed into Eppendorf® tubes containing 0.5 ml phosphate buffered saline solution and stored in –40° freezer until the test day.

Determination of CCL-28 and adrenomedullin levels by ELISA

ADM peptide specific ELISA kit (Human C-C motif chemokine 28 EIAab Science Co., Ltd., Wuhan, China) and CCL-28 peptide specific ELISA kit (Human C-C motif chemokine 28 EIAab Science Co., Ltd., Wuhan, China) were used to determine the CCL-28 and ADM levels. Prepared samples were transferred into microplates for measurement of optical density which were washed in adherence to procedures appropriate with the kit, in

automatic devices and then read by the device (BioTek® ELx800 reader 96/384 model 400-750 nm, Winooski, USA) set to 450 nm of wavelength. Values of absorbance and values of sample which were prepared according to standards were measured and recorded.

Collection of blood samples

Blood samples which were drawn from right or left antecubital region of patients at the beginning and again after the treatment were filled into sterile polypropylene tubes and centrifuged (Eppendorf safe lock tubes 1.5 mL, Eppendorf AG, Hamburg, Germany) and centrifuged at 4000 rpm for 10 min. Thus, sera was separated which was then transferred into empty sterile polypropylene tubes using micropipette and stored at –40°C until the day of analysis.

Laboratory tests

Blood values at the day before the commencement of treatment of the patients in CVD group and the patients in control group were determined with examinations and tests carried out at the Department of Cardiology.

Second set of obtained blood samples were tested using the serum which was separated for hs-CRP assay. Once serum samples were thawed at room temperature, results are evaluated and recorded with the aid of proper kits using the device with settings adjusted for hs-CRP analysis.

Statistical analysis

Statistical analysis was performed using the SPSS 15 (SPSS Inc., Chicago, IL, USA) program. Normality test of Kolmogorov-Smirnov was performed on all available data. Nonparametric tests were applied since the data did not have a normal distribution. Friedman test was employed for in-group analysis of clinical parameters. In the event of a significant in-group variance, Wilcoxon test was performed to determine which group caused such difference. Mann–Whitney U-test was used to make inter-group evaluation. Spearman's rho correlation test was performed to analyze correlation. Confidence interval of 95% and significance level of 0.05 was used to assess statistical significance of results.

Results

Evaluation of clinical periodontal parameters

Clinical periodontal parameters of the test and control groups are listed in Table 1, as pre- and post-treatment. There has been a significant decline after the treatment in clinical periodontal parameters both in test and control groups, in comparison to pretreatment. When extent of decline in pre- and post-treatment clinical periodontal parameters of the patients from the control and test groups were compared, no significant difference was found between the test and control groups (excluding BOP) ($P > 0.05$).

Evaluation of biochemical gingival crevicular fluid data

Biochemical data from test and control groups are listed in Table 2, as pre- and post-treatment. ADM has decreased in both groups after periodontal treatment. When we further examined which group had more

Table 1: Baseline and 6 months clinical periodontal indexes of the test and control groups

	Test group (atherosclerosis-chronic periodontitis)	Control group (non-atherosclerosis-chronic periodontitis)	Change between baseline and 6 month (*P*)
PI (mean±SD)			
Baseline	2.18±0.23	2.30±0.28	NS
6 month	0.31±0.18	0.27±0.14	NS
GI (mean±SD)			
Baseline	2.43±0.68	2.21±0.21	NS
6 month	0.28±0.12	0.26±0.08	NS
PD (mean±SD) (mm)			
Baseline	3.82±0.36	3.92±0.39	NS
6 month	2.18±0.29	2.12±0.30	NS
CAL (mean±SD) (mm)			
Baseline	4.25±0.59	4.93±0.46	NS
6 month	2.90±0.52	3.47±0.65	NS
BOP (mean±SD) (%)			
Baseline	78.33±13.80	47.21±11.19	<0.05
6 month	13.9±3.03	16.23±5.13	<0.05

Pl=Plaque index; GI=Gingival index; PD=Probing depth; CAL=Clinical attachment level; BOP=Bleeding on probing; SD=Standard deviation; NS=Not significant. Group significantly different from other groups ($P<0.05$)

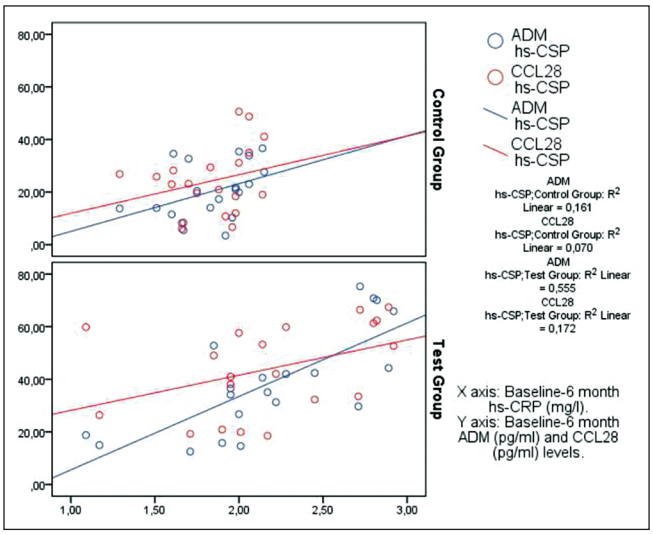

Figure 1: The correlation between adrenomedullin-chemokine (C-C motif) ligand-28 levels as detected in gingival crevicular fluid samples and high-sensitivity C-reactive protein levels as detected in serum samples in the test and control groups

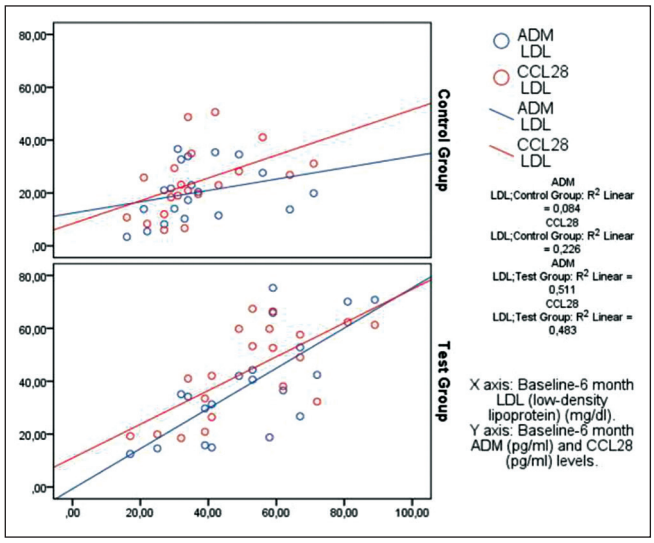

Figure 2: The correlation between adrenomedullin-chemokine (C-C motif) ligand-28 levels as detected in gingival crevicular fluid samples and low-density lipoprotein levels as detected in serum samples in the test and control groups

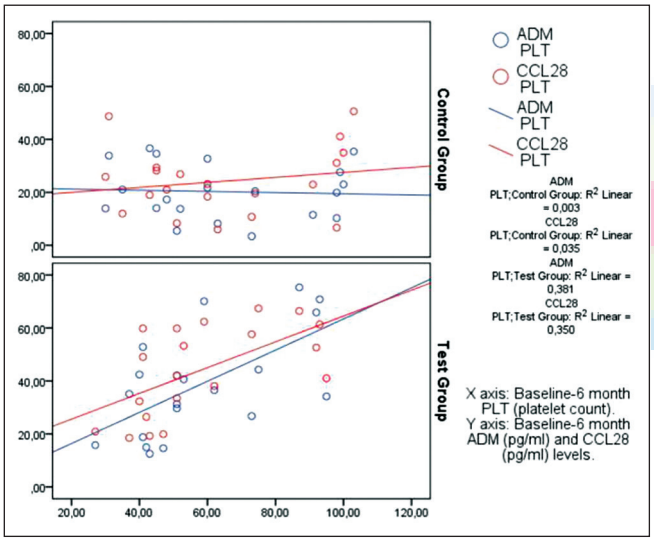

Figure 3: The correlation between adrenomedullin-chemokine (C-C motif) ligand-28 levels as detected in gingival crevicular fluid samples and platelet levels as detected in serum samples in the test and control groups

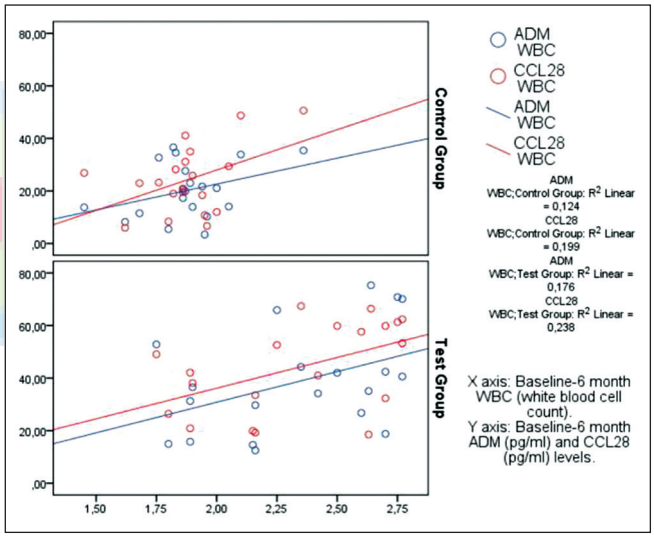

Figure 4: The correlation between adrenomedullin-chemokine (C-C motif) ligand-28 levels as detected in gingival crevicular fluid samples and white blood cell levels as detected in serum samples in the test and control groups

difference after periodontal treatment, greater difference was detected in the test group. We figured out a statistical variance between the groups in terms of this difference ($P < 0.05$).

When we compared the change in pre- and post-treatment CCL-28 data of test and control groups, we found out a significant reduction in both groups after the periodontal treatment ($P < 0.05$). When we further examined which group had more difference after periodontal treatment, greater difference was detected in the test group. We figured out a statistical variance between the groups in terms of this difference ($P < 0.05$).

Evaluation of the blood data

The difference in blood data from test and control groups are listed in Table 3, as pre- and post-treatment. When we compared the alteration in pre- and post-treatment hs-CRP data in test and control groups; it was significantly altered after periodontal treatment ($P < 0.05$). When we further examined which group had more difference after periodontal treatment, greater difference was detected in the test group ($P < 0.05$). When we compared the pre- and post-treatment data of WBC, PLT, fibrinogen, creatinine, HDL, and LDL in both groups, we determined a significant difference in both groups after periodontal

Table 2: ADM and CCL-28 levels after periodontal treatment Compared to Baseline and 6 month

	Test group (atherosclerosis-chronic periodontitis)	Control group (non-atherosclerosis-chronic periodontitis)	Change between baseline and 6 month (*P*)
ADM (pg/ml) (mean±SD)			
Baseline	86.23±37.35	42.33±9.12	<0.05
6 month	46.75±11.31	19.12±8.59	<0.05
CCL-28 (pg/ml) (mean±SD)			
Baseline	63.05±19.68	96.73±30.21	<0.05
6 month	45.31±15.62	45.93±22.92	NS

SD=Standard deviation; NS=Not significant. Group significantly different from other groups (P<0.05)

Table 3: hs-CRP, WBC, PLT, LDL, HDL, creatinine, and fibrinogen levels after periodontal treatment compared to baseline and 6 month

	Test group (atherosclerosis-chronic periodontitis)	Control group (non-atherosclerosis-chronic periodontitis)	Change between baseline and 6 month (*P*)
hs-CRP (mg/l) (mean±SD)			
Baseline	5.01±2.71	2.07±0.62	<0.05
6 month	1.14±0.57	0.78±0.37	<0.05
WBC (10^3/ml) (mean±SD)			
Baseline	9.3±1.4	7.4±1.6	<0.05
6 month	6.8±1.6	6.1±2.1	<0.05
PLT (10^3/ml) (mean±SD)			
Baseline	264.4±74.6	322.4±110.2	<0.05
6 month	222.5±64.6	268.6±84.2	<0.05
Fibrinogen (mg/dl) (mean±SD)			
Baseline	356.4±86.3	304.6±62.8	NS
6 month	296±64.5	286.2±58.8	NS
Creatinine (mg/dl) (mean±SD)			
Baseline	110.1±3 0.4	95.3±24.2	<0.05
6 month	86.3±28.4	84.2±18.3	<0.05
HDL (mg/dl) (mean±SD)			
Baseline	32.4±15.6	40.4±12.2	NS
6 month	45.6±17.2	46.2±16.8	NS
LDL (mg/dl) (mean±SD)			
Baseline	223.5±42.2	180.2±74.6	<0.05
6 month	162.5±28.2	144.2±64.4	<0.05

HDL=High-density lipoprotein; LDL=low density lipoprotein; hs-CRP=High sensitivity C-reactive protein; WBC=White blood cells; PLT=Platelet count; SD=Standard deviation; NS=Not significant. Group significantly different from other groups (P<0.05)

treatment ($P < 0.05$). When we further examined which group had more difference after periodontal treatment, greater difference was detected in the test group ($P < 0.05$).

Correlation of the data used in the study

The correlation between hs-CRP levels and ADM-CCL-28 levels in both groups are shown in Figure 1. We revealed out a positive correlation between the decrease of hs-CRP amounts and the decrease of ADM and CCL-28 amounts in the control and test groups after periodontal treatment. Correlation in test group is stronger than that in control group. The correlation between WBC levels and ADM-CCL-28 levels in test and control groups are shown in the Figure 2. We identified a positive correlation between the decrease of WBC levels and the decrease of ADM-CCL-28 levels in the control and test groups after periodontal treatment. Correlation in test group is stronger than that in control group. The correlation between LDL levels and ADM-CCL-28 levels in both groups are shown in Figure 3. We revealed out a positive correlation between the decrease of LDL value and the decrease of ADM-CCL-28 levels in the control

and test groups at after periodontal treatment. Correlation in test group is stronger than that in control group. The correlation between PLT levels and ADM-CCL-28 levels in test and control groups are shown in Figure 4. We detected a positive correlation between the decrease of PLT levels and the decrease of ADM-CCL-28 levels in the control and test groups after periodontal treatment. Correlation in test group is stronger than that in control group.

Discussion

It is known that periodontal infections may underpin formation of atheroma plaque and development of CVD.[9] The connection between periodontal inflammation and CVD can be proven by circulation of pro-inflammatory mediators such as certain bacterial endotoxins and specific cytokines in blood stream, which cause the exposure of acute-phase reactants leading to increased inflammatory activity in atherosclerotic lesions.[10] The most advocated hypothesis to explain the correlation between periodontal disease and CVD, accordingly, is systemic inflammatory process.

It is possible to deduce an assessment about inflammatory diseases depending on WBC. If WBC count is beyond the normal reference interval, i.e. there are more cells than 10–110 cells/L, we may consider leukocytosis, when the leukocyte levels in severe destructive periodontal diseases are compared with those in healthy subjects.[15] In our study, taking the numerical decrease in WBC levels after periodontal treatment into consideration, we believe that systemic inflammation might have lessened as a result of reduction in local inflammation, that is, with the periodontal therapy. Therefore, we may conclude that decreased WBC levels by periodontal mechanical treatment may have a positive effect on the prognosis of atherosclerosis and the clinical risk may be reduced. When it comes to the lipid profiles, decreased LDL levels and increased HDL levels are considered as decreased risk of disease from a CVD point of view. Consequently implying that, on blood values, clinical periodontal treatment may have an effect directed to reduce the risk of CVD. Fibrinogen, one of the markers evaluated in this study, is an acute-phase protein which is synthesized in the liver in response to IL-6. Fibrinogen level is increased in case of inflammation related to chronic periodontitis and during inflammation.[16] It is also known that fibrinogen levels significantly decrease with periodontal treatment at chronic periodontitis patients. Correspondingly, elevated fibrinogen levels might be stipulated as one of the mechanisms responsible for the correlation between periodontitis and CVD.[17] In this study, fibrinogen values in the test group were detected to be significantly higher than the control group.

The connection between CRP and atherogenesis is not limited with inflammation. High CRP levels, besides indicating an inflammation that triggers the accumulation of lipids in the plaque, also leads to endothelial dysfunction through its direct effects.[18] Due to the following trauma and bacteremia after the procedure, periodontal treatment generally gives rise to a short-term increase in systemic inflammatory/pretrombotic mediators for 24–48 h and causes a decrease in all endothelial functions.[19] Parameters, such as CRP, fibrinogen, and leukocyte count which are known as risk factors for CVD, are today also known to be associated with periodontitis. CRP is a major acute-phase protein and a marker of systemic inflammation. CRP is induced by nonspecific local or systemic tissue damage, infection, and inflammation and is effective in inflammatory mechanism of atherosclerosis.[20] We may state that periodontitis induces an acute-phase response by increasing the plasma levels of CRP, fibrinogen, and leukocyte count. Higher CRP levels have been identified in patients with coronary heart disease and periodontitis than those with coronary heart disease without periodontitis. Periodontal treatment has been reported to lessen the level of systemic inflammation.[21] Conventional measurement methods are suitable to detect high levels of CRP (40–200 mg/L) arising from acute inflammation. However, as the amount of CRP which is secreted from atheroma plaques in the event of subclinical inflammation of atherosclerosis is much lower than these levels, high-sensitivity tests are needed. Moreover, hs-CRP was used instead of CRP in this study. hs-CRP levels which offer more sensitive results than regular CRP, decreased with periodontal treatment in this study.

To date, the correlation between PLT and periodontal diseases has been investigated at a minimum level. However, PLT plays an important role in natural immunity to microorganisms. Bacteria can activate PLT. As a result, antimicrobial peptides and cytokines are secreted from PLTs.[22] In CVDs, it is possible to obtain information about systemic inflammation by examining the PLT amount. The reduction of local inflammation by reducing the amount of PLT with periodontal treatment can be explained by the reduction of systemic inflammation.

In this study, decreased values of WBC, LDL, fibrinogen, creatinine, and PLT as measured in blood samples and decreased values of ADM and CCL-28 as examined in GCF samples correlated with each other. Based on our interpretation of relevant data, we had a high-level baseline chronic systemic inflammation which might have been reduced by clinical periodontal treatment, and if this is the case, it may have a positive effect on the prognosis of atherosclerosis.

The physiological role of ADM on heart, however, is not yet fully explained.[23] Infusion of ADM in people with heart diseases was stated to reduce systolic and diastolic blood pressure, increase heart rate and plasma renin level, and to decrease plasma aldosterone level. ADM's heart-stimulating effect may be protective by preventing vascular damage during severe hypertension.[24] In this study, ADM levels in GCF were examined and its correlation with CVD was taken into account. Likewise, a study comparing ADM levels in GCF reported higher ADM levels in samples from GCF of patients with chronic periodontitis than samples from healthy patients.[9]

When the correlation between blood data and ADM-CCL-38 data is discussed, a strong positive correlation is noted among WBC, hs-CRP, LDL, and PLT in test group. In light of these results, with local reduction of the antimicrobial peptides in GCF as a result of periodontal treatment, inflammation markers in systemic circulation were decreased. Hence, periodontal treatment, in turn, is believed to cause a reduction in elements, which are risk factors for atherosclerosis.

Conclusion

Conventional periodontal diagnosis methods provide only limited information about periodontal prognosis of the patient in the future. However, determination of blood parameters and lipid profile provides information to assess the condition of the patient and to estimate the risk ratio of potential atherosclerosis and chronic periodontitis patients. We may suggest that as a result of the elimination of periodontitis, a risk factor for CVD, with clinical periodontal treatment, atherosclerosis prognosis can eventually be directed positively. A general overview of our study results suggest that a successful periodontal treatment may primarily reduce local inflammation and levels of pathogenic microorganisms followed by the decrease in antimicrobial peptides within GCF and finally relief in systemic inflammation. Reduction in systemic inflammation may have provided the decrease in markers, which are employed for determining the prognosis of CVD. Based on this implication, we believe that the risk factor can be eliminated through a successful periodontal treatment, as a result of the effect on the systemic situation by the local inflammation, which is a known risk factor for CVD. Elimination of risk factors may exert a positive effect on CVD prognosis.

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Conflicts of interest

There are no conflicts of interest.

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