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The role of HBD-2, HBD-3, and calprotectin in the relationship between chronic periodontitis and atherosclerosis

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Abstract: This study was carried out to compare individuals diagnosed with atherosclerosis and periodontal periodontitis based on the degree of change in the human beta-defensins (HBD) HBD-2, HBD-3, and calprotectin. Atherosclerosis is the most frequently observed cardiovascular disease. Dental and periodontal infections are known to provide a considerable basis for atheroma plaque formation. The study group consists of a total number of 40 subjects, with 20 patients diagnosed with atherosclerosis and chronic periodontitis and 20 systemically healthy patients diagnosed with chronic periodontitis. Clinical periodontal and blood parameters and HBD-2, HBD-3, and calprotectin biomarkers in the gingival crevicular fluid were measured. In both groups, following clinical periodontal treatment, a statistically significant decrease in white blood cells (WBC), low-density lipoproteins (LDL), fibrinogen, creatinine, and platelets (PLT), a statistically significant increase in high-density lipoproteins (HDL) in blood samples, statistically meaningful decrease in HBD-2, HBD-3, and calprotectin in the gingival crevicular fluid were achieved. Blood values and HBD-2, HBD-3, calprotectin amounts in the gingival crevicular fluid were increased significantly in the test group compared to the control group. A positive correlation was observed between decreases in HBD-2, HBD-3, calprotectin, and clinical periodontal indices. Regression in systemic inflammation was observed after clinical periodontal treatment. It is concluded that nonsurgical periodontal treatment of chronic periodontitis positively affects atherosclerosis prognosis.

Introduction

Periodontitis, a major periodontal disease, is a chronic infectious disease resulting from microbial dental plaque (MDP), affecting the gingiva, alveolar bone, and connective tissue (Beck and Offenbacher, 2005; Corretti *et al.*, 2002). Host related factors, bacteria, and environmental factors play a role in periodontal disease incidences. These factors cause variations in age at disease onset, disease progression rate and tissue destruction, treatment responsiveness and severity of disease (Ross, 1997). Bacteremia and host-related inflammation by-products caused by chronic infections damage vessel endothelium possibly resulting in susceptibility to coronary heart disease. Endothelium damage and resulting endothelial dysfunction establish the basic mechanism for the onset and development of atherosclerosis. Damaged vessel endothelium fails to function normally, and endothelial dysfunction develops (Beck and Offenbacher, 2005; Corretti *et al.*, 2002). Increased C-reactive protein (CRP) levels, erythrocyte sedimentation levels, and chemokine and cytokine (interleukin (IL)-6, IL-8, IL-10, IL-18, tumor necrosis factor-alpha (TNF-α) and human β-defensins (HBDs)) levels, are observed in individuals with periodontitis (Beck and Offenbacher, 2005; Friedewald *et al.*, 2009; Kinane and Lowe, 2000). The most frequently investigated antimicrobial peptides in the gingival area are α-defensins, β-defensins, and calprotectin. Alpha-defensins may be secreted from neutrophils, whereas β-defensins may be secreted from epithelial cells. HBDs consist of three subtypes secreted from the oral epithelium (HBD-1, HBD-2, and HBD-3) (Diamond and Ryan, 2011). They function mainly as microbial peptides and are effective in a wide area against

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Gram-positive and Gram-negative microorganisms (Yin *et al.*, 2010). Although HBD-2 and HBD-3 are secreted from the gingiva, their role in periodontal health, as well as their secretion area, differ. HBD-2 and HBD-3 are highly secreted especially in gingival inflammation areas (superficial layer of epithelium), while identified minimally on the junction epithelium (Dale *et al.*, 2001). In healthy individuals and patients with gingivitis, a lower level of HBD-2 was identified compared to patients with chronic periodontitis (Pereira *et al.*, 2013). Calprotectin as major cytosolic protein was identified primarily in leukocytes and secondarily in monocyte/macrophage and epithelium cells (Kajiura *et al.*, 2014). In systemic inflammatory diseases, calprotectin was identified primarily in gingival crevicular fluid (GCF) as well as in plasma, synovial fluid, and feces, and its level in GCF was identified to be significantly higher in individuals with chronic periodontitis compared to healthy individuals (Becerik *et al.*, 2011).

Based on this information studying the relationship between cardiovascular disease (CVD) and periodontal diseases, the objective of our study is to compare periodontal clinical index changes, changes in the amounts of HBD-2, HBD-3, calprotectin markers in GCF and high-sensitivity (hs)-CRP, platelets (PLT), white blood cells (WBC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), creatinine, fibrinogen markers in serum samples following treatment in patients diagnosed with cardiovascular atherosclerosis and with chronic periodontal periodontitis and cardiovascular healthy patients diagnosed with chronic periodontitis.

Materials and Methods

Subjects

A total of 40 patients between the ages of 35 and 65 who applied to Yuzuncu Yil University (YYU) Faculty of Medicine, Dursun Odabaş Medical Center Cardiology Department with cardiovascular complaints for whom YYU Faculty of Dentistry Periodontology Department was consulted, or who applied to YYU Faculty of Dentistry Periodontology Department for a variety of mouth and dental health complaints, the results obtained were used in evaluating the results of this study. Two groups were formed by the participants of the patients diagnosed with atherosclerosis, 20 patients were diagnosed with chronic periodontitis upon periodontal examination and included in the test group. 20 patients having no cardiovascular complaints and diagnosed with periodontitis were tested to confirm their cardiovascular health condition and included in the control group.

Test Group: Individuals diagnosed with cardiovascular atherosclerosis, and individuals diagnosed with chronic periodontal periodontitis.

Control Group: Individuals with cardiovascular health that were diagnosed with chronic periodontal periodontitis.

We took due care to ensure that control group patients enrolled in the study had no systemic diseases, did not use antibiotics and/or immune system affecting medicines in the last 6 months and did not receive any periodontal and/or medical treatment. Regarding patients in the test group, we took due care to ensure that the patients did not have any systemic diseases or conditions other than CVD, did not use antibiotics and/or immune systems effecting medicines in the last six months, and did not receive any periodontal treatment. Additional criteria applied for both patient groups included were non-smokers (no prior smoking history), no pregnancy or breastfeeding, no condition hindering periodontal treatment, available to appear steadily for controls, and stable in terms of cardiovascular health.

All patients participating in the study received non-surgical periodontal therapy for control microbial periodontal infection by removing bacterial biofilm, calculus, and toxins from periodontally involved root surfaces.

Criteria for diagnosis of atherosclerosis as a cardiovascular disease

Angiography imaging and/or blood tests were applied to 20 study subjects having a cardiovascular disease at YYU Dursun Odabaş Medical Center Cardiology Department, and upon atherosclerosis diagnosis, these patients were included in the study.

Diagnosis of chronic periodontitis

The inclusion criteria for the chronic periodontitis patients were the following: inflammation in the gingiva, supragingival versus subgingival calculus and microbial dental plaque formation, vertical and horizontal bone loss in a radiographic examination, five mm and/or more PD in at least six total sites of at least four teeth with one root, and four mm and/or more Clinic Attachment Level (CAL), diagnosed with generalized chronic periodontitis.

Diagnosis of atherosclerosis

The diagnosis was made according to clinical findings, biochemical evaluations, and coronary angiography. Biochemical evaluations were carried out on the serum samples of patients who had applied for examination to the cardiology department during the stated period of time. Following the evaluation, patients having data that might have posed a risk in terms of atherosclerosis were referred for angiography. Following angiography, the patients were divided into two groups, patients with atherosclerosis and patient not diagnosed with atherosclerosis. The patient was evaluated as having atherosclerosis if he/she had ≥50% narrowing in at least one major epicardial artery. In addition to the results of the angiography, markers in serum samples that could aid in diagnosis were evaluated. Patients who have not been diagnosed with atherosclerosis have been defined to be cardiovascular healthy.

Clinic periodontal assessment

PI (Silness and Loe, 1964), Gingival Index (GI) (Loe and Silness, 1963), PD (mm), CAL (mm) and Bleeding on Probing (BOP) (+/−) data were collected from six areas (distobuccal, mid buccal, mesiobuccal, distolingual, mid lingual and mesiolingual) of all teeth except for the wisdom teeth and recorded separately for pretreatment and post-treatment.

Obtaining GCF samples

GCF samples were obtained from a vestibular proximal area of various teeth with deepest pathological periodontal pockets

(minimum PD = 5 mm and above). This was done using specially prepared paper tapes (Periopaper Interstate Drug Exchange, Amityville, NY, USA). To prevent contamination of samples with saliva and/or supragingival plaque, the areas were isolated with cotton buffers and dried with an aspirator. In the presence of supragingival plaque, the periodontal explorer was used to carefully remove the plaque. Care was taken to avoid the mechanic trauma of gingival tissue in the process. Paper tapes were placed in the periodontal pocket mouth in selected areas in a single move and removed after 30 s.

Paper tapes soaked in GCF were recorded with Periotron (Periotron 8000, Oraflow, NY, USA) device standardized before each measurement. When measurements were completed, paper tapes were placed in Eppendorf tubes filled with 0.5 mL PBS (phosphate-buffered saline solution) fluid to be kept at −40°C until the test date.

Identification of HBD-2, HBD-3, and calprotectin levels using ELISA method

Special ELISA kits for HBD-2 peptide (Cusabio Biotech Co., Ltd., Wuhan China), HBD-3 peptide (Cusabio Biotech Co., Ltd., Wuhan China), and calprotectin peptide (HK325 Human Calprotectin ELISA KIT Hycult biotech Uden The Netherlands) were used to identify HBD-2, HBD-3, and calprotectin levels. Before testing, all solutions and standards in the tests were diluted according to the manufacturer's instructions. Samples were prepared in line with test directions and solutions needed to identify HBD-2, HBD-3 and calprotectin levels were added and kept throughout the incubation period. Prepared samples were transferred to microplates for optic density measurement. Microplates were washed in an automated device (BioTek® ELx50 Automatic Microplate Strip Washer, Winooski, United States) according to the protocol required by the kits and were read spectrophotometrically in a microplate reader (BioTek® ELx800 reader 96/384 model 400–750 nm Winooski, US) set to 450 nm wavelength. Sample values adjusted to absorbance values and standards were measured and recorded.

Blood sample collection: First-party blood samples were sent by YYU Dursun Odabaş Medical Center Cardiology Department in the beginning and after the treatment to YYU Dursun Odabaş Medical Center Biochemistry Department for routine testing. Other blood samples were taken also in the beginning and after the treatment from the right and left antecubital areas of patients and transferred to sterilized polypropylene tubes (Eppendorf safe lock tubes 1.5 mL, Hamburg, Germany) and centrifuged for 10 min at 4000 rpm. The serum was separated and transferred with a micropipette to empty sterilized polypropylene tubes and kept at −40°C until analysis. Both samples were sent to YYU Dursun Odabaş Medical Center Biochemistry Department laboratories for routine test and YYU Dursun Odabaş Medical Center Microbiology Department laboratories for hs-CRP according to procedures described below.

Laboratory tests

Blood values were identified one day before the beginning of treatment for both CVD group patients and the control group through examinations and tests at YYU Dursun Odabaş Medical Center Cardiology Department. Whole blood and biochemistry parameters were assessed according to the American College of Cardiology (AHA) and the National Cholesterol Education Program (NCEP) criteria.

The second set of blood samples was tested using serum separated for hs-CRP identification. Serum samples were thawed at room temperature and evaluated with devices adjusted for hs-CRP identification using appropriate kits and results were recorded. The evaluation was made according to the following data (Centers for Disease Control and Prevention/American Heart Association (CDC/AHA)) criteria: low cardiovascular risk: CRP ≤ 0.1; Moderate cardiovascular risk: CRP 0.1–0.3; High cardiovascular risk: CRP 0.3–1; Acute systemic inflammation: CRP ≥ 1.

Ethical statement

Study tools and methods were approved by YYU Non-Pharmacological Clinical Research Ethical Committee with committee resolution No. YYU05122013.00/06. All participants were informed about the study, and the informed consent was obtained from all participants at the beginning of the study. Our study was consistent with the Helsinki Declaration.

Statistical analysis

Clinical measurements taken at controls and data obtained from microorganisms were analyzed statistically. Statistical analyses were carried out using SPSS 15 (SPSS Inc., Chicago, IL, USA) program. Two groups with 20 patients in each group were formed. Literature research was carried out in order to determine a sample size before starting our study. The data within the studies in relation to the subject of the study were evaluated, and the nQuery Advisor program was used in order to determine a suitable patient's size for our study. We established that 20 patients from each group in our study were suitable for the study. As we had two different groups, each group consists of 20 patients. Power analysis was employed for the present study by the nQuery Advisor package program. Sample/patients size for per-group was 20, and the power of the study was 95. Kolmogorov–Smirnov normality test was applied to all data obtained. In the absence of normal distribution of data, nonparametric tests were used. Friedman's test was used for the within-group analysis of clinical parameters. When within-group changes were statistically significant, the Wilcoxon test was used to identify which group constituted the source of difference. For "between-group" assessment, the Mann–Whitney *U*-test was used. Spearman Rho correlation test was used for correlation analysis. 95% confidence interval and 0.05 level of significance were taken as a basis for assessment of the statistical significance of the results.

Results

A total of 40 patients –12 female and 28 male– were enrolled in this study. The average age of patients in the test group was 52.4 ± 7.70, and in the control group was 49.6 ± 8.31. An assessment of age data revealed no statistical difference between the two groups ($p > 0.05$).

Assessment of clinical periodontal parameters

Clinical periodontal parameters before and after treatment for test and control groups are shown in Tab. 1. After treatment, clinical periodontal parameters for both the test group and the control group showed a statistically significant decrease compared to the parameters before treatment. A comparison of clinical periodontal data of individuals in control and test groups before and after treatment revealed no statistically significant difference between the test group and the control group ($p > 0.05$).

Assessment of biochemical data in GCF

Biochemical data before and after treatment for test and control groups are shown in Tab. 2. In both groups, HBD-2 decreased after periodontal treatment. An assessment of the degree of change after periodontal treatment for both groups indicated a greater change in the test group. A statistically significant difference was observed between groups ($p < 0.05$).

A comparison of the change in HBD-3 data of individuals in the control group and the test group before and after treatment indicated a statistically significant decrease in both groups after treatment ($p < 0.05$). An assessment of the degree of change after periodontal treatment for both groups indicated a greater change in the test group. A statistically significant difference was observed between groups ($p < 0.05$).

A comparison of the change in calprotectin data of individuals in the control group and the test group before and after treatment indicated a statistically significant decrease in both groups after treatment ($p < 0.05$). An assessment of the degree of change after periodontal treatment for both groups indicated a greater change in the test group. A statistically significant difference was observed between groups ($p < 0.05$).

Assessment of blood data

Changes in blood data, before and after treatment for test and control groups, are shown in Tab. 3. A comparison of the change in hs-CRP data of individuals in the control group and the test group before and after treatment periodontal

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 months (p)
PI (Mean ± SD)	**Baseline**	2.18 ± 0.23	2.30 ± 0.28	NS
	6 months	0.31 ± 0.18	0.27 ± 0.14	NS
GI (Mean ± SD)	**Baseline**	2.43 ± 0.68	2.21 ± 0.21	NS
	6 months	0.28 ± 0.12	0.26 ± 0.08	NS
PD (Mean ± SD)	**Baseline**	3.82 ± 0.36	3.92 ± 0.39	NS
(mm)	**6 months**	2.18 ± 0.29	2.12 ± 0.30	NS
CAL (Mean ± SD)	**Baseline**	4.25 ± 0.59	4.93 ± 0.46	NS
(mm)	**6 months**	2.90 ± 0.52	3.47 ± 0.65	NS
BOP (Mean ± SD)	**Baseline**	78.33 ± 13.80	47.21 ± 11.19	<0.05
(%)	**6 months**	13.9 ± 3.03	16.23 ± 5.13	<0.05

Note: PI, 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 ($p < 0.05$).

TABLE 2

HBD-2, HBD-3, and calprotectin levels after periodontal treatment compared to baseline and 6 months

		TEST GROUP (atherosclerosis-chronic periodontitis)	CONTROL GROUP (non-atherosclerosis-chronic periodontitis)	Change between baseline and 6 months (p)
HBD-2 (pg/mL)	**Baseline**	1986.26 ± 342.88	1936.13 ± 484.40	<0.05
(Mean ± SD)	**6 months**	1026.99 ± 269.67	1078.42 ± 323.64	<0.05
HBD-3 (pg/mL)	**Baseline**	4100.07 ± 1045.6	3993.69 ± 891.21	<0.05
(Mean ± SD)	**6 months**	2337.97 ± 1502.3	2847.99 ± 1016.3	<0.05
Calprotectin (ng/mL)	**Baseline**	8.75 ± 3.33	18.84 ± 3.72	<0.05
(Mean ± SD)	**6 months**	2.66 ± 4.52	5.25 ± 2.88	<0.05

Note: 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 months

		TEST GROUP (atherosclerosis-chronic periodontitis)	CONTROL GROUP (non-atherosclerosis-chronic periodontitis)	Change between baseline and 6 months (p)
hs-CRP (mg/L)	**Baseline**	5.01 ± 2.71	2.07 ± 0.62	<0.05
(Mean ± SD)	**6 months**	1.14 ± 0.57	0.78 ± 0.37	<0.05
WBC (10^3/mL)	**Baseline**	9.3 ± 1.4	7.4 ± 1.6	<0.05
(Mean ± SD)	**6 months**	6.8 ± 1.6	6.1 ± 2.1	<0.05
PLT (10^3/mL)	**Baseline**	264.4 ± 74.6	322.4 ± 110.2	<0.05
(Mean ± SD)	**6 months**	222.5 ± 64.6	268.6 ± 84.2	<0.05
Fibrinogen (mg/dL)	**Baseline**	356.4 ± 86.3	304.6 ± 62.8	NS
(Mean ± SD)	**6 months**	296 ± 64.5	286.2 ± 58.8	NS
Creatinine (mg/dL)	**Baseline**	110.1 ± 30.4	95.3 ± 24.2	<0.05
(Mean ± SD)	**6 months**	86.3 ± 28.4	84.2 ± 18.3	<0.05
HDL (mg/dL)	**Baseline**	32.4 ± 15.6	40.4 ± 12.2	NS
(Mean ± SD)	**6 months**	45.6 ± 17.2	46.2 ± 16.8	NS
LDL (mg/dL)	**Baseline**	223.5 ± 42.2	180.2 ± 74.6	<0.05
(Mean ± SD)	**6 months**	162.5 ± 28.2	144.2 ± 64.4	<0.05

Note: 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 ($p < 0.05$).

indicated a statistically significant difference in both groups after treatment ($p < 0.05$). An assessment of the degree of change after periodontal treatment for both groups indicated a greater change in the test group ($p < 0.05$). A comparison of WBC, PLT, fibrinogen, creatinine, HDL, and LDL data in both groups before and after treatment indicated a statistically significant change in both groups after treatment ($p < 0.05$). An assessment of the degree of change after periodontal treatment for both groups indicated a greater change in the test group ($p < 0.05$).

Correlation of data used in the study

Correlation between hs-CRP values and HBD-2, HBD-3, and calprotectin values for both groups are shown in Fig. 1. In both the control group and the test group, a positive correlation was observed between the decrease in the hs-CRP count and a decrease in HBD-2, HBD-3, and calprotectin count with periodontal treatment. Correlation in the test group was stronger compared to the control group.

In both groups, the correlation between LDL values and HBD-2, HBD-3, and calprotectin values are shown in Fig. 2. In both the control group and the test group, a positive correlation was observed between the decrease in LDL count and a decrease in HBD-2, HBD-3, and calprotectin count with periodontal treatment. Correlation in the test group was stronger compared to the control group.

Correlation between PLT values and HBD-2, HBD-3, and calprotectin values in control and test groups are shown in Fig. 3. In both the control group and the test group, a positive correlation was observed between the decrease in PLT count and a decrease in HBD-2, HBD-3, and calprotectin count with periodontal treatment. Correlation in the test group was stronger compared to the control group.

Discussion

The most frequently used hypothesis to explain the association between periodontal diseases and CVD is that local inflammation resulting from periodontal disease contributes to CVD incidence inducing systemic inflammation. Periodontal disease by itself may directly or indirectly affect endothelial cell dysfunction (Mercanoglu *et al.*, 2004). The relationship of periodontal diseases with CVD has been studied for a long time. MDP, the major etiological factor in periodontal diseases, is a structure caused by polymers secreted by hosts and microorganisms. MDP is a biofilm coat housing between 700 to 800 different types of microorganisms. CVD may develop as a result of local inflammation and change of microorganism profile due to MDP (Kang *et al.*, 2003). In this study, HBD-2, HBD-3, and calprotectin levels secreted from the tissues due to microorganisms in pathological periodontal pockets in periodontal diseases were studied. Additionally, WBC, PLT, LDL, HDL, creatinine, fibrinogen, and hs-CRP levels were investigated in peripheral blood samples to evaluate CVD prognosis and risk.

There are studies indicating that periodontal pretreatment of chronic periodontitis and endothelial dysfunction patients may heal endothelial dysfunction (Mercanoglu *et al.*, 2004). Some studies, however, avoid associating chronic periodontitis and endothelial dysfunction. For example, according to the results of an 8-year observation study carried out in Sweden, a statistically significant association between CVD and chronic periodontitis could not be identified (Johansson *et al.*, 2014). Contrary to such data, there are epidemiological studies in which a statistically significant association between CVD and periodontal diseases

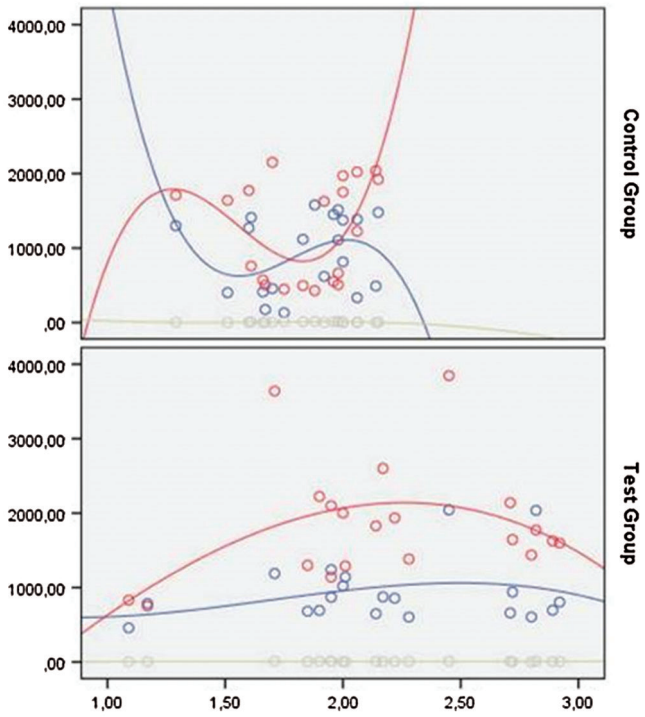

FIGURE 1. The correlation between hs-CRP level as detected in serum samples and HBD-2, HBD-3, and Calprotectin levels as detected in GCF samples at the test and control groups.
X-axis: Baseline-6 months hs-CRP (mg/L). Y-axis: Baseline-6 months HBD-2 (pg/mL), HBD-3 (pg/mL), and Calprotectin (ng/mL). **Blue circle:** HBD-2 hsCRP, **red circle:** HBD-3 hsCRP, **grey circle:** Calprotectin hsCRP, **blue line:** HBD-2 hsCRP, **red line:** HBD-3 hsCRP, **grey line:** Calprotectin hsCRP.

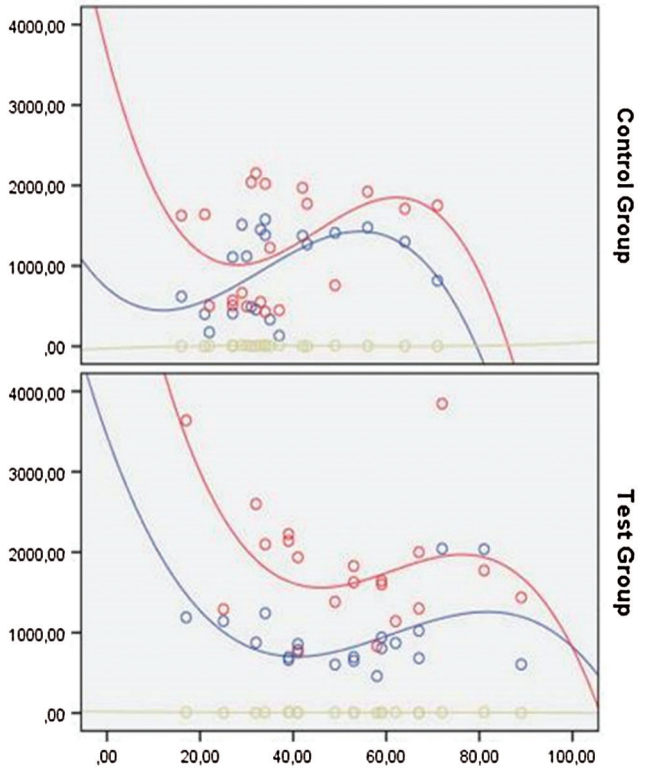

FIGURE 2. The correlation between LDL level as detected in serum samples and HBD-2, HBD-3, and Calprotectin levels as detected in GCF samples at the test and control groups.
X-axis: Baseline-6 months LDL (mg/dL). Y-axis: Baseline-6 months HBD-2 (pg/mL), HBD-3 (pg/mL), and Calprotectin (ng/mL). **Blue circle:** HBD-2 LDL, **red circle:** HBD-3 LDL, **grey circle:** Calprotectin LDL, **blue line:** HBD-2 LDL, **red line:** HBD-3 LDL, **grey line:** Calprotectin LDL.

has been observed (Mustapha *et al.*, 2007). In our study, we concluded that it is possible to change CVD indicators through a decrease in the number of antimicrobial peptides in GCF with clinical chronic periodontitis treatment and CVD conditions may improve.

In PCR based inspection of atheroma plaques, periodontal pathogen bacteria were identified in plaque composition (Untch and Schlagenhauf, 2015). Dental and periodontal infections are reported to constitute a considerable basis for atheroma plaque formation and CVD incidence (Söder *et al.*, 2014). Based on this information in literature, the decision was made to apply periodontal treatment periodontal to individuals diagnosed with cardiovascular atherosclerosis and with chronic periodontal periodontitis to obtain cardiovascular benefits in their periodontal treatment and to reduce chronic inflammation systemically present in the body. A review of studies inspecting the association between antimicrobial peptides and periodontal diseases shows that in a study carried out with patients with diabetes, calprotectin level, and glucose albumin (HbA1c) levels in GCF were studied, and HbA1c values in GCF and blood were compared. Calprotectin levels were measured and recorded in the patient population separated into four groups (diabetes–chronic periodontitis, only diabetes, only chronic periodontitis, healthy), and between-group assessments were made. Calprotectin levels in GCF in patients only with diabetes and healthy subjects were identified to be lower than levels in patients with chronic periodontitis and diabetes-chronic periodontitis (Kajiura *et al.*, 2014). In another study on calprotectin levels, calprotectin levels in chronic periodontitis patients were reported to be significantly higher compared to other groups (Becerik *et al.*, 2011). It is reported that calprotectin, which is identified to increase in the presence of inflammatory diseases, may be an indicator of inflammatory diseases. In comparison with other biochemical indicators and periodontal clinical parameters, a correlation of calprotectin levels was noted with biochemical inflammatory indicators and not with periodontal clinical parameters (Nakamura *et al.*, 2000). In this study, calprotectin was evaluated taking into account that it could be a chronic inflammatory indicator; it appears as an antimicrobial peptide in periodontal tissues; it changes in GCF, depending on chronic periodontitis; and it is insignificantly present in healthy individuals. In the two groups, namely those with both chronic periodontitis and CVD and those without, calprotectin levels were identified to decrease at the end of 6 months with clinical periodontal treatment. A decrease in these levels in GCF supports the thought that not only clinical parameters of chronic periodontitis but also of chronic inflammation are improved, and the systemic impact is reduced. Additionally, in this study, decreasing calprotectin levels were parallel with clinical periodontal parameters. A review of all biochemical data used in this

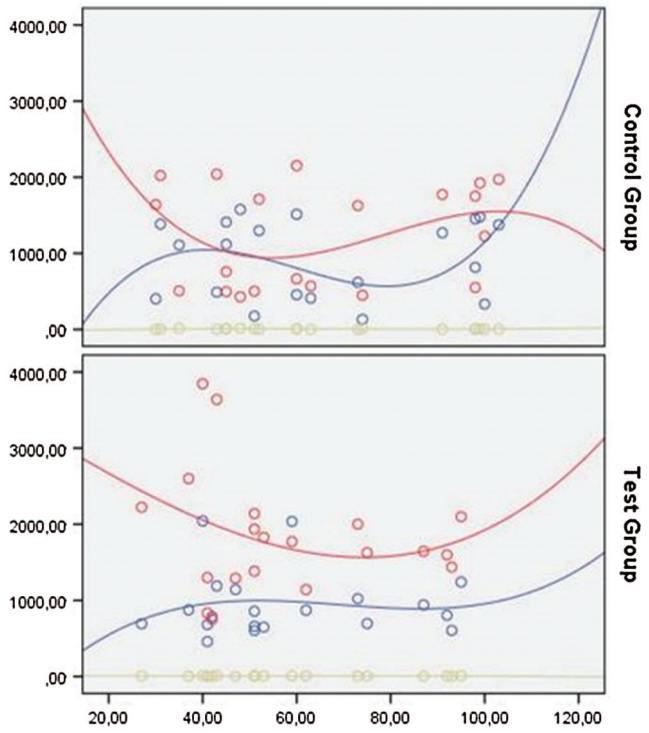

FIGURE 3. The correlation between PLT level as detected in serum samples and HBD-2, HBD-3, and Calprotectin levels as detected in GCF samples at the test and control groups.
X-axis: Baseline-6 months PLT (10^3/mL). Y-axis: Baseline-6 months HBD-2 (pg/mL), HBD-3 (pg/mL), and Calprotectin (ng/mL). **Blue circle:** HBD-2 PLT, **red circle:** HBD-3 PLT, **grey circle:** Calprotectin PLT, **blue line:** HBD-2 PLT, **red line:** HBD-3 PLT, **grey line:** Calprotectin PLT.

study at the beginning of and after completion of treatment for both groups indicates significant changes. After periodontal treatment, on the 6th month, HBD-2, HBD-3, and calprotectin changes showed statistically significant results supporting the success of periodontal treatment. Antimicrobial peptides, blood values, and hs-CRP data were found to be significantly associated, pointing out the significant association of CVD and chronic periodontitis. In addition, when the correlation between the two diseases was evaluated, a correlation was observed between CVD data and periodontitis data. These results led us to obtain data that would strengthen the link between these two diseases.

When all analyses and correlations are evaluated together, a significant decrease in CVD risk indicators can be obtained by following clinical periodontal treatment. In previous studies, various conclusions were reached in which such association was proved by some researchers, while others concluded that such association was nonexistent. Studies showing the association of chronic periodontitis with CVD and CVD are predominant. In this study, such association was investigated by evaluating those parameters not frequently used as well as those used to demonstrate the presence of an association.

In the study carried out to investigate potential paths of beta-defensins in gingival health and periodontal disease through various PCR evaluations in cell culture, results indicate that there may be an association between defensin profiles induced with oral epithelia carrying disease and pathogenicity of oral bacteria families. Moreover, they not only influence the colonization of periodontal bacteria but also constitute an indicator of the pathogenicity of these microorganisms (Vankeerberghen *et al.*, 2005). In this study, an evaluation of the role of HBD-2 and HBD-3 based on descriptions in literature is parallel with the conclusion reached. In addition to observation of a statistically significant reduction in HBD-2 and HBD-3 counts with clinical periodontal treatment, a considerable reduction was also observed in microorganism ratios. Accordingly, a decrease in HBD-2 and HBD-3 levels also indicates a reduction in pathogenicity of microorganisms reduced in ratio. It can be concluded that the impact of microorganisms, reduced both in ratio and in pathogenicity, on the atherosclerotic plaque was reduced parallel to their systemic impacts. Consequent to the elimination of periodontitis as a CVD risk factor through clinical periodontal treatment, it is possible to say that atherosclerosis prognosis has been steered positively. Through a successful periodontal treatment, individuals diagnosed with chronic periodontitis and cardiovascular atherosclerosis and individuals not so diagnosed, decrease in WBC, PLT, LDL, HDL, creatinine, fibrinogen, and hs-CRP indicators in blood, and HBD-2, HBD-3 and calprotectin levels in GCF can be observed. It is possible to say that periodontal treatment, along with cardiovascular treatment for chronic periodontitis and atherosclerosis patients, may prove beneficial for cardiovascular treatment. The periodontal treatment helped with the decrease of WBC, PLT, LDL, creatinine, fibrinogen, and hs-CRP indicator values in serum samples in patients diagnosed with cardiovascular atherosclerosis and periodontally with chronic periodontitis.

As a result, conventional periodontal diagnostic methods provide limited information about the periodontal prognosis of the patient. However, the determination of blood parameters and lipid profile provides information to assess the patient's condition and predict the risk ratio of patients with potential atherosclerosis and chronic periodontitis. An overview of our study results shows that successful periodontal treatment can first reduce local inflammation and pathogenic microorganism levels, and then decrease antimicrobial peptides in GCF and finally decrease in systemic inflammation. The decrease in systemic inflammation may have led to a decrease in the markers used to determine the prognosis of CVD.

Conclusion

Reduction in systemic inflammation may have provided a decrease in markers that are employed for determining the prognosis of CVD. Based on this implication, we believe 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. It is concluded that nonsurgical periodontal treatment of chronic periodontitis positively affects atherosclerosis prognosis.

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