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ORIGINAL ARTICLE

Determining the relationship between atherosclerosis and periodontopathogenic microorganisms in chronic periodontitis patients

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ABSTRACT

Objectives: The aim of this study is to determine the relationship between atherosclerosis and periodontopathogenic microorganisms in chronic periodontitis patients following periodontal treatment.

Materials and Methods: A total of 40 patients were included in the study. 20 of these patients diagnosed with atherosclerosis and chronic periodontitis formed the test group. The remaining 20 patients were systemically healthy patients diagnosed with chronic periodontitis and formed the control group. All patients had nonsurgical periodontal treatment. The periodontopathogenic microorganism levels were determined at baseline and at 6 months in microbial dental plaque samples and WBC, LDL, HDL, PLT, fibrinogen, creatinine and hs-CRP levels were determined by blood samples.

Results: Statistically significant reduction has been achieved in clinical periodontal parameters following non-surgical periodontal treatment in test and control groups. Following periodontal treatment, WBC, LDL, PLT, fibrinogen, creatinine and hs-CRP levels significantly decreased and HDL levels significantly increased in both test and control groups. Similarly, the periodontopathogenic microorganism levels significantly decreased following periodontal treatment in the test and control groups. A statistically significant positive correlation has been determined between the periodontopathogenic microorganism levels and WBC, LDL, PLT, fibrinogen, creatinine, and hs-CRP levels in the test group.

Conclusions: The association between hs-CRP, WBC, LDL, PLT, fibrinogen, creatinine, and the amount of periodontopathogenic microorganisms indicates the possibility that periodontal treatment could decrease the risk atherosclerosis. More studies must be conducted in order for these results to be supported.

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Atherosclerosis; chronic periodontitis; periodontopathogenic microorganisms; non-surgical periodontal treatment

Introduction

Although many external factors play a role in the formation of periodontal diseases, the primary aetiologic agent is microbial dental plaque [1]. Microbial dental plaque contains a large number of bacteria, viruses, protozoa and microplasma types [2,3]. It is known that periodontopathogenic microorganisms such as *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Porphyromonas gingivalis (P. gingivalis), Tannerella forsythia (T. forsythia), Treponema denticola (T. denticola)* and *Prevotella intermedia (P. intermedia)* play a role in the initiation and progression of chronic periodontitis following the formation of microbial dental plaque [4,5]. To examine the microbiological aetiology of chronic periodontitis, saliva or subgingival microbial dental plaque samples were analyzed. To obtain a more precise result in a microbiological diagnosis, it has been suggested that subgingival microbial dental plaque evaluation can be carried out instead of performing saliva analysis [5]. There are many methods used to determine microorganisms that have periodontopathogenic potential [6]. *micro-IDent®* (Hain Lifescience GmbH, Nehren, Germany) test and *micro-IDent PLUS®* (Hain Lifescience GmbH, Nehren, Germany) test, which provide combined molecular genetic identification, can be used to determine several microorganisms present in subgingival microbial dental plaque samples. The *micro-IDent®* test can be used to determine five microorganisms (*A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola* and *P. intermedia*), and the *micro-IDentPLUS®* test can be used to determine 11 microorganisms (*A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, P. intermedia, P. micros: Peptostreptococcus micros, F. nucleatum/periodonticum: Fusobacterium nucleatum/periodonticum, C. Rectus: Campylobacter rectus, E. nodatum: Eubacterium nodatum, E. corrodens: Eikenella corrodens, C. gingivalis/ochracea/sputigena: Capnocytophaga spec.: gingivalis/ochracea/sputigena.)* [7].

For many years the relationship between periodontal diseases and cardiovascular diseases (CVD) has been examined [8]. Studies have been carried out on biological mediators such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), and highly sensitive C-reactive proteins (hs-CRP), in order to determine the risks of CVD [9]. The most frequently observed disease in CVD is atherosclerosis. Not only is the formation of atherosclerosis dependent on several factors, such as systemic and/or local factors, but it has also

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been shown that chronic inflammation plays an important role in the progression of the disease. Thrombocytes, endothelial cells, activated monocytes, macrophages, straight muscle cells and microorganisms play a role in atheroma plaque formation [10]. Following the examination carried out on atheroma plaques, the presence of microorganisms effective in periodontal diseases has been determined [11]. The most frequently asserted hypothesis used to explain the relationship between periodontal diseases and CVDs is that the systemic inflammatory process arises from local inflammation [12,13].

The aim of this study was to relate changes in periodontal status and amount of periopathogenes *(A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, P. intermedia, P. micros, F. nucleatum/periodonticum, C. rectus, E. nodatum, E. corrodens* and *C. gingivalis/ochracea/sputigena)* to changes in established serological risk factors [HDL, LDL, hs-CRP white blood cell (WBC) levels, platelet (PLT) levels, creatinine and fibrinogen) for atherosclerosis in patients who have been diagnosed with atherosclerosis-chronic periodontitis and systemic healthy-chronic periodontitis, following non-surgical periodontal treatment.

Materials and methods

Patients

A total of 40 patients, who applied to the Department of Periodontology, Faculty of Dentistry of the Yüzüncü Yıl University (YYU) with oral and dental complaints, primarily periodontal complaints, from 2013 to 2015 were admitted to this study. Patients included in the study were divided into two groups. Once clinical periodontal examinations were completed, 20 patients among the atherosclerosis patients who had chronic periodontitis became the study test group. Also, 20 patients who had been diagnosed with chronic periodontitis but were systemically healthy, as verified by the necessary checks, became the control group.

Test group: Patients with atherosclerosis and chronic periodontitis.

Control group: Patients who were systemically healthy with chronic periodontitis.

Patients who were involved in the control group had no systemic disease, had not used any antibiotics or medications with an impact on the immune system, or had not received any periodontal and/or medical treatment within the previous 6 months. We also took care that patients in the test group had no other systemic disease or condition except for CVD, had not used any antibiotics or medications with an impact on the immune system or had not received any periodontal treatment within the previous 6 months In addition, the following criteria were established for both patient groups: patients should be non-users of any tobacco products (never used before), not pregnant or breastfeeding, able to regularly attend visits as controls regularly, and in a stable cardiovascular condition. Materials and methods of our study were approved by the Non-Drug Clinical Research Ethics Committee of YYU, and assigned ethics committee decision number YYU05122013.00/06.

Diagnosis of chronic periodontitis

The inclusion criteria for the chronic periodontitis patients were the following: inflammation in gingiva, supragingival versus subgingival calculus and microbial dental plaque formation, vertical and horizontal bone loss in 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 CAL, diagnosed with generalized chronic periodontitis [14].

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 $\geq$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.

Clinical periodontal evaluations

Recorded separately as pre- and post-treatment were the following: gingival index (GI) [15], plaque index (PI) [16], PD (mm), clinical attachment level (CAL) (mm), and bleeding on probing (BOP) (±); data were collected from six aspects (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual and mesiolingual) of all teeth, except for the third molar teeth.

Obtaining microbiological samples

Subgingival microbial dental plaque samples were taken on days 0 and 180 of this study in order to evaluate the effects of the treatment applied on subgingival microflora. The deepest pathological periodontal pocket section, which was at least a PD $\geq$5 mm in each patient, was chosen as the sampling section. First, supragingival plaque samples were removed. Following this, sterile paper points, which are included in the *micro-IDent Plus*® test box were placed inside the periodontal pocket and pushed until resistance was felt. The paper point was left in place and then removed after 10 s. Samples taken from five different regions of each patient were then placed together in a single Eppendorf tube and stored for later analysis.

Determination of microorganisms

A DNA extraction kit (QIAamp DNA Mini Kit, QIAGEN GmbH, Hilden, Germany) was used to isolate bacterial DNA from

microbial dental plaque samples. DNA isolation was performed according to the protocol suggested by the manufacturer.

The combined molecular genetic identification of periodontopathogenic microorganisms types was carried out in a microbiology laboratory using the *micro-IDent Plus*® test.

The laboratory procedure included three steps:

- Determining DNA from the subgingival microbial dental plaque samples,
- Multiple amplification of DNA with primers,
- Hybridization ('dot blot' analysis was used as a hybridization method).

The study area was cleansed from amplified DNA.

The sediment that was formed inside the 'Buffer ATL' solution, specific to the extraction kit, was dissolved in hot water. Two heating blocks were prepared, at 70 °C and 95 °C. The 180 µl 'Tissue Lysis Buffer' and 20 µl 'Proteinase K' solution for each sample were combined in a tube and mixed. Following incubation for 10 min at 70 °C, the contents were removed. The 200 µl 'Buffer AL,' which was part of the kit, was added to the samples and mixed for 15 s, following this procedure, the sample was incubated for 5 min at 95 °C.

Following the incubation, the contents were removed, and 200 µl of 96%–100% ethanol were added and mixed for 15 s.

After the labelled filter tube was added to the collection tube and transferred into the reservoir of the filter tube, the spin column was closed and was centrifuged for 1 min at 6000 × *g* (approx. 8000 rpm).

The 'Buffer AW1' solution, which was part of the 500 µl kit, was added and centrifuged. The ready solution 'Buffer AW2,' also part of the 500 µl kit, was added and was centrifuged at maximum speed for 3 min.

A filter tube was added to the 1.5 ml microcentrifuge tube. Following this, the spin column was opened and the kit's 'Buffer AE' was added at 400 µl to five paper points and was incubated for 1 min at room temperature; then, it was centrifuged for 1 min at 6000× *g*.

As a result, DNA was diluted. Then, 5 µl of the obtained solution was used for the polymerase chain reaction (PCR). The DNA's amplified by this profile was applied to a DNA-*STRIP*® (DNA-*STRIP*® Hain Life Science, Nehren, Germany) matrix containing specific probes.

A colouring reaction was observed on the DNA-*STRIP*® as a result of the interaction between the multiplied nucleic acid derivatives that were obtained from the samples by means of the probes located on the DNA-*STRIP*®.

The degree of colouring was linked to the DNA amounts in the samples obtained, and this was defined as the equivalent of a genome of the 11 bacteria levels examined in the laboratory analysis.

The biotin PCR product obtained was examined for the presence of bacteria with the reverse hybridization method in accordance with the suggestions of the manufacturer with line probes on nitrocellulose strips specific to *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola*, *P. intermedia*, *P. micros*, *F. nucleatum/periodonticum*, *C. rectus*, *E. nodatum*, *E. corrodens* and *C. gingivalis/ochracea/sputigena*.

The strips that were formed with probes specific to the bacteria being monitored were colorimetric and evaluated. The colour levels on the strips were examined by determining six main levels. All strips obtained were scanned by a computer. The scanned strips were transferred to the Adobe Photoshop® (Adobe Systems, San Jose, CA) program, and the bands related to the microorganisms on the strips were then analyzed. All of the contrast (autocontrast) luminescence amounts of the bands were measured. The presence and rates of the presence of bacteria were evaluated in accordance with the following classification.

0: No colouring (<1% no bacteria)
1: Very light coloured tinting, staining (bacteria determination from 1% to 20%).
2: Light colouring (bacteria determination from 21% to 40%),
3: Significant colouring (bacteria determination from 41% to 60%),
4: Dark colouring (bacteria determination from 61% to 80%),
5: Very dark colouring (bacteria determination from 81% to 100%).

Collection of blood samples

Routine tests were performed and analyzed at the beginning of treatment and repeated after the treatment by sending the first part of the blood samples to the YYU Medical Center, Department of Biochemistry of the YYU Medical Center, Department of Cardiology. Other blood samples, which were drawn from the right or left antecubital area of the patients at the beginning and again after the treatment, were placed into sterile polypropylene tubes (Eppendorf safe-lock microcentrifuge tubes 1.5 mL, Hamburg, Germany) and centrifuged at 4000 rpm for 10 min. Sera were separated and then transferred into empty sterile polypropylene tubes using a micropipette and stored at −40 °C until the day of analysis. Procedures for both sample types were carried out at the YYU laboratories as described below.

Statistical analysis

Clinical measurements taken during control examinations and data from microorganisms were statistically analyzed. Statistical analysis was performed using the SPSS 15 (SPSS Inc., Chicago, IL) program. The Kolmogorov–Smirnov normality test was performed on all available data. Nonparametric tests were applied because the data did not have a normal distribution. The Friedman test was employed for in-group analysis of clinical parameters. In the event of a significant in-group variance, the Wilcoxon Test was performed to determine which group caused such differences. The Mann–Whitney *U* test with Bonferroni's correction was used to make inter-group evaluations. The Spearman's Rho Correlation Test was performed to analyze correlations.

A confidence interval of 95% and a significance level of .05 were used to assess the statistical significance of the results.

Results

A total of 40 patients comprised 12 females and 28 males were involved in this study. The average age of the patients in the test group was 52.4 ± 7.70 years, while the average age of patients in the control group was 49.6 ± 8.31 years. Evaluation of the age data revealed no significant difference between the two groups ($p > .05$).

Evaluation of clinical periodontal parameters

Clinical periodontal parameters of the test and control groups are listed in Table 1, as pre- and post-nonsurgical periodontal treatment. There was a significant decline after non-surgical periodontal treatment in clinical periodontal parameters in both the test and control groups, in comparison to pre -nonsurgical periodontal treatment. When the extent of decline in pre- and post-nonsurgical periodontal 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) ($p > .05$).

Evaluation of the blood data

The difference in blood data from the test and control groups is listed in Table 2, as pre- and post-nonsurgical periodontal. When we compared the alteration in the pre- and post-nonsurgical periodontal hs-CRP data in test and control groups, we found it was significantly altered after periodontal treatment ($p < .05$). When we further examined which group had more of a difference after nonsurgical periodontal treatment, a greater difference was detected in the test group ($p < .05$). When we compared the pre- and post-nonsurgical periodontal data of WBC, PLT, fibrinogen, creatinine, HDL and LDL in both groups, we determined a significant difference in test and control groups after nonsurgical periodontal treatment ($p < .05$). When we further examined which group had more of a difference after periodontal treatment, a greater difference was detected in the test group ($p < .05$).

Evaluation of microbiological data

Periodontopathogenic microorganisms were identified via a *micro-IDent Plus*® test carried out on each patient. The microorganisms identified were as follows: *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, P. intermedia, P. micros, F. nucleatum/periodonticum, C. rectus, E. nodatum,*

Table 1. Baseline and 6-month clinical periodontal indices of the test and control groups.

		Test group (atherosclerosis-chronic periodontitis)	Control group (non-atherosclerosis-chronic periodontitis)	*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	<.05
	6 month	13.9 ± 3.03	16.23 ± 5.13	<.05

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 ($p < .05$).

Table 2. 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)	*p*
hs-CRP (mg/l) (Mean ± SD)	Baseline	5.01 ± 2.71	2.07 ± 0.62	<.05
	6 month	1.14 ± 0.57	0.78 ± 0.37	<.05
WBC (10^3/ml) (Mean ± SD)	Baseline	9.3 ± 1.4	7.4 ± 1.6	<.05
	6 month	6.8 ± 1.6	6.1 ± 2.1	<.05
PLT (10^3/ml) (Mean ± SD)	Baseline	264.4 ± 74.6	322.4 ± 110.2	<.05
	6 month	222.5 ± 64.6	268.6 ± 84.2	<.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	<.05
	6 month	86.3 ± 28.4	84.2 ± 18.3	<.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	<.05
	6 month	162.5 ± 28.2	144.2 ± 64.4	<.05

hs-CRP: high-sensitivity C-reactive protein; WBC: white blood cells; PLT: platelet count; LDL: low-density lipoprotein; HDL: high-density lipoprotein; SD: standard deviation; NS: not significant.
Group significantly different from other groups ($p < .05$).

E. corrodens, and *C. gingivalis/ochracea/sputigena*. As a result of the test, the colours on the obtained strips were divided into percentage sections according to colour density, and then the strips were evaluated.

The rates of *A. actinomycetemcomitans, T. forsythia, T. denticola, F. nucleatum, C. rectus,* and *E. nodatum* had decreased in each patient as shown by a decrease in *P. gingivalis*, which decreased in all patients except for four patients in the test group. *Prevotella intermedia* decreased in all patients in terms of rates in the control group except for in three patients. *Porphyromonas micros* showed an increase of an average of 10–20% in seven patients in the control group, whereas they showed a decrease in all of the other patients. *E*ikenella *corrodens* generally decreased except for the increased parallel with *C. gingivalis/ochracea/sputigena* in the same seven control group patients from the control group.

The decrease in the total microorganisms that was found in the microbial dental plaque samples in the study by means of clinical periodontal treatment was observed to be statistically significant in both groups ($p < .05$). Some specific microorganisms were not found in some patients in the initial stage of the treatment or following the treatment. The average total rates of change for the occurrence of microorganisms are shown in Figures 1 and 2.

The correlations of the data used in the study

In our analysis *A. actinomycetemcomitans* rates showed strong positive correlations with the PD, CAL, hs-CRP, WBC, LDL, fibrinogen, creatinine and PLT measurements and weak negative correlations with HDL, Moreover, it was observed that the relationship between them was statistically significant ($p < .05$) (Figure 3).

Porphyromonas gingivalis rates had a statistically strong positive correlation with hs-CRP, WBC, fibrinogen, creatinine and LDL data and a strong negative correlation with HDL ($p < .05$) (Figure 4). *Tannerella forsythus* showed a strong positive correlation with hs-CRP, PD, CAL, WBC, fibrinogen, creatinine and LDL data and a strong negative correlation with HDL ($p < .05$) (Figure 5). *Treponema denticola* data were not shown to have a statistically significant correlation with any of the data ($p > .05$). While *P. intermedia* did not show any type of correlation with clinical periodontal data and hs-CRP data ($p > .05$), it showed a statistically significant, strong positive correlation with WBC, PLT, fibrinogen, creatinine and LDL data ($p < .05$).

Porphyromonas micros rates showed a weak positive correlation with hs-CRP and BOP data. Moreover, they showed statistically significant strong positive correlations with WBC, PLT, fibrinogen, creatinine and LDL data ($p < .05$). *F*usobacterium *nucleatum* showed statistically significant strong positive correlations with hs-CRP and WBC, PLT, fibrinogen, creatinine and LDL data ($p < .05$). *Eikenella corrodens* showed statistically significant strong positive correlations with hs-CRP, BOP, PD, CAL data ($p < .05$). A correlation regarding *C. rectus, E. nodatum* and *Capnocytophaga spec.* and other data could not be found ($p > .05$).

Discussion

When the results of this study were discussed, it was found that the number of microorganisms decreased in comparison to prior periodontal treatment data following periodontal treatment in the test and control groups. Moreover, it was observed that WBC, PLT, LDL, creatinine, fibrinogen and hs-CRP levels in serum samples of patients following periodontal treatment increased and HDL decreased in the test group. It has been determined that in the test group there was a strong positive correlation among the microorganisms, such as *A. actinomycetemcomitans, P. gingivalis, T. forsythus,*

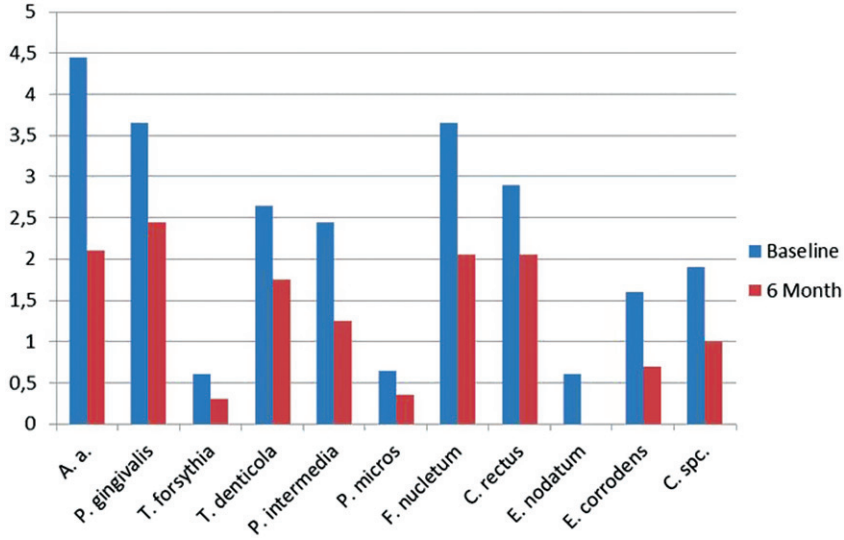

Figure 1. The distribution of the observed rates of periodontopathogenic microorganisms prior to non-surgical periodontal treatment and following non-surgical periodontal treatment in the control group: (*A. actinomycetemcomitans: Aggregatibacter actinomycetemcomitans, P. gingivalis: Porphyromonas gingivalis, T. forsythia: Tannerella forsythia, T. denticola: Treponema denticola, P. intermedia: Prevotella intermedia, P. micros: Peptostreptococcus micros, F. nucleatum/periodonticum: Fusobacterium nucleatum/periodonticum, C. Rectus: Campylobacter rectus, E. nodatum: Eubacterium nodatum, E. corrodens: Eikenella corrodens, C. gingivalis/ochracea/sputigena: Capnocytophaga spec.: gingivalis/ochracea/sputigena.*). 0: No colouring (<1% no bacteria) 1: Very light coloured tinting, staining (bacteria determination between the rates of 1% to 20%) 2: Light colouring (bacteria determination between the rates of 21% to 40%) 3: Significant colouring (bacteria determination between the rates of 41% to 60%) 4: Dark colouring (bacteria determination between the rates of 61% to 80%) 5: Very dark colouring (bacteria determination between the rates of 81% to 100%).

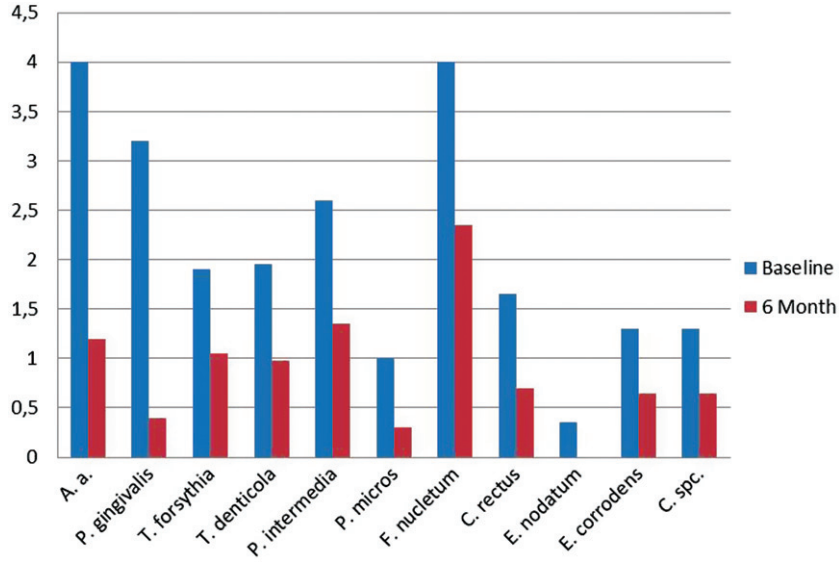

Figure 2. The distribution of the observation rates of periodontopathogenic microorganisms prior to non-surgical periodontal treatment and following non-surgical periodontal treatment in the test group: (*A. actinomycetemcomitans: Aggregatibacter actinomycetemcomitans, P. gingivalis: Porphyromonas gingivalis, T. forsythia: Tannerella forsythia, T. denticola: Treponema denticola, P. intermedia: Prevotella intermedia, P. micros: Peptostreptococcus micros, F. nucleatum/periodonticum: Fusobacterium nucleatum/periodonticum, C. Rectus: Campylobacter rectus, E. nodatum: Eubacterium nodatum, E. corrodens: Eikenella corrodens, C. gingivalis/ochracea/ sputigena: Capnocytophaga spec.: gingivalis/ochracea/sputigena.*). 0: No colouring (<1% no bacteria) 1: Very light coloured tinting, staining (bacteria determination between the rates of 1% to 20%) 2: Light colouring (bacteria determination between the rates of 21% to 40%) 3: Significant colouring (bacteria determination between the rates of 41% to 60%) 4: Dark colouring (bacteria determination between the rates of 61% to 80%) 5: Very dark colouring (bacteria determination between the rates of 81% to 100%).

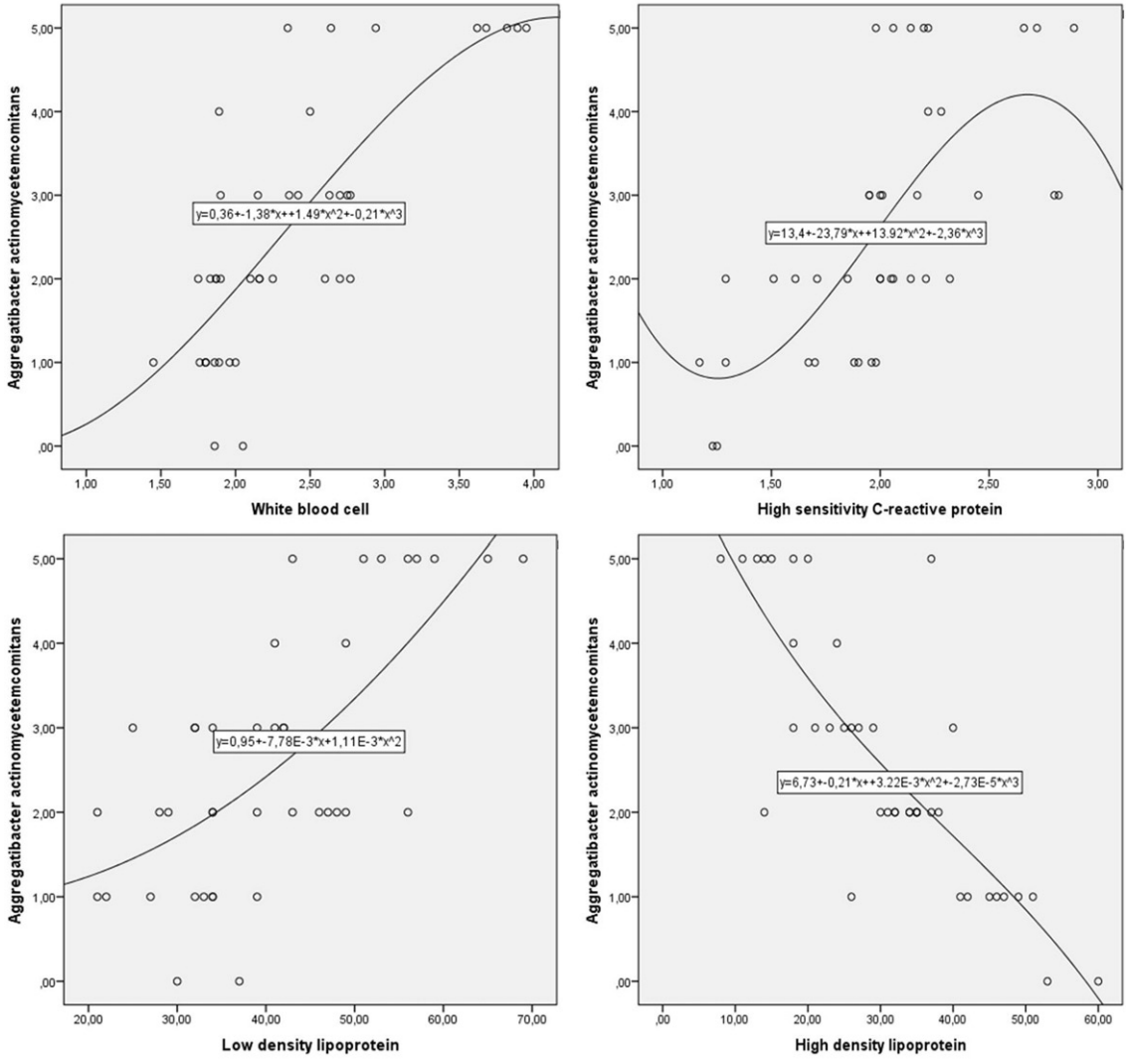

Figure 3. Correlations between *A. actinomycetemcomitans* level changes and hs-CRP, WBC, LDL and HDL levels changes in all patients.

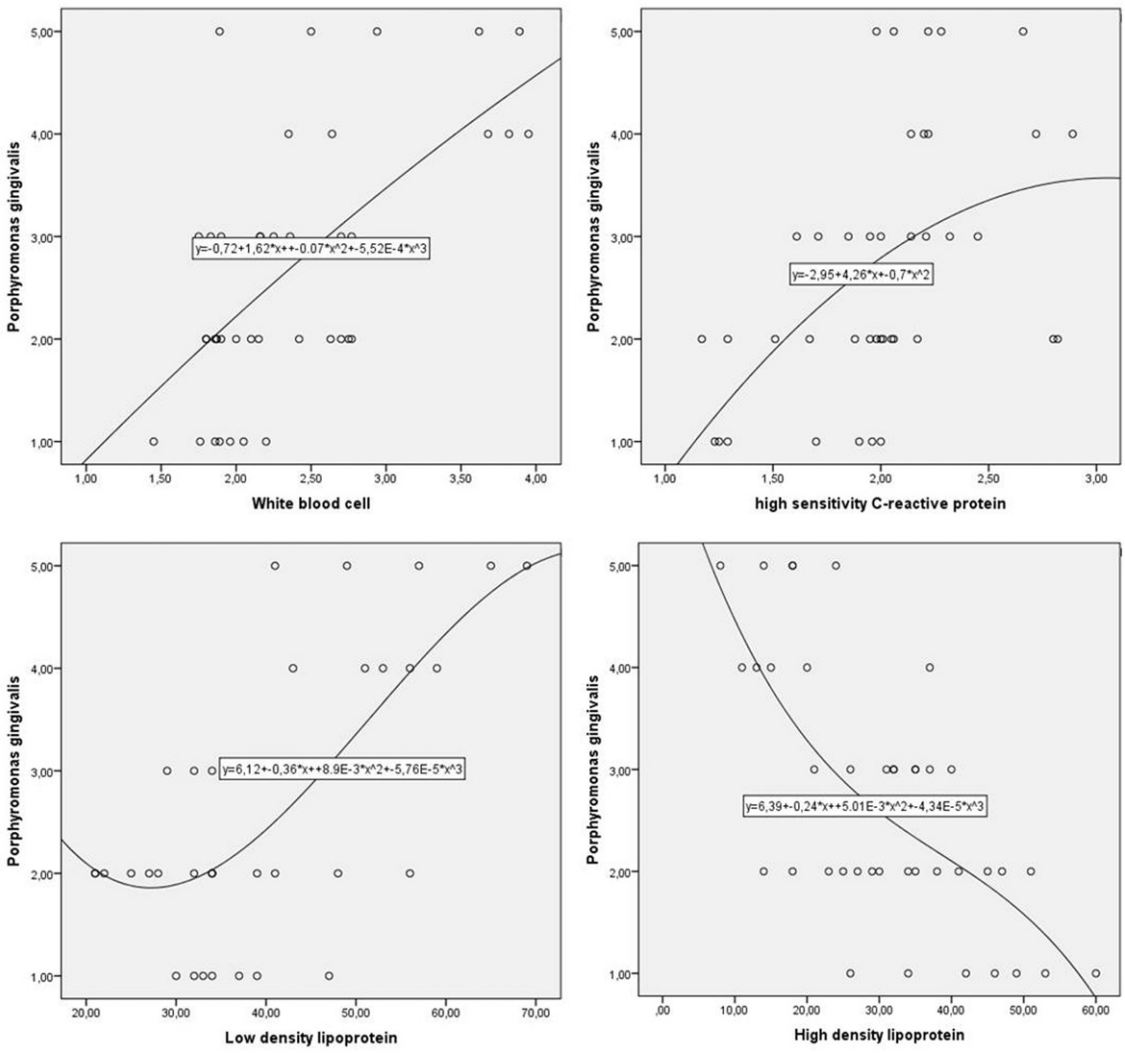

Figure 4. Correlations between *P. gingivalis* level changes and hs-CRP, WBC, LDL and HDL levels changes in all patients.

P. intermedia, F. nucleatum and *E. corrodens*, which were effective in inducing periodontal disease and hs-CRP, WBC, PLT, fibrinogen, creatinine and LDL.

When the study results found in the literature were discussed, it was observed that there are studies which show that atherosclerosis, can be healed by the initial periodontal treatment of patients who have been diagnosed with chronic periodontitis and atherosclerosis [13,17]. Some studies, however, have avoided relating chronic periodontitis to atherosclerosis [18]. It is known that dental and periodontal infections may play a significant role in the development of atheroma plaque formation and CVD [19]. Periodontopathogenic microorganisms found in microbial dental plaque are observed in blood and atheroma plaques [20]. *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* are found in atherosclerotic plaque [21] and *T. denticola, P. intermedia, T. forsythia, F. nucleatum, P. micros, E. corrodens* and *C. gingivalis/ochracea/sputigena* are related to CVD [9,22]. *Porphyromonas gingivalis* is efficient as it produces a mediator for oxidized LDL, aids in the formation of foam cells and helps to destroy atherosclerotic plaque. *Porphyromonas gingivalis*, which is shown to be very effective in CVD, has been defined as a risk factor for the formation of CVD as it can flow into the circulatory system from its own ecosystem (subgingival region) via gingival bleeding, which is one of the clinical characteristics of chronic periodontitis [23,24]. Moreover, it has been determined that *P. gingivalis* and *T. denticola* show synergic effects and together create risk factors for CVD [25]. In a study carried out to determine periodontopathogenic microorganisms in atheroma plaque, the following microorganisms were identified: *P. gingivalis* (78.57%) in 33 out of 42 samples, *A. actinomycetemcomitans* (66.67%) in 28 out of 42 samples, *T. forsythus* (61.90%) in 26 out of 42 samples, *E. corrodens* (54.76%) in 23 out of 42 samples, *F. nucleatum* (50.00%) in 21 out of 42 samples and *C. rectus* (9.52%) in 4 out of 42 samples [26]. These results prove the relationship between periodontopathogenic microorganism, CVD and periodontal diseases.

Porphyromonas gingivalis, P. intermedia, C. rectus and *B. forsythus*, have positive correlations with the blood CRP values and the levels of these microorganisms in subgingival flora. It has been noted that increased CRP increases cardiovascular risk, which, in turn, increases in parallel with the microorganisms determined to be in the subgingival flora and that this is related to the severity of chronic periodontitis [27]. It has been shown in the literature that the amount of CRP decreases following the treatment of periodontitis [28]. In this study, the

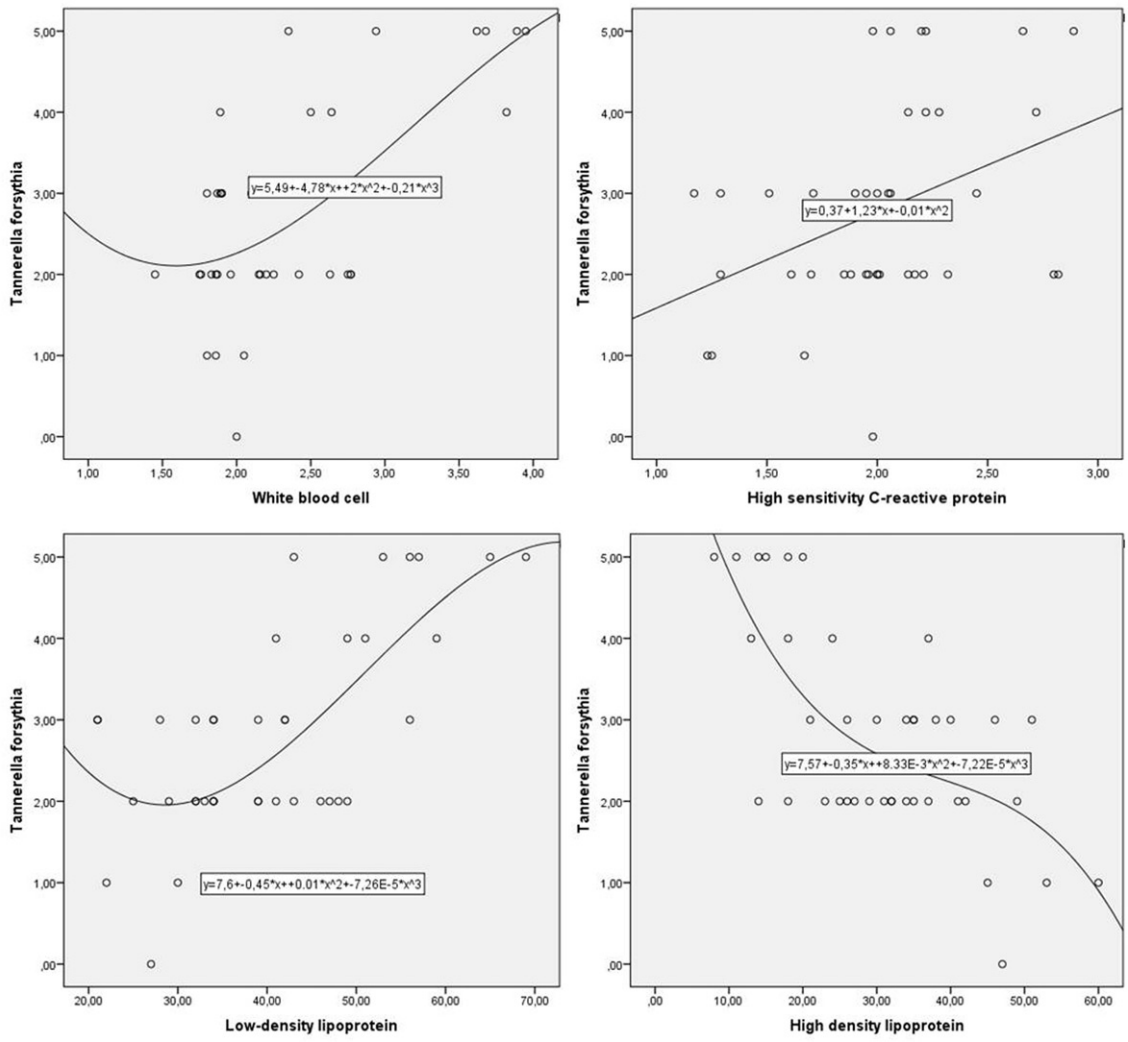

Figure 5. Correlations between *T. forsythia* level changes and hs-CRP, WBC, LDL and HDL levels changes in all patients.

relationship between CRP and periodontopathogenic microorganisms was examined. Also, the hs-CRP was preferred during the examination, as it provided precise results. There was a decrease in the rates of periodontopathogenic microorganisms in subgingival flora following periodontal treatment;at the same time, hs-CRP values decreased with statistical significance in the serum in parallel [29]. There are studies which show that HDL, LDL, WBC, triglyceride and PLT amounts such as CRP in the blood samples of patients with chronic periodontitis change with nonsurgical periodontal treatment [30]. HDL, LDL, PLT, WBC, fibrinogen and creatinine values are important data that were examined in terms of CVD. Moreover, it is known that these data change with non-surgical periodontal treatment applied to chronic periodontitis patients. It has been indicated that the results found following examination prove the relationship between CVD and chronic periodontitis [30]. Similar results were obtained in this study. The levels of WBC, PLT, LDL, fibrinogen and creatinine showed a decrease following periodontal treatment and the HDL level increased. The fact that LDL, PLT, WBC, creatinine and fibrinogen levels decrease in patients with atherosclerosis even with the initial periodontal treatment supports our hypothesis that non-surgical periodontal treatment is successful in dealing with an atherosclerosis prognosis.

When the microorganism rates and the correlation of other data that were examined in the study were discussed, a positive correlation was observed between hs-CRP and many microorganisms. The hs-CRP may be decreased due to the decrease in the amount of periodontopathogenic microorganisms following non-surgical periodontal treatment. As a result, the atherosclerosis prognosis may have been positively affected. Similarly, many microorganisms have shown a negative correlation with HDL and a positive correlation with LDL, WBC, fibrinogen, creatinine and PLT. It can be assumed that atherosclerosis symptoms may be reduced by periodontal treatment in chronic periodontitis patients when these results are taken into consideration. The microorganisms whose rates have been examined prior to the treatment and in the sixth month following non-surgical periodontal treatment in this study showed a correlation with systemic inflammation indicators and progressed in parallel with those indicators, which, in turn, proved that these bacteria had systemic effects. As a result, it can be suggested that non-surgical periodontal treatment should be carried out, ended and continued to decrease the systemic bacterial load in CVD. While periodontal parameters showed parallel development with each other, which is a significantly successful result following clinic periodontal treatment, the increase and decrease in

these parameters cannot be observed at the same level for all data.

This study has some limitations. One is that only dental plaque samples were used in determining periodontopathogen microorganisms. A sample could have been taken from the atheroma plaque and it could have been included in the study in addition to the samples that were taken. However, in this study, this process could not be carried out as the study also included cardiovascular healthy patients.

The treatment of periodontal diseases can be shown as a factor that helps in CVD treatment. It can be said that periodontal treatment of patients in addition to cardiovascular treatment of those patients who have been diagnosed with both chronic periodontitis and atherosclerosis, can be beneficial for cardiovascular treatment.

Conclusions

The association between hs-CRP, WBC, LDL, PLT, fibrinogen, creatinine and the amount of periodontopathogenic microorganism (*A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola* and *P. intermedia*) indicates the possibility that periodontal treatment could decrease the risk of atherosclerosis. Further studies must be conducted to support these results.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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