

The Role of Periodontal Disease on Acute Phase Proteins in Patients With Coronary Heart Disease and Diabetes

Gonca Çayır KELEŞ¹
Burcu Özkan ÇETİNKAYA¹
Selen Burçak ŞİMŞEK¹
Diyar KÖPRÜLÜ²
Hakkı KAHRAMAN³

Aims: Chronic infections such as periodontal disease (PD) have been suggested to be a risk factor for coronary heart disease (CHD). Elevated levels of acute phase proteins are associated with increased risk for cardiovascular events in both healthy individuals and patients with known CHD. This study aimed to investigate the role of PD on C-reactive protein (CRP) and fibrinogen levels in patients with either CHD or type 2 diabetes.

Materials and Methods: 80 subjects were evaluated in four groups: individuals with CHD+PD (group 1; n: 20), individuals with type 2 diabetes+PD (group 2; n: 20), individuals with PD without any systemic disease (group 3; n: 20), and healthy individuals (group 4; n: 20). Blood samples were taken at the time of periodontal examination.

Results: Demographic characteristics between the four groups and periodontal parameters between groups 1, 2, and 3 were not statistically different ($P > 0.05$). CRP and fibrinogen levels were increased in groups 1, 2, and 3 compared to those in group 4 ($P < 0.05$).

Conclusions: The patients in groups 1, 2, and 3 may have risk for future cardiovascular events. Findings of the present study seem to indicate that periodontitis contributes to systemic inflammation due to higher CRP and fibrinogen levels. Physicians should be aware that maintaining periodontal health might be effective in reducing cardiovascular events risk in periodontitis subjects with/without CHD and diabetes.

Key Words: Cardiovascular risk, C-reactive protein, fibrinogen, periodontal disease, periodontitis

Koroner Kalp Hastalığı ve Diabeti Olan Bireylerde Periodontal Hastalığın Akut Faz Proteinleri Üzerine Etkisi

Amaç: Periodontal hastalık (PH) gibi kronik enfeksiyonların koroner kalp hastalıkları (KKH) için risk faktörü olduğu düşünülmektedir. Sağlıklı ve KKH olan bireylerde akut faz proteinleri seviyesindeki yükselme, kardiyovasküler olayların gelişim riskinin artışı ilişkilidir. Bu çalışmada; periodontal hastalığın, KKH'ı veya tip 2 diabeti olan hastalarda C-reaktif protein (CRP) ve fibrinojen seviyesi üzerine etkisini incelemek amaçlandı.

Materyal ve Metot: 80 birey 4 gruba ayrıldı; KKH+PH'ı olan bireyler (1. grup; n:20), tip 2 diabet+PH'ı olan bireyler (2. grup; n:20), herhangi bir sistemik hastalığı olmayan PH'a sahip bireyler (3. grup; n:20), periodontal ve sistemik sağlıklı bireyler (4. grup; n:20). Kan örnekleri periodontal inceleme sırasında alındı.

Bulgular: Dört grup arasında demografik özellikler açısından, 1., 2. ve 3. grup arasında periodontal parametreler açısından istatistiksel olarak anlamlı farklılık gözlenmedi ($P > 0.05$). CRP ve fibrinojen seviyeleri; 1., 2. ve 3. grupta, 4. grup ile karşılaştırıldığında istatistiksel anlamlı artış gösterdi ($P < 0.05$).

Sonuçlar: 1., 2. ve 3. gruptaki hastalar, ileride görülecek kardiyovasküler olaylar için risk altında olabilir. Bu çalışmanın bulguları, CRP ve fibrinojen seviyesinin yüksekliği ile ilişkili olarak periodontitisin sistemik enflamasyona katıldığını göstermektedir. Hekimlere periodontal sağlığı korumanın, KKH ve diabeti olan ve/veya olmayan periodontitisli bireylerde kardiyovasküler olay riskini azaltabileceği konusunda haberdar olmaları önerilebilir.

Anahtar Sözcükler: kardiyovasküler risk, C-reaktif protein, fibrinojen, periodontal hastalık, periodontitis

Introduction

Coronary heart disease (CHD), which is an important factor of morbidity and mortality, can be a result of genetic and environmental risk factors such as diabetes mellitus, hypertension, smoking, abnormal serum lipids, and age (1-3). Periodontal disease (PD) is a slowly progressing infectious disease resulting in inflammatory

¹ Department of Periodontology,
Faculty of Dentistry,
Ondokuz Mayıs University,
Samsun - TURKEY

² Department of Cardiology,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun - TURKEY

³ Department of Internal Medicine,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun - TURKEY

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Correspondence

Gonca Çayır KELEŞ
Department of Periodontology,
Faculty of Dentistry,
Ondokuz Mayıs University
Samsun - TURKEY

goncaceles@hotmail.com

destruction of the alveolar bone as well as loss of the soft tissue attachment of the teeth (4). Although PD is a localized chronic infection of the oral cavity, it has been demonstrated that untreated PD is associated with various systemic complications (5,6). In addition, there is growing evidence that chronic infections such as periodontitis increase the risk for CHD and cardiovascular events (2,7-9).

The acute-phase response is a non-specific process, initiated and coordinated by a large number of diverse inflammatory mediators that may occur in the initial host response to injuries, infections, ischemic necrosis or malignancy (6,9). Acute phase proteins are established risk factors for CHD and have been suggested to associate with infectious diseases such as PD (10,11). C-reactive protein (CRP) and fibrinogen, acute phase proteins, are sensitive markers to evaluate the inflammatory status (6,9,12).

It is known that diabetes mellitus is an established risk factor for both PD and CHD (2,13). Elevated levels of acute phase proteins are associated with increased risk for cardiovascular events in both healthy individuals and patients with known CHD (12,14-16). However, these proteins have not yet been examined in periodontitis subjects with CHD or diabetes. Therefore, it was aimed to investigate for the first time the role of PD on CRP and fibrinogen levels regarding the possible risk for cardiovascular events in patients with either CHD or type 2 diabetes mellitus.

Materials and Methods

Subject Selection

A total of 80 individuals were enrolled in the study. Subjects were evaluated in four groups: individuals with proven CHD+PD (group 1; n: 20) from the Department of Cardiology, Faculty of Medicine; individuals with type 2 diabetes+PD (group 2; n: 20) from the Department of Internal Medicine, Faculty of Medicine; individuals with PD without any systemic disease (group 3; n: 20) from the Department of Periodontology, Faculty of Dentistry; and periodontally and systemically healthy individuals (group 4; n: 20) from the Department of Oral Diagnosis and Radiology, Faculty of Dentistry, Ondokuzmayis University. Diagnosis of CHD was made by coronary angiography. Patients with CHD had no history of recent

acute myocardial infarction. Type 2 diabetic patients had no evidence of current acute illness including clinically significant infectious disease. None had received insulin therapy. Exclusion criteria were chronic inflammatory or immunological conditions such as arthritis, gastrointestinal disorders, skin conditions, bronchitis or other chronic obstructive airway disease; acute infections (within 2 months before the entry to the study); ongoing infections; recent (<3 months) history of antibiotic usage; current corticosteroid therapy; hypertension; pregnancy; and lactation.

Only subjects who were found free from clinical evidence of systemic diseases were enrolled in the study as control subjects. Control group was matched by gender, age, geographic area, and social factors. These subjects also underwent a thorough medical examination. Control subjects with a past history of a diagnosed or unclear cardiovascular condition were excluded.

All subjects enrolled in the study were non-obese [Body Mass Index (BMI): <30 kg/m²] and non-smokers. Subjects who smoked within five years were assigned to the smokers (6).

Periodontal Examination

The same investigator performed clinical assessments of the patients in their first visit. Probing pocket depth (PPD), the distance in millimeters from the free gingival margin to the bottom of the pocket, and clinical attachment loss (CAL), the distance in millimeters from the cemento-enamel junction to the bottom of the pocket of all teeth, were measured by using William's Probe at six sites per tooth. The inclusion criteria for PD were mean CAL >3 mm and PPD ≥4 mm with periodontal sites greater than 30% (generalized periodontitis) (6,11). The healthy subjects had no history of periodontitis. They showed neither CAL nor PPD greater than 3 mm at more than one site (17).

Laboratory Analysis

Blood samples were collected from subjects at the time of clinical examination. Serum total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-cholesterol), and low density lipoprotein cholesterol (LDL-cholesterol) levels were determined by autoanalyzer in the Clinical Biochemistry Laboratory.

Serum CRP levels were quantified by latex-enhanced nephelometry in the Clinical Biochemistry Laboratory (4,11). The laboratory's reference range was 0-3 mg/L.

Plasma fibrinogen levels were determined with the modification of the Clauss method (18) in the Hematology laboratory. The laboratory's reference range was 1.8-3.5 g/L.

Statistical Analysis

The statistical analysis was performed using a commercially available software program (SPSS 12.0, SPSS Inc., Chicago, Illinois, USA). The Shapiro Wilk test was used to investigate whether or not the data was normally distributed. The Kruskal Wallis and Mann-Whitney U non-parametric tests were used for comparisons of the parameters not having a normal distribution. Significant levels were calculated for $P=0.0125$ ($0.05/4$). Data are shown as means \pm standard error of means (SEM) and minimum-maximum. One way analysis of variance (ANOVA) and Post Hoc Tukey parametric tests were used for comparisons with a normal distribution. Data are shown as means \pm SEM. Significant levels were calculated for $P < 0.05$.

Results

Table 1 shows the characteristics of the subjects. There was no significant difference in age, gender

distribution, number of standing teeth, BMI, and levels of total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol between the four groups ($P > 0.05$).

Table 2 illustrates the findings for clinical variables for groups 1, 2, and 3. There was no significant difference in PPD and CAL between the three groups ($P > 0.05$).

Serum CRP levels and plasma fibrinogen levels are depicted in Table 3. CRP levels were elevated in groups 1 ($P < 0.001$), 2 ($P < 0.012$), and 3 ($P < 0.010$) compared to those in group 4. There was also a significant difference in CRP levels between group 1 and group 2, and between group 1 and group 3 ($P < 0.001$). No significant difference was found between group 2 and group 3 ($P > 0.05$). Fibrinogen levels were increased in groups 1, 2, and 3 compared to those in group 4 ($P < 0.001$).

Discussion

PD is an inflammatory disease in which bacteria and their products are the principal etiologic agents (19). PD, a low grade local infection, is associated with a moderate systemic inflammatory response (20). It has been shown that infection and inflammation caused by PD increase the risk of CHD (2,21). The relationship between CHD and PD can be dependent on the systemic effects of PD and on the risk factors such as age, smoking, and diabetes in both diseases (2,21,22). Successful control of the

Table 1. Characteristics of the subjects (Mean \pm SEM).

	Group 1	Group 2	Group 3	Group 4
Age	50.10 \pm 0.87	48.05 \pm 1.18	47.55 \pm 0.92	48.25 \pm 0.86
Gender Distribution (Male:Female)	15:5	14:6	14:6	15:5
Number of Standing Teeth	21.85 \pm 0.66	21.15 \pm 0.74	22.90 \pm 0.62	22.35 \pm 0.47
BMI (kg/m ²)	27.05 \pm 0.14	26.92 \pm 0.22	27.04 \pm 0.20	26.86 \pm 0.23
Total Cholesterol (mg/dl)	183.55 \pm 6.87	182.40 \pm 6.21	180.70 \pm 7.61	182.45 \pm 5.19
Triglyceride (mg/dl)	157.55 \pm 8.43	158.70 \pm 10.40	156.45 \pm 11.99	159.70 \pm 6.80
HDL-Cholesterol (mg/dl)	42.09 \pm 1.52	42.45 \pm 1.78	43.97 \pm 2.41	40.35 \pm 1.17
LDL-Cholesterol (mg/dl)	119.10 \pm 4.65	118.80 \pm 3.94	120.90 \pm 4.84	120.50 \pm 3.84

No significant differences were found in any of the parameters between the groups ($P > 0.05$).

Table 2. Clinical variables of the subjects (mm).

	Probing Pocket Depth	Clinical Attachment Loss
Group 1	4.38 ± 0.12	6.52 ± 0.24
Group 2	3.91 ± 0.16	5.95 ± 0.22
Group 3	4.31 ± 0.21	6.04 ± 0.22

One way ANOVA, Post Hoc Tukey tests (Mean±SEM).
No significant differences were found in either parameter between the groups ($P > 0.05$).

Table 3. Serum CRP (mg/L) and plasma fibrinogen (g/L) levels of the subjects.

	CRP Mean ± SEM (minimum-maximum)	Fibrinogen Mean ± SEM
Group 1	5.63 ± 0.24 (3.14-7.35)	3.99 ± 0.22 ^e
Group 2	3.94 ± 0.24 (3.08-6.51) ^{abd}	4.01 ± 0.15 ^e
Group 3	3.88 ± 0.16 (3.08-5.59) ^{acd}	3.80 ± 0.11 ^e
Group 4	3.19 ± 0.04 (3.13-3.68) ^a	2.69 ± 0.06

Kruskal Wallis and Mann-Whitney U tests were used for comparisons of CRP and one way ANOVA and Post Hoc Tukey tests for comparisons of fibrinogen.

^aSignificantly different from group 1 ($P < 0.0010$).

^bSignificantly different from group 4 ($P = 0.0120$).

^cSignificantly different from group 4 ($P = 0.0100$).

^dNo significant difference between group 2 and group 3 ($P > 0.05$).

^eSignificantly different from group 4 ($P < 0.0010$).

periodontal infection has been associated with a significant reduction of serum CRP levels (12,23,24). It has been suggested that elevation of serum CRP is a significant indicator of risk of atherosclerosis, cardiovascular disease, and type 2 diabetes (14,25,26). Additionally, excessive fibrinogen production can increase proinflammatory cytokines, attract more leukocytes at the sites of inflammation, and also promote the colonization and adhesion of bacteria (6,27-29).

The results of the present study clearly showed elevated levels of CRP and fibrinogen, which are predictors of present and future cardiovascular events and diseases (30,31), in groups 1, 2, and 3. It has been reported that CRP level is influenced by many factors, including serum total cholesterol, HDL cholesterol, triglyceride, and BMI (26). In the present study, serum

total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol levels and BMI were similar between the groups. Obese subjects have been reported to exhibit higher CRP values than lean subjects (24). In our study, the mean BMI of the subjects was 26.97 ± 0.10 ; thus, they were not apparently obese.

Findings of the present study suggest that the patients in groups 1, 2, and 3 may have risk for future cardiovascular events, as serum CRP levels were increased. Increased CRP levels in the periodontitis group is consistent with the recent reports that PD may induce elevation of CRP levels (11,13,20,26). PD was considered to be a potential systemic exposure when $>15\%$ of the sites examined had a pocket depth of ≥ 4 mm (32). Only the patients with generalized periodontitis were included in the present study. PD may independently influence the CRP level, thereafter significantly greater elevation of this acute phase protein in patients with CHD+PD is probably due to the role of both diseases. Increment in CRP level could be associated with PD in diabetes patients in our study, as CRP levels were not statistically different between group 2 and group 3. Likewise, CRP level has been found to correlate with the concentration of antibody to Porphyromonas gingivalis, a microorganism responsible for the evolution of PD, in type 2 diabetic patients (15).

Our results on plasma fibrinogen levels confirm the possible risk for future cardiovascular events in patients in groups 1, 2, and 3, since increased plasma fibrinogen level is considered an independent risk factor for cardiovascular disease (6). Fibrinogen levels have been reported to be influenced by several factors such as age, gender, and smoking habits (6). Also, fibrinogen levels are higher in females and tend to be higher with increasing age (6). However, in the present study, there was no difference between the groups regarding age, gender and smoking habit.

Additionally, fibrinogen has been suggested to be a possible mediator in the pathogenesis of PD (6). This is consistent with the report that there is an independent association between PD and plasma fibrinogen levels explaining the relationship between PD and cardiovascular disease (16). It has been demonstrated that infections other than PD are associated with increased blood viscosity by raising fibrinogen levels (4). In our study, increased fibrinogen level is probably due to PD, as included patients did not have any other infections.

The association between PD and CHD has recently received considerable attention. CHD has been shown to be the most prevalent medical problem in patients with PD, accounting for 26% of the patients (33). In conclusion, the patients in groups 1, 2, and 3 associated with increased CRP and fibrinogen levels may have risk for future cardiovascular events. Serum CRP and plasma fibrinogen levels are considered as independent and useful predictors with regard to that risk. Within the limitations of the sample selection and number, findings of the present study seem to indicate that periodontitis contributes to systemic inflammation. Since increment in

acute phase proteins is associated with increased risk for cardiovascular events in patients with CHD and healthy individuals, diabetes is a risk factor for CHD, and PD can have an etiological or modulating role in CHD. After adjustment for other risk factors, assessments of serum CRP and plasma fibrinogen levels in periodontitis subjects with/without CHD and diabetes could provide important guidance in order to identify subjects also at risk for cardiovascular events like acute myocardial infarction. Physicians should be aware that maintaining periodontal health might be effective in reducing that risk in these individuals.

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