Increased Heart Failure Prevalence in Patients with a High Antibody Level Against Periodontal Pathogen

Norio Aoyama, DDS, Keitetsu Kure, DDS, Masato Minabe, DDS and Yuichi Izumi, DDS

Summary

The aim of this study was to assess whether a specific cardiovascular disease was related to an increased antibody level against a periodontal pathogen.

A strong association between cardiovascular disease and periodontitis was shown, however, the causal relationship was not proven. Increased inflammatory reaction of patients with periodontitis was a possible factor, which connected periodontal infection and vascular diseases.

We assessed medical history, blood data, and periodontal conditions in patients with cardiovascular diseases. Serum IgG antibody titers against major periodontal pathogens and existence of salivary periodontal bacteria were analyzed.

In total, 348 subjects were enrolled in this study. The patients who exhibited 10,000 counts/mL or more of salivary *Porphyromonas gingivalis* were divided into two groups according to the antibody level of the pathogen. Patients with a high antibody level against *Porphyromonas gingivalis* exhibited a high rate of heart failure compared to the low antibody group. Mean probing pocket depth and clinical attachment level significantly increased in the high antibody group. We found that the high anti-*Porphyromonas gingivalis* antibody group also experienced enhanced antibody levels against other periodontal bacteria.

An increased heart failure prevalence was found in patients with a high antibody level against a major periodontal pathogen, *Porphyromonas gingivalis*.

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**Key words:** Cardiovascular disease, Infection, Inflammation, Periodontitis

A strong association between cardiovascular diseases (CVDs) and periodontal disease was widely recognized.1-4 Periodontitis is an infective disease by many kinds of periodontopathic bacteria, such as *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), and *Prevotella intermedia* (*P. intermedia*).5 Among these bacteria, *P. gingivalis* was known as a keystone pathogen.6 The association between systemic status and infection with *P. gingivalis* has been reported.7,8 We also showed that infection of periodontal pathogens could cause or promote development of CVDs using several experimental models.9-13

While the causal relationship between periodontal disease and CVDs has not been clearly proven, systemic inflammation following continuous infection of periodontal bacteria is considered as a possible risk factor for CVDs.9 On this basis, we hypothesized that patients with an enhanced immunological reaction against periodontal pathogens, such as *P. gingivalis*, experienced an increased prevalence of some CVDs compared to those with a low reaction to periodontal bacteria who exhibited many periodontal bacteria in oral cavity. The aim of this study was to compare the prevalence of CVDs in the patients with a high antibody level against *P. gingivalis* to those with a low antibody level.

**Methods**

**Study population:** Subjects were recruited from patients with CVDs in the Tokyo Medical and Dental University Hospital from May 2012 to August 2015. All participants in this study were hospitalized patients in the cardiovascular medicine section of the hospital. Patients who did not consent to the participation or had a history and presence of other infectious diseases were excluded. The Ethics Committees of the School of Medicine and the School of Dentistry, Tokyo Medical and Dental University approved the protocol of the present study, and the protocol conformed to the Helsinki Declaration of 1975, as revised in
with a 650 nm reference wavelength. Individual antibody sorbance was read using a microplate reader at 450 nm levels (Units/mL) were calculated from the standard curve, detecting real-time polymerase chain reaction method was used to identify bacteria. A representative tooth was missing, the next tooth was used instead. Bacterial DNA was extracted from 200 μl saliva using a DNeasy Blood and Tissue kit (Qiagen, Tokyo, Japan) according to manufacturer’s instructions. A real-time polymerase chain reaction method was used to detect P. gingivalis as previously described. Prevalence of systemic and cardiovascular diseases. A chi-square test was performed to compare gender, smoker rate, and prevalence of systemic and cardiovascular diseases. A Wilcoxon test was used to compare the levels of anti-bacterial antibodies. JMP 9.0.3 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Values of P < 0.05 were considered significant.

**Results**

In total, 348 subjects were enrolled in this study. All the subjects exhibited 10,000 counts/mL or more P. gingivalis in saliva. The characteristics of the patients in the high- and low-level groups of the P. gingivalis antibody are shown in Table I. There was no statistical difference in age, sex, and smoking status between the two groups. Prevalence of DM, HT, and DL were similar in both groups. In Table II, the prevalence of several cardiovascular diseases is shown. The researchers found that a rate of heart failure statistically increased in the high antibody group compared to the low antibody group. Prevalence of the other diseases, such as coronary heart disease, cardiomyopathy, valvular disease, arrhythmia, and peripheral vascular disease, was similar among the groups. We also measured the levels of CRP, HbA1c, TC, LDL-c, HDL-c, which was obtained from the gradual dilutions of the reference.

**Medical examination:** A medical history, including diabetes mellitus (DM), hypertension (HT), and dyslipidemia (DL), was recorded, and a physical examination was performed. Cigarette smoking history was obtained by interview. Peripheral blood samples were obtained, and parameters were determined such as concentrations of C-reactive protein (CRP), hemoglobin A1c (HbA1c), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), triglyceride (TG), and B-type natriuretic peptide (BNP). Left ventricular ejection fraction (EF) was also calculated.

**Clinical periodontal assessment:** Periodontists certified by the Japanese Society of Periodontology counted the prevalence of periodontal pathogens. A medical history, including diabetes mellitus (DM), hypertension (HT), and dyslipidemia (DL), was recorded, and a physical examination was performed. Cigarette smoking history was obtained by interview. Peripheral blood samples were obtained, and parameters were determined such as concentrations of C-reactive protein (CRP), hemoglobin A1c (HbA1c), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), triglyceride (TG), and B-type natriuretic peptide (BNP). Left ventricular ejection fraction (EF) was also calculated.

**Characteristics of the Subjects**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Low antibody group</th>
<th>High antibody group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>174</td>
<td>174</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.1 ± 11.9</td>
<td>66.9 ± 10.9</td>
<td>0.1312</td>
</tr>
<tr>
<td>Sex (Female [%])</td>
<td>24.7</td>
<td>24.1</td>
<td>0.9007</td>
</tr>
<tr>
<td>Smoker [%]</td>
<td>51.7</td>
<td>43.1</td>
<td>0.1071</td>
</tr>
<tr>
<td>Diabetes [%]</td>
<td>27.0</td>
<td>27.0</td>
<td>1.0000</td>
</tr>
<tr>
<td>Hypertension [%]</td>
<td>61.5</td>
<td>66.7</td>
<td>0.3145</td>
</tr>
<tr>
<td>Dyslipidemia [%]</td>
<td>42.0</td>
<td>50.0</td>
<td>0.1319</td>
</tr>
</tbody>
</table>

Age is shown as mean ± standard deviation.

**Prevalence of Assessed Cardiovascular Diseases**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Low antibody group</th>
<th>High antibody group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary heart disease [%]</td>
<td>35.6</td>
<td>39.7</td>
<td>0.4386</td>
</tr>
<tr>
<td>Heart failure [%]</td>
<td>6.9</td>
<td>9.9</td>
<td>0.1003</td>
</tr>
<tr>
<td>Cardiomyopathy [%]</td>
<td>5.2</td>
<td>6.9</td>
<td>0.4298</td>
</tr>
<tr>
<td>Valvular disease [%]</td>
<td>9.8</td>
<td>7.5</td>
<td>0.4443</td>
</tr>
<tr>
<td>Bradyarrhythmia [%]</td>
<td>9.8</td>
<td>50.0</td>
<td>0.6679</td>
</tr>
<tr>
<td>Tachyarrhythmia [%]</td>
<td>52.3</td>
<td>50</td>
<td>1.0000</td>
</tr>
<tr>
<td>Peripheral vascular disease [%]</td>
<td>2.3</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>
The periodontal conditions of the subjects are shown in Table IV. Mean PPD and CAL were significantly high in the high antibody group, in comparison to the low antibody group. The number of the residual teeth and BOP rate were comparable between the groups. In Table V, the antibody levels of the other periodontal bacteria, \textit{A. actinomyctecomitans} and \textit{P. intermedia}, are shown. We found that the high anti-\textit{P. gingivalis} antibody group also demonstrated statistically increased antibody levels against \textit{A. actinomyctecomitans} and \textit{P. intermedia}.

We divided the patients with heart failure into a heart failure with reduced ejection fraction (HFrEF) group and a heart failure with preserved ejection fraction (HFpEF) group, according to EF. The patients with heart failure with less than 40\% of EF were categorized in HFrEF, while the heart failure patients with 50\% or more EF were classified as HFpEF. In Table VI, there are similar rates of patients with HFrEF and HFpEF in the high and low anti-\textit{P. gingivalis} antibody groups.

**Discussion**

We conducted this cross-sectional study to assess the relationship between systemic inflammation caused by periodontal infections and CVDs and demonstrated that the increased number of patients with heart failure was associated with a high \textit{P. gingivalis} antibody level.

From the proposal of periodontal medicine, many studies were performed to clarify the association between periodontal disease and systemic condition. As a scientific statement, Lockhart and colleagues indicated that the asso-
ciation between periodontitis and CVDs was important and that the link was statistically detected after the adjustment of confounders, such as age, smoking and DM. They also suggested that periodontal infection had not been proven as a risk factor of CVDs and that further studies were needed to understand the relationship between the diseases. In this review article, it is proposed that systemic inflammation could play a role of mediator. It was known that the biological reaction widely varied between individuals. This means that not only clinical and bacterial measurements but also examinations of biological and inflammatory reaction were important. Therefore, an antibody test against periodontal bacteria may be effective, particularly in assessing the effect of periodontal infection on systemic health.

A recent review article indicated that many studies showed a significant association between periodontitis and CVD; however, strong evidence that dental bacteria may serve as a causal agent for CVD has not been provided. To date, some experimental studies suggested an influence of periodontal infection on vascular diseases. Cellular hypertrophy was induced with a P. gingivalis infection via altered signaling pathways in cardiomyoblast cells. It was also showed that a P. gingivalis infection accelerated experimental cardiac hypertrophy via oxidative stress in mice. Clinical studies indicated an association between the elevated anti-P. gingivalis antibody level and myocardial infarction, carotid atherosclerosis, coronary heart disease, and stroke. These findings suggest that infection of P. gingivalis and following inflammatory reactions might influence cardiac and vascular tissues.

Among many species of periodontal bacteria, P. gingivalis is the most pronounced and experimented pathogen. P. gingivalis was one of the highly related pathogens to clinical measures of periodontal disease. The mechanism by which P. gingivalis contributes to inflammation and disease activities was assessed in many ways. In this study, we used an inclusion criterion of salivary P. gingivalis existence (10,000 counts/mL or more). The reason for this criterion was that we intended to compare the patients with the enhanced immunological reaction to those with a suppressed reaction even if they exhibited a lot of targeted bacterium. We obtained the result that the patients with a high antibody level against P. gingivalis experienced an increased prevalence of heart failure. However, no difference was found for the prevalence of other CVDs. The blood data of the present study indicated that serum inflammatory and lipid markers were comparable in both groups. All the patients experienced at least one CVD in this study, which might affect the blood data. We found that the patients with a high P. gingivalis antibody also experienced increased antibody levels of A. actinomyces and P. intermedia. Some patients might experience the same characteristic of an enhanced immunological reaction against several periodontal pathogens.

While it has been shown that several kinds of CVDs were associated with periodontal disease, the strength of the links must be different among CVDs. On this basis, we performed the present study and analyzed the patients with different immunological reaction against P. gingivalis, the most studied periodontal pathogen. As a result, an association between heart failure and a high antibody against P. gingivalis was found. Heart failure is a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood. Heart failure is caused by several underlying heart diseases such as coronary heart disease, arrhythmia, valvular disease, and cardiomyopathy. However, in this study, we could not find any difference of such heart diseases, coronary heart disease, arrhythmia, valvular disease, and cardiomyopathy, between the high and low antibody groups except for heart failure.

As a possible link between periodontal infection and cardiovascular diseases, chronic inflammation occurred by periodontitis can play a role to lead to mediator increase in blood. Local inflammation by periodontal infection continues for a long period if a patient is not dentally treated. The relationship between cardiovascular diseases and inflammatory mediators in blood is established. A well-analyzed marker for inflammation is CRP. However, we found similar CRP levels in the high and low antibody groups in this study. Periodontitis is generally characterized with chronic inflammation, thus the change of CRP, which is a major marker for acute inflammation, may be limited. Serum BNP level and EF were also similar in both groups. Further research is needed to assess the underlying mechanisms in the link of periodontal infection and heart diseases.

Recently, researchers suggested that gut microbiota might be related to heart failure. A microbial-host pathway linking dietary metabolism, the gut microbiota, and cardio-renal disease progression was proposed. Conversely, a possibility of oral pathogen's inducing systemic inflammation via alteration of gut microbiota was also suggested. Gut microbiota may be a key factor in the interrelationship between oral infection and CVDs.

There were some limitations in the present study. We obtained this epidemiological data in order to assess the relationship between periodontal condition and CVDs; however, the sample size was not calculated. Next, we did not show severity of CVDs and medication status in each patient because there were a variety of CVDs and medication. We defined heart failure according to a guideline by Japanese Circulation Society, Guidelines for Diagnosis and Treatment of Acute and Chronic Heart Failure (JCS 2017/JHFS 2017). However, information of CVDs and medication is important to assess the difference of background in patients.

In the present study, we demonstrated that the enhanced antibody level against P. gingivalis was associated with a high prevalence of heart failure in Japanese patients with CVD. We could not reveal the causal and pathological relationship in this cross-sectional study among the periodontal infection, systemic inflammatory reaction, and CVDs. Therefore, further experimental and clinical studies are needed.

Disclosures

Conflicts of interest: None.
References