High Incidence of Periodontitis in Japanese Patients With Abdominal Aortic Aneurysm

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Summary

Periodontitis is known to be a risk factor for abdominal aortic aneurysm (AAA). However, the influence of periodontitis on AAA in Japanese patients has not yet been elucidated. The aim of this clinical investigation was to assess the relationship between periodontal bacterial burden in AAA patients and the presence of the periodontal pathogens Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Prevotella intermedia in oral samples using polymerase chain reaction assays.

We found that the AAA patients had deeper pocket depth compared to the non-AAA patients (3.53 ± 0.38 mm versus 2.67 ± 0.17 mm, P < 0.05). However, the populations of periodontal bacteria were comparable between the two groups. Periodontitis may have a greater effect on aneurysm progression compared to other cardiovascular diseases. (Int Heart J 2014; 55: 268-270)

Key words: Bacteria, Periodontal disease, Pocket depth

The association between periodontal diseases and the risk of abdominal aortic aneurysm (AAA) is well known.①② Periodontitis is an infective disease caused by many different species of periodontal bacteria,③ such as Porphyromonas gingivalis (P. gingivalis), Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), and Prevotella intermedia (P. intermedia). Clinical investigations have demonstrated that each periodontal pathogen accelerates the progression of AAA.④⑤⑥ Recently, we revealed that P. gingivalis worsened AAA development while A. actinomycetemcomitans did not alter its development in experimental murine AAA models.⑦ We clarified that P. gingivalis affected AAA progression through toll-like receptors (TLRs) and matrix metalloproteinases (MMPs).⑧⑨⑩ P. intermedia is also known to be an important periodontal pathogen in cardiovascular diseases.⑪⑫ However, the clinical influence of specific periodontal bacterium on AAA has not been investigated. Thus, the aim of this study was to evaluate the condition of periodontitis and infection by periodontal bacteria in Japanese patients with AAA.

METHODS

Study population: Patients were recruited from Tokyo Medical and Dental University Hospital and the University of Tokyo Hospital between May 2012 and April 2013. The study group was composed of 12 patients with AAA. The control group consisted of 24 randomly selected age- and sex-matched non-AAA patients with various cardiovascular diseases (8 myocardial ischemia, 7 arrhythmia, 5 heart failure, and 4 valvular disease). Patients who did not consent to participation in the study or had a history and/or presence of other infections were excluded. The protocol was approved by the Ethics Committee of the School of Medicine and the School of Dentistry, Tokyo Medical and Dental University (approval number 1165) and the University of Tokyo (approval number 3058), and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. All study participants provided written informed consent.

Periodontal examination: Periodontal examinations were performed by dentists who were not familiar with the clinical backgrounds of these individuals. Full-mouth clinical measure-
ments, including probing pocket depth (PD) and bleeding on probing (BOP) were recorded using a manual probe (PCP-UNC 15, Hu-Friedy Manufacturing Co., Chicago, IL, USA) at 6 points (buccal-mesial, mid-buccal, buccal-distal, lingual-mesial, mid-lingual, and lingual-distal) on a right upper molar, an upper incisor, a left upper molar, a right lower molar, a lower incisor, and a left lower molar. We also evaluated the number of remaining teeth and the community periodontal index (CPI).

**Real-time polymerase chain reaction (PCR) to detect presence of bacteria:** Unstimulated saliva and dental plaque collected using paper points from each subject were obtained. Bacterial DNA was extracted from each sample using a DNeasy Blood and Tissue kit (Qiagen, Tokyo) according to the manufacturer’s instructions. A real-time polymerase chain reaction (PCR) method was used to detect three periodontopathic bacteria (*P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia*). Specific primers for each bacterium were used as previously described:  

- For *P. gingivalis*: 5’-cttgacttcagtggcggcag-3’ and 5’-agggaagacggttctcacca-3’;
- For *A. actinomycetemcomitans*: 5’-cttacctactcttgacatccga-3’ and 5’-atgcagcacctgtctcaaa-3’;
- For *P. intermedia*: 5’-aatacccgatgttgtccacaa-3’ and 5’-ttagccggtccttattc-3’.

The real-time PCR was performed using the Thermal Cycler Dice Real Time System (Takara Bio Co., Shiga, Japan). The real-time PCR was performed using the Sybr Green assay (25 µL) containing 12.5 µL of SYBR® Premix EX Taq II (Takara Bio Co., Shiga, Japan), 1 µL of forward and reverse primer (10 µmol/L), and 2 µL of extracted DNA. The thermocycling program was 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds with an initial cycle of 95°C for 30 seconds. At each cycle, the accumulation of PCR products was detected by monitoring the increase in fluorescence of the reporter dye from dsDNA-binding SYBR Green. After the PCR, a dissociation curve (melting curve) was constructed in the range of 60°C to 95°C.

**Data analysis:** Numerical data are presented as the mean ± standard error of the mean (SEM). Differences were examined with the Mann-Whitney U test for two group comparisons. The chi-square test was used to compare gender, underlying diseases, medications, and the bacteria present. All statistical analyses were performed with the aid of statistical software (Prism 5, versus 5.0a, GraphPad Software). Values of *P* < 0.05 were considered significant.

**RESULTS**

**Patient characteristics:** The characteristics of the study population are presented in Table I. There were no statistically significant differences in age, gender, underlying diseases, or medications between the two groups.

**Periodontal conditions:** We observed significantly deeper PD in AAA patients (3.53 ± 0.38 mm) than in the non-AAA group (2.67 ± 0.17 mm, *P* < 0.05). Although the number of remaining teeth, BOP, and average CPI were statistically comparable...
between the two groups, these factors in AAA patients tended to be more severe than in the non-AAA patients. (Table II)

**Bacterial species:** The populations of *P. gingivalis* in saliva and dental plaque were comparable between the two groups. Similarly, the populations of *A. actinomycetemcomitans* and *P. intermedeia* in saliva and dental plaque were not statistically different between the two groups.

**DISCUSSION**

Offenbacher, *et al* proposed a new field of study entitled “periodontal medicine”. Since then, many researchers have studied the association between periodontitis and systemic diseases. Lockhart, *et al* presented a scientific statement that enhanced the relationship between periodontal disease and cardiovascular diseases. Recently, we demonstrated that *P. gingivalis* deteriorated AAA and wire-injury induced arteriosclerosis in animal models. Furthermore, we demonstrated that *A. actinomycetemcomitans* aggravated ventricular remodeling after myocardial ischemia and pressure overload-induced myocardial hypertrophy. Based on the findings of our previous studies, we focused on these two bacteria in this clinical observation study on AAA.

We found that patients with AAA had significantly deeper PD in comparison to patients with non-AAA diseases in this study. Other periodontitis factors, specifically the number of remaining teeth, BOP, and average CPI, in AAA patients also tended to be more severe than in the non-AAA patients. These results support the fact that AAA patients generally have badoral and periodontal conditions. To detect specific bacterial burden, we conducted PCR using their saliva and plaque. The populations of *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedeia* in oral samples were not different between the two groups in this study population. The significance of these results can be interpreted in a number of ways. First, the specific bacterial burden that plays a key role in AAA development might be masked because the mean age of the population was too old (over 70 years) to detect a difference. Vlachojannis, *et al* demonstrated that the populations of periodontal bacteria generally increase with age. Thus, investigations with younger AAA patients should clarify the clinical pathogenesis. Secondly, other periodontal pathogens may aggravate AAA because Kurihara, *et al* showed that other periodontopathic bacteria, such as *Treponema denticola* and *Campylobacter rectus*, were present in the specimens of AAA. Therefore, surveillance of broader periodontal pathogens might be useful for elucidating the relationship between periodontitis and AAA development.

In conclusion, periodontitis may have a greater effect on aneurysm progression compared to other cardiovascular diseases. Although the types and populations of periodontal pathogens were comparable between the AAA and non-AAA patients, further clinical investigation is needed to clarify the pathological relationship between the specific bacterial burden and AAA development.

**REFERENCES**