

# Association between Periodontal Pathogens and Coronary Artery Disease: A Case-Control Study

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## Abstract

**Background and Aim-** We sought to answer the questions about the role of inflammatory factors in the formation of pathological lesions in the endothelium of the coronary vessels and also the role of host-

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based bacteria, including chronic periodontitis, in the clot formation in the blood vessels, all of which destabilize the atherosclerotic plaque.

**Methods and Materials-**This case-control study was done on 40 patients who underwent elective coronary artery bypass grafting surgery (CABG) with the need of coronary endarterectomy. In Group A, patients had chronic periodontitis and group B consisted of patients without it. Both groups were well matched regarding their demographic data. The subgingival plaque was collected by a sterile curette from periodontal pockets  $\geq 5$ mm and CAL  $\geq 4$ mm. Also, atherosclerotic plaque was collected during the coronary endarterectomy surgery from all of the 40 patients. The specimens were assessed using the PCR technique to detect the specific bacteria responsible for chronic periodontitis such as actinobacillus actinomycetemcomitans (Aa), prevotella intermedia (Pi), porphyromonas gingivalis (Pg), Tanerella forsythensis (Tf), Treponema denticola (Td), and fusobacterium nucleatum (Fn).

**Results-**In the atherosclerotic plaque of group A patients, Aa was identified in 18 (90%), Pg in 16 (80%), Tf in 13(65%),Td in 11 (55%), Pi in 10 (50%), and Fn in 6 (30%) specimens, whereas in group B the incidence was significantly lower ( $p < 0.0001$ ). In the subgingival plaque of group A, Aa and Tf were found in all 20 individuals and Pg, Pi, Td and Fn were identified in 19 specimens (95%). The differences in the incidence of Aa and Pg in two plaque samples were not significant, but the two plaque samples showed significant differences regarding the incidence of the other pathogens ( Pi :  $p < 0.001$ , Tf :  $p < 0.008$ , Td:  $p < 0.003$  and Fn : $p < 0.0001$ ).

**Conclusion-**In the present study, the same organisms were found in both coronary atherosclerotic and subgingival plaques. The findings support the potential role of the periodontopathogenic bacteria species in some steps of the atherosclerotic process as a contributor that worsens this disease. However, further studies are required to achieve more definite results regarding the role of periodontal diseases in the atherosclerotic disease, focusing on patients' background variables(*Iranian Heart Journal 2011; 12 (2):34-40*).

**Keywords:** Atherosclerosis ■ Coronary artery disease ■ Chronic Periodontitis

Periodontitis is an infective disease that is caused by the dilative topical reactions owing to aggregation of a group of mostly gram-negative anaerobic bacteria on teeth surfaces and results in the destruction of periodontal tissues.<sup>1 & 2</sup>

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ANALYSIS INVESTIGATIONS INDICATED

that the patients with chronic periodontitis are exposed to higher risk factors of coronary heart diseases as opposed to the patients having healthy periodontium.<sup>5-8</sup> A growing number of studies have been focusing on whether periodontal disease elevates the risk of coronary heart diseases or not.<sup>9, 10, 11&12</sup>

Accordingly, after equalizing the risk factors in heart diseases, epidemiological studies have indicated a 50 – 70 percent increase in the risk of coronary diseases in patients suffering from periodontitis. Similar symptoms have been observed in periodontitis and ischemic heart disease; thus, the connection between these two diseases might be also dependent on the same etiological factors.<sup>14</sup> Various potential mechanisms have been proposed for justifying the relation between periodontal and coronary heart diseases.<sup>15 & 16</sup>

Based on numerous reports, the disorder in the metabolism of plasma lipoproteins and the topical and systemic inflammatory reactions might play a role in the primary damage of coronary atheromatosis.<sup>17-19</sup> Infected and inflamed periodontium can lead to systemic inflammatory reactions such as an increase in the amount of c-reactive protein as well as other thrombogenic reactions.<sup>20-23</sup> With the onset of inflammation in periodontal tissues, the bacteria and their products enter the blood through the enlarged vessels or the ulceration of pocket epithelium.

In addition, the results of some studies have suggested that periodontal pathogens are present in the early damage of atherosclerosis and carotid atheromatosis plaque.<sup>25</sup> Therefore, periodontitis and vascular heart diseases might be related by two intermediates: (a) periodontopathogens and (b) biological mediators.

Following the focal infection theory, infection foci in remote regions can establish and spread the inflammatory diseases in other parts of the human body. Such a correlation between periodontitis and vascular heart diseases has been recently attracting more interests. Milard and Mackenzie were the pioneers to propose the relationship in 1963,<sup>26</sup> and in recent years their theory has been developed into the following form: Periodontitis, as a bacterium source in the mouth, can cause instability and progress of the atheromatosis plaque.<sup>27</sup> Unstable atherosclerotic plaque is a hazardous clinical condition that can lead to chronic incompetence of coronary arteries and finally myocardial infarction. The present study focused on finding the pathogenic relationship in patients with periodontitis versus patients without periodontitis who have undergone endarterectomy.

## Material and Methods

This case-control study was conducted on 212 elective CABG candidates in Shaheed Rajaei Cardiovascular, Medical and Research Center of Tehran from May 2009 to April 2010. Forty patients who necessarily needed coronary endarterectomy during CABG were selected. Group A included 20 patients with chronic periodontitis (mean age:56.9 years, M/F=1) and group B included 20 patients without this lesion (control group, mean age:56.7 years, M/F=1.5) The two study groups were matched well regarding age ( $p=0.36$ ) and sex ( $p=0.58$ ). The patients with diabetes mellitus, familial hyperlipidemia, history of antibiotic therapy, or scaling during the previous six months were excluded from our study. Periodontal samples were collected from two periodontal pockets without pus secretion (with a depth of 5 mm or more and Clinical Attachment Level (CAL) of 4 mm or more). The patients were recommended not to use antiseptic mouth rinse 12 hours before sampling. The selected teeth for sampling were clean from supragingival plaques and

sterile curette were put inside at least two pockets with a depth of 5 mm or more for 10 seconds for the collection of the subgingival plaques. The samples of coronary and periodontal plaques were transferred into a test tube containing normal saline. These tubes were sent for bacteriologic examination to the Dental Research Center and Cellular and Molecular biology Research center (Shaheed Beheshti Medical University). The pathogens were identified through the polymerase change reaction (PCR) techniques.

#### Laboratory steps:

**Pathogen tracing:** To each sample, 500 µl of lysis buffer (0.32 M saccharose, 10mM Tris, 1mM EDTA, 5mM MgCl<sub>2</sub>) were added and incubated overnight in 37°C. DNA was extracted using the phenol-chloroform method.

**Primer design:** Primers were designed and synthesized for each of the following bacteria:

**PCR procedure:** 0.5 µg of extracted DNA was used for the reaction. The reaction included following ingredients:

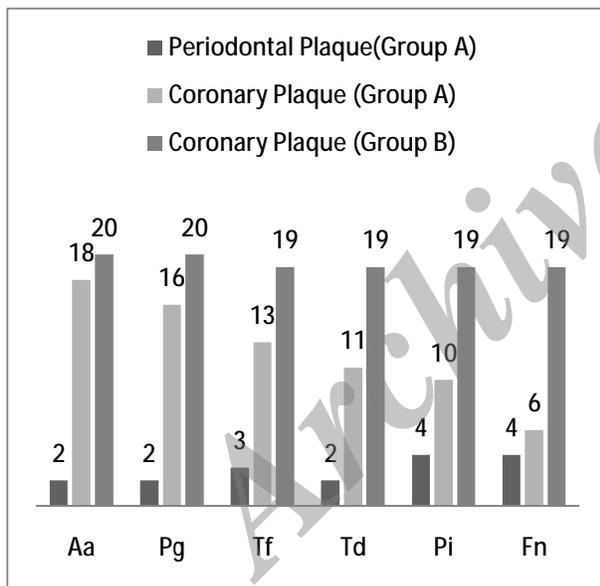
The reaction was conducted for 5 minutes in 94°C, and the 30 cycles continued in following condition:

In addition, the reaction was done in 72°C. Annealing heating of the primers was as follows:

At the end of PCR, the products were electrophoresed using % 2 agarose gel. Then, ethidium bromide was used for staining. UV Transilluminator with 26-nm wavelength was used to detect the band for the PCR product. Primers were designed to detect the microorganisms, including Porphyromonas gingivalis (Pg), Actinobacillus actinomycetem comitans (Aa), Treponema denticola (Td), Prevotella intermedia (Pi), Tanerella forsythensis (Tf), and Fusobacterium nucleatum (Fn).

### Results

In the periodontal plaque samples of group A, Aa and Tf pathogens were found in all the samples and Pg, Pi, Td, and Fn were detected in 19 cases (% 95). The incidence of these pathogens in the coronary plaques of group A was 90% for Aa, 85% for Pg, 80% for Tf, 55% for Td, 50% for Pi, and only 30% for Fn (fig. 1).



**Fig.1.** Incidence of different pathogens in periodontal and coronary plaques in the two study groups

The pathogens in the atherosclerotic plaques samples of group B included Pi and Fn in 4(% 20), Tf in 3(% 15), and Pg, Td, and Aa in 2 samples (10%) (Fig. 3).

There was a statistically significant difference regarding the incidence of pathogens in the coronary atheromatous plaque between the two study groups (Table I).

**Table I. Incidence of Different Pathogens in Coronary Atheromatous Plaques of two Groups**

\* *Actinobacillus actinomycetemcomitans* \*\* *Porphyromonas gingivalis*  
 \*\*\* *Prevotella intermedia* # *Tanarella forsythensis* ## *Treponema denticola* #S ### *Fusobacterium nucleatum*

**Table II- The incidence of different organism in periodontal and coronary plaques**

Pathogen	Periodontal Plaque No (%)	Coronary artery Plaque No. (%)	P Value
Aa*	100%	90%	0.48
Pg**	95%	80%	0.34
Pi***	95%	50%	0.001
Tf#	100%	65%	0.008
Td##	95%	55%	0.003
Fn###	95%	30%	0.0001

Pathogen	Group A No. (%)	Group B No. (%)	P Value	Odds Ratio
Aa*	90%	10%	0.0001	9
Pg**	80%	10%	0.0001	8
Pi***	50%	20%	0.05	2.5
Tf#	65%	15%	0.001	4.33
Td##	55%	10%	0.002	5.5
Fn###	30%	20%	0.47	1.5

\* *Actinobacillus actinomycetemcomitans* \*\* *Porphyromonas gingivalis* \*\*\* *Prevotella intermedia* # *Tanarella forsythensis* ## *Treponema denticola* #S ### *Fusobacterium nucleatum*

### Discussion

In recent years, many studies have been focused on the determination of the potential correlation between periodontitis and cardiovascular diseases, and numerous researchers have verified the link and indicated that the existence of periodontitis elevates the risk of heart diseases.<sup>30</sup> In most cases, periodontopathogen bacteria belong to anaerobic gram-negative group, e.g. *Actinobacillus actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg). These bacteria along with their similar species possess special virulence factors that help them colonize, avoid phagocytic digestion, increase attack power, and cause inflammation. Since the pathogenic capability of these bacteria is relatively high, their function range is not restricted to periodontium; they can function in farther regions to stimulate and even disrupt the host's immunological system in a systemic procedure.<sup>31</sup> Our results confirmed that both types of invasive bacteria have high incidence in periodontal and atheromatous plaques.

The pathogenesis of periodontitis and arteriosclerosis is similar.<sup>24</sup> In periodontitis, gradual release of cytokines causes the periodontal diseases to play a role directly in the pathogenesis of arteriosclerosis and thromboembolic diseases.<sup>31</sup> Periodontium in patients with chronic periodontitis acts as a storage location for lipopolysaccharid (LPS), which can enter the systemic blood circulation during usual daily activities, e.g. simple masticating, and results in destructive cardiovascular effects. Therefore, LPS is a systemic stimulating factor in vascular problems related to heart diseases.<sup>9</sup>

With the spread of the bacteria in the blood, inflammatory cells at the atherosclerotic plaque are also affected, and then the atherosclerotic plaque instability happens.<sup>25, 32, 33</sup> In the present study, however, this explanation was not proved since the results indicated that the frequency of the bacteria studied at the atherosclerotic plaque was lower than that of the periodontal plaque.

The current study indicated that pathogens Aa, Pg, Pi, Tf, Td, and Fn, all of which are related to chronic periodontitis, were, respectively, present in 90%, 80%, 50%, 65%, 55%, and 30% of the coronary vessel plaque samples of the patients suffering from periodontitis. Although the pathogens of the coronary artery plaques were less frequent than those in the periodontal one, their presence in the atherosclerotic plaque samples of group A was significant. Even the incidence of Aa and Pg bacteria was the same as the periodontal plaque. Madianos et al. (1996) reported the presence of Pg bacterium in periodontal pocket epithelium, and even its propagation capability in this area.<sup>34</sup> Ishihara et al. (2004) proved the increase of Pg amounts in the atherosclerotic plaque, which was linked to its presence in subgingival pockets. The results of a research by Zaremba et al. (2007) also indicated that Pg had the maximum presence frequency in the atherosclerotic plaque.<sup>36</sup> Based on the results of the present research, Pg bacterium had the second rank after Aa bacterium. The role of pathogen Pg in the development of the atherosclerotic plaque was proved in a study conducted on animals.<sup>37</sup> A study by Yaun-ming et al. (2008) for the determination of the link between microorganisms present in the atheromatous plaque and periodontal bacteria indicated that the spread of pathogens Tf, Fn, Pi, and Pg in coronary vessel plaques was respectively 31%, 12%, 18%, and 33%, and maximum frequency was for pathogen Pg.<sup>38</sup> Sakurai et al. (2007) also indicated that 33 percent of the patients with chronic coronary syndrome possessed pathogen Aa in their oral samples; they concluded that periodontopathogen species, especially Aa, might play an essential role in the progress of coronary vessels syndrome.<sup>40</sup> De Stefano et al., working on 9760 individuals with the age of 25—74 years, indicated the increased risk (a 25 %increase) of heart coronary diseases in patients with chronic periodontitis.<sup>13</sup> Mattila et al. examined the men diagnosed with heart coronary disease using angiography and oral radiography; they managed to find a significant correlation between the degree of tooth disease and the rate of coronary atheromatosis.<sup>42</sup> They also indicated that bacterial infections were the only factors that, separately from the other identified risk factors in coronary vessels, were independently involved in the severity of coronary vessels arteriosclerosis.<sup>42</sup>

Perhaps the best evidence in the exploration of the relation between periodontitis and heart disease can be originated from systematic review studies. For example, Janket et al., using the meta-analysis method, proved that general threat of cardiovascular happenings in individuals suffering from periodontitis was 19% higher than the rest.

Presence of periodontal bacteria in arteriosclerosis and periodontal plaque samples from the same patients, although with different amounts, verified the potential role of the periodontopathogen species studied in some stages of arteriosclerosis as the contributing factor with different mechanism which deteriorates the course of the disease.

#### Conclusion

The results of the present study revealed a relation between atherosclerosis and chronic periodontitis. Considering the presence of periodontal bacteria in the atherosclerosis and periodontal plaque samples which belonged to the same patients, periodontopathogen species seems to be involved in some stages of cardiovascular diseases as a contributing factor that deteriorates the course of the disease. The relation strengthens the theory that a true inflammatory link exists between chronic periodontitis and atherosclerosis. Nevertheless, to obtain conclusive evidence for verifying the influence of periodontal disease on arteriosclerosis, interventional studies must be conducted to control all the other patients' variables.

#### Conflict of Interest

No conflicts of interest have been claimed by the authors.

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