



## Presence of *Tannerella forsythia* in patients with chronic periodontal disease and atherosclerosis

### Prisustvo bakterije *Tannerella forsythia* kod bolesnika sa hroničnom periodontalnom bolešću i arterosklerozom

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#### Abstract

**Background/Aim.** Periodontal disease is an inflammatory disease that occurs in the tissues surrounding the teeth in response to bacterial biofilm accumulation (dental plaque). Among others, *Tannerella forsythia* (*Tf*) was recognized as one of the most significant and specific bacterial species in periodontal pocket („red complex“ bacteria). Atherosclerosis is a progressive narrowing of arteries that may lead to occlusion, as a consequence of lipid deposition. It underlies coronary heart disease (80%), as well as myocardial and cerebral infarctions. Increasing evidence over the past decade suggests a link between periodontal disease and atherosclerosis, where *Tf* can enter the systemic circulation directly or indirectly, and be present in atherosclerotic blood vessels. Therefore, the aim of this study was to detect the presence of *Tf* in atheromatous plaques obtained from different blood vessels in patients with chronic periodontitis. **Methods.** Ninety patients (male 61, female 29) with periodontal disease and atherosclerosis [recruited for either carotid artery stenosis requiring endarterectomy or percutaneous transluminal coronary angioplasty (PTCA)] were included in this study. Clinical periodontal examinations consisted of plaque

index (PI) (according to Silness Løe), gingival index (GI) (according to Løe Silness), sulcus bleeding index (according to Mühlemann-Son) and periodontal probing depth (PPD). Presence of *Tf* in periodontal pockets and atherosclerotic vessels was detected using polymerase chain reaction (PCR) method with positive control *Tf* ATCC 43037. **Results.** *Tf* was present in subgingival plaques of 68 (75.6%) of the patients, while its presence in atheromatous plaques were registered in 42 (53.3%) of the patients. It was significantly present in coronary blood vessels (41.7%), followed by carotid arteries (35.4%) and *a. abdominalis* aneurism (12.5%), *a. mamaria* (8.5%) and *a. femoralis* (2.1%) while in *a. iliaca* *Tf* was not detected at all. **Conclusion.** The present study suggests strong relationship between periodontal inflammation and atherogenesis; therefore, it should be considered as potential risk factor for atherosclerosis. Accordingly, it would be necessary to control periodontal disease in order to reduce mortality and morbidity associated with atherosclerosis.

#### Key words:

periapical periodontitis; *tannerella forsythia*; atherosclerosis; risk factors; comorbidity.

#### Apstrakt

**Uvod/Cilj.** Parodontopatija je zapaljensko oboljenje potpornog aparata zuba, koje se javlja kao odgovor na prisustvo dentalnog plaka. Između ostalih, *Tannerella forsythia* (*Tf*) je jedna od značajnih i veoma specifičnih bakterija koja se

nalazi u periodontalnom džepu i pripada bakterijama „crvenog kompleksa“. Ateroskleroza je progresivno sužavanje arterija koje može, konačno, dovesti do njihovog potpunog začepjenja, kao rezultat nakupljanja masti. Smatra se vodećim uzrokom koronarne srčane bolesti (80%), kao i infarkta miokarda i moždanog udara. Sve više je objavljenih

radova na temu potencijalne povezanosti parodontopatija i kardiovaskularnih oboljenja, gde se smatra da bakterije prisutne u periodontalnom džepu, između ostalih *Tf*, mogu prodrati u sistemsku cirkulaciju direktnim ili indirektnim putem. Cilj ove studije bio je utvrđivanje prisutnosti *Tf* u aterosklerotskim plakovima različitim krvnih sudova osoba obolelih od hronične parodontopatije. **Metode.** U ovu studiju je bilo uključeno 90 pacijenata (61 muškarac i 29 žena) sa dijagnozom parodontopatije i ateroskleroze koji su bili podvrgnuti endarterektomiji ili perkutanoj transluminalnoj koronarnoj angioplastici. Klinički pregled za parodontopatiju sastojao se od utvrđivanja plak indeksa (po Silness Lōu), gingivalnog indeksa (po Lōe Silnes), indeksa krvarenja iz sulkusa (po Mühleman-Son) i dubine periodontalnog džepa. Prisustvo *Tf* u periodontalnom džepu i aterosklerozom izmenjenim krvnim sudovima detektovano je metodom lančane reakcije polimeraze [*polymerase chain reaction* (PCR)]

uz *Tf* ATCC 43037 kao pozitivne kontrole. **Rezultati.** *Tf* je bila prisutna u subgingivalnom plaku kod 68 (76%) pacijenata, dok je njeno prisustvo u aterosklerotskim plakovima zabeleženo kod njih 42 (53,3%). Značajno prisustvo *Tf* je zabeleženo u koronarnim arterijama (41,7%), karotidnim arterijama (35,4%), zatim u aneurizmama *a. abdominalis* (12,5%), *a. mamaria* (8,5%) i *a. femoralis* (2,1%), dok u *a. iliaca* *Tf* nije bila prisutna. **Zaključak.** Ovom studijom utvrđena je jaka povezanost parodontopatije i aterogeneze, te se smatra da bi se parodontopatija trebala uvrstiti u potencijalne faktore rizika od nastanka ateroskleroze. Takođe, bilo bi neophodno da se kontroliše parodontopatija kako bi se smanjio rizik od oboljevanja i smrtnosti povezanih sa aterosklerozom.

**Ključne reči:**  
periodontitis, periapikalni; tannerella forsythia; ateroskleroza; faktori rizika; komorbiditet.

## Introduction

Periodontal disease is affecting up to 90% of the worldwide population<sup>1</sup>. It is an inflammatory disease that occurs in the tissues surrounding the teeth in response to bacterial biofilm accumulation (dental plaque). It is well known that more than 500 virulent microbial species from dental biofilm are mainly responsible for periodontal disease. Although several bacterial species are currently recognized as causally associated with periodontal disease, subgingival colonization is not sufficient for the disease to occur<sup>2</sup>. It is well known that abnormal host response to periodontal disease with specific genetic predisposition and detrimental environment exposures are likely important determinants of susceptibility, e.g. age, poor oral hygiene, cigarette smoking, pregnancy, obesity, stress and systemic conditions such as diabetes mellitus, osteoporosis and rheumatoid arthritis<sup>3</sup>.

Atherosclerosis is a progressive narrowing of arteries that may lead to occlusion as a consequence of lipid deposition<sup>2</sup>. It underlies coronary heart disease (80%), as well as myocardial and cerebral infarctions, therefore having a big socio-economic importance<sup>4</sup>. Onset of atherosclerosis is thought to be due to endothelium function disturbances, platelet activation, and oxidative changes in plasma lipoproteins. Several possible biological mechanisms, including common genetic variants may explain the link between cardiovascular diseases and periodontitis<sup>5</sup>. It is believed that there are multiple mechanisms underlying these links, with inflammatory, infectious, immune, and genetic components<sup>4</sup>. The link between periodontal disease and atherosclerosis was first established around 30 years ago when De Stefano et al.<sup>6</sup> reported an increased risk of atherosclerotic plaque formation in a group of patients with periodontitis (25 % higher) based on 14 years of research including 9,760 individuals aged between 25 to 74 years. Recent attention has been directed towards the potential contribution of chronic inflammatory processes that may amplify vascular inflammation in atherosclerosis, and periodontal disease is recognized as a chronic inflammatory disease<sup>4, 7, 8</sup>. Today, the American

Hearth Association defines this contribution with level A evidence.

*Tannerella forsythia* (*Tf*) is one of the most significant and specific bacterial species in periodontal pocket („red complex“ bacteria). Increasing evidence, over the past decade, suggests a link between periodontal disease and atherosclerosis, where *Tf*, mainly present in diseased periodontal pockets, can enter the systemic circulation directly, and may be present in distant organs, such as atherosclerotic blood vessels. Lypopolysaccharides and other products from *Tf* cell breakdown may stimulate inflammatory cytokines, upregulate endothelial adhesion molecules and induce a prothrombotic environment, that can enhanced risk of an atherosclerosis<sup>9</sup>. The causal relationship between periodontal disease and atherosclerosis can be detected through bacterial presence at the diseased sites detected by real-time polymerase chain reaction (PCR)<sup>10</sup>.

Therefore, the aim of the present study was to detect the presence of *Tf* in subgingival plaques and atheromatous plaques obtained from different blood vessels.

## Methods

### Patients

Ninety patients, 61 males and 20 females, aged 28-94 years (mean age: 59.2 ± 12.7 years), with periodontal disease and atherosclerosis were recruited in this study. Patients were treated in the Clinic for Vascular and Endovascular Surgery, Clinical Center of Serbia in Belgrade, the Institute for Cardiovascular Diseases „Dedinje“ in Belgrade, the Clinic of Dental Medicine, Military Medical Academy in Belgrade and Clinic of Dental Medicine, Faculty of Medicine in Kosovska Mitrovica for either carotid artery stenosis requiring endarterectomy or percutaneous transluminal coronary angioplasty (PTCA), or *a. abdominalis* aneurisms, thrombosis and occlusions of *a. femoralis* and *a. iliaca*. Patients were divided according to the localization into six groups: group 1: carotid arteries (n = 29); group 2: *a. abdominalis* aneuris-

mas (n = 10); group 3: *a. femoralis* (n = 10); group 4: *a. iliaca* (n = 4); group 5: *a. coronaria* (n = 29); group 6: *a. mammaria* (n = 8).

Patients were recruited only if they were with at least four periodontal pockets. Periodontitis was diagnosed if a patient exhibited clinical attachment level (CAL) > 1 mm and periodontal pocket depth (PPD) > 3 mm, at least at three sites in two different quadrants. According to CAL, patients with diagnosed periodontitis were classified into three subgroups: patients with moderate chronic periodontitis (CP) (CAL = 3–4 mm) and severe CP (CAL ≥ 5 mm). Periodontitis was defined as localized or generalized depending on the number of affected sites<sup>11</sup>.

Exclusion criteria were: systemic diseases, antibiotic intake and periodontal treatment in the previous three months. The medical and dental history of each subject was obtained by interview. Smoking and alcohol use were detected by self-report. Patients fulfilling the inclusion criteria were informed of the study and signed informed consent form that was approved by the Ethics Committee of the Faculty of Medicine in Kosovska Mitrovica, Serbia.

#### *Subgingival and atheromatous plaque sample*

On the same day of the surgical intervention, a complete periodontal examination was performed by a single periodontist. Clinical examinations included plaque index (PI) (according to Silness Lön), gingival index (GI) (according to Löe Silnes), sulcus bleeding index (according to Mühlemanson) and periodontal probing depth (PPD). Subgingival plaque samples were collected using the paper point technique (Periopaper, Amityville, Pro Flow, NY, USA) from the bottom of two out of four present periodontal pockets. Each sample site was isolated with cotton rolls, scaled carefully supragingivally, and air dried. A sterile paper point was inserted into the apical extent of each selected pocket, kept for 60 seconds, and transferred immediately to a sterile Eppendorf tube and kept in the refrigerator on -70°C until the analysis. Samples from the arteries were taken during the surgery (endarterectomy, PTCA) and transferred immediately to a sterile Eppendorf tube with Tris-EDTA, as transport medium, and kept in the refrigerator on -20° C until the analysis by PCR method.

#### *PCR analysis*

Presence of *Tf* in periodontal pockets and atherosclerotic vessels was detected using PCR with positive control – *Tf* American Type Culture Collection (ATCC) 43037. The negative control was sterile distilled water instead of template DNA. The positive control consisted of DNA from pure cultures of *Tf* ATCC 43037. Colonies obtained from cultures were suspended in sterile water, centrifuged and subjected to DNA extraction.

#### *Statistical analysis*

Continuous variables were presented as means ± standard deviations (SD) or median and range and categorical

variables were expressed as absolute and relative frequencies. The normality of the data distribution was confirmed by the Shapiro-Wilk tests. The Mann-Whitney *U*-test and Kruskal-Wallis test were used to compare differences between two groups and for three or more groups, respectively. Categorical data between study groups were analyzed using the chi-squared ( $\chi^2$ ) test. Results were considered statistically significant when *p*-values were less than 0.05. Statistical analysis was performed with the Statistical Package for the Social Science Program (version 22, SPSS Inc., Chicago, IL, USA).

## Results

In subgingival plaque samples, *Tf* was detected in 68 (75.3%) of the patients, while in atherosclerotic plaque samples, *Tf* was present in 48 (53.3%) of the patients (Table 1).

**Table 1**  
**Presence of *Tannerella forsythia* in subgingival and atherosclerotic plaque samples of patients with periodontal disease and atherosclerosis**

Presence of <i>Tannerella forsythia</i>	Patients, n (%)
Subgingival plaque samples	
yes	68 (75.6)
no	22 (24.4)
Atherosclerotic plaque samples	
yes	48 (53.3)
no	42 (46.7)

In subgingival and atheromatous plaque samples of patients with atherosclerotic carotid arteries, *Tf* was detected in 79.3% and 58.6% of the patients, respectively. In case of patients with *a. abdominalis* aneurisms, *Tf* was present in sublingual and atherosclerotic plaque samples of 80% and 60% of the patients, respectively. In patients with atherosclerotic *a. femoralis*, *Tf* was present in subgingival plaques of 70% of the patients, while in atheromatous plaques of this blood vessel only in 10% of the patients. At the same time, *Tf* was present in subgingival plaques in 75% of patients with atherosclerosis of *a. iliaca*, while no *Tf* was present in the *a. iliaca* plaque samples. On the contrary, *Tf* was isolated in subgingival plaques of 79.3% of patients with atherosclerotic coronary arteries, and also in atherosclerotic plaque samples of 69% of these patients. In atherosclerotic and subgingival plaque samples of patients with atherosclerotic mammary arteries, *Tf* was found in both cases in 50% of the patients (Table 2).

Among patients with atheromatous plaque positive on *Tf*, 8.3% were with moderate and 91.7% with severe gingival inflammation, which was statistically significant difference in relation to patients with no presence of the bacterium in atheromatous plaques. Further, among patients with positive atheromatous plaque on *Tf*, 22.9% and 77.1% had moderate and severe form of periodontal disease, respectively, which was also statistically significant difference in relation to patients with no presence of *Tf* in atheromatous plaques (Table 3).

**Table 2****Presence of *Tannerella forsythia* in subgingival and atherosclerotic plaque samples of patients with periodontal disease and atherosclerosis in relation to different blood vessels**

Blood vessel	Patients, n (%)		Total number of patients
	subgingival plaque	atherosclerotic plaque	
<i>A. carotis</i>	23 (79.3)	17 (58.6)	29
<i>A. abdominalis</i> aneurism	8 (80.0)	6 (60.0)	10
<i>A. femoralis</i>	7 (70.0)	1 (10.0)	10
<i>A. iliaca</i>	3 (75.0)	0 (0)	4
<i>A. coronaria</i>	23 (79.3)	20 (69.0)	29
<i>A. mammaria</i>	4 (50.0)	4 (50.0)	8

**Table 3****Clinical degree of periodontal disease, gingival inflammation and bleeding on probing (BOP) according to *Tannerella forsythia* presence in atherosclerotic plaques of blood vessels**

Parameters	Presence of <i>Tannerella forsythia</i> in atherosclerotic plaques		<i>p</i> *
	yes	no	
Total number of patients, n (%)	48 (53.3)	42 (46.7)	
Degree of periodontal disease, n (%)			
moderate	11 (22.9)	22 (52.4)	0.004
severe	37 (77.1)	20 (47.6)	
Degree of gingival inflammation, n (%)			
mild	0 (0)	3 (7.1)	0.005
moderate	4 (8.3)	12 (28.6)	
severe	44 (91.7)	27 (64.3)	
Bleeding on probing, median (range)	4.2 (2.0-5.0)	3.3 (1.0-4.9)	< 0.001

\* $\chi^2$ -test or Mann-Whitney *U* test.

Presence of *Tf* was also analyzed in relation to socio-demographic characteristics of the patients. According to our results, age, gender, level of education and presence of bad habits (smoking and alcohol use) were not statistically significant regarding presence of *Tf* (Table 4).

**Table 4****Sociodemographic characteristics of patients with periodontal disease and atherosclerosis in relation to *Tannerella forsythia* (*Tf*) presence in subgingival or atherosclerotic plaques**

Sociodemographic characteristics	Presence of <i>Tf</i>		<i>p</i>
	yes	no	
Gender, n (%)			
male	49 (72.1)	12 (54.5)	0.127
female	19 (27.9)	10 (45.5)	
Age (years), mean $\pm$ SD	59.2 $\pm$ 11.8	59.4 $\pm$ 15.7	0.937
Education, n (%)			
primary school	10 (14.7)	1 (4.5)	0.398
high school	41 (60.3)	16 (72.7)	
university	17 (25.0)	5 (22.7)	
Smoking, n (%)			
yes	38 (55.9)	14 (63.6)	0.522
no	30 (44.1)	8 (36.4)	
Alcohol, n (%)			
yes	21 (30.9)	4 (18.2)	0.248
no	47 (69.1)	18 (81.8)	
Oral hygiene, n (%)			
good	28 (41.2)	14 (63.6)	0.066
poor	40 (58.8)	8 (36.4)	

SD – standard deviation.

*Tf* demonstrated the following distribution in blood vessels of the patients with atherosclerosis: the highest prevalence was in coronary arteries (41.7%), followed by carotid arteries (35.4%) and aneurisms of *a. abdominalis* (12.5%), *a. mammaria* (8.3%) and *a. femoralis* (2.1%) while in *a. iliaca* *Tf* was not detected at all (Table 5).

**Table 5****Distribution of *Tannerella forsythia* in atherosclerotic plaques of different blood vessels**

Blood vessel	Patients, n (%)
<i>A. coronaria</i>	20 (41.7)
<i>A. carotis</i>	17 (35.4)
<i>A. abdominalis</i> aneurism	6 (12.5)
<i>A. mammaria</i>	4 (8.3)
<i>A. femoralis</i>	1 (2.1)
<i>A. iliaca</i>	0 (0)
Total	48 (100)

There were no statistically significant differences in values of PI, GI, sulcus bleeding index and PPD among patients with or with no presence of *Tf* in atheromatous plaques of different blood vessels (Table 6).

**Table 6**  
**Results of periodontal examinations in relation to *Tannerella forsythia* presence in atherosclerotic plaques of different blood vessels**

Presence of <i>Tannerella forsythia</i> in atherosclerotic plaques	Blood vessel	n	Periodontal examination, median (range)			
			PI	GI	SBI	PPD (mm)
Yes	<i>A. carotis</i>	17	2.9 (1.9–3.0)	2.7 (2.1–3.0)	4.5 (2.9–4.9)	5.0 (3.0–6.0)
	<i>A. abdominalis</i> aneurism	6	2.8 (1.9–3.0)	2.85 (1.8–3.0)	4.65 (2.8–5.0)	6.0 (3.0–7.0)
	<i>A. femoralis</i>	1	3.0 (3.0–3.0)	2.6 (2.6–2.6)	3.9 (3.9–3.9)	6.0 (6.0–6.0)
	<i>A. coronaria</i>	20	2.85 (1.5–3.0)	2.85 (1.2–3.0)	4.0 (2.0–5.0)	6.0 (3.0–8.0)
	<i>A. mammaria</i>	4	2.2 (1.9–3.0)	2.45 (1.7–2.9)	3.95 (2.8–4.8)	5.0 (4.0–8.0)
	Total	48	$p = 0.587$	$p = 0.336$	$p = 0.214$	$p = 0.655$
No	<i>A. carotis</i>	12	1.8 (0.9–2.9)	1.8 (0.9–3.0)	2.9 (1.1–4.6)	3.5 (3.0–6.0)
	<i>A. abdominalis</i> aneurism	4	2.85 (1.9–2.9)	2.3 (1.9–2.8)	4.1 (2.0–4.8)	4.5 (3.0–6.0)
	<i>A. femoralis</i>	9	2.0 (1.0–2.8)	2.1 (1.5–3.0)	3.1 (2.5–4.9)	5.0 (3.0–5.0)
	<i>A. iliaca</i>	4	2.4 (1.9–2.7)	2.5 (2.2–3.0)	3.95 (3.0–4.9)	4.5 (3.0–6.0)
	<i>A. coronaria</i>	9	2.6 (1.5–3.0)	2.8 (1.7–3.8)	4.0 (1.0–4.3)	5.0 (3.0–6.0)
	<i>A. mammaria</i>	4	2.3 (1.9–2.9)	2.2 (1.5–3.0)	2.95 (1.8–4.1)	4.0 (3.0–6.0)
Total	42	$p = 0.110$	$p = 0.469$	$p = 0.571$	$p = 0.935$	

PI – plaque index; GI – gingival index; SBI – sulcus bleeding index; PPD – periodontal pocket depth.

### Discussion

Periodontal disease represents chronic inflammation in tooth supportive tissues (periodontal ligament, connective tissue and alveolar bone), that, if left untreated, leads to periodontal pocket formation and consequent bone loss. It is unclear which pathogens initiate the disease, but several species including anaerobic Gram negative bacteria *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* are most strongly associated with the destruction of periodontium, and are routinely found in subgingival plaques in patients with chronic periodontitis<sup>12</sup>. To date, many researches are pointing on correlation between periodontal disease and systemic health. It has been suggested that periodontitis-associated bacteraemias and systemic dissemination of inflammatory mediators produced in the periodontal tissues may cause a systemic inflammation. Therefore, it is considered that periodontal disease can contribute as a risk factor for cardiovascular diseases, endocrine disturbances (e.g. diabetes mellitus), premature birth and low weight on birth, etc.<sup>13, 14</sup>. Atherosclerosis, a progressive disease of medium and large elastic and muscular arteries can lead to ischemic lesions of brain, heart or extremities and can result in thrombosis and infarction of affected vessels<sup>4</sup>. It is considered the primary cause of heart disease and stroke and is the underlying cause of around 50% of all deaths in western societies<sup>15</sup>.

Among 90 patients included in this study, 61 of them (67.8%) were males, which make a prevalence of periodontal disease and atherosclerosis higher in men. Patients' mean age was 59.2 years. That is in correlation with common understanding of periodontal disease progress. Even though, the age itself is not predetermining risk factor for periodontal disease<sup>16</sup> due to lower number of elastic and collagen fibers, and mitotic activity of fibroblasts is usually seen in adults over 40

years. Maybe the reason for the presence of periodontal disease in older persons can be partially explain by association between poor oral hygiene habits and systemic diseases<sup>17–19</sup>.

Presence of periopathogen *Tf* in atherosclerotic blood vessels showed a significant correlation in regards to degree of periodontal inflammation. Consequently, prevalence of *Tf* was statistically significantly higher in patients with moderate and severe periodontal disease when compared to patients with average PPD. Presence of *Tf* strongly correlated with level of periodontal inflammation and BOP.

Results from this study are pointing on strong correlation between periopathogens and their presence in atherosclerotic plaques, which is in accordance with many published studies on this topic<sup>20–24</sup>. So far, studies confirmed the presence of *Tf* in 30–61.9% patients with atherosclerotic *a. carotis*<sup>24, 25</sup>, and in 86% of patients with *a. abdominalis* aneurism<sup>26</sup>. In our study, among 90 patients *Tf* was detected with highest prevalence in coronary arteries (41.7%) and carotid arteries (35.4%), and *a. abdominalis* aneurisms (12.5%), followed by *a. mammaris* (8.3%) and *a. femoralis* (2.1%), while in *a. iliaca* *Tf* was not detected at all. Therefore, our results are pointing that most probably the periopathogens after entering the systemic circulation are located in the circulation nearby heart blood vessels. *Tf* presence in far away blood vessels are less, but not unimportant.

### Conclusion

The present study suggests strong relationship between periodontal inflammation and atherogenesis, therefore it should be considered as potential risk factor for atherosclerosis. It is, however necessary to control periodontal disease in order to reduce mortality and morbidity associated with atherosclerosis and myocardial infarction.

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Received on March 21, 2018.

Revised on June 5, 2018.

Accepted on July 2, 2018.

Online First July, 2018.