Hrvat. čas. zdr. znan. 2025; 5: 32-40
https://doi.org/10.48188/hczz.5.1.5

Izvorni rad
Original articles

THE ASSOCIATION OF THE PRESENCE OF PERIODONTOPATHOGENIC BACTERIA WITH GENDER, AGE, SYSTEMIC DISEASES, AND SNORING ANALYZED IN PATIENTS FROM SPLIT-DALMATIA COUNTY

EVA VUKELIĆ[1], NINA KALAJŽIĆ[1], FERDINAND JOSIP BUŠELIĆ[2], SENDI KURET[1]

Objectives: Periodontal disease is a chronic inflammatory condition that significantly impacts oral and systemic health. Age, gender, systemic diseases, sleep disordered breathing, and specific periodontopathogenic bacteria are the risk factors for developing periodontal disease. This study aimed to assess the presence of five periodontopathogenic bacteria (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola) in a sample and analyze their association with variables such as age, gender, systemic disorders, and snoring.

Materials and methods: This cross-sectional study included 149 adult patients (62 male and 87 female) with clinical symptoms of localized chronic periodontitis. Participants filled out a questionnaire to provide data on their sociodemographic and clinical characteristics, and the presence of five periodontopathogenic bacteria in subgingival plaque samples was analyzed with real-time PCR.

Results: Statistically significant results were obtained regarding the association between age and periodontopathogenic bacteria. Positive results for Porphyromonas gingivalis and Tannerella forsythia were associated with ages between 41 and 60, and over 60. For the bacterium Aggregatibacter actinomycetemcomitans, the statistical results in the distribution of patients regarding snoring were borderline significant. More individuals who did not snore had positive results.

Conclusions: Older age had the greatest impact, and the majority of patients positive for the tested bacteria were females.

Keywords: PERIODONTAL DISEASE, PERIODONTOPATHOGENIC BACTERIA, SYSTEMIC DISEASES, SNORING, SPLIT-DALMATIA COUNTY

Introduction

Periodontal disease is a prevalent chronic inflammatory condition that affects the supporting structures of the teeth. It is characterized by the destruction of periodontal ligaments and alveolar bone, which, if left untreated, ultimately leads to tooth loss (1, 2). This disease has far-reaching implications for both oral and systemic health. Various risk factors, including gender, age, systemic diseases (e.g., rheumatoid arthritis (RA), cardiovascular diseases (CVDs), diabetes), sleep disordered breathing (e.g., snoring, obstructive sleep apnea), and specific periodontopathogenic bacteria, have been identified as significant contributors to the onset and progression of periodontitis (3-5).

Researchers have identified age as a significant risk factor for periodontitis. Epidemiological studies consistently show an increase in the prevalence and severity of periodontal disease with advancing age (6). Furthermore, the incidence and severity of periodontal disease show a gender predilection, with males appearing to have a higher incidence and severity than females (7).

The oral cavity is rich in bacteria, and the oral microbiome is composed of a core microbiome that is present in all individuals and a variable microbiome that is specific to each individual. Maintaining the balance of the oral microbiome impacts both oral and general health, and the presence of periodontopathogenic Gram-negative bacteria impacts this homeostasis (8). Notably, species that have been implicated in periodontitis include Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Porphyromonas gingivalis (P. gingivalis), Prevotella intermedia (P. intermedia), Tannerella forsythia (T. forsitia), and Treponema denticola (T. denticola) (9).

Due to changes in the oral microbiota and the prevalence of anaerobic bacteria in periodontitis, they have been previously categorized, with the primary category in periodontitis being the red complex, which includes P. gingivalis, T. forsitia, and T. denticola (9, 10). These opportunistic pathogens share the same characteristics, which include extracellular proteolytic activity, complex anaerobic fermentation of amino acids, production of toxic metabolites, and possession of outer membrane vesicles. Through these features, the bacteria invade the periodontal tissue by adhering to the oral epithelium, degrading both soft and hard tissues, and circulating through the bloodstream to organs distant from the mouth (9). Microorganisms entering the circulation cause transient bacteraemia, which could induce or exacerbate systemic inflammatory responses and endothelial injury. The inflammatory mediators produced in response to periodontal pathogens, such as C-reactive protein (CRP), interleukins (IL-1β, IL-6), and tumour necrosis factor-alpha (TNF-α), are also elevated in patients with cardiovascular diseases (9, 11, 12).

Olsen et al. identified oral bacteria in cardiovascular tissues, such as aneurysms, heart valves, and atherosclerotic plaques (13). The ability to destroy tissue explains the observations of bacteria in the cardiac muscle tissue and arterial walls, suggesting a potential pathogenic causal connection among periodontal pathogens and CVD. Periodontal treatment may also reduce systemic inflammatory markers and improve endothelial function (14). According to recent research, periodontitis may contribute to systemic inflammation and endothelial dysfunction, key mechanisms in which lead to atherosclerosis and other CVDs (15, 16).

Additionally, the European Federation of Periodontology and WONCA Europe's consensus report summary indicates that periodontitis increases the risk of the first cardiovascular event and the risk of developing diabetes. Red complex bacteria may induce chronic inflammation, oxidative stress, and insulin resistance, all associated with diabetes pathogenesis (17).

Recent research showed that periodontal disease is associated with an increased risk of RA in individuals. This disease's pathophysiology is complex, with genetic and environmental variables contributing to its development. The microbiome has been identified as an environmental component contributing to the development of RA. Furthermore, it has been demonstrated that the severity of periodontal disease correlates with the activity of the disease process in RA patients and that treating periodontal disease symptoms reduces RA symptoms (5).

Other relevant oral bacteria that do not belong to the red complex but are present in chronic and aggressive periodontitis are A. actinomycetemcomitans and P. intermedia.

A. actinomycetemcomitans is a Gram-negative, facultative anaerobic coccobacillus present in subgingival plaque in patients with aggressive periodontitis. It has 10 subtypes and different characteristics. Its pathogenicity is primarily attributed to several virulence factors that increase and facilitate colonization, evade the host immune system, and cause tissue damage. A. actinomycetemcomitans can evade immune responses and establish infection by producing leukotoxin, which targets leukocytes and monocytes. It also forms immunoglobulin proteases and collagenase. These properties can contribute to the survival of bacteria within the oral cavity, influence their pathogenic potential, and impact clinical outcomes in infected individuals (9, 18).

P. intermedia is a Gram-negative, strictly anaerobic, short rod-shaped bacterium that produces lipase when involved in bacterial invasion of the periodontium. Given the high diversity of the Prevotella genus, some of the 30 subspecies are members of the orange complex (10). P. intermedia produces lipopolysaccharide, which invades human oral epithelial cells, inhibits the phagocytic and chemotactic activities in human dental stem cells, and promotes alveolar bone resorption. It also induces the expression of IL-8 in human gingival fibroblasts (19).

According to recent studies, there may be an association between periodontitis and sleep disordered breathing, with inflammation as a common underlying mechanism. Snoring, a primary symptom of obstructive sleep apnea (OSA), results from the vibration of respiratory structures due to turbulent airflow and is often considered an early indicator of potential airway obstruction. The intermittent hypoxia associated with these conditions can lead to systemic inflammation, which may adversely affect periodontal health (12, 20).

The aim of this study was to assess the presence of five periodontopathogenic bacteria in patients from the Split-Dalmatia County and to analyze the association between the frequency of bacteria and investigated variables (age, gender, systemic diseases, and snoring) among patients with periodontitis.

Materials and Methods

Study Design and Participants

The research was designed as a cross-sectional study and was carried out on a sample of adults with stage II/III grade B periodontitis, between the ages of 20 and 80 (21). Dental examination and microbiological sampling were performed by a dentist in a private dental office in Split, Croatia, who also assessed the degree of inflammation in the oral cavity during the examination. Sample collection and analysis, and data collection from individuals were obtained between September 2022 and October 2024. Patients were not receiving antibiotic therapy before sampling. Pooling method was used for harvesting bacteria from the pocket using 6 paper points of moderate strength (average size, # 30-50) and "pooling" several probes from various pockets equally distributed in both jaws, following which the paper point (samples) would be placed in a cold transport container, delivered to the laboratory and kept at a temperature of +4 °C until the start of the analysis. For this study, dental examination was performed on 492 Caucasian patients, of which 240 did not have periodontitis and were excluded. From 252 patients with periodontitis, 53 of them who were younger than 20 or older than 80 were also excluded. Of 199 patients with periodontitis, 50 patients tested negative for all tested bacteria and were excluded from the study. The study included 149 patients with periodontitis who were positive for at least one bacterium and met our study requirements. The flow chart in Figure 1 shows all phases of the study.

[1]Faculty of Health Sciences,
University of Split
[2]Department of Dental Medicine,
School of Medicine, University of Split

Corresponding author:
Eva Vukelić, student
Faculty of Health Sciences,
University of Split
21000 Split, Croatia
E-mail: ev10163@ozs.unist.hr

The study was approved by the Ethics Committee of the University of Split, University Department of Health Study on April 23, 2025. (Class: 029-03/25-08/01; Reg. No.: 2181-228-103/1-33). All procedures conformed to the Declaration of Helsinki.

All participants signed a consent form agreeing to provide personal information relevant to the study at the time of sample collection. Data regarding systemic disorders (CVD, diabetes, and RA) and snoring were obtained from the questionnaire that patients completed while providing informed consent to participate in the study. The patients were questioned about their medication, dietary habits, and lifestyle to avoid confounding factors. Out of the total number of patients included in the study, 55 had a systemic disease (42 patients presented with cardiovascular difficulties, 8 patients with RA, and 5 patients with diabetes).

Sample Collection and Storage

Cotton rolls were used to isolate the sampling area. The tooth surface was cleaned with 70% ethanol and dried with sterile cotton swabs. Samples were obtained from the deepest pockets of the diseased areas with five sterile paper points inserted into the gingival crevice for 15 seconds and then transferred to a sterile 1.5 ml vial for molecular analysis.

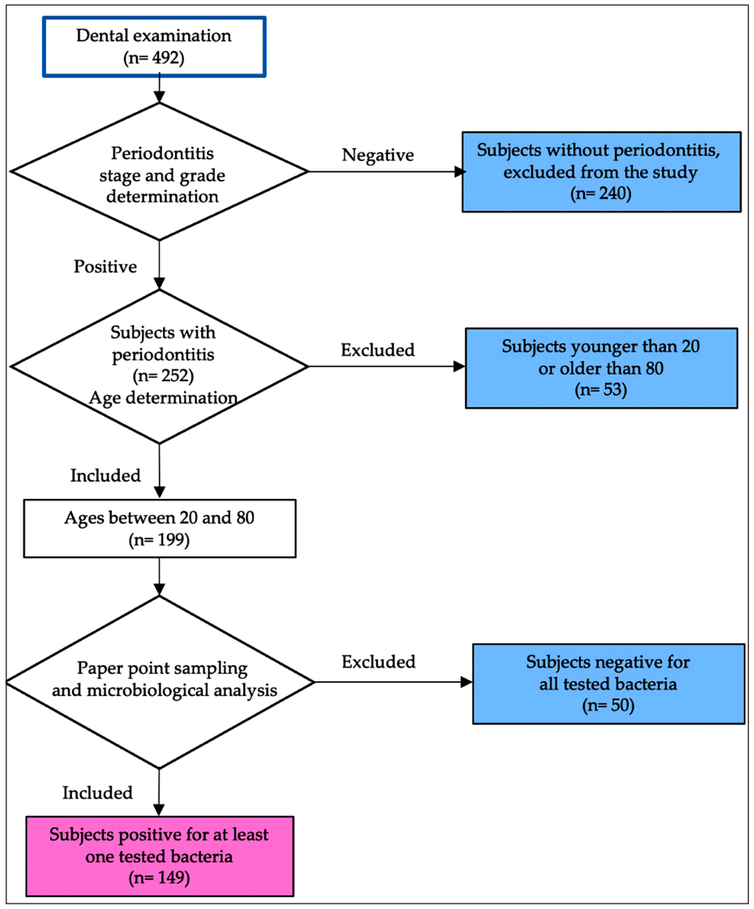

Figure 1.
Flow diagram for each stage of the study

The samples were sent to a molecular laboratory and remained stable dor 7-days at temperatures ranging from 2 to 8 °C.

DNA Extraction

To extract the DNA from the periodontal sample, 500 µl of saline was added to the tube and mixed vigorously for 10 seconds to remove bacterial cells from the paper points. The DNA was then extracted using the NucleoSpin®-Microbial kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. The quantity of extracted DNA was quantified spectrophotometrically at 260 nm with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA), and its purity was calculated as the ratio of absorbance observed at 260 and 280 nm.

Real-time PCR

Real-time PCR was carried out using a real-time analyser, the ABI Prism 7500 Real-Time PCR System (Applied Biosystems, USA). Primers for Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, and Tannerella forsythia were used as previously described (22).

The RT-PCR was performed in a total reaction volume of 50 µL, which included 5.0 µL of isolated DNA as the template, 25 µL of Power SYBR Green PCR Master Mix (Thermo Fisher, USA), 18 µL of sterile water, and 2 µL (20µM) of a bacteria-specific primer pair. Primerconcentrations were identical for all assays. Negative and positive controls were included in each batch of specimens. Negative controls contained ultrapure water instead of sample DNA. The positive control consisted of the genomic DNA of the five positive targeted bacteria. The 5.0 µL of negative and 5.0 µL of positive control were included in each analysis run. All amplifications and detections were carried out in a MicroAmp optical 96-well reaction plate. The cycling conditions were initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 5 seconds, and annealing at 60°C for 34 seconds each. The accumulation of PCR products was observed at each cycle by monitoring the increase in fluorescence of the reporter dye from dsDNA binding SYBR Green. After the PCR, the specificity of the amplification was assayed with the use of melting curves which were constructed in the range of 60°C to 95°C (23).

Statistical Analysis

Categorical variables are expressed as frequencies and percentages. Differences in categorical variables were analyzed using the Pearson chi-square test or Fisher's exact test. Furthermore, we performed logistic regression analyses to assess the association of positive findings of analyzed bacteria with the odds ratios of predictors that had p-value <0.1 in univariate models. The statistical significance was considered for two-sided p-values less than 0.05. Statistical analysis was conducted using JASP (Version 0.18.3).

Sample size was calculated using an on-line sample size calculator for estimating a single proportion: https://www.statulator.com/SampleSize/ss1P.html. Data on the total number of respondents and the proportions of negative/ positive respondents (0.18/ 0.82) were taken from the previous study and established as the expected proportions in the research (23). Level of confidence was set to 0.95 and precision or margin of error is set to 0.1. The calculation determined that the study required a number of 57 subjects, and 149 subjects were included in this study.

Table 1.
Sociodemographic and clinical characteristics of the patients.

		Number	Percentage (%)
Gender	Male	62	42%
	Female	87	58%
Age	20-40	33	22%
	41-60	66	44%
	61-80	50	34%
Systemic diseases	Yes	55	37%
	No	94	63%
Snoring	Yes	56	38%
	No	93	62%

Results

A total of 149 individuals met the inclusion criteria. Table 1 shows the sociodemographic and clinical characteristics of the patients. There were more females (58%) than males (42%). Most patients were aged between 41 and 60 years (44%), and over 60 years (34%). The 63% of participants did not have any systemic diseases, compared to 37% of them who did. 62% of the participants reported no snoring .

The detection of five periodontopathogens: Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola in subgingival plaque samples of periodontal disease patients were tested by real-time PCR. The results are summarized in Table 2.

The data in Figure 2 represent the distribution of subjects by gender and the analyzed microorganisms. No statistically significant difference between genders was observed for any tested bacteria. Using the chi-square test, the p-values were between 0.131 and 0.942.

The results obtained for the bacteria P. gingivalis and T. forsythia showed a statistically significant association between negative and positive results and age categories (p = 0.021, p = 0.034, respectively). Participants aged 41-60 years had the highest percentage of positive findings for both bacteria (48.5% for P. gingivalis and 42.1% for T. forsythia).

The association between positive findings for bacterium A. actinomycetemcomitans and snoring (p = 0.095) was borderline significant. More patients who did not snore had positive results. According to the Chi-square test, about

Table 2.
The results of the real-time PCR analysis of five periodontopathogenic bacteria in plaque samples from 149 patients.

	Detection by Real-Time PCR (copies/plaque sample)			
	Score			
Bacterial Species	0	$<10^4$	10^4–10^6	$>10^6$
Aggregatibacter actinomycetemcomitans (A.a.)	108 (72.5%)	36 (24.1%)	4 (2.7%)	1 (0.7%)
Porphyromonas gingivalis (P.g.)	86 (57.7%)	28 (18.8%)	31 (20.8%)	4 (2.7%)
Prevotella intermedia (P.i.)	27 (18.1%)	52 (34.9%)	67 (45%)	3 (2%)
Tannerella forsythia (T.f.)	27 (18.1%)	26 (17.5%)	54 (36.2%)	42 (28.2%)
Treponema denticola (T.d.)	54 (36.2%)	29 (19.5%)	55 (36.9%)	11 (7.4%)

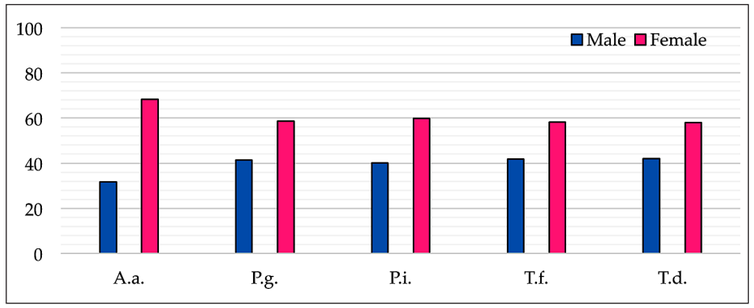

Figure 2.
A graphical representation of the results of periodontopathogenic bacteria according to gender (A.a. = A. actinomycetemcomitans, P.g. = P. gingivalis, P.i. = P. intermedia, T.f. = T. forsythia, T.d. = T. denticola.). The percentage of the number of positive patients is shown on the y-axis.

systemic diseases, no statistically significant association was observed with any of the analyzed bacteria. The results are shown in Table 3.

Furthermore, we performed a logistic regression analysis for positive findings of P. gingivalis with age categories as a predictor (p <0.016, Nagelkerke R^2 = 0.072). Results showed that age categories 41-60 years (OR = 3.43, 95% CI (0.23, 2.23), p = 0.011) and 61-80 years (OR = 3.43, 95% CI (0.23, 2.23), p = 0.016) had higher odds for positive results compared to age category 20-40 years. A logistic regression analysis model for T. forsythia was also statistically significant (p = 0.031, Nagelkerke R^2 = 0.074) and revealed that participants aged 41-60 years had higher odds for a positive result (OR = 3.2 (95% CI (1.006, 10.175), p = 0.049) compared to participants aged 20-40 years. No statistically significant result was observed for the age category 61-80 years (OR = 0.911, 95% CI (0.330, 2.517), p = 0.857). We also performed a logistic regression analysis for A. actinomycetemcomitans with snoring as an independent variable (p = 0.09, Nagelkerke R^2 = 0.028). Snoring showed borderline significance (OR = 1.948, 95% CI (-0.123, 1.457), p = 0.098).

Table 3.
Results of analyzes of periodontopathogenic bacteria (A.a. = A. actinomycetemcomitans, P.g. = P. gingivalis, P.i. = P. intermedia, T.f. = T. forsythia, T.d. = T. denticola.) and their relationship with regard to negative and positive results classified by age, systemic diseases, and snoring. The statistical significance was considered for two-sided p-values less than 0.05 obtained using the Pearson chi-square test or Fisher's exact test.

	A.a.			P.g.			P.i.			T.f.			T.d.		
	neg	poz	p*	neg	poz	p*	neg	poz	p*	neg	poz	p*	neg	poz	p*
Age															
20-40	23 (69.7%)	10 (30.3%)		26 (78.8%)	7 (21.2%)		4 (12.1%)	29 (87.9%)		4 (23.5%)	18 (23.7%)		17 (51.5%)	16 (48.5%)	
41-60	45 (68.2%)	21 (31.8%)	0.340	34 (51.5%)	32 (48.5%)	0.021	13 (19.7%)	53 (80.3%)	0.597	2 (11.8%)	32 (42.1%)	0.038	20 (30.3%)	46 (69.7%)	0.108
61-80	40 (80%)	10 (20%)		26 (52%)	24 (48%)		10 (20%)	40 (80%)		11 (64.7%)	26 (34.2%)		17 (34%)	33 (66%)	
Systemic diseases															
Yes	43 (78.2%)	12 (21.8%)	0.233	27 (49.1%)	28 (50.9%)	0.103	11 (20%)	44 (80%)	0.649	10 (18.2%)	45 (81.8%)	0.988	17 (30.9%)	38 (69.1%)	0.300
No	65 (69.1%)	29 (30,9%)		59 (62.8%)	35 (37.2%)		16 (17%)	78 (83%)		17 (18.1%)	49 (77.9%)		37 (39.4%)	57 (60.6%)	
Snoring															
Yes	45 (80.4%)	11 (19.6%)	0.095	30 (53.6%)	26 (46.4%)	0.427	9 (16.1%)	47 (83.9%)	0.614	12 (21.4%)	44 (78.6%)	0.416	16 (28.6%)	40 (71.4%)	0.131
No	63 (67.7%)	30 (32.3%)		56 (60.2%)	37 (39.8%)		18 (19.4%)	75 (80.6%)		15 (16.1%)	78 (83.9%)		38 (40.9%)	55 (59.1%)	

The distribution of subjects by gender, age, systemic diseases, snoring, and the intensity of identified microorganisms is shown in Table 4. It was observed for bacterium T. forsythia that the distribution of patients regarding age (p = 0.080) and snoring (p = 0.089) was borderline significant. For bacterium T. denticola, the results showed statistically significant differences between examined groups in the distribution of patients regarding age (p = 0.029).

Discussion

The results of our study showed that the prevalence and intensity of bacteria increase with age. Huang and Dong evaluated the prevalence of periodontal disease in middle-aged and elderly patients. They concluded that age is an influencing factor on the incidence of periodontitis (24). In the cross-sectional study, Motoc et al. analyzed the association between age, gender, diet, and 11 periodontopathogenic bacteria in children and adolescents. The study included 60 participants and showed that age had a statistically significant influence on four bacteria (8). In our study, there were statistically significant differences between the groups for P. gingivalis and T. forsythia, where the presence or absence of bacteria varied with regard to age. The participants over 60 years old (48%) and the group between 41 and 60 years old (48.5%) had the highest prevalence of P. gingivalis. The group between 41 and 60 years old had a higher prevalence (42.1%) of T. forsythia compared to the group between 20 and 40 years old (Table 3). For the same bacterium a borderline significance was observed for the intensity and age groups. For T. denticola, the results demonstrated statistically significant differences between examined groups in the distribution of patients regarding age and the intensity of the bacterium (Table 4).

Table 4.
Results of analysis of periodontopathogenic bacteria (A.a. = A. actinomycetemcomitans, P.g. = P. gingivalis, P.i. = P. intermedia, T.f. = T. forsythia, T.d. = T. denticola.) classified according to their concentration (0 = negative, 1 = <104, 2 = 104 – 106, 3 = >106) and according to gender, age, systemic diseases, and snoring of patients (N = 149). The statistical significance was considered for two-sided p-values less than 0.05 obtained using the Pearson chi-square test or Fisher's exact test.

Bacteria	Score	Gender			Age				Systemic disease			Snoring		
		Male	Female	p*	20-40	41-60	61-80	p*	Yes	No	p*	Yes	No	p*
A.a.	0	49 (45.4%)	59 (54.6%)		23 (21.3%)	45 (41.7%)	40 (37%)		43 (39.8%)	65 (60.2%)		45 (41.7%)	63 (58.3%)	
	1	11 (30.6%)	25 (69.4%)	0.352	8 (22.2%)	19 (52.8%)	9 (25%)	0.538	11 (30.6%)	25 (69.4%)	0.605	10 (27.8%)	26 (72.2%)	0.374
	2	2 (50%)	2 (50%)		2 (50%)	1 (25%)	1 (25%)		1 (25%)	3 (75%)		1 (25%)	3 (75%)	
	3	0 (0%)	1 (100%)		0 (0%)	1 (100%)	0 (0%)		0 (0%)	1 (100%)		0 (0%)	1 (100%)	
P.g.	0	36 (41.9%)	50 (58.1%)		26 (30.2%)	34 (39.5%)	26 (30.3%)		27 (31.4%)	59 (68.6%)		30 (34.9%)	56 (65.1%)	
	1	12 (42.9%)	16 (57.1%)	0.924	2 (7.1%)	16 (57.1%)	10 (35.8%)	0.116	14 (50%)	14 (50%)	0.268	8 (28.6%)	20 (71.4%)	0.251
	2	13 (41.9%)	18 (58.1%)		5 (16.2%)	13 (41.9%)	13 (41.9%)		13 (41.9%)	18 (58.1%)		16 (51.6%)	15 (48.4%)	
	3	1 (25%)	3 (75%)		0 (0%)	3 (75%)	1 (25%)		1 (25%)	3 (75%)		2 (50%)	2 (50%)	
P.i.	0	13 (48.1%)	14 (51.9%)		4 (14.8%)	13 (48.2%)	10 (37%)		11 (40.7%)	16 (59.3%)		9 (33.3%)	18 (66.7%)	
	1	21 (40.4%)	31 (59.6%)	0.682	15 (28.8%)	17 (32.7%)	20 (38.5%)	0.415	17 (32.7%)	35 (67.3%)	0.126	20 (38.5%)	32 (61.5%)	0.727
	2	26 (38.8%)	41 (61.2%)		14 (20.9%)	34 (50.7%)	19 (28.4%)		24 (35.8%)	43 (64.2%)		25 (37.3%)	42 (62.7%)	
	3	2 (66.7%)	1 (33.3%)		0 (0%)	2 (66.7%)	1 (33.3%)		3 (100%)	0 (0%)		2 (66.7%)	1 (33.3%)	
T.f.	0	11 (40.7%)	16 (59.3%)		8 (29.6%)	6 (22.2%)	13 (48.2%)		10 (37%)	17 (63%)		12 (44.4%)	15 (55.6%)	
	1	9 (34.6%)	17 (65.4%)	0.591	9 (34.6%)	10 (38.5%)	7 (26.9%)	0.080	7 (26.9%)	19 (73.1%)	0.324	9 (34.6%)	17 (65.4%)	0.089
	2	21 (38.9%)	33 (61.1%)		10 (18.5%)	26 (48.2%)	18 (33.3%)		18 (33.3%)	36 (66.7%)		14 (25.9%)	40 (74.1%)	
	3	21 (50%)	21 (50%)		6 (14.3%)	24 (57.1%)	12 (28.6%)		20 (47.6%)	22 (52.4%)		21 (50%)	21 (50%)	
T.d.	0	22 (40.7%)	32 (59.3%)		17 (31.5%)	20 (37%)	17 (31.5%)		17 (31.5%)	37 (68.5%)		16 (29.6%)	38 (70.4%)	
	1	12 (41.4%)	17 (58.6%)	0.994	8 (27.6%)	9 (31%)	12 (41.4%)	0.029	9 (31%)	20 (69%)	0.470	13 (44.8%)	16 (55.2%)	0.326
	2	23 (41.8%)	32 (58.2%)		7 (12.8%)	28 (51%)	20 (36.2%)		24 (43.6%)	31 (56.4%)		24 (43.6%)	31 (56.4%)	
	3	5 (45.5%)	6 (54.5%)		1 (9.1%)	9 (81.8%)	1 (9.1%)		5 (45.5%)	6 (54.5%)		3 (27.3%)	8 (72.7%)	

However, Li et al. reported contradictory findings in their cross-sectional study involving 9803 participants aged 20 and older. They analyzed the association between biological aging and periodontitis. The study showed no association between biological age and the development of periodontitis (25). Additionally, Victor et al. in their cross-sectional study evaluated the periodontal status and the presence of three periodontopathogenic bacteria in 132 non-smoking adult subjects. The presence of P. gingivalis, A. actinomycetemcomitans, and F. nucleatum was not significantly associated with gender or age (26). In our study of five tested bacteria, age was not associated with two bacteria, A. actinomycetemcomitans and P. intermedia. It remains unclear if the higher prevalence and severity in older people are the result of the lifetime accumulation of local risk factors that include tooth plaque and microbial deposits or an inherent greater predisposition to periodontal disease (27-29).

Several studies showed that the prevalence and severity of periodontal disease exhibit a gender predilection. The results of our study, contrary to our expectations based on previous studies, showed that out of the total number of positive patients, female patients predominated. In the study conducted by Zhao et al., which evaluated gender variations in the subgingival bacteria microbiome of elderly patients and identified differences in the composition of the oral microbiome between males and females (7). The study showed that males had a greater variety and number of bacteria than females. A small number of participants (32) were included in their research (7). In 2012, Eke et al. observed that gender had a significant association with prevalence, with an 18% difference between males and females (56.4% as opposed to 38.4%). Additionally, in 2019, they reported that males had a two-fold higher probability of having periodontitis compared to females, with severe periodontitis having the highest prevalence (adjusted prevalence ratio aPR = 2.68; 2.22; 3.23) (30, 31). However, their studies included more subjects than ours.

The results of the study conducted by Motoc et al. showed that gender did not have a statistically significant influence on the estimated periodontopathogenic bacteria, however, the participants in their study were children and adolescents, while in our study, the patients were adults (8). In our study, despite the higher prevalence of bacteria, which was observed in women, the difference was not statistically significant. One possible reason could be that more females (58%) than males (42%) were included in our study. Also, other studies included more subjects in their research, which may be another possible explanation for the findings in our study.

Recent studies led to an increased interest in the oral microbiome and its association with the development of periodontitis and systemic diseases; hence, Larvin et al. included 30 longitudinal cohort studies in their meta analysis. They found that CVD risk was significantly higher in patients with periodontitis compared to those without periodontitis (RR 1.20; 95% CI 1.14-1.26) (32). Haraszthy et al. found that 44% of carotid endarterectomy specimens obtained from patients with atherosclerosis were positive for at least one of the target periodontal pathogens (A. actinomycetemcomitans, T. forsythia, P. gingivalis, and P. intermedia) (33). Hernández-Ruiz et al. in their systematic review reported that red complex bacteria present in patients with periodontitis can translocate into the circulation and cause a local and systemic immune response, which supports the hypothesis that periodontopathogenic bacteria may have an impact on lipid metabolism and the atherosclerotic plaque formation (34).

Although much is known about the individual pathogens associated with periodontitis and the prevalence of CVD in periodontitis patients, the mechanisms underlying the association between periodontitis and CVDs remain unclear (35).

Studies have shown that periodontal disease is associated with a higher risk of developing RA. According to Nik-Azis et al. and De Smit et al., there was a higher incidence of RA in patients with periodontal disease (36, 37). De Aquino et al. reported that P. gingivalis may have an important role in the development of inflammation in RA patients (38). However, Konig et al. were not able to identify a mechanism relating RA to patients with detected P. gingivalis (39). Furthermore, Schmickler et al. in their study evaluated periodontopathogenic bacteria and their influence on patients with RA. Of the 11 bacteria tested, only the presence of T. denticola was related to periodontal condition in RA patients (40). In our study, there was no association between periodontopathogenics and systemic diseases. Although there was no statistical significance for the bacterium P. intermedia, the intensity of bacteria varied with concerning the presence or absence of systemic disease, as shown in Table 4.

The influence of sleep disorders, such as snoring, has been studied in the last decade (41, 42). According to the studies, periodontitis is a dental condition that may be related to sleep disorders like obstructive sleep apnea and snoring (43, 44). To our knowledge, the association between snoring and the presence of periodontopathogenic bacteria has not been researched; however, given that the presence of bacteria is the most common cause of periodontitis, we compared our results to studies that observed the relationship between snoring and periodontitis.

Acar et al. included in their study 291 patients (41 patients presented with snoring and 250 with obstructive sleep apnea). There was no association between obstructive sleep apnea and periodontitis, while snoring complaints were positively associated with periodontal disease (45). Our study showed that the association between snoring and the intensity of T. forsythia was borderline significant. Conversely, the bacterium was more prevalent in subjects who do not snore, as shown in Table 4. Also, the results regarding the presence or absence of A. actinomycetemcomitans and snoring were borderline significant, and the bacterium was more prevalent in subjects who do not snore (Table 3). The obtained result is possible due to the constant presence of air, which dries out and changes the conditions in the oral cavity.

The environment with the presence of oxygen may not be suitable for anaerobic bacteria.

Therefore, our ongoing research will incorporate a greater number of subjects and variables, enabling us to yield more results that will elucidate the previously discussed issues.

Conclusions

According to the results of our research, the presence and intensity of periodontopathogenic bacteria are more common in the elderly. Our study point to the conclusion that more women were positive for the presence of all tested bacteria.

NOVČANA POTPORA/*FUNDING*
Nema/*None*

ETIČKO ODOBRENJE/*ETHICAL APPROVAL*
Nije potrebno/*None*

SUKOB INTERESA/*CONFLICT OF INTEREST*
Autori su popunili *the Unified Competing Interest form na www.icmje.org/coi_disclosure.pdf (dostupno na zahtjev)* obrazac i izjavljuju: nemaju potporu niti jedne organizacije za objavljeni rad; nemaju financijsku potporu niti jedne organizacije koja bi mogla imati interes za objavu ovog rada u posljednje 3 godine; nemaju drugih veza ili aktivnosti koje bi mogle utjecati na objavljeni rad./ *All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.*

Literature

1. Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017; 75 (1): 7-23.
2. Sedghi LM, Bacino M, Kapila YL. Periodontal Disease: The Good, The Bad, and The Unknown. Front Cell Infect Microbiol. 2021; 11: 766944.
3. Liu Y, Yu Y, Nickel JC, Iwasaki LR, Duan P, Simmer-Beck M, et al. Gender differences in the association of periodontitis and type 2 diabetes. Int Dent J. 2018; 68 (6): 433-40.
4. Al-Jewair TS, Nazir MA, Al-Masoud NN, Alqahtani ND. Prevalence and risks of habitual snoring and obstructive sleep apnea symptoms in adult dental patients. Saudi Med J. 2016; 37 (2): 183-90.
5. Kozak M, Pawlik A. The Role of the Oral Microbiome in the Development of Diseases. Int J Mol Sci. 2023; 24 (6).
6. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. J Periodontol. 2015; 86 (5): 611-22.
7. Zhao J, Zhou YH, Zhao YQ, Feng Y, Yan F, Gao ZR, et al. Gender Variations in the Oral Microbiomes of Elderly Patients with Initial Periodontitis. J Immunol Res. 2021; 2021: 7403042.
8. Motoc GV, Juncar RI, Moca AE, Motoc O, Vaida LL, Juncar M. The Relationship between Age, Gender, BMI, Diet, Salivary pH and Periodontal Pathogenic Bacteria in Children and Adolescents: A Cross-Sectional Study. Biomedicines. 2023; 11 (9).
9. Lund Håheim AL. Oral anaerobe bacteria-a common risk for cardiovascular disease and mortality and some forms of cancer? Front Oral Health. 2024; 5: 1348946.
10. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998; 25 (2): 134-44.
11. Loos BG. Systemic markers of inflammation in periodontitis. J Periodontol. 2005; 76 (11 Suppl): 2106-15.
12. Gamsiz-Isik H, Kiyan E, Bingol Z, Baser U, Ademoglu E, Yalcin F. Does Obstructive Sleep Apnea Increase the Risk for Periodontal Disease? A Case-Control Study. J Periodontol. 2017; 88 (5): 443-9.
13. Olsen I, Progulske-Fox A. Invasion of Porphyromonas gingivalis strains into vascular cells and tissue. J Oral Microbiol. 2015; 7: 28788.
14. Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al. Treatment of periodontitis and endothelial function. N Engl J Med. 2007; 356 (9): 911-20.
15. Reyes L, Herrera D, Kozarov E, Roldá S, Progulske-Fox A. Periodontal bacterial invasion and infection: contribution to atherosclerotic pathology. J Periodontol. 2013; 84 (4 Suppl): S30-50.
16. Rahimi A, Afshari Z. Periodontitis and cardiovascular disease: A literature review. ARYA Atheroscler. 2021; 17 (5): 1-8.
17. Bui FQ, Almeida-da-Silva CLC, Huynh B, Trinh A, Liu J, Woodward J, et al. Association between periodontal pathogens and systemic disease. Biomedical journal. 2019; 42 (1): 27-35.
18. Åberg CH, Kelk P, Johansson A. Aggregatibacter actinomycetemcomitans: virulence of its leukotoxin and association with aggressive periodontitis. Virulence. 2015; 6 (3): 188-95.
19. Könönen E, Fteita D, Gursoy UK, Gursoy M. Prevotella species as oral residents and infectious agents with potential impact on systemic conditions. J Oral Microbiol. 2022; 14 (1): 2079814.
20. Franklin KA, Lindberg E. Obstructive sleep apnea is a common disorder in the population-a review on the epidemiology of sleep apnea. J Thorac Dis. 2015; 7 (8): 1311-22.
21. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. Journal of clinical periodontology. 2018; 45 Suppl 20: S1-s8.
22. Shet UK, Oh HK, Kim HJ, Chung HJ, Kim YJ, Kim OS, et al. Quantitative analysis of periodontal pathogens present in the saliva of geriatric subjects. J Periodontal Implant Sci. 2013; 43 (4): 183-90.
23. Kuret S, Kalajzic N, Ruzdjak M, Grahovac B, Jezina Buselic MA, Sardelić S, et al. Real-Time PCR Method as Diagnostic Tool for Detection of Periodontal Pathogens in Patients with Periodontitis. Int J Mol Sci. 2024; 25 (10).
24. Huang Q, Dong X. Prevalence of periodontal disease in middle-aged and elderly patients and its influencing factors. Am J Transl Res. 2022; 14 (8): 5677-84.
25. Li S, Wen C, Bai X, Yang D. Association between biological aging and periodontitis using NHANES 2009-2014 and mendelian randomization. Sci Rep. 2024; 14 (1): 10089.
26. Victor LV, Cortelli SC, Aquino DR, de Carvalho Filho J, Cortelli JR. Periodontal profile and presence of periodontal pathogens in young African-Americans from Salvador, Ba, Brazil. Brazilian journal of microbiology : (publication of the Brazilian Society for Microbiology). 2008; 39 (2): 226-32.
27. Marcenes W, Kassebaum NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, et al. Global burden of oral conditions in 1990-2010: a systematic analysis. J Dent Res. 2013; 92 (7): 592-7.
28. Eke PI, Wei L, Borgnakke WS, Thornton-Evans G, Zhang X, Lu H, et al. Periodontitis prevalence in adults ≥ 65 years of age, in the USA. Periodontol 2000. 2016; 72 (1): 76-95.
29. Baelum V, López R. Periodontal disease epidemiology - learned and unlearned? Periodontol 2000. 2013; 62 (1): 37-58.
30. Eke PI, Borgnakke WS, Genco RJ. Recent epidemiologic trends in periodontitis in the USA. Periodontol 2000. 2020; 82 (1): 257-67.
31. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of periodontitis in adults in the United States: 2009 and 2010. J Dent Res. 2012; 91 (10): 914-20.

32. Larvin H, Kang J, Aggarwal VR, Pavitt S, Wu J. Risk of incident cardiovascular disease in people with periodontal disease: A systematic review and meta-analysis. Clin Exp Dent Res. 2021; 7 (1): 109-22.

33. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. Journal of periodontology. 2000; 71 (10): 1554-60.

34. Hernández-Ruiz P, González-Pacheco H, Amezcua-Guerra LM, Aguirre-García MM. Relación entre la disbiosis de la microbiota oral y la enfermedad cardiovascular aterosclerótica. Archivos de cardiologia de Mexico. 2022; 92 (3): 371-6.

35. Scannapieco FA, Dasanayake AP, Chhun N. "Does periodontal therapy reduce the risk for systemic diseases?". Dent Clin North Am. 2010; 54 (1): 163-81.

36. Nik-Azis NM, Mohd N, Baharin B, Said MSM, Fadzilah FM, Haflah NHM. Periodontal disease in seropositive rheumatoid arthritis: scoping review of the epidemiological evidence. Germs. 2021; 11 (2): 266-86.

37. de Smit M, Westra J, Vissink A, Doornbos-van der Meer B, Brouwer E, van Winkelhoff AJ. Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study. Arthritis Res Ther. 2012; 14 (5): R222.

38. de Aquino SG, Abdollahi-Roodsaz S, Koenders MI, van de Loo FA, Pruijn GJ, Marijnissen RJ, et al. Periodontal pathogens directly promote autoimmune experimental arthritis by inducing a TLR2- and IL-1-driven Th17 response. J Immunol. 2014; 192 (9): 4103-11.

39. Konig MF, Paracha AS, Moni M, Bingham CO, 3rd, Andrade F. Defining the role of Porphyromonas gingivalis peptidylarginine deiminase (PPAD) in rheumatoid arthritis through the study of PPAD biology. Ann Rheum Dis. 2015; 74 (11): 2054-61.

40. Schmickler J, Rupprecht A, Patschan S, Patschan D, Müller GA, Haak R, et al. Cross-Sectional Evaluation of Periodontal Status and Microbiologic and Rheumatoid Parameters in a Large Cohort of Patients With Rheumatoid Arthritis. Journal of periodontology. 2017; 88 (4): 368-79.

41. Carra MC, Schmitt A, Thomas F, Danchin N, Pannier B, Bouchard P. Sleep disorders and oral health: a cross-sectional study. Clin Oral Investig. 2017; 21 (4): 975-83.

42. Schroeder K, Gurenlian JR. Recognizing Poor Sleep Quality Factors During Oral Health Evaluations. Clin Med Res. 2019; 17 (1-2): 20-8.

43. Movahed E, Moradi S, Mortezagholi B, Shafiee A, Moltazemi H, Hajishah H, et al. Investigating oral health among US adults with sleep disorder: a cross-sectional study. BMC Oral Health. 2023; 23 (1): 996.

44. Pereira D, Progiante P, Pattussi M, Grossi P, Grossi M. Study on the association between sleep disorders versus oral health related variables. Med Oral Patol Oral Cir Bucal. 2021; 26 (2): e164-e71.

45. Acar M, Türkcan İ, Özdaş T, Bal C, Cingi C. Obstructive sleep apnoea syndrome does not negatively affect oral and dental health. J Laryngol Otol. 2015; 129 (1): 68-72.

Sažetak

PARADONTOPATOGENE BAKTERIJE U PACIJENATA SPLITSKO-DALMATINSKE ŽUPANIJE: POVEZANOST IZMEĐU SPOLA, DOBI, SISTEMSKIH BOLESTI I HRKANJA

Eva Vukelić, Nina Kalajžić, Ferdinand Josip Bušelić, Sendi Kuret

Cilj: Parodontna bolest kronično je upalno stanje koje značajno utječe na oralno, ali i sistemsko zdravlje. Rizični čimbenici za razvoj parodontitisa uključuju dob, spol, sistemske bolesti, poremećaje disanja tijekom spavanja te prisutnost specifičnih parodontopatogenih bakterija. Cilj ovog istraživanja bio je ispitati prisutnost pet parodontopatogenih bakterija (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia i Treponema denticola) u uzorcima bolesnika te analizirati njihovu povezanost s dobi, spolom, sistemskim bolestima i hrkanjem.

Materijali i metode: U ovom presječnom istraživanju sudjelovalo je ukupno 149 odraslih ispitanika (62 muškarca i 87 žena) s kliničkim znakovima lokaliziranog kroničnog parodontitisa. Ispitanici su ispunili upitnik kojim su prikupljeni podaci o njihovim sociodemografskim i kliničkim obilježjima, a prisutnost pet parodontopatogenih bakterija u subgingivnom plaku analizirana je metodom real-time PCR-a.

Rezultati: Dobiveni su statistički značajni rezultati koji upućuju na povezanost dobi i prisutnosti parodontopatogenih bakterija. Statistički značajni rezultati za bakterije Porphyromonas gingivalis i Tannerella forsythia najčešće su zabilježeni kod ispitanika u dobi između 41 i 60 godina te onih starijih od 60 godina. Za bakteriju Aggregatibacter actinomycetemcomitans dobiveni su granično značajni rezultati u odnosu na hrkanje, pri čemu je više pozitivnih nalaza zabilježeno kod osoba koje ne hrču.

Zaključak: Dob se pokazala najznačajnijim čimbenikom, a većina pacijenata s pozitivnim nalazima testiranih bakterija bile su žene.

Ključne riječi: PARODONTITS, PARODONTOPATOGENE BAKTERIJE, SISTEMSKE BOLESTI, HRKANJE, SPLITSKO-DALMATINSKA ŽUPANIJA

Primljeno/Received: 25. 4. 2025.
Prihvaćeno/Accepted: 16. 5. 2025.