

Relationship between past myocardial infarction, periodontal disease and *Porphyromonas gingivalis* serum antibodies: A case-control study

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Abstract

Background: *The relationship between chronic periodontitis (CP) and increased risk for cardiovascular disease (CVD) is known but quantitative assessments and mechanisms are not fully understood. The aim of this study was to assess the relationship between past myocardial infarction (MI) and the severity of CP, and the level of serum antibody titer against Porphyromonas gingivalis gingipains.*

Methods: *The study sample consisted of 97 patients after MI and 113 high risk controls with no history of coronary heart disease (CHD) matched with age, sex and place of residence (urban vs. rural). Data on the history of CHD and presence of risk factors were collected. Periodontal status was assessed using the Community Periodontal Index (CPI), clinical attachment loss (CAL), bleeding on probing (BOP) and pocket depth.*

Results: *After adjustment for potential confounders patients with BOP = 20–50% and BOP > 50% had more than four times higher odds of past MI (OR = 4.56; 95% CI 2.03–10.27). Patients with CPI code = 4 had a three times higher odds of past MI (OR = 3.18, 95% CI 1.01–10.06). CAL ≥ 6 was related to higher odds of past MI (OR = 1.28, 95% CI 1.11–1.49). Patients with moderate antibody titer levels had an almost 3 times higher odds of past MI (OR = 2.82, 95% CI 1.02–7.84).*

Conclusions: *There was an association between CP and past MI, which was independent of classical CVD risk factors and confirmed by an association between past MI and immunological reaction against P. gingivalis gingipains. (Cardiol J 2018; 25, 3: 386–392)*

Key words: periodontal disease, myocardial infarction, *Porphyromonas gingivalis* gingipains, cardiovascular disease risk factors

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Introduction

An increasing number of studies have shown a relationship between chronic periodontitis (CP) and increased risk of cardiovascular disease (CVD) even after controlling for possible confounders [1, 2]. CP has been postulated to be a CVD risk factor, which is independent of well-known risk factors, such as smoking, hypertension, obesity, diabetes and hypercholesterolemia [3, 4]. CP is an inflammatory disease of tooth supporting tissues and is commonly prevalent among the middle-aged and the elderly in the general population. The prevalence of CP in the general European population is estimated to be over 50%, and the percentage of patients with severe forms of CP is over 20% [5]. Its prevalence increases with age. According to a recent nationwide Polish study, almost 20% of the population from large Polish cities aged 35–44 years and almost 32% aged 65–74 years had severe CP [6].

The most pathogenic and etiologic bacteria for CP are: *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Treponema denticola*, *Eikenella corrodens*, *Campylobacter rectus*, *Prevotella intermedia*, and *Fusobacterium nucleatum* [7]. *P. gingivalis* is the most frequent bacteria and is identified in 85% of sites of CP [8]. *P. gingivalis* has a number of potential virulence factors, such as cysteine proteinases — gingipains (GPs), lipopolysaccharide, capsules, and fimbriae [9]. GPs contribute to 85% of the total proteolytic activity of *P. gingivalis* [10] and are crucial in inducing CP since they have multi-directional adverse impacts on the immune system. The pathomechanism of the relation between CP and coronary heart disease (CHD) is still unclear, but in general two mechanisms have been postulated. The first is the general inflammatory host response in CP, which is supported by the findings of elevated inflammatory markers (C-reactive protein) in CP patients and their decrease after successful treatment of periodontitis [11]. Furthermore, it has been found that *P. gingivalis* may increase overall inflammatory response through its virulence factor, especially GPs [12, 13]. The second postulated mechanism involves direct invasion of arteries and endothelial cells by periodontal pathogens, especially *P. gingivalis*. The latter is supported by findings that endothelial dysfunction, which plays a key role in the development of arteriosclerotic plaques, was associated with CP [14]. Furthermore, antibodies against *P. gingivalis* have been found in human arteriosclerotic plaques [15] and experimentally induced *P. gingivalis* bacteremia

accelerated formation of arteriosclerotic plaques in pigs [16]. In addition, defects in endothelial cells and arteriosclerotic plaques that appear as the effect of CP may be a background for myocardial infarction (MI) [15].

In most studies showing an association between CP and CHD, periodontal status was based on clinical examination or self-reporting. Such methods do not allow for the assessment of systematic immunological response to CP. The level of antibodies against periodontopathogenic bacteria, especially their major virulence factors as a better indicator of the CP [17] could be a more accurate measure of the link between CP and systemic diseases. Therefore the aim of this study was to assess the association between past MI and the clinical parameters of CP in the context of the level of serum antibody titer against *P. gingivalis* gingipains.

Methods

Study design

A frequency-matched case-control study was performed to identify factors which are associated with past MI that are related to the periodontium health status. The potential characteristics explored were: pocket depth (PD), clinical attachment loss (CAL), bleeding on probing (BOP), community periodontal index (CPI), and the titer of antibody (AT) directed against gingipains. The study group consisted of 220 patients, including 97 cases and 113 controls.

For a type I error of 5% and power of 80%, 98 cases were sufficient to detect an odds ratio > 2.2 for factors with a prevalence of 35% in the general population [18].

Studied groups

Patients after MI were recruited from five cardiology departments, serving the city of Krakow and the surrounding rural area. Patients aged ≥ 18 years and ≤ 80 years, with definite clinical diagnosis of MI, were identified from medical records, excluding those who died during their in-hospital stay and were asked to undergo dental examination.

Controls were persons with no diagnosis of MI or CHD, who were selected randomly from three general practices: two in Krakow and one outside of Krakow. Inclusion criteria were: high CVD risk identified by attempted treatment for hypertension, hypercholesterolemia or diabetes mellitus. Information on treatment was collected from medical records using a standardized data collection form. Then, controls were matched ac-

ording to their age (5-year groups), sex and place of residence (rural vs. urban) and were asked to undergo dental examination. However, of the 113 controls, 91 (80.5%) were comprised of men and 22 (19.5%) women, i.e. 5 women less than in the previous MI group. Those 5 women were excluded because they had less than 6 teeth.

In both groups, the exclusion criteria were: use of antibiotics during the 2 weeks prior to the examination, and immunosuppressive therapy or chemotherapy.

Data collection

Data on demographic characteristics, personal history of CHD, smoking status, blood pressure, fasting glucose, plasma lipids and prescribed medications were obtained using a standardized data collection form. Patient height and weight were measured in a standing position without shoes and heavy outer garments using standard scales with a vertical ruler. Blood pressure was measured twice, on the right arm in a sitting position after at least 5 min of rest. For plasma lipid and glucose measurements, a fasting venous blood sample was taken between 7:30 AM and 8:30 AM. Biochemical analyses were carried out no later than 4 h after blood collection. All analyses were performed at one central laboratory.

Dental examination

Periodontal condition was assessed in participants with at least 6 teeth. Dental clinical examination was carried out according to World Health Organization recommendations [19]. Participants were examined by one qualified dentist. Standard intraoral examination was performed starting from the first to the fourth quadrant. Pocket depth was measured on 6 sites around each tooth using a PCPUNC15-type periodontal Hu-Friedy probe. BOP was evaluated according to Ainamo and Bay [20]. For each patient, the percentage of teeth with bleeding after gentle probing was calculated. Results were interpreted as follows: BOP > 50% severe, advanced CP, BOP = 20–50% moderate CP, BOP = 19–10% mild CD, BOP < 10% no CP. CAL was defined as the distance between the pocket bottom and the cement-enamel junction. The CPI was used to record periodontal status [21]. CPI ranged from 0 to 4, representing healthy (CPI 0), bleeding on probing (CPI 1), calculus or conditions for dental plaque retention (CPI 2), 4–5 mm pocket depth (CPI 3) and ≥ 6 mm pocket depth (CPI 4). Participants were classed into CPI groups according to the worst measurement in any quadrant.

Serum samples for antibody determination

Serum samples for antibody determination were stored at –80°C immediately after centrifugation. Immunoglobulin G antibody titers against *P. gingivalis* GPs were determined using the enzyme-linked immunosorbent assay (ELISA), in all samples using the method described by Zdzalik et al. [22]. Antibody titers are given in ELISA units.

Definition of CVD risk factors

Participants were considered smokers, if they declared they had smoked at least one cigarette during 1 month prior to the interview or had ≥ 10 ppm carbon monoxide in their exhaled air. The concentration of carbon monoxide in exhaled air was measured using Smokerlyzer Micro+ (Bedfont Scientific Ltd, England). Hypertension was defined as blood pressure ≥ 140/90 mmHg or antihypertensive drugs taken by a patient. Hypercholesterolemia was defined as total cholesterol ≥ 5.0 mmol/L or low density lipoprotein ≥ 3.0 mmol/L or lipid lowering drugs taken by a patient. Diabetes mellitus was defined as fasting glucose ≥ 7 mmol/L or self-reported history. Obesity was defined as body mass index ≥ 30 kg/m².

Statistical analysis

All analyses were performed together for males and females. Cases and controls were compared for demographic characteristics, health status variables and oral health indices using t-Student, Mann-Whitney or χ^2 tests as appropriate. Unconditional logistic regression was used to assess the relation between periodontal indices, level of antibody titer and past MI. Three models were examined: a univariate model (model A), and two multivariate models in which the matching variables were forced into model. Model B was adjusted for age, gender and education. The final model (model C) included additional covariates: smoking, diabetes, hypertension, hypercholesterolemia, and body mass index. Antibody titer was included in the logistic regression as a categorical variable. Based on a frequency distribution of all subjects, antibody titer values were divided into three categories and then compared between cases and controls. The reference category included the lowest 10 percent of values (AT < 900), titers above 900 to the median value (= 8,100) formed the second category, values above the median were included in a third category.

Sensitivity analysis that restricted cases and controls to 1:1 matching was also performed. The frequency-matched case-control analysis included 92 pairs of observations. Five cases were excluded

Table 1. General descriptive statistics and cardiovascular disease risk factors in the study groups.

	MI (n = 97)	Control group (n = 113)	P
Age [years]*	60.5 ± 8.7	60.4 ± 8.7	0.94 ^c
Men	72.2%	80.5%	0.15 ^a
Years of education [years]**	12.0 (10.0–15.0)	13.0 (11.0–17.0)	0.005^b
Body mass index [kg/m ²]*	29.0 ± 4.7	28.6 ± 4.7	0.61
Arterial hypertension	80.4%	73.0%	0.21
Diabetes	23.4%	24.3%	0.88
Hyperlipidemia	71.3%	79.7%	0.16
Obesity	36.1%	31.8%	0.52
Smoking	17.7%	17.9%	0.98

*Mean ± standard deviation; **Median (upper-lower quartile); ^aχ² test; ^bMann-Whitney U test; ^cStudent T test; MI — myocardial infarction; n — number of participants

due to lack of appropriately matched controls within the gender.

Results are presented as odds ratios (OR) with 95% confidence intervals (95% CI). Analyses were performed using SPSS Software (IBM, Armonk, NY, USA). Statistical significance was accepted at the level of $\alpha = 0.05$.

Ethics

All participants were informed about the study protocol and gave their informed consent. The study was approved by the Bioethics Committee of the Jagiellonian University.

Results

There were no significant differences in mean age and distribution by sex between the studied groups. Patients after MI were slightly less educated than controls. However, the prevalence of CVD risk factors, i.e. smoking, hypertension, hyperlipidemia, obesity, and diabetes, were similar (Table 1).

Patients after MI had a lower mean number of teeth (15 vs. 18). On average, they also had their teeth affected by periodontal disease more frequently than the controls. Cases were more likely than the controls to have some periodontal pocketing of 6 mm or more (29.9% vs. 17.7%, respectively) and had a significantly higher median value of CAL (7 mm compared to 6 mm for controls, $p < 0.001$). They were also more likely to have a higher percentage of bleeding teeth and to have some teeth with calculus deposits or conditions for dental plaque retention. Cases had a higher percentage of participants with CPI stage 4 and BOP > 50%. However,

differences in antibody titer against *P. gingivalis* gingipains between the cases and the control group were not observed when compared as a quantitative variable or a categorical variable (Table 2).

Table 3 shows clear and consistent ORs of past MI for various periodontal health indicators.

After adjustment for age, sex, years of education, smoking, hypertension, hypercholesterolemia, body mass index, diabetes mellitus, and number of teeth, those patients with BOP 20–50% and BOP ≥ 50% had a more than 4 times higher odds of past MI. Patients with CPI 4 code had a 3 more times higher odds of past MI and those with CAL ≥ 6 mm had a 1.28 odds of past MI. Association between PD ≥ 6 mm and past MI was attenuated after adjustment for possible confounders. A clear relationship between antibody titer and the odds of past MI was not apparent in the univariate model. When some potential confounding factors were entered into regression models, those patients from antibody titer group 2,700–8,100 had an almost 3 times higher odds of past MI.

In a separate model (not shown in the tables), the sample was limited to 92 cases and their 92 matched controls. The relations between past MI, CP and antibody titer in the unconditional and conditional models were similar.

Discussion

These results indicate that impaired periodontal health is associated with prevalence of past MI which was confirmed also by increased antibody titer against *P. gingivalis* gingipains in patients after MI. The relation was independent of classic CVD risk factors.

Table 2. Descriptive statistics for oral and periodontal health in the study groups.

	MI (n = 97)	Control group (n = 113)	P
Number of teeth	15 ± 6	18 ± 7	0.0006 ^A
Pocket depth [mm]	5 (4–6)	5 (4–5)	0.12
Pocket depth ≥ 6 mm	29.9%	17.7%	0.04
CAL [mm]	7 (6–9)	6 (5–7)	< 0.0001
BOP less than 20% teeth	29.47%	69.03%	< 0.001
BOP = 20–50% teeth	35.79%	15.93%	
BOP above 50% teeth	34.74%	15.04%	
CPI 0	0%	8.9%	< 0.001
CPI 1	18.6%	8.9%	
CPI 2	33%	23.8%	
CPI 3	21.7%	52.2%	
CPI 4	26.8%	6.2%	
Antibody titer [median, Q1–Q3]	8100 (2700–24300)	8100 (2700–24300)	0.36
Low AT ≤ 900	9.3	14.2	0.13
Moderate AT 2700–8100	52.6	38.9	
high AT > 8100	38.1	46.9	

AT — antibody titer; BOP — bleeding on probing; CAL — clinical attachment loss; CPI — community periodontal index; MI — myocardial infarction; n — number of participants; Q — quartile; ^AStudent T test

Table 3. Relation between periodontal health parameters, antibody against *P. gingivalis* gingipains and myocardial infarction status.

	Model A OR (95% CI)	Model B OR (95% CI)	Model C OR (95% CI)
Pocket depth < 6 mm	1	1	1
Pocket depth ≥ 6 mm	1.98 (1.03–3.81)	1.95 (0.99–3.82)	1.91 (0.95–3.84)
CAL ≥ 6	2.35 (1.19–4.67)	1.31 (0.14–1.51)	1.28 (1.11–1.49)
BOP less than 20% teeth	1	1	1
BOP = 20–50% teeth	5.26 (2.56–10.81)	5.06 (2.42–10.58)	4.34 (2.00–9.44)
BOP above 50% teeth	5.41 (2.60–11.24)	5.02 (2.29–11.01)	4.56 (2.03–10.27)
CPI 0 or 1	1	1	1
CPI 2	1.32 (0.58–3.00)	1.04 (0.43–2.47)	0.96 (0.39–2.40)
CPI 3	0.40 (0.18–0.89)	0.33 (0.14–0.79)	0.27 (0.11–0.68)
CPI 4	4.13 (1.44–11.87)	4.03 (1.34–12.13)	3.18 (1.01–10.06)
Low AT ≤ 900	1	1	1
Moderate AT 2700–8100	2.06 (0.82–5.15)	2.76 (1.05–7.24)	2.82 (1.02–7.84)
High AT > 8100	1.24 (0.49–3.13)	1.48 (0.57–3.87)	1.37 (0.50–3.76)

Model A non adjusted

Model B adjusted for: sex, age and years of education

Model C adjusted for: sex, age, years of education, smoking, hypertension, hypercholesterolemia, body mass index, diabetes mellitus and number of teeth

AT — antibody titer; BOP — bleeding on probing; CAL — clinical attachment loss; CI — confidence interval; CPI — community periodontal index; n — number of participants; OR — odds ratio

The relationship between CP and MI was first reported by Matilla et al. [1] and later confirmed in the studies which showed 1.2–2.0 times increased

odds for MI [23, 24]. In contrast, some studies showed negative relationships between periodontal disease and CHD. Hujoel et al. [25] and Tuominen et al. [26]

reported that CP was not associated with CHD after controlling for CVD risk factors. Depending on the indicator, in our study we showed increased odds of past MI in CP patients by 1.2–5.0 times compared to non CP patients. The inconsistencies in the strength of the relation may be explained partially at least that various indices have been adopted to assess CP. However, a unified indicator for CP in population studies is not yet defined. Also, definitions of CHD varied among the studies. Some studies included only patients with history of acute MI, whereas others included patients with stable or unstable angina. It was decided to include only patients with verified definite clinical diagnosis of acute MI.

The evidence for the relationships between CHD and the level of antibody against *P. gingivalis* is inconsistent. In our study we found that high antibody titer against *P. gingivalis* gingipains is related to an almost 3 times increased chance of MI. This confirms the finding of another other study in which males aged 45 to 75 years who were seropositive for *P. gingivalis* had more frequent CHD than those who were seronegative for *P. gingivalis* [27]. Alternatively, some cross-sectional studies have shown no association between antibodies against *P. gingivalis* and CHD, after adjustment for classic CVD risk factors [28, 29].

The present study has several strengths. The main one is the use of antibody titer to investigate associations between CP and MI. The use of definition of CP based on clinical parameters, i.e. pocket depth, to assess relationships between CP and other chronic diseases has been criticized. Clinical parameters might not be a good indicator of the effect of long-lasting influence of bacterial infection. Thus, antibody titer seems to be a better marker of immunological response for periodontal bacteria. Another strength of this study is that cases and controls were highly comparable. Patients after MI were selected from patients from 5 clinical cardiology wards serving a defined geographical area and the controls were sex, age place of residence matched, and constituted of a random sample of high risk persons registered in selected primary practices serving the same residential area. Restriction of the control group to persons at high risk allowed us to limit confounding by CVD risk factors which was controlled further by adjustment for these in the multivariate analyses.

However case-control design which does not allow for firm conclusions on causality remains

the main limitation of the study. Furthermore, the study sample had limited statistical power, although it appeared to be sufficient to find a strong association. Also, the strong relation between BOP and past MI might be explained at least partially by the use of antiplatelet agents. We were not able to adjust for this this effect as nearly all MI participants were taking these agents. However, the relation between BOP and antiplatelet treatment was not confirmed unequivocally [30]. It is also possible that patients after MI had been paying less attention to oral hygiene as a result of their history of serious disease and as in a consequence had developed more severe CP than healthy persons. Antibody levels for periodontopathic bacteria are considered to be stable over time, but it remained unclear whether antibody level is an indicator of disease history or active infection. The other limitation is that the present study assessed only antibody for one periodontopathogenic bacteria. It is possible that if the presence of antibodies against other periodontitis-associated bacterial species were included in the analysis the relationships could have been stronger.

Conclusions

In conclusion, an association was found between chronic periodontal disease and past MI which was independent of classical CVD risk factors and confirmed by the association between past MI and immunological reactions against *P. gingivalis* gingipains.

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