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PORHYROMONAS GINGIVALIS AND ACUTE MYOCARDIAL INFARCTION

SUMMARY

The classic dental disease, caries and periodontal disease might have an effect on systemic health. These diseases result from infections by microbes with highly specific adhesion mechanisms in the mouth. Over the last decade, accumulating evidences have linked dental infections to an increased risk of atherosclerosis and thrombosis. The aim of our study was to investigate whether Porhyromonas gingivalis might play a role in the development of acute myocardial infarction (AIM) as well as its possible relation with traditional risk factors.

The study enrolled 124 participants, 74 of whom were patients with AIM (63 years old, 44 males) and 50 were controls (60.3 years old, 31 males). Blood was sampled and sent on dry ice to Immunosciences Lab Inc (USA). We determined circulating levels of IgG antibodies against Porhyromonas gingivalis by using the ELISA method.

A high proportion of patients had circulating levels of IgG antibodies against Porhyromonas gingivalis above the reference range (98% vs 22%, P <0.001). The titers were significantly higher in patients compared to controls (P<0.001). Circulating concentrations of antibodies were higher in men and smokers. The higher the titers, the higher the monocytes and white blood cells count.

In view of our results, patients with AIM have evidence of chronic infection caused by Porhyromonas gingivalis. Chronic dental infection could be considered as an independent risk for atherosclerotic disease.

Key words: **Porhyromonas gingivalis, infection, inflammation, myocardial infarction**

INTRODUCTION

Atherosclerosis, the main underlying disease responsible for cardiovascular and cerebrovascular morbidity and mortality around the world, results from multifactorial etiology. Traditional risk factors such as obesity, hyperlipidemia, diabetes, hypertension and cigarette smoking accounts only for a proportion of cases of cardiovascular diseases.

For more than a century, it has been postulated that infectious agents are responsible for atherosclerotic diseases (1). The association between pathological substrate of acute myocardial infarction (AIM) and infection seems to be the rational one, as the processes of development of atherothrombosis involve a low-grade inflammation. Moreover, markers of inflammation, such as C-reactive protein, leukocyte count, fibrinogen and cytokines are regarded as predictors of present and future cardiovascular events and diseases (1).

Renewed interest in this topic has taken

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place since the late 1980s when as association between patients who had had acute myocardial infarction and the presence of Chlamydia pneumoniae antibodies were noted by Saikku and colleagues (2). Further, there is evidence that infection with Helicobacter pylori, Cytomegalovirus and periodontopathic bacteria (such as Porhyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, etc. are associated with heart diseases. For years, dentists have noticed that certain characteristics are common to patients with periodonitis and patients with AIM (3 - 7).

Such reports emphasize that maintaining oral health might be more important than healthy teeth. The mouth is a portal of entry as well as the site of disease for microbial infections that affect general health status. The biological mechanisms that may explain the relationship might involve several different ways: bacteria from periodontal disease may enter the circulation and contribute directly to atherothrombotic processes, or systemic factors alter the immune-inflammatory processes involved in both periodontal and cardiovascular disease.

Based on this data and our own interest, we decided to investigate the question if dental infection caused by Porhyromonas gingivalis plays a role in development of AIM and if there is its possible correlation with traditional risk factors for heart disease.

MATERIAL AND METHODS

Study participants

The prospective study we conducted enrolled a total of 124 participants, 74 of whom were patients with AIM and 50 were volunteers without documented coronary artery disease (CAD) who made a control group. Patients (63 ± 12 years of age, 44 males) with the diagnosis of AIM were admitted to Coronary Care Unit (CCU) of Clinic for Cardiovascular Disease, Clinical Center of Nis, (Serbia) between December 2002 and May 2004. The inclusion criteria were chest pain due to AIM with or without electrocardiographic evidence of ST- segment elevation. The exclusion criteria included: major surgery or trauma within previous months, suspected thrombotic disorders, malignancy or inflammatory diseases as well as concomitant heart diseases (dilated cardiomyopathy, valvular heart disease etc). Traditional risk factors for CAD were noted: cigarette smoking, hypertension, hyperlipidemia, diabetes, obesity, family history of CAD. We noted previous history of UA or myocardial infarction (MI).

Blood donors - a total of 50 volunteers (60.3 years of age, 31 males) with unknown coronary disease represented the control group.

Pieces of information were obtained from all participants in accordance with the guidelines of the Ethical Review Committee of Clinical Center in Nis.

Blood sampling and analyses

Blood was sampled on admission before administration of therapy. Standard biochemical analyses were performed in the Central Lab, by using standard methods. Sera (10 ml) were kept frozen and sent on dry ice with the permission of Center for Disease Control of United States to Immunosciences Lab where advanced analysis were performed.

Sera were tested for auto-antibodies by using an in-house enzyme linked immunosorbent assay (ELISA).

Peptides: Strains of oral bacteria were purchased from American Type Culture Collection in Rockville, Maryland (USA). The bacteria were lysed by sonication and the purified antigens were immobilized by attachment to a solid surface microtiter plate.

ELISA: Patients and controls' sera were used in dilutions 1:200. Sera were added to microtiter plate and incubated for 60 minutes. Unreacted material was removed in a washing step and then alkaline phosphatase - labelled goat anti - human IgG (KPI, Gaithersburg, MD) was added to each well in an optimal dilution. After washing five times with Phosphate - buffer saline (PBS) containing Tween 20, the enzyme reaction was started by adding paranitrophenylphosphate in 0.1ml diethanolamine buffer containing 1mM $MgCl_2$ and sodium azide, pH 9.8. The reaction was stopped 45 minutes later with 50 l of 1N NaOH. The optical density was read at 405nm (OD_{405}) with a microtiter reader.

Calculations: ELISA values for specimen were determined by using the following formula:

ELISA values= (Values of calibrator x Absorbance of test specimen) / (Absorbance of calibrator)

Statistical analyses

Results of normally distributed continuous variables are expressed as the mean value ± standard deviation. Continuous variables with a non - normal distribution are presented as median values (interquartile interval) and qualitative variables are presented as frequencies. Analysis of normality of the continuous variables was preformed with the Kolmogorov Smirnov test. Differences between patients and controls were assessed by the unpaired t - test

and the Mann Whitney U test for continuous variables. χ^{2} testing was used for discrete variables. Correlations between continuous variables were analyzed with the two-way Pearson correlation test. Univariate analysis had been performed. The SPSS 10.0 statistical software package was used for calculations.

RESULTS

Baseline characteristics of examined groups

Baseline characteristics of 124 study participants are shown in Table 1.

levels of total cholesterol ($p < 0.001$) and triglycerides ($p < 0.001$) were significantly higher in the patients' group. Total white blood cells count (WBC), monocytes and fibrinogen were above the referent range and significantly higher in patients ($p < 0.001$).

Clinical characteristics of patients with acute myocardial infarction

Table 2 outlines clinical characteristics of patients with AIM. Out of 74 patients involved in the study, the presenting ECG showed ST segment elevation in 50 patients.

Mean value of systolic blood pressure was 130 ± 38 mmHg and diastolic 77 ± 24mmHg. Duration of hospital stay was 10.8 ± 5.4 days to

Table 1: Baseline characteristics of study participants

Characteristics	Patients (n=74)	Controls (n=50)	p
Age at entry (years)	63	60.03	0.
Male sex, n (%)	44	31 (62)	53
Current or ex smokers, n (%)	(59.45)	22 (44)	0.
Dyslipidemia, n (%)	33 (44.5)	3 (6)	77
Hypertension, n (%)	30 (40.5)	13 (26)	0.
Diabetes, n (%)	42	0 (0)	61
Familial history of CAD, n (%)	(56.75)	13 (26)	<
BMI, mean (SD), kg/m^2	20	24.88	0.001
Total cholesterol, mean (SD),	(27.02)	(1.48)	<
mmol/L	40	4.82	0.001
Triglycerides, mean (SD),	(54.05)	(0.50)	<
mmol/L	26.98	1.35	0.001
WBC count, mean (SD), x10^9/L	(2.01)	(0.3)	<
Monocytes, median (range),	5.82	6.68	0.001
x10^9/ L	(1.45)	(1.04)	<
Fibrinogen (g/l)	2.21	0.5 (0.3	0.001
	(1.36)	– 0.7)	<
	10.50	3.07 (0.6)	0.001
	(3.49)		<
	1.1 (0.7		0.001
	– 1.4)		<
	6.63		0.001
	(1.37)		<
			0.001
			<
			0.001

CAD indicates coronary artery disease; WBC, white blood cells; BMI, body mass index

The majority of the study participants were male (59.45% of patients). The mean age of patients was 63 years and of controls 60.03 years ($p = 0.53$). A high proportion of patients as well as controls were smokers ($p = 0.61$). As expected, the patients who suffered from ACS were more likely to have a higher prevalence of hypertension ($p < 0.001$), diabetes ($p < 0.001$), dyslipidemia ($p < 0.001$) and a family history of CAD ($p < 0.001$) compared to controls. There was a significant difference in BMI ($p < 0.001$). The complete medical treatment. Previous MI was recorded in 17 patients (22.97%) and previous CABG in 7 (9.45%). Aspirin had been used before hospital admission by 19 (25.67%) patients. Fibrinolytic therapy (chiefly streptokinase) had been received by 44 (59.45%) patients just after the admission to CCU. During the hospital stay, 71 patients (95.94%) received aspirin and 72 (97.2%) received anticoagulant therapy (chiefly low molecular weight heparin). Intravenous nitrates had

Table 2: Clinical characteristics of patients with ACS

Characteristics of 74 patients with AIM	N (%)
ECG abnormalities at discharged	
AIM with q	59 (79.72)
Non q AIM	15 (20.27)
Systolic BP, mmHg, mean (SD)	130 (38)
Diastolic BP, mean (SD), mmHg	77 (24)
Heart rate, heartbeats/min, mean (SD)	84 (24)
Previous disease and drug use	
Previous MI	17 (22.97)
Previous CABG	7 (9.45)
Aspirin before admission	19 (25.67)
Duration of staying in hospital, mean (SD)	10.8 (5.4)
Treatment during hospital stay	44 (59.45)
Fibrinolytic agents	71 (95.94)
Aspirin	5 (6.7)
Ticlopidin	72 (97.2)
Anticoagulant	
β blocker	66 (89.18)
ACEI	48 (64.86)
Nitrate (intravenous)	69 (93.24)
Antiarrhythmics	23 (31.08)
Statins	38 (51.35)
LVEF, mean (SD)	52.49
New event (12.77)	
Death during first 48 hours	32 (43.24)
	11 (14.86)

ECG indicates electrocardiogram; BP, blood pressure; MI myocardial infarction; CABG coronary artery by pass grafting; ACEI, angiotensin converting enzyme inhibitors; LVEF, left ventricle ejection fraction

Values are % unless otherwise indicated

been received by 69 patients, beta blockers by 66, ACEI by 48 and statins by 38. Five patients had persistent sinus bradycardia which required atrial pacing. In our group of patients, echo was performed in 91.2% of patients.

For the primary composite outcome of death, 11 (14.86%) patients died during the first 48 hours in hospital. During the follow up, 32 patients had new event (death, new MI or episodes of UA).

Prevalence of dental infectious in study participants

Porhyromonas gingivalis was detected in 95% of patients and in 22% of controls. The proportion of patients with positive IgG antibodies was significantly different compared to controls (RR. 4.32, 95% CI (2.98 6.26), $p < 0.001$), *Figure 1*.

With regard to circulation concentrations of IgG antibodies against Porhyromonas gingivalis, there was a significant difference in patients with acute myocardial infarction and controls, $p < 0.001$, *Figure 2*.

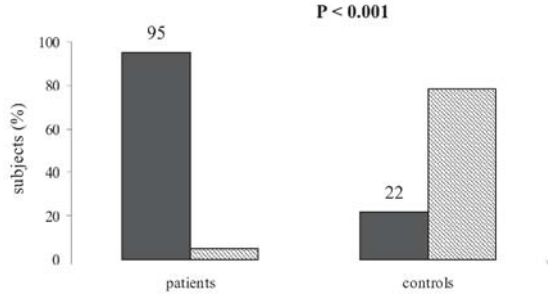

Figure 1: The proportion of subjects with positive circulating titers of IgG antibodies against Porhyromonas gingivalis in examined groups (%)

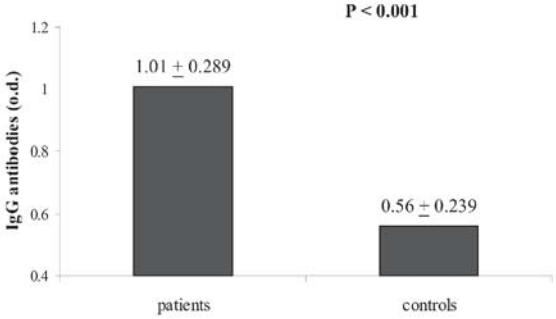

Figure 2: Comparison of IgG antibodies against Porhyromonas gingivalis between patients with AIM and controls. Values are expressed as mean and standard deviation

Clinical predictors of serum IgG antibodies distribution in patients with AIM

Baseline serum concentration of circulation IgG antibodies against Porhyromonas gingivalis were higher in men (p = 0.006) and patients who were exposed to cigarette smoking (P = 0.02) in a univariate analysis. We did not find the correlation in regard to age (p = 0.13), previous myocardial infarction (p = 0.16) and previous coronary artery by pass grafting (p = 0.53). IgG antibody titers did not differ in regard to the presence of family history of coronary artery disease (p = 0.68). Also, circulating concentrations of IgG antibodies were not significantly different in regard to the presence of diabetes (p = 0.210) and dysplidemia (p = 0.477).

Baseline serum concentrations of IgG antibodies showed a strong correlation with monocytes (p = 0.008) and leucocytes count (p = 0.02) in the univariate analysis.

DISCUSSION

Study findings indicate that high proportion of patients with AIM had been previously exposed to Porhyromonas gingivalis infection. In addition, the circulating concentrations of IgG antibodies against this dental infectious agent were significantly higher in patients than controls with unknown coronary artery disease.

Dental infections appear as cardiovascular risk factors in some cross-sectional studies and in follow-up studies (8). This association is independent of the "classic" coronary risk factors. In the meta-analysis of five cohort studies, an increased relative risk of CAD due to periodontal disease was reported (9). A recent meta- analysis (10) of nine studies also yielded a modest but significant increase in a relative risk similar to the result of previous meta-analysis among individuals with periodontal disease compared to those without it.

It is hypothesized that this association may be due to an underlying inflammatory response trait, which exposes an individual to a high risk for development of both periodontal disease and arteriosclerosis. Furthermore, periodontal disease which represents a chronic infection by oral bacteria that affects the supporting structures of teeth might provide a biological burden of inflammatory cytokines that promote atherosclerosis and thromboembolic events.

Porhyromonas gingivalis, which is involved in periodonitis, may play a role in the pathogenesis of atherosclerosis and may directly infect vascular endothelial cells (11). Upon colonization of the subgingival plaque, this pathogenic microorganism probably invades and lyses mucosal epithelial cells and digests a pathway to connective tissues (12). In response to infection and inflammation, susceptible individuals may exhibit greater expression of local and systemic mediators and may thereby be at high risk for a future myocardial infarction or even stroke.

The antibody response varies between individuals and immune system response to an organism can be influenced by individuals' genetic and immunological background, previous exposure to the organism and immunogenic characteristics of the antigenic challenge. Systemic exposure that originates from local infection may imply as insufficient local response to prevent systemic entry as well as factors that promote acute episodes of periodontal disease activity such as smoking.

High antibody titers could represent a response to a chronic phase of infection in subjects but could also reflect the host response to an oral pathogen that confers protection of an individual without disease. The premise for this study was an assumption that an antibody response merely indicates systemic exposure to an oral organism. Increases of a systemic antibody response to organisms have also been associated with systemic inflammatory activity. Not only periodonitis but also many other lesions in oral cavity may generate inflammatory mediators, such as severe gingivitis. Matilla et al. incorporate several oral lesions and create Total Dental Index by summing several oral pathogens (13). Several years later, Janket et al. investigated the role of Asymptotic Dental Score and coronary artery disease (14).

Univariate analysis confirmed the strong correlation between white blood cells count and monocytes with serum concentrations IgG antibodies.

In regard to traditional risk factors, the study did not find the correlation with the presence of diabetes, hypertension or dyslipidemia. Furthermore, there was no difference concerning age and family history of CAD. The determinate of circulating levels were gender and cigarette smoking.

Moreover, smoking is a risk factor for both periodontal disease and heart disease and must be considered as a confounder. Because periodontal disease is itself heavily influenced by cigarette usage (15), controlling for the confounding effects of smoking is a prerequisite for credible claims of causality in these associations.

CONCLUSION

Results of our study suggest possible relation between Porhyromonas gingivalis and AIM. If so, the mouth serves as a mirror of health or disease, as a sentinel of early warning system and as a potent ional source of pathology affecting other

systems and organs. Appropriate primary and secondary prevention might have benefit of cardiovascular health.

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PORHYROMONAS GINGIVALIS I AKUTNI INFARKT MIOKARDA

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SAŽETAK

Dentalna oboljenja, karijes i periodentalna bolest, mogu imati efekat na sistemsko zdravlje a javljaju se kao rezultat oralne infekcije izazvane mikrobima sa visoko specifičnim adhezionim mehanizmima. Poslednjih godina, intenzivno se proučava uloga dentalnih infekcija u nastanku aterotromboze.

Naša studija imala je za cilj da ispita moguću ulogu oralnog patogena - Phorphyromonas gingivalis u nastanku akutnog infarkta miokarda kao i njegovo međudejstvo sa tradicionalnim faktorima rizika.

Studija je uključila 124 ispitanika, 74 bolesnika hospitalizovanih sa dijagnozom akutnog infarkta mokarda (prosečne starosti 63 godine, 44 pripadnika muškog pola) i 50 pripadnika kontrolne grupe (prosečne starosti 60.3 godine, 31 pripadnik muškog pola). Serum je na suvom ledu transportovan u Immunoscieces Lab (USA) gde su urađene analize. Određivali smo titar antitela klase IgG ELISA metodom.

Visok procenat pacijenata imao je detektibilna antitela protiv Phorphyromonas gingivalisa (98% vs 22%, P<0.001). Titri antitela bili su signifikantno viši kod bolesnika u odnosu na kontrolnu grupu (P<0.001). Pripadnici muškog pola i pušači imali su značajno više titre. Porast titra bio je praćen porastom broja monocita i leukocita.

Naši rezultati ukazuju da dentalna infekcija prouzrokovana Phorhyromonas gingivaliom može igrati ulogu u nastanku AIM. Hronične dentalne infekcije mogu predstavljati faktor rizika za nastanak aterosklerotskog oboljenja.

Ključne reči: **Phorphyromonas gingivalis, infekcija, inflamacija, infarkt miokarda**