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Original Article

Detection of infectious agents by polymerase chain reaction in human aortic wall

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Abstract

Introduction: Several studies have been suggested that infectious agents may induce or progress the process of atherosclerosis in humans. In the present study, the samples of visually healthy human aortic wall were examined for the presence of *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, Helicobacter *pylori*, herpes simplex virus (HSV), and cytomegalovirus (CMV). **Methods:** Bacterial DNA of *C. pneumoniae*, *M. pneumoniae*, and *H. pylori* and viral DNA of HSV and CMV were analyzed by polymerase chain reaction. The specimens were obtained from 40 patients with atherosclerotic three-vessel stable coronary artery disease referred to surgical revascularization (coronary group) and 20 controls referred to aortic valve replacement (valve group). **Results:** *C. pneumoniae* was detected in 11 of 40 samples of aorta in coronary group (27.5%) and 5 of 20 in valve group (25%). *M. pneumoniae* was found in 6 of 40 (15%) and 5 of 20 (25%) samples, and CMV was found in 22 of 40 (55%) and 10 of 20 (50%) samples. The most frequent detected pathogens were *H. pylori* and HSV. *H. pylori* was found in 32 of 40 samples of aortic wall in coronary group (80%) and 17 of 20 samples in valve group (85%), whereas HSV was found in 27 of 40 (67.5%) and 17 of 20 (85%) aortic wall specimens. **Conclusion:** Results demonstrate that *C. pneumoniae*, *M. pneumoniae*, *H. pylori*, CMV, and HSV can be detected in macroscopically healthy aortic wall of coronary and valve patients in similar frequency, which do not support hypothesis concerning the role of microorganisms in atherosclerosis etiology.

Keywords: Atherosclerosis; Aortic wall; PCR; *C. pneumoniae*; *M. pneumoniae*; *H. pylori*; HSV; CMV

1. Introduction

The hypothesis that the most popular infectious agents may induce or progress the process of atherosclerosis in humans has been extensively tested in epidemiologic, clinical, and experimental in vivo and in vitro studies. Animal model study, in which infection of herpes simplex virus (HSV) caused induction of atherosclerotic lesions [1], and human study, in which association between acute myocardial infarctions and *Chlamydia pneumoniae* antibodies was found [2], indicate that both viral and bacterial infectious agents are worth to consider in atherosclerosis development.

The most investigated initiators of atherosclerotic injury include *C. pneumoniae*, *Mycoplasma pneumoniae*, Helicobacter *pylori*, cytomegalovirus (CMV), HSV-1 and HSV-2, and common human pathogens. However, seroepidemiologic reports have not confirmed well the association between infectious agents and atherosclerosis risk [3,4]. More direct analysis for atheroma formation in coronary vessels, caused by viral and/or bacterial infections, has been given when polymerase chain reaction, in situ hybridization, and electron microscopy techniques were applied, but also with conflicting results. In tested aortic tissues from 33 autopsies, 10 atherosclerotic aortic tissues were positive for HSV-1 in 80% and for CMV in 40%, whereas 23 nonatherosclerotic tissue samples were positive for HSV-1 in 13% and for CMV

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in 4% [5]. *C. pneumoniae* DNA was present in 16 of 52 specimens of carotid artery atherosclerotic plaques (30.8%), and *H. pylori* DNA was detected in 9 of 52 (17.3%) atherosclerotic plaques. In 52 specimens obtained from the macroscopically healthy regions of ascending aorta wall, 1 of 52 (1.9%) was positive for *C. pneumoniae* and none of samples were positive for *H. pylori* [6]. Other investigations did not confirm this difference in the frequency of pathogen DNA in atheromatous and healthy aorta. In German study, none of investigated 29 atherosclerotic plaque specimens showed presence of *C. pneumoniae* DNA and CMV DNA [7]. In a study performed in Ireland, *Chlamydia* species were not detected in any of the atheromatous carotid specimens that are normal or with atheromatous lesions [8].

In the present study, we investigated the occurrence of *C. pneumoniae*, *M. pneumoniae*, *H. pylori*, HSV, and CMV DNA in aortic wall in patients with coronary artery disease and in patients with aortic valve disease by means of polymerase chain reaction (PCR) techniques.

2. Methods

2.1. Patients' population

The study groups consisted of people living in central Poland. Macroscopically healthy aortic wall specimens were obtained from 40 patients with clinical presentation of stable angina caused by three-vessel coronary artery disease, referred to coronary artery bypass grafting (CABG) on cardiopulmonary bypass. As controls, 20 specimens of aortic wall from patients referred to aortic valve replacement (AVR) were taken. In all patients included in the study, aorta was neither aneurysmatic nor dilated (size, 32–47 mm). The patients referred to AVR were preoperatively subjected to coronary angiography, and no significant changes were observed. None of the patients had previously undergone CABG or percutaneous coronary intervention. All specimens obtained during surgery were frozen at −70°C. The Regional Ethics Committee for Scientific Research approved the study protocol, and a written consent was obtained from each participant of the study. All volunteers were informed about the purpose of the investigation and a possible withdrawal from the study. Demographic characteristics of studied groups, smoking habits, fibrinogen concentration, and lipid profile for each patient were also recorded. Study group characteristics are presented in Table 1.

2.2. Polymerase chain reaction

DNA from 25 to 30 mg of tissue samples was isolated by DNeasy Tissue Mini kit (Qiagen, Syngen, Poland) according to protocol's recommendations. For bacterial and viral DNA amplification, we used 100 ng of isolated genomic DNA. The quality of isolated DNA from each specimen was analyzed spectrophotometrically and by means of amplification for human housekeeping *GAPDH* gene. Primers sequences for specific microorganisms are presented in Table 2.

In *C. pneumoniae* DNA detection in tissue specimens, nested PCR was applied according to LaBiche et al. [9]. After initial denaturation step at 95°C for 9 min, 40 cycles (first amplification) or 35 cycles (second amplification) of denaturation at 95°C for 30 s, annealing for 1 min, and elongation at 72°C for 30 s were conducted. In *M. pneumoniae*, DNA amplification [10] and seminested PCR were used. After 12 min of polymerase activation in 95°C, 40 cycles (first and second amplification) of denaturation at 95°C for 1 min, annealing at respectable temperature for 1 min, and elongation step at 72°C for 1 min were applied, followed by additional elongation step for 72°C. For detection of *H. pylori*, complementary primers for *glmM* gene were used [11]. Amplification conditions were as follows: initial denaturation step at 95°C for 15 min, 45 cycles of DNA denaturation at 95° for 1 min, respective annealing temperature for 1 min, and DNA elongation at 72°C for 1 min. An additional elongation at 72°C for 10 min was added to the last step. Cytomegalovirus DNA detection in analyzed tissue specimens was based according to Sanchez and Storch [12], and after initial denaturation at 95°C for 12 min, 40 cycles of amplification conditions similar to these, used in *C. pneumoniae* amplification, were

Table 1
Characteristics of study participants

	Valve group, *n*=20	Coronary group, *n*=40	*P*
Mean age ± SD	49.90 ± 9.50	54.23 ± 7.61	NS
Gender: women/men	2 (10%)/18 (90%)	8 (20%)/32 (80%)	NS
Smoking: yes/no	12 (60%)/8 (20%)	38 (95%)/2 (5%)	.001
SI [a]	395.17	617.08	.04
BMI [b] (kg/m^2)	28.8 ± 4.24	27.53 ± 3.62	NS
Fibrinogen (g/L)	3.61 ± 1.29	4.23 ± 1.10	.05
Total cholesterol level (mean mg/dl)	206.80 ± 38.22	204.9 ± 56.7	NS
HDL level (mean mg/dl)	57.15 ± 16.38	52.20 ± 12.28	NS
LDL level (mean mg/dl)	110.10 ± 27.9	120.18 ± 44.92	NS
Triglyceride level (mean mg/dl)	119.20 ± 45.8	163.75 ± 122.37	NS

[a] Smoking index was calculated according to daily cigarette consumption and duration of smoking.
[b] Body mass index.