Chlamydia pneumoniae in the Atherosclerotic Plaques of Coronary Artery Disease Patients

Article *in* Acta medica Iranica · December 2013

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Jpn. J. Infect. Dis., 62, 195-197, 2009

Short Communication

Detection of *Chlamydia pneumoniae* in Atherosclerotic Plaques of Patients in Tehran, Iran

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(Received July 16, 2008. Accepted January 29, 2009)

SUMMARY: Persistent infection of arteries with organisms such as *Chlamydia pneumoniae* was previously found to contribute to the development of atherosclerosis. We investigate the presence of *C. pneumoniae* in atherosclerotic plaque by polymerase chain reaction and direct immunofluorescence assay, and we examine the correlation between clinical status and the presence of this bacterium in Iran. The study group consisted of 33 atherosclerotic plaque specimens from the arteries (26 coronary and 7 abdominal aorta) of patients who had undergone coronary artery bypass grafting surgery (CABG). The control group consisted of 31 specimens: 12 from biopsies of macroscopically healthy regions of the ascending aorta in patients who had undergone CABG and 19 autopsy specimens of normal coronary arteries. *C. pneumoniae* DNA and antigen were found in 6 (18%) and 7 (21%) of 33 endarterectomy specimens, respectively. *C. pneumoniae* was not detected in the control group by either method. The presence of *C. pneumoniae* in atherosclerotic plaques and its absence in healthy vessels supports the idea that *C. pneumoniae* may have a role in the development of atherosclerosis, especially in countries where infection is prevalent and where conventional risk factors fail to explain the exact reason for the high prevalence of atherosclerotic vascular disease.

Atherosclerosis, a disease affecting arterial blood vessels, has been considered a public health problem since it is the leading cause of morbidity and mortality in the developed and some developing countries, such as Iran (1,2). None of the known risk factors, such as genetic predisposition, hypercholesterolemia, hypertension, smoking, or diabetes mellitus, can fully explain the pathogenesis of atherosclerosis (3). The possible role of some microorganisms, such as *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, or herpes simplex virus has been proposed in the pathogenesis of atherosclerosis, but the issue is unresolved (4,5). The frequency of *C. pneumoniae* infection and that of atherosclerotic heart disease in Iran are rather high (6,7). The aims of the current study are to investigate the prevalence of *C. pneumoniae* in atheromatous and nonatheromatous areas of the arteries of Iranian patients, based on polymerase chain reaction (PCR) and direct immunofluorescence assay (DIF), as well as to examine the correlation between clinical status and both the DNA and antigen (outer membrane protein) positivity of this bacterium.

The study included 33 consecutive patients who were admitted to the Department of Cardiovascular Surgery of the Tehran University of Medical Science hospitals between November 2006 and November 2007 with various manifestations of ischemic vascular disease, and who underwent surgery while there. In addition, 31 specimens–12 from biopsies of macroscopically healthy regions of the ascending aorta in patients who had undergone coronary artery bypass grafting surgery (CABG) and 19 autopsy specimens of normal coronary arteries–were collected at the National Forensic Medicine Department. Data on demographics, smoking habit, lipid profile, and medical history were recorded for each case. This study was approved by the Institutional Review Board (IRB) and the ethics committee of Tehran University of Medical Sciences (TUMS). All subjects provided written informed consent to participate in the study and were assured of both anonymity and confidentiality of data obtained.

Tissue samples were dissected in the operating room under sterile conditions. Artery segments approximately 4 to 5 mm in length were placed in microcentrifuge tubes without any buffer. Transport vials were sealed in the operating room and opened only in the laminar airflow safety cabinet at the microbiology laboratory. Tissue samples were divided into two parts, one for DIF and the other for DNA extraction. All of the specimens were kept at −20°C until processing. For preparation of genomic DNA and PCR, DNA was extracted from endoarterectomy specimens by using the QIAamp tissue method (Qiagen, Hilden, Germany). PCR was carried out for *C. pneumoniae* using primers, HL1 (5′-GTTGTTCATGAAG GCCTACT-3′) and HR1 (5′-TGCATAACCTACGGTGTGTT-3′), which amplify a 437-bp fragment of the *Pst*I cloned gene (8). PCR products were visualized by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and examined under UV illumination. To carry out DIF, atherosclerotic tissues were homogenized and vortexed to yield a milky suspension. A 5-μl amount of suspension was placed on a single well of a Teflon-coated slide (Multispot Microscope Slides; CA Hendley, Essex, UK), dried and fixed in acetone

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for 5 min, drained and finally air-dried before staining. The staining method was described by Garnett et al. (9). Each slide was read by two persons independently, each of whom was experienced in the diagnosis of *C. pneumoniae* by DIF. A test was deemed positive when four or more elementary bodies appeared as apple-green fluorescent disc-shaped bodies about 300 nm in diameter. Positive and negative control slides were included in each test. Statistical analysis was performed with SPSS 11.5 for Windows (SPSS, Chicago, Ill., USA). Continuous variables were analyzed with the two-sample *t* test (in the case of normal distribution) or the Mann-Whitney U rank-sum test. Binary data were analyzed with Fisher's exact test. All tests were two-tailed. A value of $P < 0.05$ was considered statistically significant.

Thirty-three specimens from atherosclerotic plaques (26 coronary and 7 abdominal aorta) as a lesion group and 31 control specimens (12 from biopsies and 19 from autopsies) were examined for the presence of *C. pneumoniae* by PCR and DIF.

C. pneumoniae DNA was positive in 18% (6 of 33) and outer membrane antigen in 21% (7 of 33) of the atherosclerotic samples. All DNA-positive samples were positive in DIF too. The additional positive sample with DIF may be related to nonspecific fluorescence and cross-reaction (10). The detection rate of *C. pneumoniae* varies around the world; for example, it is 10% in India, 15% in Turkey, 27% in Poland, and 62% in Japan (11-14). Our finding was close to that of Turkey, a neighbor of Iran. In the control group, *C. pneumoniae* was not detected by either method. There were no statistically significant differences between the lesion group and the well-matched control group in terms of age, sex, hypertension, hyperlipidemia, smoking, and diabetes mellitus (Table 1). As shown in Table 2, none of the demographic characteristics of the patients, such as age, sex, smoking, hypertension, hyperlipidemia, or diabetes mellitus, differed significantly with respect to *C. pneumoniae* DNA/antigen positivities and DNA/antigen negativities. In addition, we found no significant correlation between chronic heart disease and myocardial infarction in patients with *C. pneumoniae* infection in studied patients ($P > 0.05$).

Table 1. Demographic characteristics of lesion and patient groups

Parameter	Lesion group ($n = 33$)	Control group ($n = 31$)	*P*
Mean age ± SD	59 ± 9	45 ± 9	
Sex (no. of M/F)	22/11	22/9	0.79
No. of A/C specimens	7/26	12/19	0.17
No. of patients (%) with			
Hypertension	15 (45)	12 (39)	0.62
Diabetes	11 (33)	7 (22)	0.41
Smoking	12 (36)	15 (48)	0.45
Hyperlipidemia	13 (39)	11 (35)	0.80
CHD	11 (33)	9 (30)	0.79
Myocardial infarction	11 (33)	8 (26)	0.58

M/F, male/female; A/C, aortic/coronary; CHD, chronic heart disease.

Two of 7 abdominal aorta specimens and 5 of 26 coronary specimens were found to be positive for the organism of interest by either PCR or DIF. Statistically, however, *C. pneumoniae* did not show any location preference ($P > 0.05$) (Table 2). Following angiography, disease severity was assessed by the number of coronary vessels diseased (>50% atherosclerotic plaque stenosis). All of the *C. pneumoniae*-positive samples were in patients with three or more vessels diseased; this suggested the organism is probably a factor in the severity of atherosclerosis. However, we could not find any significant correlation between the number of diseased arteries and stenotic segments on the one hand and, on the other, the positivity of *C. pneumoniae* ($P = 0.2$). The presence of *C. pneumoniae* in more than 18% of the atherosclerotic lesions and its absence in the healthy vessels supports their possible role in atherogenesis. However, their absence in more than three-fourths of the lesions shows that they are not an obligate component of the atherosclerotic process. They might have a role in the initiation and/or acceleration of atherogenesis. Neither PCR nor DIF revealed an association between clinical instability, defined as acute coronary syndrome or transient ischemic attack, and the presence of *C. pneumoniae* ($P > 0.05$), although this situation was more prevalent in the *C. pneumoniae*-positive group. We found that *C. pneumoniae* DNA was more common in the coronary arteries of smokers than in those of nonsmokers. This may be attributable to the fact that smokers are more vulnerable to the colonization of *C. pneumoniae* as a respiratory system organism. In addition, hypertension, hyperlipidemia, and myocardial infarction rates were high in the *C. pneumoniae*-positive group, but statistically the differences were not significant ($P > 0.05$). Interestingly, one of six patients positive for *C. pneumoniae* had none of the conventional risk factors for atherosclerosis. However, the others had several risk factors. In conclusion, considering that there are so many risk factors and genetic influences, it seems unlikely that infection will be the only or main cause of atherosclerosis. However, the role of these newly emerging risk factors and

Table 2. Characteristics of lesion group according to the *C. pneumoniae* status by PCR and DIF

Parameter	*C. pneumoniae* status by PCR		*P*	*C. pneumoniae* status by DIF		*P*	Total
	Positive ($n = 6$)	Negative ($n = 27$)		Positive ($n = 7$)	Negative ($n = 26$)		
Mean age ± SD	62 ± 8	58 ± 10	0.40	61 ± 8	58 ± 10	0.39	59 ± 10
Sex (no. of M/F)	3/3	19/8	0.37	3/4	19/7	0.18	22/11
No. of A/C specimens	2/4	5/22	0.58	2/5	5/21	0.62	7/26
No. of patients (%) with							
Hypertension	3 (50)	12 (44)	0.80	4 (57)	11 (42)	0.67	15 (46)
Diabetes	1 (17)	10 (37)	0.64	2 (29)	9 (34)	0.76	11 (33)
Smoking	4 (67)	8 (30)	0.08	4 (57)	8 (30)	0.37	12 (36)
Hyperlipidemia	3 (50)	10 (37)	0.66	4 (57)	9 (35)	0.39	13 (39)
CHD	1 (17)	10 (37)	0.63	1 (14)	10 (38)	0.21	11 (33)
Myocardial infarction	3 (50)	8 (30)	0.37	3 (43)	8 (31)	0.66	11 (33)

Abbreviations are in Table 1.

their relationships with traditional risk factors, such as hypertension or lipids, remain unexplored. The uncertainty of their role and the types of infection or types of patients that should be treated must be explored in properly conducted, prospective studies in different geographical regions.

ACKNOWLEDGMENTS

We would like to thank Miss F. Aminharati for her coordination of laboratory. The authers express their sincere appreciation to Dr M.A. Pourhosseingholi for his assistance in data analysis.

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