Demonstration of *Chlamydophila pneumoniae, Mycoplasma pneumoniae,* Cytomegalovirus, and Epstein-Barr virus in atherosclerotic coronary arteries, nonrheumatic calcific aortic and rheumatic stenotic mitral valves by polymerase chain reaction

Aterosklerotik koroner arterler, romatizmal olmayan kalsifik aort ve romatizmal stenotik mitral kapaklarda Chlamydophila pneumoniae, Mycoplasma pneumoniae, Cytomegalovirus ve Epstein-Barr virüsün polimeraz zincir reaksiyonu ile gösterilmesi

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ABSTRACT

Objective: The aim of this study was to investigate whether bacterial and viral infectious agents can be demonstrated in atherosclerotic lesions of patients with coronary artery disease (CAD) as well as in stenotic aortic and mitral valves from patients undergoing heart valve replacement.
Methods: In this cross-sectional study, the presence of *Chlamydophila pneumoniae, Mycoplasma pneumoniae*, Cytomegalovirus (CMV), and Epstein-Barr virus (EBV) was investigated by polymerase chain reaction in atherosclerotic and non-atherosclerotic vascular samples taken from patients undergoing coronary artery bypass surgery due to CAD, and from patients undergoing aortic (AVR) and/or mitral valve replacement (MVR) secondary to valvular stenosis. For statistical analyses ANOVA, Chi-square test or Fisher's exact test were used.
Results: The presence of *C. pneumoniae, M. pneumoniae,* and CMV in atherosclerotic versus non-atherosclerotic samples was as follows: 30% vs. 16.7% (p=0.222), 6.7% vs. 3.3% (p=0.554), and 10% vs. 0% (p=0.076), respectively. In valve group, same pathogens were present in AVR and MVR patients as follows: 24.2% vs. 21.4% (p=0.773), 9.1% vs. 7.1% (p=0.758), and 21.2% vs. 11.9% (p=0.275). EBV DNA was not detected in any of vascular specimens, but in one (3%) patient with AVR (p=0.256).
Conclusion: Our results suggest that *C. pneumoniae, M. pneumoniae,* and CMV are present with similar frequency both in atherosclerotic and non-atherosclerotic vessels. We conclude that although non-atherosclerotic, vascular samples of CAD patients are invaded by infectious agents as like as atherosclerotic vessels. We further conclude that *C. pneumoniae, M. pneumoniae,* and CMV are present in stenotic aortic and mitral valves and atherosclerotic tissues with similar frequency indicating that atherosclerosis and valvular stenosis might share a common etiology related to infection. *(Anadolu Kardiyol Derg 2011; 11: 237-43)*
Key words: Atherosclerosis, coronary artery, aortic valve, mitral valve, *Chlamydophila pneumoniae, Mycoplasma pneumoniae*, Cytomegalovirus, Epstein-Barr virus

ÖZET

Amaç: Bu çalışmanın amacı, koroner arter hastalığı (KAH) olan kişilerin aterosklerozlu vasküler örnekleri ile stenotik aort ya da mitral kapak nedeni ile kapak replasmanı yapılan hastaların kalp kapaklarında bakteriyel ve viral enfeksiyon ajanlarının varlığının araştırılmasıdır.
Yöntemler: Bu kesitsel çalışmada, KAH nedeni ile koroner arter baypas operasyonu yapılan hastalardan alınan aterosklerotik olan ve olmayan vasküler örnekler ile kapak stenozu nedeni ile aort (AVR) ve/veya mitral kapak replasmanı (MVR) yapılan hastalardan alınan örneklerde *Chlamydophila pneumoniae, Mycoplasma pneumoniae,* Cytomegalovirus (CMV) and Epstein-Barr virüs (EBV) varlığı polimeraz zincir reaksiyonu yöntemi ile araştırıldı. İstatistiksel analizlerde ANOVA, Ki-kare testi ya da Fisher's exact testi kullanıldı.

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Bulgular: Aterosklerotik olan ve olmayan örneklerde *C. pneumoniae, M. pneumonia* ve CMV'nin varlığı sırası ile %30 ve %16.7 (p=0.222), %6.7 ve %3.3 (p=0.554), 10% ve %0 (p=0.076) olarak bulundu. Aynı etkenlerin AVR ve MVR hastalarında tespit edilme sıklığı sırası ile %24.2 ve %21.4 (p=0.773), %9.1 ve %7.1 (p=0.758), %21.2 ve %11.9 (p=0.275) olarak bulundu. EBV DNA vasküler örneklerin hiçbirinde saptanmazken, AVR yapılan bir (%3) hastada tespit edildi (p=0.256).
Sonuç: Çalışmadan elde edilen sonuçlara göre *C. pneumoniae, M. pneumoniae* ve CMV'nin aterosklerotik olan ve olmayan vasküler örneklerde benzer sıklıkta bulundukları gözlendi. KAH olan kişilerde, aterosklerotik olmayan vasküler yapıların, aterosklerotik vasküler yapılarda olduğu gibi enfeksiyon ajanları tarafından invaze edildiği saptandı. *C. pneumoniae, M. pneumoniae* ve CMV'nin stenotik kalp kapaklarında aterosklerotik dokularla benzer sıklıkta bulunması, ateroskleroz ile kapak stenozunun enfeksiyona bağlı ortak bir etiyolojiye sahip olduğunu düşündürdü.
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Anahtar kelimeler: Ateroskleroz, koroner arter, aort kapağı, mitral kapak, *Chlamydophila pneumoniae, Mycoplasma pneumoniae*, Cytomegalovirus, Epstein-Barr virüs

Introduction

The hypothesis that several bacterial and viral agents may induce the progression of atherosclerosis in coronary arteries has been extensively studied for two decades. Results of these studies have provided evidence implicating direct pathogenic involvement of infectious agents in the process of atherogenesis, especially in the development of coronary artery disease (CAD) (1).

On the other hand, the association between infectious agents and calcified aortic valve stenosis (CAS) has been investigated in a limited number of studies (2-11). For many years, degenerative aortic stenosis was thought to be caused by the passive accumulation of calcium on the surface of the aortic valve leaflet. Recent studies have demonstrated, however, that the etiology of aortic valve disease has a similar pathophysiology to that of vascular atherosclerosis (11, 12). Stenosis of the mitral valve is usually observed as a late complication of rheumatic fever. Mitral stenosis due to reasons other than rheumatic fever is an uncommon occurrence and is usually encountered in sporadic cases secondary to infective endocarditis which causes functional stenosis from obstructive vegetations (13-15).

Aortic valve replacement surgery is usually needed if the heart valve leaflets have become damaged or narrowed due to aortic valve calcification (adult type calcific aortic stenosis) or rheumatic heart disease (rheumatic aortic stenosis) (16). On the other hand, mitral valve replacement is mostly performed due to damage of the valve by rheumatic fever, which is a condition resulting from untreated infection by group A streptococcal bacteria. Damage to valve leaflets from post-rheumatic heart disease causes mitral stenosis with commissural fusion and irregular thickening and calcification of the leaflets (17).

Chlamydophila pneumoniae (2-4, 10, 11) and *Mycoplasma pneumoniae* (10) have been found to be associated with stenotic aortic valves in patients undergoing aortic valve replacement. A high prevalence of cytomegalovirus (CMV) DNA was detected in aortic walls of aortic valve replacement patients (18). Although the existence of Epstein-Barr virus (EBV) DNA in the human aortic wall (19) and coronary plaques (20) of patients with atherosclerosis was shown before, there is no evidence of the presence of EBV in stenotic aortic valves. The association of infectious agents such as *C. pneumoniae, M. pneumoniae*, CMV, and EBV with mitral stenosis has not been reported before.

The aim of the present study was to investigate whether bacterial infectious agents such as *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* and viral agents such as cytomegalovirus and Epstein-Barr virus, which are supposed to be associated with the progress of atherosclerosis in coronary arteries, can be demonstrated in atherosclerotic lesions of patients with coronary artery disease (CAD) as well as in stenotic aortic and mitral valves from patients undergoing heart valve replacement. The presence of bacterial and viral pathogens in coronary and valve groups were compared.

Methods

Patients

In this cross-sectional study, the study group consisted of 105 patients admitted to the Department of Cardiovascular Surgery at Sanko Hospital in Gaziantep, Turkey between March-October 2007. Thirty patients underwent coronary bypass surgery (coronary group) and 75 patients were referred to aortic and/or mitral valve replacement (valve group). Atherosclerotic samples from coronary arteries were taken by endarterectomy during bypass surgery of CAD patients. Decisions for thromboendarterectomy were always made intraoperatively on the basis of coronary morphology. As controls, non atherosclerotic tissues from the respective bypass grafts were used (n=30; 27 specimens from internal mammary arteries and one specimen from each saphenous vein, superficial femoral artery and radial artery). Ten to twelve millimeters coronary artery segments with advanced atherosclerotic lesions from the CAD patients were obtained. Similar sizes of segments from macroscopically healthy non-atherosclerotic vessels from respective bypass grafts were dissected as controls.

Thirty-three of 75 valve patients had aortic valve replacement (AVR) and 42 had mitral valve replacement (MVR). All of the patients undergoing AVR (n=33) had aortic valve stenosis (AS) as the result of adult type calcific aortic valve stenosis (CAS) and all of the patients with MVR (n=42) have been referred to surgery due to mitral valve stenosis (MS) secondary to rheumatic heart disease (RHD). Nine patients (12%) had AVR and MVR at the same time. All valves studied were removed solely for patient treatment purposes. The excised valves were macroscopically examined to determine the suitability for the study. Only valves showing definite areas of calcification and thickening with evi-

dence of clinical obstruction were included in the study. Coronary and valve samples were stored in sterile bottles and immediately frozen at -20˚C after harvesting. A written consent was obtained from each patient before surgery and the Regional Board of Ethics Committee approved the study.

Cardiovascular risk factors for atherosclerosis were assessed for each patient. Hypertension was defined as systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg or being administered antihypertensive medication. Diabetes mellitus was defined as fasting blood glucose ≥120 mg/dL and non fasting blood glucose ≥200 mg/dL or being administered antidiabetic medication. Hypercholesterolemia was defined as total cholesterol concentration ≥220 mg/dL. Smoking habit was defined as presently smoking or cessation within four years. Characteristics of patients with CAD and AVR and/or MVR are presented in Table 1.

Polymerase chain reaction

Coronary and other vascular segments approximately 10 mm in length and 25 to 30 mg of valvular tissues were used for DNA isolation using QIAamp Tissue kit (Qiagen GmbH, Hilden, Germany) according to protocol's recommendations. For bacterial and viral DNA amplification 100 ng of genomic DNA was used. The quality of the isolated DNA from each specimen was analyzed by means of amplification for human beta-actin gene. DNA extraction, PCR, and analysis of PCR products were performed in separate laboratories.

Detection of *C. pneumoniae* and *M. pneumoniae*

For the qualitative detection of *C. pneumoniae* and *M. pneumoniae* in coronary and valve samples a real-time amplification kit was used (*Mycoplasma pneumoniae/ Chlamydia pneumoniae* Real-TM; Sacace Biotechnologies, Caserta, Italy). For the detection of *C. pneumoniae* the gene coding for the major outer membrane protein (*omp* A) and for the detection of *M. pneumoniae* the gene coding for 16s RNA region were amplified. The sensitivity of the PCR assay was evaluated by amplification of serial 10-fold dilutions of both positive controls. The assay was able to detect *C. pneumoniae* and *M. pneumoniae* DNA with a sensitivity of <100 copies/ml. The analytical specificity of the primers and probes, which was validated with negative samples, was 100%.

Real-time PCR of the samples was performed with Rotor-Gene 3000 real-time analyzer (Corbett Research, Australia). Amplification conditions were as follows: initial denaturation step at 95˚C for 5 min, 45 cycles of DNA denaturation at 95˚C for 10 sec, annealing at 63˚C for 30 sec and elongation at 72˚C for 10 sec. An additional elongation at 72˚C for 10 min was added to the last step.

Detection of CMV DNA

Detection of CMV DNA in coronary and valve samples was performed by qualitative PCR. The UL55 gene encoding glycoprotein B of CMV was amplified using the primer set described before (21). Amplification conditions were as follows: 3 min at 95°C, 40 cycles (30 s at 95°C, 30 s at 45°C, 45 s at 72°C), and 5 min at 72°C. Amplification was performed by thermal cycler (iCycler, Bio-Rad Laboratories, California, USA). The 150 bp PCR products were visualized by 1.5% agarose gel electrophoresis and ethidium bromide staining.

Detection of EBV DNA

Detection of EBV DNA was performed by qualitative PCR assay using nested primers as previously described (22). A single cycle consisted of denaturation (94°C for 1 min), annealing (60°C for 2 min), and primer extension (72°C for 3 min); this was followed by a 30 min extension period at 72°C in the final cycle. Amplifications were carried out for 30 cycles with a PCR thermal cycler (iCycler, Bio-Rad Laboratories, California, USA). The amplified product (122 bp) was separated on a 1.5% agarose gel stained with ethidium bromide.

Statistical analysis

All statistical analyses were performed with the Statistical Program for Social Sciences (SPSS, version 15.0 for Windows;

Table 1. Characteristics of patients with coronary artery disease and heart valve replacement

Variables	Patients with CAD (n=30)	Patients with AVR (n=33)	Patients with MVR (n=42)	p
Mean age, years	55.6 (34-74)±9.5	53.9 (21-73)±13.0	48.7 (17-70)±11.1	0.028[a],*
Sex, male/female, n	24/6	22/11	17/25	0.002[b]
Hypertension, n(%)	20 (66.7)	27 (81.8)	34 (81.0)	0.270[b]
Diabetes mellitus, n(%)	15 (50.0)	13 (39.4)	11 (26.2)	0.113[b]
Hypercholesterolemia, n(%)	17 (56.7)	22 (66.7)	29 (69.0)	0.535[b]
Smoking history, n(%)	16 (53.3)	20 (60.6)	25 (59.5)	0.819[b]

Data are presented as mean+SD and number (percentage)

[a]ANOVA (F=3.711)

*posthoc Bonferroni test

[b]Chi-square test

AVR - aortic valve replacement, CAD - coronary artery disease, MVR - mitral valve replacement

SPSS Inc., Chicago, IL, USA). For comparison of continuous variables analysis of variance (ANOVA) was used. As the posthoc ANOVA test Bonferroni test was used. Categorical variables were tested by the Chi-square test or Fisher's exact test. Statistical significance was defined by a p value of less than 0.05.

Results

The study population with CAD consisted of 30 patients with a mean age of 55.6 years. Demographic data of patients with CAD is shown in Table 1. A total of 60 vascular samples from CAD patients consisted of 30 atherosclerotic specimens from coronary arteries plus 30 control specimens from non-atherosclerotic bypass grafts were investigated by PCR. The main finding of this study indicated that DNA from *C. pneumoniae* was detected in 9 (30%) of atherosclerotic coronary arteries and in 5 (16.7%) of non-atherosclerotic vessels of CAD patients (p=0.222). Four patients were positive for *C. pneumoniae* in both their atherosclerotic and non-atherosclerotic vessels. The prevalence of *M. pneumoniae* within atherosclerotic lesions and non-atherosclerotic tissues found by the detection of genomic DNA were 6.7% (n=2) and 3.3% (n=1), respectively (p=0.554). CMV DNA was found in 10% (3 of 30) of the atheromatous tissues and in none of the healthy vascular specimens (p=0.076). Evidence of EBV DNA specimens was shown neither in atheromatous nor in non-atheromatous tissues (Table 2). In the coronary group, both of patients who were positive for *M. pneumoniae* were also positive for *C. pneumoniae*. Among the three CMV positive coronary specimens, two were positive for *C. pneumoniae* too. There was no triple infection in the coronary group.

The patients from group CAS (n=33; mean age=53.9) were older than that of RHD (n=42; mean age=48.7) and the difference was statistically significant (p=0.028, F=3.711). Demographic data of patients with heart valve replacement are shown in Table 1. In AVR group, all patients had tricuspid aortic valves and none had a history of rheumatic fever. In MVR group, all patients had a history of RHD. In both AVR and MVR groups, none of the patients had any systemic infection at the time of the operation.

A total of 17 (22.7%) out of 75 patients were positive for *C. pneumoniae*, of which 8 (24.2%) were from AVR and 9 (21.4%) were from MVR group (p=0.773). Three patients from both (AVR and MVR) groups were positive for *M. pneumoniae* (9.1% and 7.1%, respectively) (p=0.758). CMV DNA has been detected in 7 (21.2%) of AVR patients and in 5 (11.9%) of MVR patients (p=0.275). Two CMV-positive AVR patients and one CMV-positive MVR patient had coinfection with *C. pneumoniae.* EBV DNA was positive only in one patient of the valve group and this was an AVR patient (3%) (p=0.256). Two of the patients who had AVR and MVR at the same time were positive for *C. pneumoniae* in both of their aortic and mitral valves. In one patient with AVR, genetic material of *C. pneumoniae*, *M. pneumoniae*, and EBV were demonstrated simultaneously. PCR results of AVR and MVR patients are shown in Table 3.

Table 2. Presence of bacterial and viral DNA in atherosclerotic and nonatherosclerotic vascular samples of patients with coronary artery disease

Patients with CAD	*C. pneumoniae*, n(%)	*M. pneumoniae*, n(%)	CMV, n(%)	EBV, n(%)
Atherosclerotic group (n=30)	9 (30)	2 (6.7)	3 (10)	-
Non-atherosclerotic group (n=30)	5 (16.7)	1 (3.3)	-	-
p	0.222[a]	0.554[b]	0.076[b]	-

Data are presented as number (percentage)
[a]Chi-square test
[b]Fisher's exact test
CAD - coronary artery disease, CMV - cytomegalovirus, EBV - Epstein-Barr virus

Table 3. Presence of bacterial and viral DNA in aortic and mitral valves of patients with heart valve replacement

Patients with valve replacement	*C. pneumoniae*, n(%)	*M. pneumoniae*, n(%)	CMV, n(%)	EBV, n(%)
AVR group (n=33)	8 (24.2)	3 (9.1)	7 (21.2)	1 (3)
MVR group (n=42)	9 (21.4)	3 (7.1)	5 (11.9)	-
p	0.773[a]	0.758[b]	0.275[a]	0.256[b]

Data are presented as number (percentage)
[a]Chi-square test
[b]Fisher's exact test
AVR - aortic valve replacement, CMV - cytomegalovirus, EBV - Epstein-Barr virus, MVR - mitral valve replacement

Discussion

In the present investigation, *C. pneumoniae*, *M. pneumoniae*, and CMV were detected in patients with stenotic aortic and mitral valves and in patients with coronary atherosclerosis with similar frequencies. *Chlamydophila pneumoniae* was detected with a rate of 24.2% in stenotic aortic valves, 21.4% in stenotic mitral valves, and 30% in coronary arteries. *Mycoplasma pneumoniae* was observed with a rate of 9.1% in aortic vs 7.1% in mitral valves, and 6.7% in atherosclerotic vessels. CMV DNA was detected in 21.2% of calcified aortic valves, 11.9% of rheumatic stenotic mitral valves, and 10.0% of coronary arteries.

The cause of calcific aortic stenosis is largely unknown, but one typical characteristic is an active inflammatory process that bears some similarities to atherosclerosis (23-25). Mohler et al. (26) found that 88% of surgically excised heart valves from patients who underwent cardiac valve replacement contained atherosclerotic plaques. The hypothesis that both viral and bacterial infectious agents may induce the process of atherosclerosis in humans (18) forced us to investigate whether these agents were responsible for the non rheumatic calcific stenosis of the aortic valve and rheumatic stenosis of the mitral valve from patients undergoing valve replacement.

Bacterial and viral DNA in cardiac tissues has been detected by means of different techniques, e.g., in situ hybridization, enzyme-linked immunosorbent assays, electron microscopy, culturing, and PCR (27). In this study we used PCR technique because this method has been adapted for use with most types

of clinical material, including valvular specimens, and has proven to be both easy and reliable when applied to surgically removed heart valves (28).

Chlamydophila pneumoniae has been implicated in the pathogenesis of atherosclerotic lesions in several vascular regions (29-33). However, some other studies have presented lack of occurrence of this pathogen in atherosclerosis (34-37). Chlamydiasis can damage heart tissue, causing valvular and other heart infections (38) and persistence is a well-known feature of Chlamydia infections (39). *Chlamydophila pneumoniae* was encountered in non rheumatic calcified aortic valves between 26% to 86% (2-4, 6-9). In the present investigation, *C. pneumoniae* was detected by PCR in nearly every fourth stenotic aortic valve (24.2%), and in more than every fifth stenotic mitral valve (21.4%) (p=0.773).

Mycoplasma spp. has been considered as typical parasites of respiratory and genitourinary tract epithelium, however Higuchi et al. (10) speculated that *M. pneumoniae* together with *C. pneumoniae,* were frequently present in atherosclerotic plaques also. Same authors hypothesized also that these agents might play an important role in the development of aortic valve calcification and they found higher concentration of both agents in calcific aortic valves compared to normal aortic valves. In this study *M. pneumoniae* was present less than *C. pneumoniae* in stenotic heart valves and it was observed almost equally in both aortic and mitral valves (9.1% and 7.1%, respectively) (p=0.758).

The role of CMV in atherogenesis has been supported by some authors with detection rates of 10% to 90% (20, 29, 40, 41), but others have failed to detect CMV in atherosclerotic tissue (35, 42, 43). Xenaki et al. (44) detected CMV DNA in both atherosclerotic plaques and non-atherosclerotic tissues from the same patients with similar frequency. They denoted that they did not support a direct causative role of CMV in the development of atherosclerosis. A high prevalence of CMV was detected in aortic walls of aortic valve replacement patients (18) and in degenerated stenotic aortic valves (6), however Kennedy et al. (45) and Radke et al. (5) could not demonstrate an association between CMV and aortic stenosis. In the present study CMV DNA was detected 21.2% in calcified aortic valves and 11.9% in rheumatic stenotic mitral valves (p=0.275).

According to Reszka et al. (18) *C. pneumoniae, M. pneumoniae,* and CMV can be found in aortic wall specimens of patients with or without coronary atherosclerosis by similar frequency, which may suggest that these pathogens can normally occur in arterial walls. In this study the difference between the presence of all three agents in atherosclerotic versus non-atherosclerotic samples was statistically nonsignificant (p=0.222, 0.554, 0.076, respectively).

The role of EBV in the pathogenesis of atherosclerosis is as speculative as CMV. While some authors detected EBV DNA at a high percentage (60%-80%) (19, 46), others could not demonstrate EBV DNA even in advanced atheromatous tissue (47, 48). The presence of EBV in valve tissue has not been investigated before. Here, EBV DNA was found only in one aortic valve (3%) and in none of the mitral valves (p=0.256).

Mohler et al. (26) denoted that patients with calcified valves had an increased prevalence of coronary artery disease compared with those without valve calcification. Additional analyses revealed no significant associations between valvular bone tissues and other concurrent cardiovascular diseases or risk factors. Also, stratification by valve origin (ie, rheumatic, bicuspid, or degenerative) failed to reveal any hidden associations. Concurrent with this finding, we did not find any association between the presence of infectious agents and origin of valve pathology among non rheumatic (AVR) and rheumatic (MRV) patients (p>0.05 for all).

The present report is the first of its kind in several aspects; the authors tried to evaluate the presence of two bacterial (*C. pneumoniae* and *M. pneumoniae*) and two viral (CMV and EBV) pathogens in heart valves at the same time. Further, presence of bacterial and viral agents in calcific valves from both non rheumatic and rheumatic etiology was investigated for the first time in this study. Lastly, presence of *C. pneumoniae*, *M. pneumoniae*, CMV, and EBV in stenotic mitral valves was demonstrated for the first time in this study.

The evolution of degenerative AS does not simply result from a 'wear and tear mechanism' and aging, but probably from an active inflammatory process similar to that of atherosclerosis (49). The pathogen burden may contribute to valvular degeneration by promoting further deleterious inflammatory and (auto) immune processes (50). This assertion is supported by the findings of epidemiological studies, which suggest that risk factors are common to both coronary disease and degenerative, AS (51). In pathologic terms, the presence of infectious agents in atherosclerotic tissues clearly does not necessarily imply a causal relationship. They might simply be present or carried there by mononuclear phagocytes without playing an active role. *Chlamydia pneumoniae* has been shown to disseminate systemically from the lungs through infected peripheral blood mononuclear cells and to localize in arteries where it may infect endothelial cells, vascular smooth muscle cells, monocytes/macrophages and promote inflammatory atherogenic process (52). The presence of multiple infectious agents may suggest nonspecific trapping of these microorganisms in areas of tissue damage, such as atherosclerotic plaques (29). This observation is with agreement with the results of this study, indicating occurrence of *C. pneumoniae*, *M. pneumoniae*, and CMV in patients with stenotic aortic and mitral valves and in patients with coronary atherosclerosis with similar frequencies. The presence of EBV DNA in atherosclerotic vessels and stenotic heart valves needs further investigation.

Study limitations

The limitation of the present study is that authors did not analyze the presence of viral and bacterial pathogens in normal heart valves, which could be obtained from cadavers. The rea-

son is that, in both institutes where this study was performed, autopsy was not a routine procedure.

The number of patients with concurrent infections with *C. pneumoniae* and *M. pneumoniae* (n=8), *C. pneumoniae* and *M. pneumoniae* (n=5), and *C. pneumoniae*, *M. pneumoniae* and EBV (n=1) was too small to allow us to draw definite conclusions with regard to pathogenic impact on atherosclerosis.

Conclusion

Our results suggest that *C. pneumoniae, M. pneumoniae,* and CMV are present with similar frequency both in atherosclerotic and non-atherosclerotic vessels. We conclude that although non-atherosclerotic, vascular samples of CAD patients are invaded by infectious agents as like as atherosclerotic vessels. We further conclude that *C. pneumoniae, M. pneumoniae*, and CMV are present in stenotic aortic and mitral valves and atherosclerotic tissues with similar frequency indicating that thero-sclerosis and valvular stenosis might share a common etiology related to infection.

Conflict of interest: None declared.

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