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The Presence of *Porphyromonas Gingivalis*, *Chlamydia Pneumonia*, *Helicobacter Pylori*, *Mycoplasma Pneumonia* and *Enterobacter Hormaechei* DNA in the Atherosclerotic Plaques

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ABSTRACT

Cardiovascular disease is the most common illness in the developed and developing countries. Atherosclerosis is a cardiovascular inflammatory disease, which causes tissue destruction and fibrosis in the long run. On the other hand, atherosclerosis is a very common disease and is a multifactorial process. A potential role of some infectious agents has been suggested in the pathogenesis of atherosclerosis. This study aimed to determine the presence of Porphyromonas gingivalis, Chlamydia pneumonia, Helicobacter pylori, Mycoplasma pneumonia and Enterobacter hormaechei DNA in coronary artery atherosclerotic plaques by PCR analysis and the study of association between the presence of bacterial DNA and atherosclerosis, clinical, and demographic features of patients. Twenty-eight patients with atherosclerotic diseases who had undergone coronary artery bypass grafting surgery were included. Cinna Pure DNA Kit was used for extraction of total bacterial genomes. The presence of bacterial DNA in endarterectomy specimens were detected by the PCR using 16S rRNA and conserved specific primers. The significant statistic association between presence of different bacterial DNA and clinical and laboratory features of included patients were evaluated by χ2 and Fisher's exact tests. C. pneumoniae and H. pylori DNA was detected in 4 out of 28 (14.3%) and 1 of 28 (3.6%) atherosclerotic plaques, respectively. DNA of Mycoplasma pneumonia, Enterobacter hormaechei and Porphyromonas gingivalis were not detected. There was no considerable difference between the presence of bacterial DNA and clinical and demographic features of patients. The higher rate of Chlamydia pneumoniae and Helicobacter pylori DNA in atherosclerotic damages suggest that these pathogens may play an important role in the pathogenesis of atherosclerosis progression.

Key words: Atherosclerosis, *Chlamydia pneumonia*, *Helicobacter pylori*

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INTRODUCTION

Cardiovascular disease is the most common illness in the developed and developing countries [1]. Atherosclerosis is a cardiovascular inflammatory disease, which causes tissue destruction and fibrosis in the long run [2, 3]. Atherosclerosis is a very common disease and is a multifactorial process. It is considered to be one of the chief causes of morbidity and mortality [4-6]. Several studies have reported evidence indicating that infection is a risk factor for atherosclerosis and plays an important role in chronic inflammatory progressions either via direct or indirect

mechanisms [7, 8]. The association of bacterial and viral pathogens with atherosclerosis has been illustrated by sero-epidemiological assessments that indicate a higher risk of atherosclerosis with increased levels of antibodies against specific pathogens as well as the detection of infectious agents in atherosclerotic lesions. However, the burden of evidence for a contributing role of infectious agents lies in establishing biological plausibility that the pathogen contributes to atherosclerotic progression and therefore, that therapeutic strategy or protective approaches influence disease development [2]. The detailed mechanism of atherosclerosis, which is the main cause of this disease, is the progression of chronic inflammation has been established as a common theory, but its association with the particular infectious agent (which can induce such chronic inflammations) has not been known until now[1]. Several individual bacterial pathogens have been detected more commonly in atherosclerotic legions by means of electron microscopy, immunocytochemistry, and polymerase chain reaction (PCR) that suggest their role as agents of the endothelial injury result in atherogenesis [7]. In the present study, the relationship between the presence of several bacterial DNA in atherosclerosis plaque and cardiovascular risk factors in Iranian adult patients who had undergone surgery were investigated.

MATERIALS AND METHODS

Patients and sample collection

The subjects, included in this study, were patients attending Shahid Madani Medical Research and Training Hospital, Tabriz who had undergone surgery and the atherom was taken. Information about epidemiological data and other risk factors were recorded and 28 subjects with atherosclerosis were included. We used a piece of non-atherosclerotic artery (grafts) as a control of the study. This study was approved by the Ethics Commission of Tabriz University of Medical Sciences. Atheromatous plaque samples and non-atherosclerotic artery were dissected in the operating room and placed in a 5 ml TE buffer (50 mM Tris/ HCl, pH 7.6; 1 mM EDTA, pH 8.0) under sterile conditions. Samples were immediately frozen at -20°C and were stored at -80°C until processing and were manipulated by the same investigator.

DNA extraction

After homogenization with a manual homogenizer, samples were incubated for 2 h in a SDS/proteinase K solution at 37°C. The total DNA was extracted by Cinna Pure DNA Kit.

Detection of bacterial DNA

The universal bacterial 16sRNA was used for the detection of bacterial DNA. Conserved specific primers were used for the detection of *Porphyromonas gingivalis* (*P. gingivalis*), *Chlamydia pneumonia (C. pneumonia*), *Helicobacter pylori (H. pylori), Mycoplasma pneumonia (M. pneumonia)* and *Enterobacter hormaechei (E. hormaechei)*. PCR conditions and primer sequences were shown in table-1. Positive and negative controls, obtained by the addition of genomic DNA of bacteria or sterile distilled water, respectively, were used in every set of reactions. The PCR products were submitted to electrophoresis in 1/5% agarose gel.[9]The PCR products were sequenced for confirmation of the presence of bacterial DNA.

Statistical analysis

The relationship in the presence of different kinds of bacterial DNA and clinical and laboratory characters were evaluated by using $\chi 2$ and Fisher's exact tests by the SPSS software (Washington, the USA), version 19. A P-value of ≤ 0.05 was considered statistically significant.

RESULTS

In the present study, 28 specimens of atheroma plaques were collected from 22 male and 6 female patients with a mean age of 55±10.41 years and were examined for the presence of bacterial DNA. The total amount of 80 to 230 ng of genomic DNA was extracted from each specimen. By using the universal bacterial 16sRNA primers it was found that 39% of inspected specimens were positive for bacterial DNA presences. In the present study, H. pylori and *C. pneumoniae* DNA was detected in specimens one (3.6%) and 4 (14.3%) through the use of conserved specific primers for the PCR (figure 1 and 2). All of them were positive for 16sRNA. DNA of Mycoplasma pneumonia, *Enterobacter hormaechei* and *Porphyromonas gingivalis* was not detected by PCR using specific conserved primers. In our study, there was no significant difference between the presence of bacterial DNA and clinical and demographic features of patients (Table-2). Interestingly, hyper-triglyceridemia (P-value 0.42) was found in

all (100%) infected and non-infected patients. Hypercholesterolemia (P-value 0.2) and hyperglycemia (P-value 1) was observed in 5 (45.5%) and 8 (72.7%) of infected patients and 11 (64.7%) and 11 (64.7%) in non-infected patients, respectively.

Table 1: PCR assays for detection of bacterial DNA

PCR target	Primers sequences	PCR condition	Ref
C. pneumoniae	F: 5'- GTTGTTCATGAAGGCCTACT-3' R: 5'-TGCATAACCTACGGTGTGTT-3'	94ºc-45s 50ºc-30s 72ºc-30s (40 cycles)	[11]
H. pylori	F: AAGCTTTTAGGGGTGTTAGGGGTT-3' R: AAGCTTACTTTCTAACACTAACGC-3'	94ºc-1 min 56ºc-1 min 72ºc-1 min (35 cycles)	[11]
M. pneumonia	F: 5'-AAGGACCTGCAAGGGTTCGT-3' R: 5'-CTCTAGCCATTACCTGCTAA-3'	94ºc-1 min 58ºc-1 min 72ºc-2 min (40 cycles)	[24]
E. hormaechei	F: 5'-TTGACGTTACCCGCAGAAGA-3' R: 5'-ACCGCTACACCTGGAATTCTAC-3'	94ºc-45s 58ºc-45s 72ºc-1 min (35 cycles)	[25]
P. gingivalis	F: 5′-GTAGATGACTGATGGTGAAAACC-3′ R: 5′-ACGTCATCCCCACCTTCCTC-3′	94ºc-1 min 59ºc-45s 72ºc-1 min (34 cycles)	[26]

Table 2: The demographic and clinical data of the patients

Characteristics	Infected patients	Non-infected patients	P-value
Age	58±12.5	55.76±9.1	0.67
Gender (male)	8(72.7%)	14 (82.4%)	0.43
Height	170±19.76	165.23±6.3	0.19
Weight	78±6.7	81.64±14.36	0.36
Cigarette smoking	6(54.5%)	7 (41.2%)	0.38
Blood pressure	120±5.6	125.48±13.3	0.64
Blood cholesterol	130±35.7	129.3±28.0	0.39
Triglyceride	120±42	105±30.9	0.28
Fasting blood sugar	105±27	118.52±30.67	0.42
Antibiotics use	1(9.1%)	2 (11.8%)	0.66
Body mass index	27.8±3.4	29.84±4.4	0.35

DISCUSSION

Atherosclerosis is a chronic inflammatory disease, which finally leads to tissue injury and fibrosis. Bacterial pathogens may induce chronic inflammation via direct mechanisms or indirect mechanisms. Bacterial pathogens, such as respiratory pathogens (*C. pneumoniae*), periodontal pathogens (*P. gingivalis*), a gastric pathogen (*H. pylori*), have been demonstrated to induce atherosclerotic lesion in vivo to offer evidence of a significant role in the pathogenesis of atherosclerosis. [2]

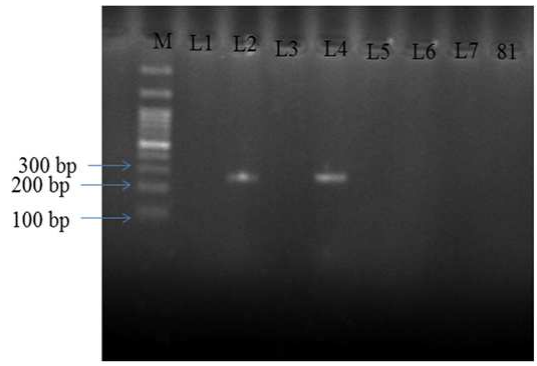

Figure 1: The PCR products for detection of H. pylori (294 bp): M: DNA size marker (100bp), L1: (-) negative control, L2: (+) control, L4: H. pylori (+), L3 and L5-L8: H. pylori (-)

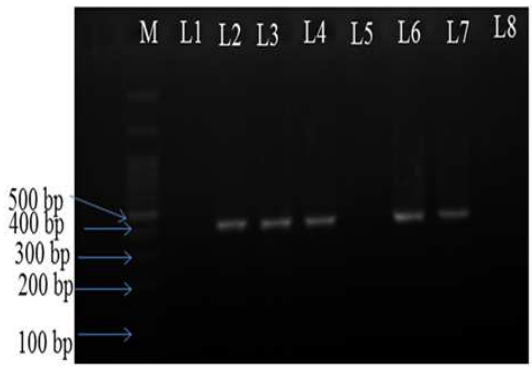

Figure 2: The PCR products for detection of C. pneumoniae (463 bp): M: DNA size marker (100bp), L1: (-) negative control, L2: (+) control, L3, L4, L6 and L7: C. pneumoniae (+), L5 and L8: C. pneumoniae (-)

An association of infections progressions and atherosclerosis has been described in several studies in recent years. [10, 11] C. pneumoniae and H. pylori are among the most frequently involved pathogens in the progression of atherosclerosis. By using the universal bacterial 16SrRNA primers, we detected the presence in atherosclerotic plaques of DNA bacteria. According to the PCR results, 39% of inspected specimens were positive for bacterial DNA presences. Several studies have shown differences in the presence of bacterial DNA in atheromatous plaques using the PCR technique. Stephan et al reported bacterial DNA in 100% of coronary heart disease by conserved PCR but not in control materials or in any of the normal/unaffected coronary arteries [11]. [12, 13] found bacterial DNA in 31 out of 33 carotid atheromatous plaque

specimens. [14] Reported bacterial DNA in 8 out of 18 specimens by primers to amplify the conserved bacterial 16SrDNA nucleotide sequence. These large variations may be due to a lack of uniformity in the distribution of bacteria in the atherosclerotic lesions or to a lack of standardization of the methods used, the presence of DNA polymerase inhibitors, the different origins of the samples, or contamination with bacteria from the oral, genital or fecal human commensally flora or environmental bacteria. [14]

By using the conserved specific primers, we detected the presence of *C. pneumoniae*, *H. pylori*, *M*. *pneumonia*, *E. hormaechei* and *P. gingivalis* DNA in atherosclerotic plaques. Several studies have demonstrated a role of Chlamydia pneumoniae, as a significant respiratory pathogen for humans, in the progressions of atherosclerosis [15]. A potential relationship between *C. pneumoniae* and atherosclerosis was reported in most of the studies which used the PCR technique with noticeably different frequency. PCR results can vary largely; for the detection of *C. pneumoniae* in atheromatous tissue, some studies described positivity rates of 2% while others reported that PCR completely failed to detect the organism [15]. reported that positivity results for atheroma specimens varied between 0 and 60% for the different test techniques, with the maximum concordant rate for positivity being only 25% of one carotid artery sample. There was no consistent pattern of positive rates among the different laboratories, and there was no association of the detection rates with the sensitivity of the test used. Another bacterium that is reported to play a role in the progressions of atherosclerosis is H. pylori and its contributing role in duodenal ulcers has been established.[16]In the present study, H. pylori DNA was detected in one (3.6%) of the specimens. In recent years, the association between infections caused by H. pylori and coronary atherosclerosis has been described by several studies. However, subsequent reports presented conflicting results in the association between H. pylori infection and atherosclerosis progression [11, 17]. There is no strong evidence, as of yet, to suggest an association between H. pylori and atherosclerosis as there is for *C. pneumonia*,[11, 18]. Several studies reported no presence of H. pylori DNA in vessel walls and atherosclerotic plaques obtained at endarterectomy by PCR [19-21]. The positivity rates of PCR for the detection of H. pylori DNA In others studies varied from 17.3% to 53%.[11, 22]In a few studies, some clinical signs of atherosclerosis were described to be associated with the presence of bacterial DNA in atherosclerotic specimens. In our study, there was no difference between the presence and absence of bacterial DNA with clinical and demographic characteristics of patients [22, 23].

The limitations of this study included factors such as a small sample size and not the deification of control group for cross-sectional design because sampling from healthy people is not possible. The severity of coronary artery involvements and its association with nature of microorganism and the role of viral pathogens in the pathogenesis of atherosclerotic are suggested subjects for further study. Previously, there have been reports of the association of bacteria, especially *C. pneumoniae* and atherosclerosis. However, the studies less worked directly on the atheromatous tissue. Geographic differences are epidemiologically important. The strength of this study is a regional importance and this was done for the first time in this area. *C. pneumoniae* and H. pylori were detected as most frequent pathogens in coronary artery atheroma plaques. These infectious agents may be associated with the pathophysiology of atherosclerotic.

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Authors' contributions
The authors contributed equally.

Conflict of interest
The authors have reported no conflict of interest.

REFERENCES

1. Sung KC, Rhee EJ, Ryu SH, Beck S-H. Prevalence of Helicobacter pylori infection and its association with cardiovascular risk factors in Korean adults. Int J Cardiol 2005; 102: 411-17.
2. Campbell LA, Rosenfeld ME. Infection and atherosclerosis development. Arch Med Res 2015; 46:339-50.
3. Ghotaslou R, Aslanabadi N, Ghojazadeh M. Hepatitis B virus infection and the risk of

coronary atherosclerosis. Ann Acad Med Singapore 2008; 37:913.
4. Sawayama Y, Ariyama I, Hamada M, et al. Association between chronic Helicobacter pylori infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). Atherosclerosis 2005; 178:303-9.
5. Kalay N, Kutukoglu I, Ozdogru I, et al. The relationship between Chlamydophila pneumoniae IgG titer and coronary atherosclerosis. Cardiol J 2008;15:245-51.
6. Ghotaslou R, Nasser Aslanabdi M. Hepatitis A Virus and Coronary Artery Diseases. J Cardiovasc Thorac Res 2009;1:43-6.
7. Elkind V, Mitchell S. Infectious burden: a new risk factor and treatment target for atherosclerosis. Infect Disord Drug Targets 2010;10:84-90.
8. Safaie N, Ghotaslou R, Ghaem HM. Seroprevalence of cytomegalovirus in patients with and without coronary artery diseases at Madani Heart Center, Iran. Acta Med Iran 2010; 48:403-6.
9. Akhi MT, Ghotaslou R, Alizadeh N, et al. High frequency of MRSA in surgical site infections and elevated vancomycin MIC. Wound Medicine 2017; 17:7-10.
10. Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97:1837-47.
11. Kaplan M, Yavuz SS, Cinar B, et al. Detection of Chlamydia pneumoniae and Helicobacter pylori in atherosclerotic plaques of carotid artery by polymerase chain reaction. Int J Infect Dis 2006; 10:116-23.
12. Ott SJ, El Mokhtari NE, Musfeldt M, et al. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. Circulation 2006; 113:929-37.
13. Aimetti M, Romano F, Nessi F. Microbiologic analysis of periodontal pockets and carotid atheromatous plaques in advanced chronic periodontitis patients. J Periodontol 2007; 78:1718-23.
14. Watt S, Aesch B, Lanotte P, et al. Viral and bacterial DNA in carotid atherosclerotic lesions. Eur J Clin Microbiol Infect Dis 2003; 22:99-105.
15. Apfalter P, Blasi F, Boman J, et al. Multicenter comparison trial of DNA extraction methods and PCR assays for detection of Chlamydia pneumoniae in endarterectomy specimens. J Clin Microbiol 2001; 39:519-24.
16. Huang B, Chen Y, Xie Q, et al. CagA-positive Helicobacter pylori strains enhanced coronary atherosclerosis by increasing serum OxLDL and HsCRP in patients with coronary heart disease. Dig Dis Sci 2011; 56:109-14.
17. Jha HC, Prasad J, Mittal A. High immunoglobulin A seropositivity for combined Chlamydia pneumoniae, Helicobacter pylori infection, and high-sensitivity C-reactive protein in coronary artery disease patients in India can serve as atherosclerotic marker. Heart Vessels 2008; 23:390-6.
18. Farsak B, Yildirir A, Akyön Y, et al. Detection of Chlamydia pneumoniae and Helicobacter pylori DNA in Human Atherosclerotic Plaques by PCR. J Clin Microbiol 2000; 38:4408-11.
19. Dore MP, Sepulveda AR, Bacciu PP, et al. Detection of Chlamydiae pneumoniae but not Helicobacter pylori DNA in atherosclerosis plaques. Dig Dis Sci 2003;48:945-51.
20. Blasi F, Denti F, Erba M, et al. Detection of Chlamydia pneumoniae but not Helicobacter pylori in atherosclerotic plaques of aortic aneurysms. J Clin Microbiol 1996; 34:2766-9.
21. Kaklikkaya I, Kaklikkaya N, Buruk K, et al. Investigation of Chlamydia pneumoniae DNA, chlamydial lipopolisaccharide antigens, and Helicobacter pylori DNA in atherosclerotic plaques of patients with aortoiliac occlusive disease. Cardiovasc Pathol 2006; 15:105-9.
22. Ameriso SF, Fridman EA, Leiguarda RC, Sevlever GE. Detection of Helicobacter pylori in human carotid atherosclerotic plaques. Stroke 2001; 32:385-91.
23. Shmuely H, Passaro DJ, Vaturi M, et al. Association of CagA+ Helicobacter pylori infection with aortic atheroma. Atherosclerosis 2005; 179:127-32.
24. Van Kuppeveld F, Van der Logt J, Angulo A, et al. Genus-and species-specific identification of mycoplasmas by 16S rRNA amplification. Appl Environ Microbiol 1992; 58:2606-15.
25. Rafferty B, Dolgilevich S, Kalachikov S, et al. Cultivation of Enterobacter

hormaechei from human atherosclerotic tissue. J Atheroscler Thromb 2011; 18:72-81.
26. Tran SD, Rudney JD. Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. J Clin Microbiol 1996; 34:2674-8.