



The Presence of *Helicobacter Pylori* DNA in Coronary Artery Diseases (CAD)

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Abstract

Background: Cardio-vascular illness is the most common disease in the world. Coronary Artery Diseases (CAD) are cardiovascular inflammatory diseases. CAD is a multifactorial disease. Recently, some infectious agents such as *Helicobacter pylori* (*H. pylori*) have been suggested that have a key role in the pathogenesis of CAD. The current study aimed to investigate the presence of *H. pylori* acid nucleic atherosclerotic plaques in coronary blood vessel by the PCR and the relationship between the existence of *H. pylori* DNA and CAD.

Methods: Eighty-four patients with CAD that had experienced bypass grafting surgery were involved in Azerbaijan, Iran. The existence of *H. pylori* DNA in samples was distinguished by the PCR. The statistical relationship between the bacterial DNA presence, clinical and laboratory findings of CAD was assessed by SPSS software (version 20).

Results: *H. pylori* DNA was detected in 9 of 84 (10.7%) atherosclerotic plaques. We found a significant alteration among the bacterial DNA existence, clinical, and demographic topographies of patients (such as gender, weight, cigarette smoking, blood pressure, cholesterol, triglyceride, blood sugar, and BMI).

Conclusions: The presence of *H. pylori* DNA in atherosclerotic plaques recommends that this bacterium can play a role in the pathogenesis of CAD.

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Introduction

Coronary Artery Disease (CAD) is the most common disorder in the world [1]. Atherosclerosis due to be an inflammatory disease, cause soft tissue damage, narrowing, and obstruction of the vessels [2,3]. However, atherosclerosis is a multifactorial illness, male gender; cigarette smoking, genetic, family history, hyperlipidemia, diabetics' mellitus, hypertension, and obesity

may be the cause of CAD. It is well thought out to remain one of the principal causes of ill health and death worldwide [4-6]. Numerous scholarships have described confirmation representing that some infections may be a risk factor for CAD and theatres in chronic inflammatory progressions of CAD [7,8]. The association of some pathogens with CAD has been demonstrated

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by serological tests in atherosclerotic lesions. So, in the future, therapeutic strategies or protective approaches may influence disease development and decrease, and protect [2]. The comprehensive mechanism of CAD is chronic inflammation, but its relationship with a specific infectious mediators for instance *H. pylori* has not well been recognized up till today [1]. Several bacterial and viral pathogens have been discovered in atherosclerotic masses by electron microscopy, immunocytochemistry, and Polymerase Chain Reaction (PCR) that recommend these microbial agents may have a role in the endothelial injury give rise to atherogenesis [7].

H. pylori is a Gram-negative, spiral-shaped, urease-positive bacterium, and found in the stomach and duodenum in the human. Infections due to *H. pylori* is the commonest infection disease, half of the general population in the world are infected by this bacterium. *H. pylori* infections in the industrialized nations are lower than the developing countries. The rate of *H. pylori* infection in Iran is about 80%. *H. pylori* are the cause of the peptic ulcer, gastric cancer, MALT lymphoma, as well as, have been related to various extra-gastrointestinal diseases [9-10]. In the previous article, we worked on some microorganisms as a pilot study. According to a statistically significant relationship between *H. pylori* and CAD, we decided to work on more samples [11]. In the current study, the association between the existence of *H. pylori* DNA in atherosclerosis plaque in Azerbaijani patients, Iran were examined.

Materials and methods

Patients and sample collection

The patients who had experienced bypass operation, the atheroma was engaged. The data of epidemiological and hazard factors of 84 subjects with atherosclerosis were included in Azerbaijan, Iran. In the current study, a part of the healthy artery (grafts) as a controller was used. This study was accepted by the Ethics Commission of Tabriz University of Medical Sciences. Plaques and non-atherosclerotic artery (grafts) were cut up and sited in a TE buffer (pH 8.0) under sterile situations in the operating room, and sections were directly frozen and stored at -80°C until processing.

DNA extraction

We followed the methods of S S Abibiglou et al. 2018 [11]. The samples were homogenized and were keep warm for two h in a SDS/proteinase K at 37.5°C. The DNA was take out by a Kit (Cinna Pure DNA, Iran).

Detection of DNA of *H. pylori*

To detect *H. pylori* DNA, we used the universal bacterial 16sRNA. To discover DNA of *H. pylori*, well-preserved specific primer was used (F: AAGCTTTTAGGGGTGTTAGGGGTT-3' and R: AAGCTTACTTTCTAACACTAACGC-3' [12]. To control and diminish the contamination, we used positive and negative controls in every PCR reaction (DNA of *H. pylori* or germ-free purified water), respectively. The products of PCR were electrophoresed in 1/5% agarose gel. Finally, to confirm the presence of *H. pylori* DNA, PCR products were sequenced.

Statistical analysis

The relationship between the attendance of *H. pylori* DNA, medical, demographic, and laboratory data were assessed by the SPSS (Washington, the USA), type 22, and a P-value of less than 0.05 was reflected statistically noteworthy.

Results

In the current study, 84 atheroma samples were composed from 60 males and 24 females with a mean age of 55±10 years. The specimens were studied for the presence of *H. pylori* DNA. The genomic DNA was extracted from each specimen. *H. pylori* DNA was discovered in nine specimens (10.7%) by the PCR (Figure 1). In our study, there was a noteworthy alteration between the existences of *H. pylori* DNA, clinical and demographic characterizes of patients (Table 1). Remarkably, there is a statistically noteworthy association between the existence of *H. pylori* DNA with gender, weight, cigarette smoking, blood pressure, cholesterol, triglyceride, blood sugar, and BMI.

Table 1: The association of bacterial DNA presence and clinical or demographic patient's data.

Characteristics	Infected patients (9 cases)	Non-infected patients (75 cases)	P-value
Age	58 ± 12.5	55.76 ± 9.1	0.67
Gender			0.04
Male	6 (66.6%)	70 (93.3%)	
Female	3 (33.3%)	5 (6.7%)	
Height	170 ± 19.76	165.23 ± 6.3	0.19
Weight	78 ± 6.7	67.64 ± 14.36	0.03
Cigarette smoking	7 (77.7%)	30 (40%)	0.05
Blood pressure	130 ± 5.6	120 ± 13.3	0.06
Blood cholesterol	150 ± 35	120 ± 28	0.03
Triglyceride	270 ± 42	220 ± 30	0.02
Fasting blood sugar	135 ± 27	118.52 ± 30	0.04
Body Mass Index (BMI)	28 ± 3	23 ± 4	0.05

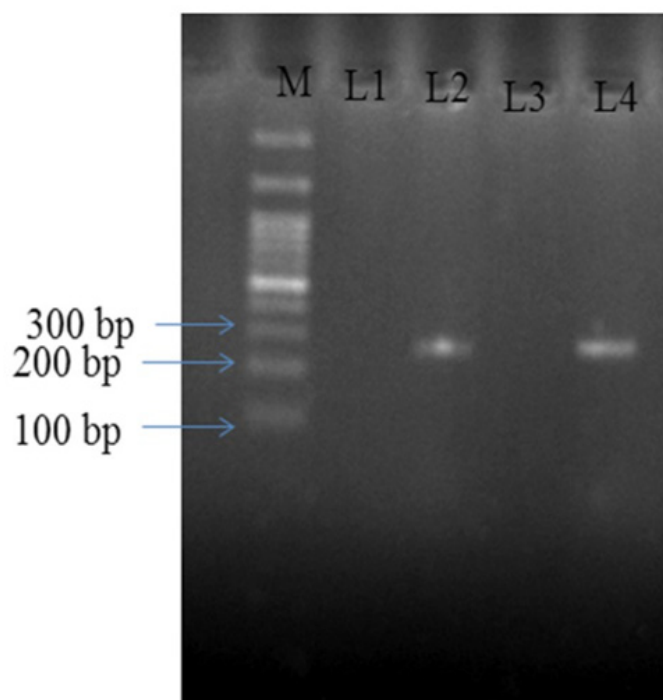


Figure 1: The electrophoresis of PCR products for discovery of *H. pylori* (294 bp): M: DNA size marker (100bp), L1: (Negative Control), L2: (+) Control, L3 (-), and L4 (Positive Control).

Discussion

CAD is a long-lasting and advanced provocative disease, which result in fibrosis, tissue damage, myocardial infarction, unstable angina, and stable angina. Bacterial pathogens such as *H. pylori* can prompt and enhance inflammation through direct or indirect mechanisms. *H. pylori* may create certain mediators, until now the nature of these materials which is unidentified, finally reach the cardiovascular system. *H. pylori* have been shown to make atherosclerotic injuries in vivo, so this bacterium may have a significant role in the pathogenesis of atherosclerosis [2].

Recently, a power relationship between infections and atherosclerosis has been defined [10,12]. *H. pylori* is one of the most often involved microorganisms in the development of CAD [12]. Some studies have exposed alterations in the occurrence of bacterial DNA in atheromatous lesions by the PCR [9,13-14]. Stephan et al described that the presence of bacterial DNA in 100% of CAD by the PCR, however, not in the control group or somewhat of the normal/natural coronary blood vessels [15]. Aimetti reported that the presence of bacterial DNA in 31 on view of 33 carotid artery plaques [16]. Watt et al found bacterial DNA in 8 out of 18 samples by specific primers to increase the well-maintained bacterial 16SrDNA sequence [17]. These great differences in the rate of bacteria presence can be owing to a nonappearance of homogeneity in the diffusion of microorganism in the atherosclerotic injuries, use of various primers, or to use not a standard method, the existence of PCR inhibitors, the diverse origins of the tested samples, various geography, antibiotic use and contamination [18]. We found *H. pylori* DNA in 10.7% of tested CAD patients. In recent years, a probable association between *H. pylori* and atherosclerosis was described in the greatest of the studies, which recycled the PCR method, but with a distinctly various rate [18,11,19]. There is not robust documentation to suggest a strong association between *H. pylori* and atherosclerosis yet [11,20]. However, some studies reported no association between *H. pylori* and CAD by PCR [22]. The presence of *H. pylori* DNA in CAD were different from 17.3% to 53% [11,23]. Despite several studies that have demonstrated a significant role for *H. pylori* in the progress of CAD [9,12-14,16-17,19], interestingly, some studies were described a relationship between clinical manifestations of CAD with the existence of bacterial DNA [23,24]. In the present study, there was an important alteration between the occurrence and absence of *H. pylori* DNA with gender, weight, cigarette smoking, blood pressure, cholesterol, triglyceride, blood sugar, and BMI.

Our study had some limitations comprising low sample size and absence of a control set for cross-sectional project since test group from hale and hearty people is not promising. The association between CAD and severity of *H. pylori* infections in the pathogenesis of atherosclerotic are recommended in future study. However, the fort point of our study was to work directly on the atherosclerotic atheroma, in the previous searches less toiled straight on the damaged material. Topographical, ethnic, and genetic variances may be significant; this study was done for the first time in Azerbaijan, Iran.

Conclusion

H. pylori were detected in coronary artery atheroma. *H. pylori* infections may be associated with the pathophysiology of CAD. It is recommended more studies weigh the mechanisms of association between *H. pylori* with CAD as well as its relationship with time.

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Conflict of interest

The authors have reported no conflict of interest.

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