Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Differential identification of atypical pneumonia pathogens in aorta and internal mammary artery related to ankle brachial index and walking distance

Erkan Iriz, MD,[a,*] Meltem Yalinay Cirak, MD,[b] Mustafa Hakan Zor, MD,[a] Doruk Engin, MD,[b] Levent Oktar, MD,[a] and Yusuf Unal, MD[c]

[a] Department of Cardiovascular Surgery, School of Medicine, Gazi University, Ankara, Turkey
[b] Department of Microbiology, School of Medicine, Gazi University, Ankara, Turkey
[c] Department of Anesthesiology and Reanimation, School of Medicine, Gazi University, Ankara, Turkey

ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form
10 January 2013
Accepted 25 January 2013
Available online 15 February 2013

Keywords:
Chlamydia pneumoniae
Cardiovascular
Atherosclerosis
Peripheral artery disease
Nested PCR
Ankle brachial index

ABSTRACT

Background: We studied the existence of agents in aorta biopsies, such as *Chlamydia pneumoniae*, cytomegalovirus, and *Mycoplasma pneumoniae*, that are thought to have a role in atherosclerosis etiopathogenesis role, and their association with peripheral artery disease.
Materials and methods: We examined aorta wall and internal mammarian artery (IMA) biopsies taken from two different places in 63 patients in whom coronary artery bypass was performed. In these biopsies, we evaluated the deoxyribonuclease (DNA) of these microorganisms using polymerase chain reaction. From the same patients, we recorded the ankle brachial index, road walking distance information, lipid profile, C-reactive proteins, blood parameters such as fibrinogen, and the patient's operation data.
Results: In the nine aorta biopsies taken from 63 patients, we isolated *C pneumoniae* DNA. In IMA biopsies taken from the same patients, we detected no microorganism DNA ($P < 0.001$). In the same aorta biopsies, we found no cytomegalovirus or *M pneumoniae* DNA. We examined 12 patients using an index value of 0.9 in the ankle brachial index evaluation; eight had *C pneumoniae* in the aorta biopsies ($P < 0.001$).
Conclusions: We found a significant relationship between *C pneumoniae* DNA and the existence of peripheral artery disease. In the development of atherosclerosis with *C pneumoniae*, there may be a determinant pathogen in both the aorta and the peripheral arteries. The nonexistence of *C pneumoniae* DNA in the IMA biopsies may indicate infectious agents because of the predominant endothelial functions in this artery, and thus its resistance to atherosclerosis.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Peripheral artery disease (PAD) and coronary artery disease (CAD) related to atherosclerosis are among the major causes of mortality and morbidity in developed countries. Factors such as hypertension, increased blood lipid level, smoking habit, diabetes mellitus, and family history explain the etiopathogenesis of 50%–70% of atherosclerotic diseases. However, the mechanisms responsible for the start and advance of atherosclerosis are not fully known [1]. In recent years, the

* *Corresponding author*. Gazi Üniversitesi Tıp Fakültesi Kalp ve Damar Cerrahisi AD, Beşevler 06500, Ankara, Turkey. Tel.: +90 312-2025619-2025637; fax: +90 312 2129014.
E-mail address: erkaniriz@gmail.com (E. Iriz).
0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.01.053

role of chronic infections has been underlined in the development of atherosclerotic diseases. It is accepted that atherosclerotic incidents develop as a response to damage [2–4]. Inflammation is considered to cause atherosclerotic incidents, and atherosclerosis is thought to be a chronic inflammatory disease in which macrophages have a role [5].

Peripheral artery disease is also a systemic disease that is thought to develop with the existence of atherosclerotic risk factors [6–8]. Although symptomatic patients with lower extremity PAD, a subgroup of PAD, apply to hospitals with intermittent claudication, if they are in a worse condition, because they are asymptomatic it can only be determined through a good physical examination and ankle brachial index (ABI) evaluation [6]. According to the guidelines for peripheral artery diseases, for the condition to be considered important, the critical value should be below 0.9 in the ABI used [6]. In the test, values between 0.9 and 1.1 indicate normal, between 0.4 and 0.9 slight to medium, and below 0.4, serious disease [6].

Chlamydia pneumoniae (CP) is a compulsory intracellular microorganism that causes gram-negative lower and upper respiratory tract infections. *Chlamydia pneumoniae* causes chronic infections. Studies published the recent years have shown the relationship between CP and atherosclerotic heart diseases. *Chlamydia pneumoniae* has been found in the atheromatous lesions of coronary arteries [9–11].

Chlamydia pneumoniae infection begins with the determinant infecting the monocytes in the respiratory tract. *Chlamydia pneumoniae* corpuscles, which have the ability to reproduce in these cells as a result of the persistent infection of alveolar macrophages, begin general circulation. These corpuscles turn the reticulate corpuscles into elementary corpuscle without causing the destruction of the infected cells, and cause atheromatous changes by infecting new mononuclear cells and endothelium cells. In the antigens of CP, such as lipopolysaccharides, synthesis of cytokines such as tumor necrosis factor-α, interleukin-1β, interleukin-6, reproduction factors, and matrix metalloproteins. These factors cause endothelium damage, change the lipid metabolism, increase acute-phase proteins, and tear the atheroma plaque's capsule, thus creating new thrombus and preparing the ground for atherosclerosis.

Iriz *et al.* [12] demonstrated CP in the human ascending aorta with nested polymerase chain reaction (PCR) in CAD, and Higushi *et al.* [13] and Watt *et al.* [14] revealed the deoxyribonuclease (DNA) of *Mycoplasma pneumonia* (MP) and cytomegalovirus (CMV) with the PCR method. It was reported that in MP and CMV infections, histopathologic cases emerge similar to the ones mentioned [13,14].

In many studies done after Saikku *et al.* [15] first determined the serologic relationship between CP and coronary heart disease in 1988, this relationship was also shown through methods such as electron microscopy, immunohistochemical staining and PCR.

In the diagnosis of acute CP infection, serology has an important place; however, its value in the diagnosis of chronic CP infection should be discussed. Nested and real-time PCR are sensitive methods that can detect the DNA of the PCR determinant in small amounts [11,12].

In this study, we analyzed the DNA of bacterial and viral microorganisms, such as CP, CMV, and MP, which are atypical pneumonia determinants, in the aorta tissue and internal mammary artery (IMA) tissues. We also attempted to determine the relationship between peripheral artery disease intensity in the same patients and the association of these microorganisms in the aorta wall.

2. Methods

2.1. *Taking preserving tissue biopsies*

We received the required permission from the ethical board committee of our university. All patients signed permission forms stating that they were informed about the study. We took tissue biopsies from 63 patients who decided to undergo coronary artery bypass operation because of coronary artery disease. We performed aorta biopsy and IMA biopsy of the same patients. For the aorta biopsy, we performed puncher biopsies opened for the purpose of attaching the proximal anastomosis of coronary bypasses. The IMA biopsy was achieved by taking a biopsy from the end of this artery prepared for coronary bypass.

All biopsy specimens were immediately sent to the microbiology department in normal saline and were preserved in the deep freezer at −70°C until the days the tests were performed with PCR.

2.2. *Deoxyribonuclease extraction from biopsy samples*

We minced aortic and IMA biopsy samples using sterile razorblades. We digested the samples by incubation in 500 μL digestion buffer (10 mmol/L Tris [pH 8.5], 1 mmol/L ethylenediaminetetraacetic acid, 0.5% sodium dodecyl sulfate) containing 300 μg/mL proteinase K for 3 h at 52°C. We performed DNA purification from digested tissues using a spin column DNA purification kit (Invitek, Berlin, Germany).

2.3. *Detection of* C pneumoniae *DNA*

We detected the presence of CP DNA in the biopsy samples using a nested PCR procedure described by Tong and Sillis [16]. In the first round of amplification, each 25-μL reaction tube included 10 mmol/L Tris-HCl, pH 8.3; 50 mmol/L KCl, 0.001% gelatin; 1.5 mmol/L $MgCl_2$; 200 μmol/L each of dNTP; 0.026 U/μL Taq DNA polymerase (AmpliTaq DNA polymerase; Applied Biosystems, Foster City, CA); 0.4 μmol/L of outer primers CP1 (5′-TTA CAA GCC TTG CCT GTA GG-3′) and CP2 (5′-GCG ATC CCA AAT GTT TAA GGC-3′); and 5 μL DNA extract. The thermal cycle consisted of denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min. We also performed the second round of amplification in 25-μL reaction volume, which included 10 mmol/L Tris-HCl, pH 8.3; 50 mmol/L KCl; 0.001% gelatin; 3 mmol/L $MgCl_2$; 200 μmol/L of each dNTP; 0.026 U/μL Taq DNA polymerase (AmpliTaq DNA polymerase); 1 μmol/L of inner primers CPC (5′-TTA TTA AT GAT GGT ACA ATA-3′) and CPD (5′-ATC TAC GGC AGT AGT ATA GTT-3′); and 1 μL of PCR product from the first round. The amplification products were separated in 2% agarose gel, stained with ethidium bromide, and visualized (Syngene, Seoul, South

Download English Version:

https://daneshyari.com/en/article/4300854

Download Persian Version:

https://daneshyari.com/article/4300854

Daneshyari.com