Internal Mammary Artery Atherosclerosis in Segments Removed During Coronary Artery Bypass Grafting Surgery and C.Pneumoniae Infection

Koroner Baypas Sırasında Çıkarılan İnternal Mammaryan Arter Segmentlerinde Ateroskleroz ve Klamidya Pnömoni Enfeksiyonu

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Abstract

Objective: Recent studies suggest the association of atherosclerotic cardiovascular disease with Chlamydia pneumoniae infection. We investigated C. pneumoniae DNA in internal mammarian artery (IMA) (used as a coronary bypass conduit) and its relationship with atherosclerosis.
Methods: Sixty-six consecutive patients who underwent coronary artery bypass grafting (CABG) during an eight-month period were included in this study. From all patients, we attempted to obtain surplus segments of harvested IMA grafts. The vessels were examined histopathologically, and presence of C. pneumoniae DNA in IMA grafts was assessed by polymerase chain reaction (PCR).
Results: C. pneumoniae DNA was found in 7 (10.6%) of 66 IMA specimens. The light microscopic examinations of IMA segments from the C. pneumonia positive group showed atherosclerotic intimal changes in four of the seven patients. These atherosclerotic changes were type II in three patients and type III in one patient according to the AHA classification. The rest of the IMA segments from 62 patients did not show any discernible atherosclerotic lesion.
Conclusion: The IMA graft examination by PCR and histopathology may be helpful in the determination of future graft patency for IMA bypass surgery. *(Anadolu Kardiyol Derg 2004; 4: 144-8)*
Key Words: Chlamydia pneumoniae, atherosclerosis, coronary artery bypass, internal mammary artery, polymerase chain reaction

Özet

Amaç: Son çalışmalar klamidya pnömoni ile aterosklerotik kardiyovasküler hastalık arasındaki ilişkiyi desteklemektedir. İnternal mammaryan (İMA) arterde (koroner baypas greft olarak kullanılan) C. Pneumoniae DNA sını ve onun aterosklerozla ilişkisini araştırdık.
Yöntem: Sekiz aylık süre içinde koroner arter baypas greft cerrahisi uygulanan 68 ardışık hasta çalışmaya alındı. Bütün hastalarda hazırlanan İMA greftinin arta kalan kısmında çalışıldı. Damarlar histopatoloijk olarak incelendi ve İMA greftlerinde C. Pneumoniae DNA'sının varlığı polimeraz zincir reaksiyon (PCR) ile değerlendirildi.
Bulgular: C. Pneumoniae DNA'sı 66 İMA spesimeninin 7 (%10.6) sinde bulundu. C. Pneumoniae pozitif gruptaki İMA segmentlerinin ışık mikroskop incelemesinde, 7 hastanın 4'ünde aterosklerotik intimal değişiklikler görüldü. Amerikan Kalp Cemiyeti sınıflamasına göre bir hastada tip III üç hastada tip II aterosklerotik değişiklik vardı. Geri kalan 62 hastanın İMA segmentlerinde belirgin her hangi bir aterosklerotik lezyon gösterilemedi.
Sonuç: İnternal mammaryan arter greftinde; PCR ile C. Pneumoniae ve histopatolojik olarak ateroskleroz tespit edilmesi İMA greftinin gelecekteki açıklığını belirlemede yardımcı olabilir. *(Anadolu Kardiyol Derg 2004; 4: .144-8)*
Anahtar Kelimeler: K.Pneumoniae, koroner arter baypas cerrahisi, internal mammaryan arter, polimeraz zincir reaksiyonu

Introduction

Atherosclerosis is associated with several risk factors such as smoking, hypertension, dyslipidemia, diabetes, positive family history, male sex, and age. However, only 50% of the coronary artery atherosclerosis patients have these factors, and therefore, the other risk factors must contribute. Another risk factor that has been proposed for coronary atherosclerosis is chronic infection (1). At present, several lines of evidence suggest that atherosclerosis may be regarded as a chronic inflammatory disease and that infections may play an important role in perpetuating this inflammatory status (2).

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Chlamydia pneumoniae is a gram-negative obligate intracellular bacterium that is a common cause of respiratory disease (3). Infection with C. pneumoniae seems to be geographically widespread and approximately 10% of community-acquired pneumonias are due to this microorganism (4). High prevalence of antibodies against C. pneumoniae has been found in different populations suggesting that most people are infected (5).

Increasing evidence exists that C. pneumoniae might play a role in atherosclerosis. Animal studies show that C. peumoniae can promote lesion initiation and progression, and antibiotic treatment can prevent the development of arterial lesions (6). An association between the microorganism and atherosclerosis was first demonstrated in seroepidemiological studies (7). In addition, C. pneumoniae has been detected in human atherosclerotic lesions by various techniques like polymerase chain reaction (PCR), immunocytochemistry (ICC), electron microscopy, and microbiological culture (8). C. pneumoniae frequently invade the arterial system (9). C. pneumoniae was detected in the 50-80% of atherosclerotic plaques and it is found in 2-12 % of non-atherosclerotic vessels (10, 11).

In this study we investigated C. pneumoniae DNA in internal mammarian artery (IMA) (used as a coronary bypass conduit) and its relationship with atherosclerosis.

Materials and Methods

Sixty-six consecutive patients who underwent coronary artery bypass grafting (CABG) during an eight months period were included in this study. Demographic characteristics, smoking habits and medical history, clinical and angiographic data were recorded for each patient. A total of 55 patients were men, and 11 were women. Coronary artery disease risk factors were smoking in 35 patients (53.8%), diabetes mellitus in 9 patients (13.6%), hypertension in 20 patients (30.3%), hypercholesterolemia in 29 (43.9%), and family history of coronary artery disease in one patient (1.5%). Selection criteria for CABG in our 66 patients were; left anterior descending artery stenosis in 7 patients (10.6%), triple-vessel disease in 33 patients (50%) and double vessel disease with proximal left anterior descending artery stenosis in 26 patients (39.4%). All patients underwent elective coronary artery bypass grafting surgery. From all patients, we attempted to obtain surplus segments (distal part) of harvested left IMA grafts. Vessel specimens (3 to 5 mm) were collected in the operating room under sterile conditions and processed immediately by dividing them into two portions, one for histopathological examination, other for PCR amplification.

Sample preparation and amplification: The specimens in the tube containing Tris EDTA buffer were cut with a sterile blade, as multiple sections and frozen at –20°C. The specimen sections were treated with a solution containing 10 mM Tris-HCl (pH 8.3), 1 mM EDTA, and 100 mg of proteinase K per ml and incubated at 60 °C for 1 h and heated at 96 °C for 10 min. DNA was extracted with phenol-chloroform-isoamylic alcohol and precipitated with absolute alcohol. The precipitate was washed with 70% ethanol. The pellet was dried and dissolved in 25 ml of sterile, double distilled water, and 5 ml of the DNA suspension were used for amplification. Each PCR reaction mixture (50 µl) contained 5 ml of genomic DNA, 20 pmol of HL-1 primer (5'-GTT GTT CAT GAA GGC CTA CT-3'), 20 pmol of HR-1 primer (5'-TGC ATA ACC TAC GGT GTG TT–3'), 2.5 unit of Taq DNA polymerase (Promega Corporation, USA), 200 µM deoxynucleoside triphosphate mix, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM $MgCl_2$. The reaction mixture was amplified with Thermal Cycler MJ Research Inc. PTC-200, Peltier Thermal Cycler Massachusetts, USA) for 40 cycles at 94 °C for 1 min, 48 °C for 1 min, and 72 °C for 1 min (12). Amplification products were electrophoresed by 1.5% agarose gel containing ethidium bromide, and were visualized under UV illumination.

Histopathological examination: IMA biopsies were fixed in the neutral 4% formalin solution overnight and processed in graded alcohol solutions then cleared in xylene and embedded in paraffin wax. Paraffin tissue sections were stained by Hematoxylin and Eosin, and then examined under the light microscope. The lesions were graded as outlined by AHA (13).

Statistical analysis was performed with SPSS 8.0 Windows. Binary data were analyzed with Fischer's exact test. A value of $p<0.05$ was considered to indicate statistical significance.

Informed consent was obtained from the patients and, the study approved by our institutional ethics committee on human research.

Results

C. pneumoniae DNA were found in 7(%10.6) of 66 IMA specimens, that were assessed by PCR. The

characteristics of the 66 patients according to risk factors are shown in Table 1. There was no any statistical difference in mean age, hypertension, diabetes, family history, and high cholesterol levels between PCR positive and negative groups. Table 2 summarizes the clinical status of the patients in both groups. Two of the C. pneumoniae positive cases had one vessel disease, one had 2 vessels disease, and 4 had 3 vessels disease.

The light microscopic examinations of IMA segments from the C. pneumonia positive group showed atherosclerotic intimal changes in four of the seven patients. These were type II atherosclerosis in three and, type III atherosclerosis in one patient according to the AHA classification (13). Three of the patients who were PCR(+) for C. pneumoniae were male, and two of them had type II and the other one had type III atherosclerosis in their IMA grafts. The fourth patient was a female patient and she had type II atherosclerosis in her IMA graft. The rest of the IMA segments from 62 patients did not show any discernible atherosclerotic lesion.

Discussion

In explanation of coronary atherosclerosis, the known risk factors are not satisfactory for nearly 50% of cases. Infections with some microorganisms such as C. pneumoniae, Helicobacter pylori, cytomegalovirus have been put forward as possible risk factors in the development of atherosclerosis (14). Among the microbiological agents under investigation, C. pneumoniae has been associated with atherosclerotic cardiovascular disease more extensively: the organism was detected by electron microscopy immunocytochemistry, direct immunofluorescence, and PCR in coronary arteries (10, 14, 15). Besides coronary arteries C. pneumoniae was also detected in the aorta, carotid arteries and even IMA reflecting an affinity of the microorganism to the arterial system (17). It is well known that IMA is the most frequently used graft in CABG surgery. In our study, we aimed to investigate the atherosclerotic lesions of IMA grafts, and their relation with possible C. pneumoniae infection. In a former study, we detected IMA atherosclerosis in 9.6 % of patients having four or more risk factors and 6 % of patients with three or less risk factors (18).

Wong et al.(19) found that two of five IMA's were C. pneumoniae positive by PCR in the first CABG surgery whereas in redo CABG surgery patients they found new IMA grafts were infected in four of fifteen grafts (26%) by PCR. In this group of patients C. pneumoniae involvement was high. In our series of 66 patients only seven (%10.6) IMA were PCR positive. Polymerase chain reaction positivity for C. pneumonia was detected in 38.5% of coronary artery endarterectomy specimens, and 11% in new saphenous veins grafts (19). In atherectomy specimens this positivity may be as high as 79% (11). Davidson (20) identified C. pneumoniae organism in 37% of coronary arteries by PCR and ICC. Kuo et al. (4) detected C. pneumoniae in coronary artery atheromas by ICC (15/36) and by PCR (13/30) in autopsy cases from Johannesburg, South Africa (4). The organism has

Table 1. Risk factors distribution in patients with respect to C. pneumoniae PCR positivity

Parameters	C. pneumoniae		P
	PCR (+) positive (n= 7)	PCR (-)negative (n=59)	
Mean age±SD, years	59 ± 7	60 ± 8	0.774
Sex(male/female)	4/3	51/8	0.394
Smoking history, n%	2/7(28.6)	33/59 (55.9)	0.569
Hypertension, n%	3/7(42.9)	17/59 (28.8)	0.622
Diabetes, n%	1/7 (14.3)	8/59 (13.6)	0.361
Family history, n%	0	1 (1.7)	0.955
High cholesterol level, n%	4/7(57.1)	25/59 (42.4)	0.408

Table 2. Clinical characteristics of the patients

Parameters	C. pneumoniae	
	PCR (+)	PCR (-)
Inferior MI, n%	2 (28.57)	15 (25.43)
Extended anterior MI, n%	2 (28.57)	4 (6.77)
Anteroseptal MI, n%	1 (14.28)	6 (10.16)
Anterolateral MI, n%	1 (14.28)	8 (13.55)
Unstable angina MI, n%	-	7 (11.86)
Stable angina, n%	1 (14.28)	19 (32.20)
Total, n	7	59

MI: myocardial infarction

been detected frequently by ICC and PCR in atheromatous tissues (approximately 50% of subjects) but rarely in normal arteries (approximately 1% of subjects) (21). Taylor-Robinson (10) found C. pneumoniae in the aorta, femoral, and iliac arteries. In a subsequent study, the organism was detected in arteries of subjects as young as 15 years. In this collaborative investigation, 71% of atheromatous arteries taken at autopsy from white South African subjects were C. pneumoniae positive compared with 9% of non-atheromatous arteries. Of interest, the organisms were detected in 67% of vessels that showed only early atherosclerotic lesions (fatty streaks). The presence of C. pneumoniae organisms within foam and smooth muscle cells of atherosclerotic plaques is beyond doubt, but their role in atherosclerosis remains enigmatic (10,15). C. pneumoniae is found in coronary lesions in young adults with atherosclerosis but is not found in normal-appearing coronary arteries of both persons with and without other evidence of atherosclerosis (22).

Ouchi et al. (23) studied 67 atheromatous plaques from Japanese symptomatic patients and 110 nonatherosclerotic tissues and organs, of these 62% of atherosclerotic plaques from symptomatic patients were infected with C. pneumoniae compared with just 2% of non-atherosclerotic tissues.

In a study of Turkish people the atherosclerotic material was taken from 8 cases by directional atherectomy and from 23 cases by surgical endarterectomy. C. pneumoniae positivity was 32.3% (10/31) by indirect immunofluorescence (IIFA) and 29.0% (9/31) by PCR while the evaluation of the methods together yielded a positivity of 35.5% (11/31) (24). In another similar study C. pneumoniae DNA was found in 12 (%26) of 46 endarterectomy specimens and none of the healthy vascular-wall specimens by PCR ($p<0.001$) (25).

Atherosclerotic plaques contain a lipid-related, immune-mediated inflammation, with release of secretory products capable of changing plaque morphology. Plaques that prone to complications contain large numbers of inflammatory cells; stable plaques contain little inflammation. Similarly, atherectomy specimens from patients with coronary syndromes revealed more inflammatory cells in unstable than those in stable patients. These observations, and the fact that acute coronary syndromes are associated with increased blood levels of inflammatory markers, have renewed interest in the possible relationship between infection and atherogenesis. Of all potential candidate antigens, C. pneumoniae presently is considered the most likely because a substantial number of patients with unstable syndromes contain C. pneumoniae reactive T cells, both in blood and within the atherosclerotic plaque, suggesting enhancement of intraplaque inflammation (26). In our series, reflecting the possible complicated status of atherosclerosis, six of 7 C. pneumoniae positive patients had acute coronary syndromes.

C. pneumoniae seems to be preferentially located in atherosclerotic arteries (11) and it exacerbates rather than causes atherosclerosis (27). Also, C pneumoniae has been found in vessels not usually associated with atherosclerosis, such as IMA and saphenous vein (19).

We found C. pneumoniae PCR positivity as 10.6% (7/66) in our series. In 4 of 7 (57.14%) PCR positive cases, IMA atherosclerosis was confirmed by histopathological examination. Pathogenesis of atherosclerosis in human remains unclear. We cannot rule out the effect of C. Pneumoniae in the pathogenesis of atherosclerosis, and coronary artery disease since we have demostrated C. Pneumoniae positivity with PCR in the left IMA grafts used in CABG surgery. Infection of the left IMA grafts by C. Pneumoniae may have a role in the iniation or progression of atherosclerosis.

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