ROLE OF CHLAMYDIA PNEUMONIA AND HELICOBACTER PYLORI IN THE PATHOGENESIS OF ATHEROSCLEROSIS AND ABDOMINAL AORTIC ANEURYSM

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ABSTRACT

Chlamydia pneumonia and *Helicobacter pylori* can cause persistent infections of the respiratory and gastrointestinal tract, respectively. It has been suggested that persistent infection of arteries with these bacteria can contribute to the development of atherosclerosis and aneurysm. The aim of this study was to investigate the association between PCR evidence of infection with C. pneumonia and H. pylori, and the occurrence of abdominal aortic aneurysm and atherosclerosis. Sixty patients; 30 with abdominal aortic aneurysm (mean age 67.4: 27 male, 3 female) and 30 with aortoiliac arteriosclerosis (mean age 58.5; 29 male, 1fe male) undergone surgery of the abdominal aorta for atherosclerotic obstructive lesions were the study group. Ten specimens from the macroscopically healthy regions of aortic wall from cadaver were the control group. The presence of microorganism DNA in specimens was assessed by PCR. Chlamydia Pneumonia DNA discovered in 47/60 (78.3%) of the study group cases in the wall of aorta and/or its atheromatous plaque whereas none of them showed H. pylori DNA. The control group showed no detection of C. Pneumonia DNA. The presence of *C. pneumonia* and *H. pylori* DNA in a considerable number of atherosclerotic plaques but their absence in cadaver healthy vascular wall supports the idea that they may have a role in the development of atherosclerosis, especially in countries where infection is prevalent and where conventional risk factors fail to explain the high prevalence of atherosclerotic vascular disease.

KEY WORDS: Chlamydia Pneumonia, Helicobacter Pylori, Atherosclerosis, Aneurysm.

INTRODUCTION

Conventional risk factors, including hyperlipidemia, hypertension, diabetes, tobacco use, sex, and family history of premature vascular disease, account only for approximately half of the patients with clinically apparent atherosclerosis[(1)]. The possible role of some organisms such as C. pneumoniae, Helicobacter pylori, mycoplasma pneumoniae and herpes virus family has been suggested in atherosclerotic disease (D. Pietro, Filardo, D. Santis, & Sessa, 2013; Data obtained from several seroepidemological studies has given rise to the hypothesis that an infection can initiate or maintain the atherosclerotic process[(2)]. Pathophysiological mechanisms by which this may occur have been described in experimental studies[(3,4)] and include effects on lipid metabolism, leukocyte–endothelial-cell interaction, coagulation factors, and platelet activation. Infections caused by *Chlamydia pneumonia* and *Helicobacter pylori* have been postulated to be of interest[(5)]. Seroepidemiological evidence, immunohistochemistry, and molecular biology studies have suggested an association between *C. Pneumoniae* and coronary artery disease. However, epidemiological and serological data suggesting an association between *H. Pylori* and atherosclerosis are conflicting. Furthermore, seropositivity does not correlate with the presence and extent of atherosclerosis. In the present study, the presence of *C. pneumonia* and *H.* pylori DNA were investigated by PCR in endarterectomy and vascular-wall specimens. So the aim of the present study was to investigate the association between PCR evidence of infection with C. pneumonia and H. pylori, and the occurrence of abdominal aortic aneurysm and atherosclerosis. Significant differences compared with the control population would either support or rule out the hypothesis of a relationship between C. pneumonia and H. pylori infection and this disease.

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MATERIALS AND METHODS

Study group: sixty consecutive patients operated on in our department, thirty patients operated for Abdominal Aortic Aneurysm (AAA) and thirty patients for aortoiliac occlusive disease: Leriche's syndrome (LS). Each research group assessed separately, each with various manifestations of ischemic vascular diseases of lower limbs. All cases were operated on electively. Demographic characteristics, smoking habits, and medical history (arterial hypertension, ischemic heart diseases, diabetes, and hypercholesteremia) were recorded for each patient (table 1). Polymerase chain reaction (PCR) test for the presence of Chlamydia pneumonia (C. pneumonia) and helicobacter pylori bacteria were done in all patients. For PCR test biopsy specimen of 2X3mm in size taken from anterior wall of abdominal aorta between the origin of renal arteries and bifurcation of aorta, under complete aseptic condition, divided into 2 specimens and kept in -40C until processing. Control group : 10 specimens from the macroscopically healthy regions of aortic wall from cadaver with a maximum aortic diameter of 30mm of cadaver; their age range from 11 to 27 years for its five cadavers and from 61-71 years for 2nd five cadavers. PCR test for detection of Pneumonia has been done for all

specimens. Specimen collection. All specimens were dissected in the operating room under sterile conditions. Specimens sizes approximately 2X3 mm in length were placed in micro-centrifuge tubes containing Tris-EDTA buffer. Transport vials were sealed in the operating room and opened only in the laminar air flow safety cabinet at the microbiology laboratory. All of the specimens were kept at -40°C until processing. Dissected arterial materials were ground by a sterile glass grinder. Chromosomal DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method according to the DNA Miniprep protocol of Wilson[30]. This method is known to remove complex polysaccharides which may inhibit PCR amplification. PCR amplification: (i) *C. pneumonia.* For the detection of *C. pneumonia* by PCR, primers that amplify 463-bp fragment of the 16S rRNA gene were used. After amplification, 1.2% agarose gel electrophoresis at 100 V and ethium bromide staining were used to visualize the PCR products. (ii) *H. pylori.* The primers HPU1 and HPU2 were used to amplify a 411-bp internal fragment of the urease A gene of *H. pylori.* This assay has been assessed previously for its specificity for the urease A gene of *H. pylori* and found not to cross-react with other *Helicobacter* species or other known urease-producing organisms. Agarose gel (1.2%) electrophoresis at 100 V and ethidium bromide staining were used to visualize the PCR products. Statistical tests: standard statistical tests e.g. mean, standard deviations, cofactor correlation using Pearson test.For all analytic statistical tests ; P value less than or equal to 0.05 was used to test level of significance. Ethical approval: This study has got approval of the local ethical community.

RESULTS

The demographic data of the patients in the lesion group and the control group are listed in (table 1) No significant differences were found between the two groups with respect to age, sex, known risk factors, and cholesterol levels.

(Table 1) characteristics of patients with abdominal aortic aneurysm (AAA) and aortoiliac obstruction (LS).

Parameter	AAA (n = 30)	LS (n = 30)	P
Gender M/F	27/3	29/1	NS
Mean age ± SD	67.4 ± 8.1	58.5 ± 7.3	P<0.001
Aneurysm diameter	60.9±11.6		
Ischemic heart disease	15(50%)	17(56.7%)	NS
Hypertension	16 (53.3%)	157 (50%)	NS
Diabetes	3 (10%)	5 (16.7%)	NS
Hypercholesteremia	3(10%)	3(10%)	NS
Smoking history	23(76.7%)	21 (70%)	NS

NS=not significant.

In the study group both(AAA and LS); C. Pneumonia DNA discovered in 47/60 (78.3%) cases in the wall of aorta and/or its atheromatous plaque whereas none of them showed H. pylori DNA. The control group showed no detection of Pneumonia DNA as shown in (table 2).

(Table 2) showed results of PCR test regarding detection of C. pneumonea and H. pylori in the study and control group.

	C.pneumonea DNA (%)	H.pylori DNA (%)
AAA	24/30(80%)	0/30(00%)
Lerich syndrome	23/30(76.6%)	0/30(00%)
Control group	0/10(00%)	-

DISCUSSION

Chlamydia pneumonia is one of the leading causes of acute respiratory tract infections in humans worldwide[6,7,8]. Known risk factors for atherosclerosis (e.g., smoking, hypercholesterolemia, hypertension, etc.) do not completely explain the pathogenesis of the disease[9]. Chronic infections and atherosclerosis showed unknown associations for years[10,11]. and for the first time, Shore et al described the presence of C. pneumoniae in atheroma plaques, by the PCR method[12]. Chlamydia pneumonia has also been indicated as a possible cause of atherosclerosis[13]. Later on, researchers looked for a possible association between C. pneumoniae and atherosclerosis, because of the high prevalence of C. pneumoniae infection in the general population[14]. Therefore, the study of atherosclerosis has taken new directions, one of the most intriguing of these being the infective theory of atherosclerosis that has been addressed in recent years. Epidemiological studies have suggested a role of infections in the pathogenesis of atherosclerosis[14,15,16,17,18] and bacterial DNA and viral structures have been identified in atherosclerotic lesions[19]. Our study showed nearly 80% (24/30 & 23/30) of study group patients were positive for Chlamydia pneumonia detected with the PCR technique in the wall of aortic aneurysm and iliac atheromatous plaques but none of the healthy vascular wall is infected. Our results are consistent with many other studies suggesting a chronic C. pneumonia infection as a possible risk factor for atherosclerosis as (Rostami et al.[20], Bahrmand et al.[21], Dabiri et al.[22], while others could not (Sadeghian et al.[23], Zibaeenezhad et al.[24], Pooria et al.[25], Hosseinian, Habibzadeh, Ahari, & Mokhtarpoor[26]. In 1993, Kuo et al[27] in South Africa detected Chlamydia pneumonia for the first time in atherosclerotic lesions originated from autopsies using the PCR technique and found a positivity of 43%. Juvonen et al[28] showed 100% positivity for Chlamydia pneumonia in aneurysms of the abdominal aorta with PCR and immunohistochemical techniques. Disparity in the prevalence of C. pneumoniae could be due to selection of samples[29] & differences in the size of tested samples[30]. Conversely, our results tend to rule out the possibility of a direct involvement of H. pylori infection in the pathogenesis of atherosclerosis.

CONCLUSION

The high incidence of C. pneumonia DNA detected in study group patients suggests that C. pneumonia infection may play a role in the pathogenesis of aneurysm and atherosclerosis.

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