Antibodies to Various Mycoplasmas in Patients with Coronary Heart Disease

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ABSTRACT: **Background:** Clinical and epidemiologic features of coronary heart disease may not be explained solely by established risk factors. The role of infectious pathogens in the development and rupture of atherosclerotic plaques remains elusive but an association between *Chlamydia pneumoniae, Mycoplasma pneumoniae* and CHD has been reported previously.

Objectives: To determine whether there is an association between mycoplasmal infections and CHD.

Methods: We conducted a prospective cohort analysis of 150 consecutive hospitalized patients with CHD (85 with acute coronary syndrome and 65 admitted for unrelated reasons) and 98 healthy blood donors. Antibody titers for *Mycoplasma pneumoniae, M. fermentans, M. hominis* and *Ureaplasma urealyticum* were measured with the agglutination test or specific enzyme-linked immunosorbent assay in all three groups of patients.

Results: Analysis of the antibody titers did not reveal any significant difference in the presence of mycoplasmal antibodies between the patients with ACS, patients with known stable CHD hospitalized for non-CHD reasons, and healthy blood donors.

Conclusions: Determination of specific antibodies did not reveal a significant association among different types of mycoplasmal infection and CHD.

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KEY WORDS: *Mycoplasma pneumoniae, Mycoplasma fermentans, Mycoplasma hominis, Ureaplasma urealyticum,* acute coronary syndrome, coronary heart disease, atherosclerosis

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Factors other than the conventional risk factors may contribute to the development of atherosclerosis [1-3]. Since inflammation plays an important role in the pathogenesis of atherosclerosis [1,3,4], the potential role of infectious agents in the initiation or modulation of atherosclerosis has been investigated. Several seroepidemiologic and immunohistochemical studies have demonstrated an association between microbial infections, particularly *Chlamydia pneumoniae,* and atherosclerosis [1,5]. Mycoplasmas, the smallest self-replicating wall-less bacteria, are parasites in humans, animals and plants. They are ubiquitous and tend to establish chronic infections which are accompanied by alterations of immune reactivity [6]. The involvement of *Mycoplasma pneumoniae* in cardiovascular diseases has been previously reported, and several investigators have pointed to a possible association, along with *Chlamydia pneumoniae*, as risk factors for embolization and myocardial infarction [7]. However, the results are controversial [8-11]. There have been no reports regarding other human mycoplasmas. In the present study a possible relationship between mycoplasmal infections and CHD was evaluated by measuring the level of specific antibodies to several human mycoplasmas in CHD patients.

CHD = coronary heart disease
ACS = acute coronary syndrome

PATIENTS AND METHODS

A prospective cohort study of 150 consecutive patients with coronary heart disease hospitalized in Soroka University Medical Center, and 98 healthy blood donors was performed. Of the 150 patients with CHD, 85 were admitted with an ACS and 65 for reasons unrelated to CHD. The discharge diagnoses (ICD-9) for identifying subjects with coronary heart disease was used. Patients with active atherosclerotic cerebral and peripheral arterial disease (admitted due to stroke or peripheral vascular disease) were excluded. The study was approved by the Institutional Review Board.

MEASUREMENT OF ANTI-MYCOPLASMAL ANTIBODIES

Anti-*Mycoplasma pneumoniae* antibodies in patients' sera were measured by the microagglutination test (Fujirabo Kit, Japan). Titers of 1:80 and above were considered positive (Fujirabo Kit). Anti-*Ureaplasma urealyticum* and anti-*Ureaplasma parvum* antibodies were measured by a specific enzyme-linked immunosorbent assay routinely used in our National Center for Mycoplasma (Clalit Health Services). A titer above 1:160 was considered positive. Anti-*Mycoplasma fermentans* and anti-*Mycoplasma hominis* antibodies were measured with a highly specific ELISA used in our center.

ELISA = enzyme-linked immunosorbent assay

Table 1. Comparison of clinical characteristics of patients with ACS and stable CAD

Characteristics Mean (SD)/ n (%)	Acute coronary syndrome (n=85)	Stable coronary heart disease (n=65)	*P* value
Age (yrs)	62.1 (8.1)	61.3 (7.2)	0.5
Gender (male)	68 (80)	47 (72.3)	0.4
Diabetes mellitus	33 (38.8)	26 (40)	1
Chronic renal failure	10 (11.8)	8 (12.3)	1
MI in the past	17 (20)	18 (27.7)	0.3
History of CABG	13 (15.3)	21 (32.3)	0.023
History of PCI	9 (10.6)	17 (26.2)	0.02
Heart failure	17 (20)	9 (13.8)	0.4
Atrial fibrillation	5 (5.9)	4 (6.2)	1
Significant valvular disease	4 (4.7)	9 (13.8)	0.09
ICD	1 (1.2)	2 (3.1)	0.6
History of stroke	4 (4.7)	4 (6.2)	0.7
Hypertension	47 (55.3)	38 (58.5)	0.8
Rheumatologic disease	1 (1.2)	3 (4.6)	0.3
Malignancy	5 (5.9)	5 (7.7)	0.7
COPD	3 (3.5)	6 (9.2)	0.18
Pneumonia	2 (2.4)	1 (1.5)	1
Other lung disease	4 (4.7)	2 (3.1)	0.7
Recurrent urinary tract infection	1 (1.2)	2 (3.1)	0.6

Numbers with a *P* value of < 0.05 were considered significant

CABG = coronary artery bypass graft, COPD = chronic obstructive pulmonary disease ICD = implantable cardioverter defibrillator, MI = myocardial infarction, PCI = percutaneous coronary intervention

The cutoff values (for each mycoplasma) were previously determined by comparing infected patients with the healthy population [12,13]. The cutoff values for *M. fermentans* and for *M. hominis* were 0.687 and 0.876 optical density, respectively. These values were calculated as the mean plus 2 SD (standard deviations) in the healthy population.

STATISTICAL ANALYSIS

The results are presented as the mean (SD) for continuous variables and as the total number of patients (percentage of total patients) for categorical data. The *t*-test was used for comparison of the continuous variables or chi-square test for categorical data with the use of Fisher's exact test if needed. A two-sided *P* value < 0 .05 was considered statistically significant.

RESULTS

Characteristics of the patients with coronary disease are shown in Table 1. As shown in Table 2, there were no significant differences in the prevalence of anti-mycoplasmal antibodies (four different species of human mycoplasmas) between the patients with coronary heart disease and the group of healthy donors. Also, as shown in Table 3, there was no difference in positive antibody response between patients hospitalized due to ACS and patients with stable CHD.

Table 2. Prevalence of anti-mycoplasma antibodies in patients with coronary heart disease and healthy donors

Antibodies	Coronary heart disease (n=150)	Healthy donors (n=98)	*P* value
Ureaplasma urealyticum	26/145 (17.9%)	13/98 (13.3%)	0.43
Mycoplasma pneumonia	20/150 (13.3%)	21/98 (21.2%)	0.14
Mycoplasma fermentans	5/150 (3.3%)	3/98 (3.1%)	1.0
Mycoplasma hominis	9/150 (6.0%)	10/97 (10.3%)	0.32

Table 3. Prevalence of anti-mycoplasma antibodies in patients with acute coronary syndrome and stable coronary heart disease

Antibodies	Acute coronary syndrome (n=85)	Stable coronary heart disease (n=65)	*P* value
Ureaplasma urealyticum	15/81 (18.5%)	11/63 (17.5%)	1.0
Mycoplasma pneumonia	12/85 (14.3%)	8/65 (12.3%)	0.91
Mycoplasma fermentans	2/85 (2.4%)	3/65 (4.6%)	0.65
Mycoplasma hominis	5/85 (6.0%)	4/65 (6.2%)	1.0

DISCUSSION

It has been previously demonstrated that inflammation plays an important role in the pathogenesis of atherosclerosis [1,3,4] and activated macrophages and T lymphocytes are present in atherosclerotic plaques, resulting in an immune-mediated process [1].

Several seroepidemiologic and immunohistochemical studies have demonstrated an association between microbial infections and atherosclerosis [1,5,14]. In addition, infectious agents were detected in atheromas by molecular techniques (mainly polymerase chain reaction) [14,15]. A specific T cell response, both in atheromatous plaque and in peripheral blood, was also detected [1,7].

Several microorganisms have been associated with atherosclerotic diseases [5,14,15], but the results have been controversial and it is not clear whether infectious agents play an active role in atherogenesis or whether they are passive inhabitants of the plaque [6,16,17]. Previous reports have noted the involvement of *Mycoplasma pneumoniae* in cardiovascular diseases [8-11,18], although cardiac complications

associated with *M. pneumoniae* are relatively uncommon (1%–8.5% in persons with serologic evidence of infection) [19]. Pericarditis, myocarditis and pericardial effusion with and without cardiac tamponade have all been described [18], and the organism has been detected by PCR in pericardial fluid as well as in ruptured atherosclerotic plaques and stenotic heart valves [8,11,19]. According to one study, almost half the patients with *M. pneumoniae* infection had symptoms or signs of heart abnormalities at an average of 16 months later [19]. There were no reports on other human mycoplasmas.

Mycoplasma species are possible candidates to be involved in diseases due to their ability to induce chronic disease states. Chronicity develops due to several reasons: First, they adhere tightly to the host cell membrane, or alternatively become intracellular residents, and clearing of the organism is therefore extremely difficult. Second, mycoplasmas are notorious for their fast and elaborated variation of their surface lipoproteins, and therefore evade the immune system [18]. Third, these bacteria exert immunomodulatory effects, namely, altering the functions of several cells of the immune machinery, e.g., activation of macrophages and T lymphocytes [6,20]. These cells are known to play an important role in the atheromatous plaque formation [21,22]. Also, some mycoplasmas (*Ureaplasma urealyticum*) also possess phospholipase A [23], an enzyme that plays a pivotal role in the transformation of macrophages into foam cells, a major component of the atheromatous plaque [24]. In addition, some mycoplasmas cause accumulation of H_2O_2 and other oxygenated radicals that may increase the oxidation in the plaque. Mycoplasmas induce cytokine production by macrophages, especially tumor necrosis factor-alpha, interleukin-6 and IL-1β [6] – cytokines that are involved in atheromatous plaque formation [21, 22]. All the above may suggest that mycoplasmal infection might act in a pro-atherogenic fashion. In one study *M. pneumoniae* was found in almost all coronary atheromas and it was suggested that large amounts of *M. pneumoniae* and *Chlamydia pneumoniae* may be necessary for the development of plaque instability [11].

In the present study we measured antibodies in patients' sera. There were no significant differences in the prevalence of positive antibody response to any mycoplasma among patients with CHD and healthy subjects or between patients with acute coronary syndrome and stable coronary heart disease. These results do not exclude the possibility that mycoplasmas are involved in the development of atheromas. It merely suggests that determining the level of antibody to mycoplasma, usually used to identify mycoplasmal infection, is not the appropriate method to evaluate an association between the infectious agent and CHD. This is probably due to the intracellular localization of mycoplasmas in the macrophages, which render them less exposed to the immune humoral response, namely production of specific antibodies [25].

PCR = polymerase chain reaction
IL = interleukin

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