DETERMINATION OF CHLAMYDIA PNEUMONIAE ANTIBODIES IN ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE RUNNING TITLE: C. PNEUMONIAE AND COPD

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Chlamydia pneumoniae is one of the common causative agents of respiratory system infections. The present study aims to find out the role of Chlamydia pneumoniae in the aetiology of infection-related chronic obstructive pulmonary disease (COPD) exacerbations.

Chlamydia pneumoniae IgM and IgG antibodies were studied in 77 sera samples. Out of 77 sera, 21 patients had COPD in the exacerbation period, 17 had stable COPD and 18 were healthy controls. Enzyme-linked immunosorbent assay technique was used. The acute infection was indicated by IgM seropositivity and seroconversion of IgG antibody.

We found five reactivation cases out of 21 patients (24%) in the COPD-exacerbation group. Among stable COPD cases there was only 1 positive IgM out of 17 cases (6%) and in the healthy group there was not any IgM positivity. The findings were statistically different between the COPD-exacerbation group and the healthy group.

We suggest that Chlamydia pneumoniae may be responsible for infection-related exacerbations in COPD cases according to our results.

Key words: Chlamydia pneumoniae, chronic obstructive pulmonary disease, exacerbation.

The significance of chronic obstructive pulmonary disease (COPD) as a major cause of morbidity and mortality and the need for a better insight into its aetiology and pathogenesis are well recognised (1). Smoking has been

identified as the principal risk factor for this disease. In spite of the strong relationship between cigarette smoking and COPD, only a minority of cigarette smokers develops COPD. Similarly, not all patients with COPD smoke or have smoked in the past. The significance of persistent or recurrent respiratory infections in adult life for the development of COPD is still to a large extent unknown (2).

Chlamydia pneumoniae, the third member of the genus Chlamydia, causes upper and lower respiratory tract infections, including at least 10% of all community-acquired pneumonia cases, bronchitis, pharyngitis, and sinusitis (3,4). Although C. pneumoniae infections are mostly mild or may even be asymptomatic, the importance of persistent infections has recently been recognized. A prolonged or even chronic course is a typical feature of all chlamydial infections. An association between C. pneumoniae infection and asthmatic bronchitis and adult-onset asthma has been suggested, as has also an association between C. pneumoniae infection and prolonged bronchitis (2). Chronic C. pneumoniae infection has been associated with several common chronic diseases, quite recently with chronic obstructive pulmonary disease. Chronic C. pneumoniae infection may amplify smoking-associated inflammation in the bronchi and may be a contributory factor in the development of irreversible pathological changes (5).

Acute exacerbation of COPD is characterized by increased breathlessness, cough and sputum, change of the colour and/or tenacity of sputum, and fever (6). The most common causes of exacerbation are infections of the tracheobronchial tree, which account for around 50-75% of acute COPD exacerbations and air pollution (7,8,9,10).

Recent seroepidemiologic studies have

Table 1. Clinical characteristics of study groups*.

	Group 1	Group 2	Group 3
No. of subjects (n)	21	17	18
Male/Female	13/8	11/6	14/4
Age, yr			
Mean ± SD	65.0 ± 11.4	70.3 ± 13.3	56.5 ± 11.9
Median	68 (42-85)	70 (41-99)	57 (37-80)
Smoking			
Nonsmokers	6	4	9
Ex-smokers	11	10	7
Current smokers	4	3	2
Leukocytes (n> 10.500)	5	3	1
Respiratory function tests (Mean ± SD)			
VC (Liter)	2.4 ± 1.3	2.5 ± 0.9	3.1 ± 1.3
FVC (Liter)	1.5 ± 0.7	1.8 ± 0.7	2.5 ± 1.2
FEV_1 (Liter / 1 sec)	1.4 ± 1.4	1.3 ± 0.5	2.3 ± 1.0
Symptoms			
Dyspnea	21	16	-
Cough	19	15	2
Increased sputum	19	11	-
Fever	7	1	-
Weakness	14	5	1
Chest pain	8	3	-
Lack of appetite	6	3	-
Weight loss	2	-	-
Hemoptysis	-	1	-
Other organism(s) in sputum culture	7	3	-
Single microorganism positive	4	3	-
Two microorganisms positive	2	-	-
Three microorganisms positive	1	-	-

* Group 1: COPD with exacerbation, Group 2: Stable COPD, Group 3: Healthy subjects

suggested a possible association between C. pneumoniae infection and acute exacerbations of COPD (11). The present study aims to find out the role of C. pneumoniae in the aetiology of infection-related COPD exacerbations in Istanbul, Turkey.

MATERIALS AND METHODS

Study subjects

Thirty-eight hospitalised adults, who had been diagnosed as COPD by physical examination and spirometry previously, were admitted to the Department of Respiratory Diseases at GMMA Camlica Chest Diseases Hospital. This study was conducted on three groups. Group 1 consisted of patients with COPD in the acute exacerbation period; Group 2 consisted of patients with stable COPD and Group 3 healthy people. In Group 1, there were 21 patients with COPD exacerbation with an age median 65 (42-85). In this group five patients lacked paired serum samples and were excluded from the study. In Group 2, there were 17 patients with stable COPD with age median 70 years (range, 41-99). In Group 3, there were 18 healthy people with an age median 57 years (range, 37-80). The median age of the 38 patients was 67.5 years (range, 41-99). Of these patients, 25 (65.78%) were male and 13 (34.21%) were female. Patient demographical characteristics are in Table 1.

Patients in the acute respiratory failure were not included in this study. Patients with conditions that may mimic the symptoms of an acute exacerbation including pneumoniae, congestive heart failure, pneumothorax, pleural effusion, pulmonary embolism, and arrhythmia were excluded. Recent respiratory illness other than COPD, congestive heart failure, and recent antibiotic therapy were other exclusion criteria. The healthy group was selected among the age-matched patient relatives without known respiratory and any other systemic diseases.

Patients in all groups were interviewed by the study coordinator at the time of entry into the study. Demographic characteristics, medical history, smoking habit, drug therapy, and physical signs were recorded by the admitting medical staff and later they were confirmed by postal questionnaires. Pertinent laboratory values and chest radiographs were obtained and then radiographs were interpreted independently by two of the investigators. The study protocol was approved by the local ethics committee and patients gave their consent.

Methods

Blood was taken twice from all study subjects on arrival in hospital and they were asked to attend the outpatients' department 6 weeks after discharge for convalescent blood sampling.

Enzyme-linked immunosorbent assay (ELISA): Chlamydial group-specific antibodies were measured by ELISA according to manufacturer's directions. ELISA (A192-01M and A191-01M, Savyon Diagnostics, Israel) was used for detection of anti-chlamydial IgM and IgG antibodies sequentially. The optical density was read at 450 nm with an ELISA reader (Bio-Rad Laboratories).

Statistical analysis: Fisher exact probability test was used for statistical analysis.

RESULTS

In the Group 1 three patients were IgM-positive and two other patients had a seroconversion of IgG antibody, suggesting reinfection or reactivation with C. pneumoniae in five out of 21 patients (24%). In Group 2 there was only one positive IgM out of 17 cases (6%) and there was not any positive IgM in Group 3. (Table 2) There was no significant difference between Group 1 (exacerbation group) and Group 2 (stable group) (Fisher, p=0.145). Also there was no significant difference between Group 2 (stable group) and Group 3 (healthy group) (Fisher, p=0.486). In the healthy group there was no sign of an infection. When Group 1 and healthy group were compared statistically, reinfection in Group 1 was significantly higher than the healthy group (Fisher, p=0.035).

On the other hand, IgG positivity has been evaluated in each group separately. According to these data, out of 21 patients in the first patient group, there were 18 IgG positive patients (86%) either in the first or second

Table 2. Antibodies against C. pneumoniae in subjects of each group*.

Antibodies	Group 1 (n= 21)		Group 2 (n= 17)		Group 3 (n= 18)	
	(n)	(%)	(n)	(%)	(n)	(%)
Acute (IgM)	3	14	1	6	0	
Preexisting (IgG)	18	86	15	88	17	9

* Group 1: COPD with exacerbation, Group 2: Stable COPD, Group 3: Healthy subjects

sera. Fifteen out of 17 patients (88%) were IgG positive in the second group. In healthy group, 17/18 (94%) was IgG positive.

DISCUSSION

We evaluated the reactivation or reinfection as IgM seropositivity or seroconversion of IgG by ELISA technique. In COPD-exacerbation group there were three IgM-positive and also two IgG-seroconversion patients, suggesting C. pneumoniae infection in five patients. In stable COPD group, only one patient had IgM positivity indicating C. pneumoniae infection. On contrary, there was no sign of infection in healthy group. The present study shows that C. pneumoniae may play a role in the aetiology of COPD exacerbations.

Hertzen et al. reported that due to the frequent occurrence of C. pneumoniae-specific IgG antibodies in adult and elderly populations worldwide, the value of IgG antibodies alone as a diagnostic marker in chronic infections, where seroconversions are not to be expected between paired sera, is minimal (2). Similarly our results indicate a high prevalence of IgG antibodies in all groups when tested by the ELISA kit. Out of 21 patients in the first patient group, there were 18 IgG positive patients (86%) either in the first or second sera. Fifteen out of 17 patients (88%) were IgG positive in the second group. In healthy group, 17/18 (94%) was IgG positive.

Infection is the most common observable cause of death in prospectively followed-up COPD patients.(12) In general, the causal relationship between infection of the respiratory tract and acute exacerbations of COPD is difficult to prove. C. pneumoniae is an emerging pathogen that has occupied a higher rank in the aetiology of COPD in recent studies (13). Miyashita et al showed that repeated or prolonged exposure to C. pneumoniae may be associated with acute exacerbations of COPD, and that the patient should be noted as a possible source of C. pneumoniae infection (14). In order to investigate the role of C. pneumoniae in acute purulent exacerbations of COPD, Mogulkoc et al. examined sputum specimens and acute and convalescent sera experiencing an acute purulent exacerbation of COPD and their results suggested reinfection with C. pneumoniae (15). Schafer et al. reported that around 25% of patients with stable COPD show some evidence of tracheobronchial colonisation. The vast majority of patients become colonised at some time during the course of the disease. A variety of factors including current smoking and viral infections predispose to bacterial colonisation and subsequently acute exacerbations. Bacterial pathogens are present in around 50% of patients, Haemophilus influenza and Streptococcus pneumoniae being the most frequently encountered pathogens. Only recently, evidence for infection by C. pneumoniae has been found in 5-20% of patients (7). It is important to remind that several respiratory viruses are associated with 30% of exacerbations, with or without a superimposed bacterial infection (16). Viral infections are present in around 10-20%, with influenza virus representing the most frequent viral pathogen (10).

CONCLUSIONS

In conclusion, our findings support the importance of C. pneumoniae as an aetiological agent among hospitalised adults with COPD during exacerbation in Istanbul, Turkey. However, the frequency of mixed infection is an important consideration for determination of appropriate therapy.

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