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Original Article / *Klinik Çalışma - Araştırma*

Respiratory Viruses and Atypical Agents in Acute Exacerbations of Chronic Obstructive Pulmonary Diseases in İzmir District, Turkey

Türkiye'nin İzmir İlinde Kronik Obstrüktif Akciğer Hastalığı Akut Alevlenmelerinde Solunumsal Virüsler ve Atipik Etkenler

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Objectives: Infections are the major causes of the acute exacerbations of chronic obstructive pulmonary disease (AE-COPD). The aim of the study was to detect the incidence of common respiratory viruses and atypical agents in hospitalized patients with AE-COPD.

Patients and Methods: A prospective study was conducted in tertiary regional thoracic center. Serum samples of 86 consecutive severe AE-COPD patients were studied by ELISA method for detection humoral response of respiratory syncytial virus (RSV), *Mycoplasma pneumoniae*, adenovirus, *Chlamydia pneumonia* and *Legionella pneumophilia*. Four-fold increase in antibody titers of double serum samples obtained at two- to four-week intervals for IgG or positive result for IgM were considered diagnostic for infection. The severity of COPD was interpreted by spirometry. Patients whose forced expiratory volume in one second (FEV_1) was below 50% were evaluated as having severe COPD.

Results: Positive sera were detected in 55 patients (63.9%). The incidence of RSV, *M. pneumoniae*, adenovirus, *C. pneumoniae* and *L. pneumophilia* infections were found as 29%, 15%, 7%, 3%, and 2.5%, respectively. Combined infections were also detected.

Conclusion: The findings suggest that respiratory viral and atypical infections seem to have substantial frequency in acute exacerbations.

Key words: Respiratory syncytial virus; *Mycoplasma pneumoniae*; adenovirus; *Chlamydia pneumoniae*; *Legionella pneumophilia*; COPD.

Amaç: İnfeksiyonlar kronik obstrüktif akciğer hastalığının akut alevlenmesinin (KOAH-AA) başlıca nedenidir. Bu çalışmanın amacı, bölgemizde hastaneye yatırılan KOAH-AA hastalarında yaygın görülen solunumsal virus ve atipik etkenlerin insidansını belirlemektir.

Hastalar ve Yöntemler: Çalışma prospektif olarak üçüncü basamak bölgesel göğüs hastalıkları merkezinin mikrobiyoloji laboratuvarında yapıldı. Toplam 86 ardışık ağır KOAH-AA hastalarının serum örnekleri respiratuar sinsityal virüs (RSV), *Mycoplasma pneumoniae*, adenovirus, *Chlamydia pneumoniae* ve *Legionella pneumophilia* saptanması için ELISA yöntemi ile ticari serolojik kitler kullanılarak çalışıldı. İki ile dört hafta ara ile alınan çift serum örneğinde IgG titresinde en az dört misli artış veya pozitif IgM sonucu infeksiyon için tanısal olarak kabul edildi. KOAH ağırlığı spirometrik olarak değerlendirildi. Bir saniyedeki zorlu ekspriyum hacimleri %50'nin altında olan hastalar ağır KOAH olarak değerlendirildiler.

Bulgular: Elli beş hastada (%63.9) pozitif seroloji saptandı. RSV, *M. pneumoniae*, adenovirus, *C. pneumonia* ve *L. pneumophilia* infeksiyonları sıklığı sırasıyla %29, %15, %7, %3 ve %2.5 olarak bulundu. Ayrıca kombine infeksiyonlar da saptandı.

Sonuç: Bulgular viral ve atipik infeksiyonların akut ataklarda önemli bir sıklığı olduğunu düşündürmektedir.

Anahtar sözcükler: Respiratuar sinsityal virüs; *Mycoplasma pneumoniae*; adenovirüs; *Chlamydia pneumonia*; *Legionella pneumophilia*; KOAH.

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Chronic obstructive pulmonary disease (COPD) is associated with frequent exacerbations causing considerable morbidity and mortality.[1-4] These exacerbations may lead to further worsening of symptoms and increased airway resistance. Acute exacerbation of chronic obstructive pulmonary disease (AE-COPD) is a common cause of hospital admission. Many exacerbations are believed to be due to upper and/or lower respiratory tract viral and atypical bacterial infections with or without bacterial infections.[4]

The aim of this prospective study was to document the presence and prevalence of respiratory syncytial virus (RSV), Mycoplasma *pneumoniae*, adenovirus, *Chlamydia pneumoniae* and *Legionella pneumophilia* infections in hospitalized patients with AE-COPD in spring and summer months. Although several studies have been worked out for the incidence of *M. pneumonia*, *C. pneumonia* and *L. pneumophilia* infections in AE-COPD adult patients, the incidence of adenovirus and RSV infections were studied for the first time in Turkey. This knowledge will be important for planning the prevention and treatment strategies.

PATIENTS AND METHODS

This study was conducted in microbiology laboratory of a regional reference center for respiratory diseases at the Western Turkey.

Consecutive severe (FEV_1<50%) COPD patients who were hospitalized in the respiratory diseases clinic ward between March and December 2003 were enrolled in the study. Spirometric tests were performed for the classification of severity by using ZAN 100 BetterFlow (Bavaria, Germany) spirometry. Forced expiratory volume in one second (FEV_1) and forced and inspiratory vital capacity (FVC, IVC) were assessed for defining the stage of COPD. Chronic obstructive pulmonary disease, its stages and acute exacerbations were defined according to the Turkish Thoracic Society and American Thoracic Society (ATS) criteria as follows:[5,6]

- Defining of COPD by spirometry (FEV_1 / FVC): <%88 (expected)
- Stage of COPD (FEV_1):
  1. Mild >%70 (expected)
  2. Moderate %50-70 (expected)
  3. Severe <%50 (expected)

Routine posterior-anterior chest radiographs were evaluated on admission by experts for all subjects. Patients with infiltration suggesting pneumonia were excluded from the study. Those with coexisting asthma, bronchiectasis and nosocomial pneumonia were also excluded. Twenty healthy blood donor serums were used for control group. Spirometry was not performed for control subjects. Detailed clinical history and physical examinations were taken for subjects in the control group to exclude the presence of COPD. Smoking habits were evaluated in pack years for both patient and control groups.

Bacteriological examination was done in patients expectorating sputum. Sputum samples were evaluated macroscopically and purulent sputum samples were sent to microbiology laboratory. Sputum samples which were <10 squamous epithelial cells and >25 polymorphonuclear leukocytes per low power field (x100) in Gram smear were accepted well-qualified and studied.[7]

Serologic evidence of *M. pneumonia*, *C. pneumoniae*, *L. pneumophilia*, RSV and adenovirus was determined by comparing specific IgG titers in pre- and post-illness serum and presence of IgM by using enzyme immunoassay. Four-fold increase in antibody titers of double serum samples for IgG or positive result for IgM were considered diagnostic for infection. *M. pneumonia* IgM and IgG (Novum Diagnostica GgmbH, Germany), *C. pneumoniae* IgM and IgG (Novatec Immundiagnostica GmbH, Germany), *L. pneumophilia* IgM and IgG (Novatec Immudiagnostica GmbH, Germany), RSV IgM and IgG (Immunlab GmbH, Germany), adenovirus IgM and IgG (Immunlab GmbH, Germany) EIA commercial kits were used. PCR and culture for viral and atypical agents were not used due to the insufficiency of laboratory conditions.

Ethical approval was taken from hospital ethics committee for this study.

Table 1. Demographical and spirometric data of the patients and controls

	Patients	Controls
Age (years, mean±SD)	64.7±5.2	60.5±4.2
Gender		
Male (n, %)	68 (79%)	14 (70%)
Female (n, %)	18 (21%)	6 (30%)
Hospital stay days (mean±SD)	14.8	–
Smokers (n, %)	85 (98.8%)	16 (80%)
FEV_1<50 (predicting at admission)	86	–

Data were analyzed using SPSS Statistical Software, version 11.0 for Windows. Proportions were compared using Fisher exact test. Significance was set at $p<0.05$ using two-sided comparisons.

RESULTS

Eighty-six patients (20.9% female and 79.1% male) and 20 controls (30% female and 70% male) were enrolled. The two groups were comparable in terms of age, sex, and smoking habits ($p>0.05$). All study subjects were negative for anti-HIV and immunocompetent.

Demographical and spirometric data of the patients and controls can be seen in Table 1.

Out of 86 patients, positive sera were detected in 55 (63.9%) patients for the viral and atypical agents tested. Numbers of seropositive cases are seen in Figure 1.

For bacteriological examination, sputum samples which were evaluated well-qualified for culture were obtained 44 (51.1 %) patients.

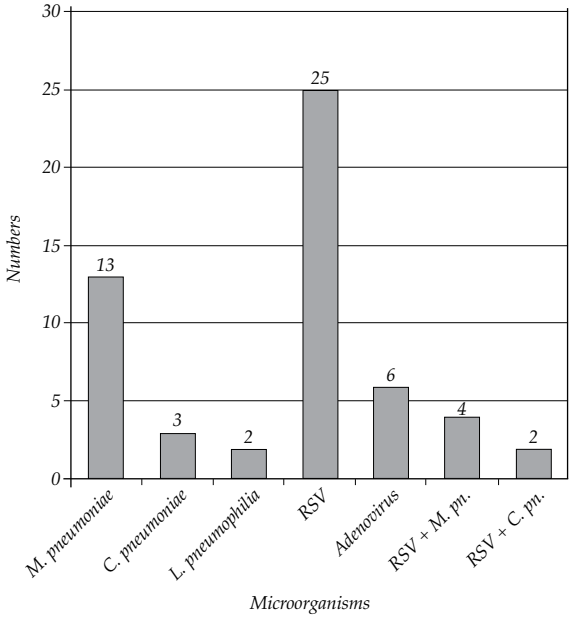

Fig. 1. *The numbers of detected seropositive subjects.*

Nineteen bacterial agents were isolated from 18 (20.9%) patients. *Haemophilus influenzae* (n=7, 36%; 8.1% in all patients) was the most often found bacterial pathogen followed by *Streptococcus pneumoniae* by (n=5, 26%; 5.8% in all patients). Number and percentages of bacterial isolates associated with viral and atypical agents are given in Table 2.

Among our cases, RSV was the most common agent (29.5%). *M. pneumoniae*, adenovirus, *C. pneumoniae* and *L. pneumophilia* were detected as 15.1%, 6.9%, 3.4%, and 2.3%, respectively. Combined infections were obtained as RSV + *M. pneumoniae* and RSV + *C. pneumonia* in four (4.6%) and two (2.3%) cases. Table 3 gives the

Table 2. Bacterial pathogens isolated from COPD patients

Bacterial pathogen*	Number	%**	Associated viral and atypical agent
H. influenzae	7	8.1	2 *Mycoplasma*, 1 RSV
S. pneumoniae	5	5.8	1 *Mycoplasma*, 1 RSV
E. coli	2	2.3	-
P. aeruginosa	2	2.3	1 *Mycoplasma*, 1 RSV
A. baumanii	2	2.3	-
S. pyogenes	1	1.1	1 adenovirus
Total	19	22	

* *S. pneumoniae* and *H. influenzae* were isolated in one patient associated with RSV.
** Percentages are given the total patient number as denominator.

Table 3. Incidence of viral and atypical agents in patients with AE-COPD

	n	%
Number of patients	86	100
Number of patients with seroconversion	55	63.9
Each organism		
RSV	31	36
Adenovirus	6	6.9
M. pneumoniae	17	19.7
C. pneumoniae	5	5.8
L. pneumophilia	2	2.3
Combined organisms		
RSV + *M. pneumoniae*	4	4.6
RSV + *C. pneumoniae*	2	2.3

numbers and percentages of viral and atypical agents.

In controls, one adenovirus (5%) and one RSV (5%) infection were diagnosed. Total seropositivity rate was significantly higher in AE-COPD patients than control group in the same time period ($p < 0.001$). Adenovirus infection incidence did not significantly different in control group in all infectious agents ($p > 0.05$). Difference was significant in other agent groups ($p < 0.001$).

Two patients in which *S. pneumoniae* and RSV infection were detected died during hospitalization.

DISCUSSION

This study has investigated the relationship among RSV, adenovirus, *M. pneumonia*, *C. pneumoniae*, and *L. pneumophilia* infections in severe COPD exacerbations in our region of Turkey in spring and summer months. We have shown that almost 64% of acute exacerbations of COPD are associated with respiratory virus and atypical bacteria infection. This is a much higher detection rate than has been found in other studies in which serological methods have been used in Turkey.[8-10]

In recent years, we have seen an increasing recognition of the role of atypical bacterial pathogens in AE-COPD. Several older and recent studies have implicated atypical bacteria in 5 to 10% of episodes.[11,12] Although *M. pneumoniae* infection has been shown to be a rare cause of AE-COPD by some several studies, some investigators have indicated 6.7% and even 14.2% rates in AE-COPD patients.[13,14] In Turkey, the incidence of *M. pneumoniae* infection in AE-COPD patients were detected as 7.6%-12% in recent studies.[8-10] Our data (15%) suggested a higher incidence of *M. pneumoniae* in Turkey.

Although *Legionella* spp. does not appear to cause an isolated bronchial infection, *Legionella* spp. should be considered, especially in the summer months and in patients with COPD.[12] Alleged etiological role of *Legionella* spp. in AE-COPD has been recently discussed by different authors.[15-17] Lieberman et al.[15] detected 3.75% *L. pneumophilia* infection in their AE-COPD patients. Torres et al.[18] reported 5.5% incidence in Spain in COPD cases. In Turkey, *L. pneumophilia* was reported as 2.7% recently by Uzun et al.[8] which is quite similar with our results (2.5%).

Evidence for infection by *C. pneumoniae* was found in 5-28% of AE-COPD patients.[12,13,18-20,22,23] Mogolkoç et al.[9] found *C. pneumoniae* as the etiologic agent in 22% of the cases in Turkey. In different studies, 14%, and even 35.9% rates were reported in the country, contrary to our results (3%).[8,10] However, according to local publications, the high incidence of infection with *C. pneumoniae* provides insight into the importance of this organism among agents leading to exacerbations of COPD in Turkey.[8,9]

About 20-40% of the episodes of AE-COPD seem to be associated with viral infections.[12,19,24] Asymptomatic viral infections (diagnosed by four-fold rise in specific antibody titers) were also seen, but the incidence of viral infection in association with exacerbations was significantly greater than that during stable periods. Liebermann et al.[24] reported that in 48.8% AE-COPD hospitalizations, at least one viral etiology was identified. Respiratory syncytial virus is one of the most frequent viral agents in AE-COPD patients as we found in our serial (29%). The frequency of RSV infection reported in

several studies ranged widely (6%-51%).[1,11,25-30] But, in Turkey, no data were found about RSV prevalence in COPD patients.

Adenoviruses most commonly cause respiratory illness, however, depending on the infecting serotype. Outbreaks of adenovirus-associated respiratory disease have been more common in the late winter, spring, and early summer; however, adenovirus infections can occur throughout the year. Adenovirus infections among AE-COPD were reported between 1.5-5% in different studies.[1,8,27] We detected a little higher rate of adenovirus as causative agent than expected (7%), probably due to seasonal variation.

Concomitant viral and bacterial pathogens were mentioned about AE-COPD as were found in our study.[8,10,24,31] Multiple viruses were detected in 6.5-21% in various studies.[1,25,30] *C. pneumoniae* was found as the sole causal agent in 16% of cases, and the causal agent with other agents in 6% by Mogolkoc et al.[9] Liebermann[14,15] indicated that there was serological evidence of infection with at least one other respiratory pathogen in quite high rates as 65% and 71% for *L. pneumophilia* and *M. pneumonia*, respectively. We found RSV infection associated with *M. pneumoniae* in four (5%) and *C. pneumoniae* in two (2.5%) cases. At least one bacterial agent was isolated in seven (8.1%) cases along with viral and atypical agents in our study.

We have shown that serological evidence for RSV, *M. pneumoniae*; adenovirus, *C. pneumoniae* and *L. pneumophilia* infections are common in descending order in our patients hospitalized for COPD exacerbations. High prevalence of atypical agents suggests that empirical antibiotic therapy should be arranged on the local microbiological prevalence in the management of AE-COPD.

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