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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

Differences of viral panel positive versus negative by real-time PCR in COPD exacerbated patients

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SUMMARY

Differences of viral panel positive versus negative by real-time PCR in COPD exacerbated patients

Introduction: Exacerbations of chronic obstructive pulmonary disease (COPD) are often caused by respiratory tract infections. The aim of this study was to investigate the clinical, laboratory and computed tomography features of patients with hospitalized COPD exacerbations in which respiratory viruses were detected using a real-time polymerase chain reaction (PCR) technique.

Materials and Methods: This retrospectively planned study included patients hospitalized in the chest diseases clinic due to exacerbation of COPD between November 2018-February 2019. The study included patients who had virus-specific real-time PCR, and computed tomography scans of the chest.

Results: A total of 110 patients were included in the study. Respiratory viruses were identified in the nasopharyngeal swabs of 50 patients (45.5%) using the real-time PCR method, with rhinovirus (25%), influenza A (13.1%) and coronavirus (11.8%) being the most commonly isolated agents. The mean age of the patients was 68.28 ± 9.59 years in the virus-positive group and 68.20 ± 8.27 years in the virus-negative group (p= 0.963). Gender distribution, rate of smokers, exposure to biofuels, blood leukocyte count, neutrophil percentage, C-reactive protein (CRP) level, FEV₁/FVC ratio did not significantly differ between the two groups (p> 0.05). Procalcitonin (PCT) and FEV₁ values were significantly lower (p= 0.001 and p= 0.028, respectively) and the number of exacerbations was significantly higher in the virus-positive group (p= 0.001). The length of hospital stay was longer in the virus-positive group than in the virus-negative group (p= 0.012). Among the findings of computed tomography (CT) of the chest, bronchial wall thickening, cystic

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bronchiectasis, and emphysema did not differ significantly ($p > 0.05$). The rate of infiltrative lesions (tree-in-bud opacity, ground-glass opacity, atypical pneumonia) was significantly higher in the virus-positive group ($p = 0.020$).

Conclusion: Viral respiratory tract infections should be considered in hospitalized patients with an exacerbation of COPD who have a history of frequent exacerbations, normal PCT value, and the absence of consolidation in CT scan of the chest. The use of broad-spectrum antibiotic therapy should be avoided in patients with these features.

Key words: Chronic obstructive pulmonary disease; procalcitonin; viral respiratory panel

ÖZET

KOAH alevlenmeli hastalarda gerçek zamanlı PCR tekniğiyle viral panel pozitif olanların negatif olanlardan farklılığı

Giriş: Kronik obstrüktif akciğer hastalığı (KOAH)'nın alevlenmesi genellikle solunum yolu enfeksiyonlarından kaynaklanır. Bu çalışmanın amacı, gerçek zamanlı polimeraz zincir reaksiyonu (PCR) tekniği kullanılarak solunum yolu virüslerinin tespit edildiği hastane yatışı olan KOAH alevlenmeli hastaların klinik, laboratuvar ve bilgisayarlı tomografi özelliklerini araştırmaktır.

Materyal ve Metod: Retrospektif olarak planlanan bu çalışmaya, Kasım 2018-Şubat 2019 tarihleri arasında KOAH alevlenmesi nedeniyle göğüs hastalıkları kliniğinde yatan hastalar alındı. Çalışma, virüse özgü gerçek zamanlı PCR tekniği ile viral panel bakılmış ve akciğer tomografisi olan hastaları içermektedir.

Bulgular: Toplam 110 hasta çalışmaya dahil edildi. Elli hastanın (%45.5) nazofarengeal sürüntülerinde solunum virüsleri, gerçek zamanlı PCR yöntemi kullanılarak tanımlandı, rinovirüs (%25), influenza A (%13.1) ve coronavirüs (%11.8) en sık izole edilen ajanlardı. Hastaların yaş ortalaması virüs pozitif grupta 68.28 ± 9.59 ve virüs negatif grupta 68.20 ± 8.27 ($p = 0.963$) idi. Cinsiyet dağılımı, sigara içenlerin oranı, biyoyakıt maruziyeti, kan lökosit sayısı, nötrofil yüzdesi, C-reaktif protein (CRP) düzeyi, FEV₁/FVC oranı bakımından iki grup arasında anlamlı fark görülmedi ($p > 0.05$). Virüs pozitif grupta, Prokalsitonin (PCT) ve FEV₁ değerleri anlamlı derecede düşüktü (sırasıyla $p = 0.001$ ve $p = 0.028$) ve alevlenme sayısı anlamlı olarak daha yüksekti ($p = 0.001$). Hastanede kalış süresi virüs pozitif grupta virüs negatif gruptan daha uzundu ($p = 0.012$). Bilgisayarlı tomografi (BT), bronş duvarı kalınlaşması, kistik bronşektazi ve amfizem bulguları arasında anlamlı fark bulunmadı ($p > 0.05$). Virüs pozitif grupta infiltratif lezyonların oranı (tomurcuklanmış ağaç opasitesi, buzlu cam opasitesi, atipik pnömoni) anlamlı olarak yüksekti ($p = 0.020$).

Sonuç: Sık alevlenme öyküsü mevcut, normal PCT değeri olan ve göğüs tomografisinde konsolidasyon bulunmayan KOAH alevlenme nedeniyle hastane yatışı olan hastalarda viral solunum yolu enfeksiyonları düşünülmelidir. Bu özelliklere sahip hastalarda geniş spektrumlu antibiyotik tedavisi kullanılmaktan kaçınılmalıdır.

Anahtar kelimeler: Kronik obstrüktif akciğer hastalığı; prokalsitonin; viral respiratuvar panel

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease that is characterized by airflow restriction and respiratory symptoms resulting from severe exposure to harmful particles or gases (1). COPD is associated with episodes of acute deterioration that are termed as "exacerbations", and which are characterized by worsening symptoms (worsening dyspnea, cough, increased volume of sputum and/or changes in color, increased wheezing, chest discomfort) from the usual stable state (1,2). COPD exacerbation is a global problem due to accelerated disease progression, a decline in quality of life, increased risk of mortality and increased healthcare costs (1,3,4).

COPD exacerbations have various infectious and non-infectious causes. Approximately 70% of exacerbations in COPD are caused by respiratory tract infections brought on by bacteria, viruses and atypical bacteria (5,6). Respiratory viruses are considered to be among the most important triggers of exacerbations.

Between 15% and up to 64% of COPD exacerbations have been found to be associated with symptomatic colds precipitated by viruses (5,7,8). The prevalence of respiratory viruses in COPD patients can vary widely depending on geography and local epidemiologic trends (9).

Virus-induced COPD exacerbations occur with much greater frequency during the winter months when respiratory viral infections are prevalent in the community (10). Co-infection by bacteria and viruses has been described in up to 25% of hospitalized patients, suggesting a certain susceptibility to bacterial infection after a viral process (11). The management of exacerbations of COPD depends on the severity and the cause of infection (bacterial or viral). It would be a logical approach for countries to update their epidemiological data on a regular basis as a guide for empirical antibiotherapy in COPD exacerbations. In this study, we aimed to determine the frequency of viral infections and to evaluate the clinical, radiological and laboratory characteristics of these hospitalized COPD patients due to exacerbation.

MATERIALS and METHODS

Subject Selection

The study was planned retrospectively and COPD patients who were hospitalized due to exacerbation between November 2018-February 2019 were included in the study. We selected patient files containing nasopharyngeal swabs for the panel of respiratory viruses, CRP, PCT, and scans of the chest for the study. Patients with comorbid diseases such as diabetes mellitus, hypertension, thyroid disease and coronary artery disease that would not affect the laboratory markers of infections were included in the study. Patients with one or more conditions that may cause dyspnea, such as asthma, pulmonary embolism, thromboembolism, pneumothorax, congestive heart failure or lung cancer were excluded from the study, even if they had been hospitalized due to COPD exacerbation. Furthermore, patients with an active fungal infection that may affect PCT levels and those with systemic infections other than a lung infection were also excluded. Patients with an appearance of pneumonic consolidation on a chest X-Ray were also excluded. The study was approved by the ethical committee of the Selcuk University Medical School.

Real-Time PCR

The nasopharyngeal swabs are taken before antibiotic treatment and stored at -20 degrees until the time of analysis. In the multiplex PCR analysis, which was performed in eight tubes, the following agents were investigated: influenza A virus, influenza B virus, influenza C virus, influenza A (H1N1)-swl virus, human parainfluenza viruses 1-4, human coronaviruses NL63, 229E, OC43 and HKU1, human metapneumoviruses A/B, human rhinovirus, human respiratory syncytial viruses A/B, human adenovirus, enterovirus, human parechovirus, human bocavirus, *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Bordetella* spp. (excluding *Bordetella parapertussis*), *Klebsiella pneumoniae*, *Legionella pneumophila longbeachae*, *Salmonella* spp. and *Haemophilus influenzae*. Due to the specificity of the test, the test results were include viruses together with bacteria. Therefore, only isolated virus-identified panels were evaluated as viral panel-positive.

Pulmonary Function Test

All spirometric examinations were carried out using a single pulmonary function testing system (Viasys Master Scope, Germany) by a certified and experienced technician, in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) criteria. According to the rules of our clinic, inpatient spirometry is performed when respiratory distress is reduced.

CT Examination

The low dose CT scans of the chest without contrast enhancement are performed in hospitalized patients due to exacerbation of COPD for phenotype assessment and lung cancer screening according to our clinical rules. All CT examinations were performed using a 128 multidetector CT scanner (Siemens Healthcare, Erlangen, Germany) at a radiation exposure of 100 mAs. The thoracic CT scans were interpreted by dividing them into five groups, being: bronchial wall thickening according to the parenchyma window, bronchiectasis, emphysema, infiltrative lesion and normal.

Statistical Analysis

The first hematological, CRP and PCT values were taken into account in the statistical calculation. A Shapiro-Wilk test was used for each parameter to test for the normal distribution of data. An independent samples t-test was used to compare two independent groups with normal distribution, a Mann-Whitney U-test was used to compare two independent groups without normal distribution, and a Chi-square test with a Yates's continuity correction and Fisher's exact test were used for the analysis of categorical variables. All analyses were evaluated with an alpha level of 0.05 (95% confidence interval).

RESULTS

Included in the study were 110 patients. A respiratory virus was identified in the viral panel in 50 patients (45.5%), and the patients were divided into two groups as viral panel-positive and viral panel-negative. The panels of bacteria identified together with respiratory viruses were considered as viral panel-negative. Therefore, only isolated virus-identified panels were evaluated. A comparison of the variables between the two groups is presented in Table 1. The mean age was 68.28 ± 9.59 years in the viral pan-

Table 1. Between-group comparison of variables

	VP (50)	VN (60)	p
Age	68.28 ± 9.59	68.20 ± 8.27	0.963
Gender, n (%)			
Female	10 (20)	20 (33.3)	0.117
Male	40 (80)	40 (66.7)	
Smoking (package/year)	33.12 ± 28.92	32.33 ± 32.88	0.651
Biofuel (years)	40.42 ± 22.00	43.73 ± 16.42	0.456
FEV ₁ /FVC	51.94 ± 10.90	57.62 ± 9.85	0.474
FEV ₁ (%)	46.96 ± 21.12	56.33 ± 16.42	0.028
Number of exacerbations (Last one year)	3.14 ± 1.21	2.17 ± 0.98	0.001
Length of hospital stay	10.12 ± 4.60	8.53 ± 3.95	0.012
WBC (K/uL)	12.68 ± 5.97	12.00 ± 4.58	0.831
Neutrophil (%)	79.27 ± 8.82	76.97 ± 10.92	0.232
CRP (mg/dL)	5.36 ± 4.76	5.90 ± 5.89	0.918
Procalcitonin (ug/L)	0.12 ± 0.10	0.051 ± 0.020	0.001
Blood eosinophil (K/uL)	0.031 ± 0.03	0.15 ± 0.16	0.001

VP: Virus-positive, VN: Virus-negative.

el-positive group and 68.20 ± 8.27 years in the viral panel-negative group, with no significant difference being identified between the two groups (p= 0.963). There was no significant difference between the two groups in terms of gender distribution, rate of smokers (pack/year), exposure to biofuels (years), blood leukocyte count (K/uL), neutrophil percentage, CRP level (mg/dL) or FEV₁/FVC ratio (p> 0.05). The mean blood eosinophil count was significantly higher in the viral panel-negative group than in the other group (p= 0.001). PCT and FEV₁ (%) values were significantly lower (p= 0.001 and p= 0.028, respectively) and the number of exacerbations was significantly higher in the virus-positive group (p= 0.001). The length of hospital stay was longer in the virus-positive group than in the virus-negative group (p= 0.012).

A total of 76 viral pathogens were identified in the viral panel-positive group, with the most frequently isolated pathogens being rhinovirus, influenza A and coronavirus (Table 2). Rhinovirus was positive in 19 patients and constituted 25% of all agents. Among the findings of the computed chest tomography, no significant difference was found between bronchial wall thickening, cystic bronchiectasis and emphysema (p> 0.05). The rate of infiltrative lesion (tree-in-bud opacity, atypical pneumonia) was significantly higher in the viral panel-positive group (p= 0.020) (Table 3).

Table 2. Number and percentage of the causative agents isolated from the virus-positive group

Agents	n (%)
Rhinovirus	19 (25)
Influenza A	10 (13.1)
Coronavirus	9 (11.8)
Influenza B	8 (10.5)
RSVA	7 (9.2)
RSVB	7 (9.2)
H1N1	4 (5.2)
Human metapneumovirus A	3 (3.9)
Human metapneumovirus B	3 (3.9)
Parainfluenza B	2 (2.6)
Enterovirus	2 (2.6)
Adenovirus	1 (1.3)

DISCUSSION

The main findings of this study were that respiratory viruses were frequently detected during exacerbations of COPD patients admitted to hospital. Rhinovirus, influenza virus and coronavirus were higher in viral panel-positive group. PCT and FEV₁ were lower and the number of exacerbations over the past one year were higher in viral panel-positive group.

Table 3. Between-group comparison of radiological findings

	VP (50)	VN (60)	p
Bronchial wall thickening, n (%)	21 (42)	29 (58)	1.000
Bronchiectasis, n (%)	10 (20)	40 (80)	0.850
Emphysema, n (%)	31 (62)	28 (46.7)	0.157
Infiltrative lesion, n (%)	18 (36)	9 (15)	0.020
Normal, n (%)	5 (10)	11 (18.3)	0.336

VP: Virus-positive, VN: Virus-negative.

The prevalence of respiratory viruses in COPD exacerbation has been underestimated. Older studies using cultures or serological methods reported rates of isolation for respiratory viruses that range from 10-30% (12,13). The weighted mean prevalence value of respiratory viral infections detected by PCR and/or Reverse Transcriptase PCR in COPD exacerbations in a systematic review reported 34.1% (14). The lowest prevalence rate for respiratory viral infections in literature has been reported in the United States (15). In this study, respiratory viruses were identified from nasal swabs using the PCR method in 25% of patients who presented to the emergency room with COPD exacerbation. Rohde et al. detected respiratory viruses in the sputum and nasal lavage samples of 56% (48/85) of patients with COPD that were hospitalized with exacerbation (16). Respiratory viruses were detected by a microarray technique in 53.5% (107/200) of hospitalized patients with COPD exacerbation in Greece (17).

Rhinovirus, RSV and influenza are the most common viral agents associated with COPD exacerbation and rhinovirus was the most common agent identified in the present study (5,8). Studies of COPD in Western countries have identified rhinovirus as the most common infectious agent (7,16,18). Rhinovirus is responsible for the common cold, and respiratory viral infections have been identified as important triggers of COPD exacerbations. In a study of 83 patients conducted by Seemungal et al. in the United Kingdom, rhinovirus was detected in 58.2% of the patients (7). Rohde et al. detected rhinovirus in 36% and influenza in 25% of patients hospitalized with exacerbation of COPD (16). The rate of rhinovirus was 20.1% and the rate of influenza was 8.2% in a US study that involved patients hospitalized with exacerbation of COPD (18). In a study by Dimopoulos et al., RSV (40.5%), influenza virus (11%) and rhinovirus (8%) were the most commonly identified virus-

es (17). In an Asian study, influenza A (7.3%) was the most commonly detected viral agent (9). In their study, a prevalence rate of 4.6% was reported for coronavirus and 3.1% for rhinovirus. The differences between studies may be attributed to the different rates of influenza vaccination.

Exacerbations associated with viruses tend to have greater effects on the airway and greater systemic inflammatory effects than non-viral infections (14). Viral exacerbations appear to be more severe, as reflected in the length of hospitalization, the decrease in FEV₁, FEV₁%, FEV₁/FVC% and diffusion capacity, and with a trend towards greater hypoxemia (14). Dai et al. showed that the length of hospital stay associated with exacerbations of COPD was higher in the coinfection group from which viruses and bacterial agents are isolated simultaneously than in exacerbations caused by bacterial agents and non-infectious exacerbations (19). Restrictions in the airway were more severe and the length of hospital stay was longer in the viral panel-positive group. Seemungal et al. reported that exacerbations associated with respiratory viruses had a longer median symptom recovery time than non-viral exacerbations (13 and 6 days, respectively) (7). Viral positivity has also been associated with a greater degree of systemic inflammation. The mean plasma fibrinogen level was two-times higher in the viral exacerbations than in the non-viral exacerbations (7). CRP levels indicating systemic inflammation were higher than normal in both the positive and negative viral panel groups, and no difference was identified between the two groups. Cals et al. demonstrated that plasma CRP level cannot predict the presence of a potentially pathogenic microorganism in the sputum (20).

The role and choice of antibiotics in the treatment of exacerbations remains as a matter of controversy, and not all patients are advised to start antibiotherapy (21,22). Antibiotics are the mainstay treatment for

patients with moderate to severe COPD with exacerbations that include increased purulence sputum (12). The criteria proposed by Anthonisen et al. seem to be the most useful means of estimating the probability of success with antibiotics, since their use seems to be beneficial in both type I and type II exacerbations of bacterial origin (23,24). On the other hand, PCT has been accepted as a more sensitive marker of bacterial infection, and it has been suggested to assist in selection of patients that will benefit most from antibiotherapy (25,26).

The CT signs of pulmonary viral infection depend on the underlying pathologic process and histopathologic features. These include diffuse alveolar damage (intraalveolar edema, fibrin, and variable cellular infiltrates with a hyaline membrane), intraalveolar hemorrhage and interstitial (intrapulmonary or airway) inflammatory cell infiltration (27). Radiological findings are often bilateral in viral pneumonia. Reticular and reticulonodular opacities, patchy alveolar infiltrates with ill-defined contours, peribronchial thickening, tree-in-bud and ground-glass appearance are observed that are more prominent in the perihilar region (27). Radiological findings suggesting viral pneumonia were more common in the viral panel-positive group.

There are a number of limitations to this study. First, the respiratory viruses which is detected from nasopharyngeal swabs by PCR technique is indistinguishable from colonization.

Secondly, all of the respondents in the study were inpatients. Patients presenting to outpatient clinics or those treated in the emergency departments could also have been included, as all COPD exacerbations would have been evaluated and the number of patients would be higher. Thirdly, another potential source of bias is the date of the study because of seasonality of respiratory viral infections.

In summary, this study supports the hypothesis that a significant proportion of hospitalized patients with exacerbations of COPD have respiratory viral infections. Rhinovirus was the most common viral infection detected, followed by influenza and coronavirus. Viral respiratory tract infections come to mind in first place if the procalcitonin level is low in a patient with radiological findings suggestive of viral pneumonia who is hospitalized with an exacerbation of COPD and who is suffering from severe airway

restriction, frequent exacerbations and prolonged hospitalizations. The use of broad-spectrum antibiotics must be avoided in patients exhibiting such characteristics.

CONFLICT of INTEREST

All authors have no conflict of interests.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: BY

Analysis/Interpretation: MS

Data Acquisition: DF

Writing: BY

Critical Revision: MS

Final Approval: BY

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