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Original Article

Enhanced detection of respiratory pathogens using PCR in COPD and asthma exacerbations

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Abstract

Aim: This study aimed to identify bacterial pathogens causing chronic obstructive pulmonary disease (COPD) exacerbations and asthma attacks by evaluating nasopharyngeal swabs tested by polymerase chain reaction (PCR) and simultaneous sputum cultures from hospitalized patients, and to assess the contribution of PCR to causative agent identification.

Materials and methods: Patients hospitalized with COPD exacerbations or asthma attacks from September 2022 to May 2023 were included. Bacterial pathogens were evaluated using PCR on nasopharyngeal swab samples, with simultaneous sputum culture results recorded. Analyzed pathogens included *Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Bordetella pertussis, Legionella pneumophila,* and *Chlamydia pneumoniae*. Frequencies of pathogens detected by PCR and culture were compared.

Results: The study included 911 patients. Nasopharyngeal swabs and simultaneous sputum cultures were obtained from 448 patients with COPD exacerbations and 127 patients with asthma attacks. In COPD exacerbations, *H. influenzae* was detected in 52 (8.7%) patients and *S. pneumoniae* in 32 (5.3%) by PCR, compared to 2 (0.4%) and 3 (0.7%) patients by sputum culture, respectively. In asthma attacks, *H. influenzae* was detected in 20 (6.5%) patients and *S. pneumoniae* in 11 (3.5%) patients by PCR, with *S. pneumoniae* detected by sputum culture only in 1 (0.8%) patient. *H. influenzae* and *S. pneumoniae* were significantly higher in COPD exacerbations and asthma attacks using PCR ($p<0.05$).

Conclusion: PCR may detect bacterial pathogens in COPD exacerbations and asthma attacks more effectively than sputum culture within the context of this study.

Keywords

asthma, COPD, PCR, sputum culture

Introduction

Chronic obstructive pulmonary disease (COPD) and asthma are common respiratory conditions characterized by persistent symptoms that often lead to acute exacerbations, resulting in hospitalization and loss of lung function.[1] These exacerbations are associated with decreased lung function, worsened quality of life, and increased mortality. Infections are a major cause of asthma attacks and COPD exacerbations.[2] Although viral infections predominate, bacterial infections are also significant contributors.[3]

Sputum culture is commonly used to identify infectious agents but has low sensitivity and specificity in determining etiology.[4] Polymerase chain reaction (PCR) is an alternative method for detecting pathogens, widely used for viral agents identification but increasingly applied to bacterial detection. PCR is more sensitive and faster than conventional diagnostics, particularly when culture is challenging, although it has limitations in distinguishing colonization from infection. It also allows detection of multiple pathogens from a single microbiological sample and identification of agents in patients receiving antibiotics.[5,6]

Aim

In this study we aimed to identify the bacterial pathogens causing COPD exacerbations and asthma attacks by evaluating nasopharyngeal swabs tested by PCR and simultaneous sputum cultures in hospitalized patients, and to assess the contribution of PCR to causative agent identification.

Materials and methods

This retrospective, cross-sectional study included 911 patients hospitalized for COPD exacerbations or asthma attacks at the Samsun Education and Research Hospital Chest Diseases Clinic between September 2022 and May 2023. Nasopharyngeal swabs were collected from all 911 patients, while sputum cultures were obtained from 575 patients that were able to produce sputum simultaneously. Sputum culture and nasopharyngeal swab PCR results were retrieved from the hospital information system. Bacteria evaluated by PCR in nasopharyngeal swabs included *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *B. pertussis*, *L. pneumophila*, and *Chlamydia pneumoniae*. Frequencies of pathogens detected by PCR and culture were compared.

Respiratory samples were analyzed using the Biospeedy® Respiratory Tract RT-qPCR MX-24 Panel (Bioeksen R&D Technologies Inc., Türkiye).

Data were analyzed using SPSS version 23 (SPSS Inc., Chicago, IL, USA). Frequencies and percentages were calculated for categorical variables and means and standard deviations for numerical variables. Categorical variables were compared using the chi-square test.

Results

Between September 2022 and May 2023, 911 patients were evaluated at the Chest Diseases Clinic (female: 311 [34.1%], male: 600 [65.9%]). The mean age was 65.8±13.7 years (female: 67±15.3; male: 65.2±12.8). Simultaneous sputum cultures were obtained from 448 patients with COPD exacerbations and 127 patients with asthma attacks. Comorbidities were present in 90.3% of patients, with hypertension (59.4%), ischemic heart disease (36.4%), and cerebrovascular disease (14.6%) being the most common. Demographic characteristics are shown in **Table 1.**

In COPD exacerbations, *H. influenzae* was detected in 52 (8.7%) patients and *S. pneumoniae* in 32 (5.3%) by PCR, compared to 2 (0.4%) and 3 (0.7%) patients by sputum culture, respectively. In asthma attacks, *H. influenzae* was detected in 20 (6.5%) patients and *S. pneumoniae* in 11 (3.5%) by PCR, while *S. pneumoniae* was detected only in 1 (0.8%) patient by sputum culture. *H. influenzae* and *S. pneumoniae* were significantly higher in COPD exacerbations and asthma attacks by PCR than in sputum culture ($p<0.05$). *M. catarrhalis*, *B. pertussis*, *L. pneumophila*, and *C. pneumoniae* were not detected by either method **(Table 2)**.

Other microorganisms detected by sputum culture in COPD exacerbations and asthma attacks are shown in **Table 3**.

Table 1. Demographic characteristcs and comorbidity

Age (mean ± SD)	65.8±13.7
Sex (n, %)	
Male	600 (65.9)
Female	311 (34.1)
COPD exacerbation	
PCR	601
Sputum culture	448
Asthma attack	
PCR	310
Sputum culture	127
Comorbidity (n, %)	
Hypertension	541 (59.4)
Ischemic heart disease	332 (36.4)
Cerebrovascular disease	133 (14.6)
Malignancy	57 (6.3)
Bronchiectasis	51 (5.6)
Chronic renal failure	40 (4.4)
Alzheimer	19 (2.1)
Diabetes	13 (1.4)
Diffuse interstitial lung disease	8 (0.9)

Discussion

Bacterial infections in COPD and asthma contribute to exacerbations, prolonging hospital stays and increasing mortality.[7] Recent studies suggest bacteria cause 30%–50% of COPD exacerbations, based on sputum culture and serological methods.[8] However, detection rates of respiratory viruses and atypical bacteria by these methods are low, particularly in patients recently treated with antibiotics. Respiratory viruses, typical bacteria, and atypical bacteria are all successfully detected by PCR.[9] It has been shown by PCR that viral and atypical factors are more prominent than bacteria in asthma attacks. Although exacerbations caused by bacteria are observed in asthma attacks, there are few studies in the literature about its incidence because cul-

Table 2. Microorganisms detected as causative agents in COPD exacerbations and asthma attacks by sputum culture and PCR

Pathogenic microorganisms	COPD exacerbation n=601		Asthma attack n=310		*p*
	PCR n=601 (%)	**Sputum culture n=448 (%)**	**PCR n=310 (%)**	**Sputum culture n=127(%)**	
Haemophilus influenzae	52 (8.7)	3 (0.7)	20 (6.5)	0 (0)	<0.05
Streptococcus pneumoniae	32 (5.3)	2 (0.4)	11 (3.5)	1 (0.8)	<0.05
Moraxella catarrhalis	0	0	0	0	
Bordetella pertussis	0	0	0	0	
Legionella pneumophila	0	0	0	0	
Chlamydia pneumoniae	0	0	0	0	

COPD: chronic obstructive pulmonary disease; PCR: polymerase chain reaction

Table 3. Microorganisms detected as causative agents in COPD exacerbations and asthma attacks by sputum culture

Pathogenic microorganisms	COPD exacerbation n (%)	Asthma attack n (%)
Pseudomonas aeruginosa	17 (3.8)	6 (4.7)
Acinetobacter baumannii	10 (2.2)	0
Escherichia Coli	14 (3.1)	0
Klebsiella pneumoniae	5 (1.1)	1 (0.8)
Burkholderia cepacia	3 (0.7)	0
Staphylococcus aureus	2 (0.4)	0

tures are not always performed, and viruses are important organisms in asthma exacerbations.

This study evaluated a larger cohort than most reported studies to determine bacterial etiology in COPD exacerbations and asthma attacks.[4-13] We found that sputum culture detected a low rate of agents (10.7%) and asthma attacks (5.5%), consistent with the literature, and that the PCR method detected *H. influenzae* and *S. pneumoniae* at a higher rate than sputum culture.[4,10-13]

There have been few studies published that compare bacterial detection by PCR and sputum culture in COPD exacerbations and asthma episodes, underlining the need for more research in this field. In a prospective study, Shimizu et al. found that PCR detected pathogens in 21 (42%) patients with COPD exacerbations, compared to 23 (46%) patients by sputum culture. The presence of *S. pneumoniae* was identified in seven patients through nasopharyngeal swab PCR, in 11 patients through sputum PCR, and in 10 patients through sputum culture. *H. influenzae* was detected in three patients by nasopharyngeal swab PCR, in eight by sputum PCR, and in two patients by sputum culture. *Mycoplasma pneumoniae* was detected in three patients in nasopharyngeal swab PCR, in one in sputum PCR, but not in sputum culture. *C. pneumoniae* was detected in one patient in nasopharyngeal swab PCR, but not in sputum PCR and sputum culture. *Staphylococcus aureus* (5), *Pseudomonas aeruginosa* (4), *Enterobacter cloacae* (3), *Klebsiella pneumoniae* (1), *Serratia marcescens* (1), *Proteus mirabilis* (1), *Acinetobacter baumannii* (1), and *Stenotrophomonas maltophilia* (1) were detected in sputum culture but not in nasopharyngeal swab PCR and sputum PCR. The study emphasized that sputum PCR evaluation is more valuable than nasopharyngeal swab, and *H. influenzae* is detected at a higher rate by PCR than by sputum culture. Both methods should be used simultaneously to detect causative agents in COPD exacerbation.[11]

Akin et al.'s study on hospitalized patients for COPD exacerbation found that *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were detected with PCR, and compared these results with those in sputum culture. PCR detected at least one of these microorganisms in 47 (75.8%) patients, while bacterial cultures were positive in only 11 (17.7%) patients. Additionally, multiple pathogens could be detected by PCR, while culture did not allow this. The PCR detection rates for the three microorganisms were *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were 53.2%, 17.7%, and 35.5%, respectively. Other factors detected in sputum culture were *Moraxella catarrhalis* (6), *Enterobacter cloacae* (2), *Acinetobacter baumannii* (1), *Serratia marcescens* (1), and *Pseudomonas aeruginosa* (1). The study emphasized that bacterial detection rates with PCR were significantly higher than those with sputum culture.[4]

Wark et al. found that 35% of patients hospitalized for asthma episodes (96) and COPD exacerbations (103) had a viral etiology, 19% had a bacterial cause, and 8% had both. Bacterial culture identified agents in six (5%) asthma attack patients and 20 (17%) COPD exacerbation patients. The most common bacteria found were *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Stenotrophomonas maltophilia*.[12]

In Yoshii et al.'s study, PCR (nasopharyngeal swab and sputum) results were compared with sputum culture in 64 asthma attack patients. PCR detected pathogens in 29 (45.3%) patients, while sputum culture detected pathogens in eight (12.5%) patients. *Haemophilus influenzae* was detected in four patients (6.3%) in nasopharyngeal swab PCR, 17 patients (26.6%) in sputum PCR, and two patients (3.1%) in sputum culture. *Streptococcus pneumoniae* was detected in three patients (4%) in nasopharyngeal swab PCR, six (9.4%) in sputum PCR, and three (4.7%) in sputum culture. *Streptococcus pyogenes* was detected in sputum and nasopharyngeal swab PCR in two (3.1%) patients and sputum culture in one (1.6%) patient. *M. pneumoniae* was detected in one (1.6%) patient in nasopharyngeal swab PCR and in four (6.3%) patients in sputum PCR, but not in sputum culture.[10]

In the study by Iikura et al., they detected in the nasopharyngeal PCR swabs from 48 asthma attack patients *S. pneumoniae* in nine (18.7%) patients, *H. influenzae* in six (12.5%), *M. catarrhalis* in one (1.2%), *K. pneumoniae* in one (2%), *B. pertussis* in one (2%), and *C. pneumoniae* in one (2%).[13] The present study identified similar microbial agents in sputum culture as those rported in the literature, with *S. pneumoniae* and *H. influenzae* being detected at significantly higher levels using PCR than in sputum culture.

The limitations of the present study were as follows: it was retrospective, each patient did not have a history of sputum production, sputum culture could not be evaluated one-to-one with PCR, atypical and other pathogenic bacteria were not evaluated with the PCR method, and PCR was not performed on sputum. Another limitation was that the antibiotic use status of the patients before hospitalization could not be determined. This situation may have caused the agents to be detected in PCR but not to grow in sputum culture.

Conclusions

In COPD exacerbations and asthma attacks, sputum culture alone may be less effective than PCR for detecting common bacterial pathogens, such as *H. influenzae* and *S. pneumoniae*. Incorporating PCR into diagnostic procedures may improve detection of bacterial factors. This study highlights the potential of PCR as a complementary tool to sputum culture in detecting bacterial pathogens during COPD exacerbations and asthma attacks. Further prospective studies are needed to confirm these results and investigate their generalizability across different patient populations.

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Competing Interests

The authors have declared that no competing interests exist.

Author contributions

S.G.: conceptualization, methodology, investigation, data curation, writingoriginal draft, visualization, and project administration, writing-review and editing. A.C.P.: conceptualization, formal analysis, writing-original draft review and editing. B.Ö.: supervision; writing-review and editing. L.Ö.: methodology, investigation, data analysis, validation, writing-review and editing. E.A.A.: investigation, data curation, writing-review and editing. Final approval of manuscript: all authors.

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