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Original Research Article

A Study on Diagnostic Modalities of Acute Exacerbation of Bronchial Asthma, Chronic Obstructive Pulmonary Disease at Government General and Chest Hospital, Hyderabad

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD), a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients. Aim and Objectives: 1. To observe the prevalence of infection with Mycoplasma pnemoniae, Chlamydia pneumoniae, Legionella pneumophilia in diagnosed patients of Acute Exacerbation of Bronchial Asthma, Chronic Obstructive Pulmonary Disease. 2. To subject sputum samples to sanger sequencing.

Material and Methods: It was an observational study conducted at Government General & Chest Hospital, Erragadda, Hyderabad Between March 2015 to March 2016. Total 60 Patients with Acute exacerbation of Chronic Obstructive Pulmonary Disease, Acute exacerbation of Bronchial asthma with age 16 to 65 years and Patients presenting with symptoms and signs of fever, cough, dyspnoea and extrapulmonary symptoms were included in the study. No exacerbation in the month prior to enrolment (Bronchial Asthma & COPD). Bronchiectasis, Interstitial lung disease and Pulmonary Tuberculosis were excluded from the study.

Results: Most (30%) Bronchial asthma cases were seen in younger age groups i.e, 16-26 years whereas later ages saw almost equal distribution was observed. On the contrary old ages i.e, 47 years and above acute exacerbation observed. Majority 63.33% cases of males had Acute exacerbation of Bronchial Asthma. Only males had Acute Exacerbation of COPD. Majority (83.3%) had mucoid sputum, 16.7% had Mucopurulent Sputum.

Conclusion: Sanger sequencing can be used as a useful tool to substantiate the evidence of infection by atypical organisms when used along with IgM ELISA. Hence, it is useful in identification of bacteria at species and strain level and could be used when affordable.

Keywords: Chronic Obstructive Pulmonary Disease, Bronchial Asthma, Sanger sequencing, IgM ELISA.

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Introduction

Chronic Obstructive Pulmonary Disease (COPD), a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients[1].

An exacerbation of COPD is an acute event characterized by a worsening of the patient‟s respiratory symptoms that is beyond normal day to day variations and leads to a change in medication[1]. Exacerbations of COPD can be precipitated by several factors. The most common causes appear to be respiratory tract infections[1]. Regarding microbial patterns and their possible involvement in the aetiology of Acute exacerbation of COPD, it is a common view that H. influenzae, S. pneumoniae and M. catarrhalis are the leading pathogens. In several studies, serological evidence of C. pneumoniae, Legiollane spp. and M. pneumoniae playing a role as a pathogen or co-pathogen in acute exacerbations has been reported[2].

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation[1]. Exacerbations represent an acute or subacute worsening in symptoms and lung function from the patients usual status, or in some cases, the initial presentation of asthma[2]. Its etiology is complex, involving interactions between genetic susceptibility, exposure to allergens and external aggravating factors, such as smoking, air pollution and RTIs. A study found that 37% of adults admitted to hospital over a 12-month period with an acute asthma exacerbation had evidence of recent RTI.[3]Evidence currently available supports a role for C. pneumonia and M. pneumoniae infection as a trigger for 5–30% episodes of wheezing or acute asthma exacerbations[1].

Aim and Objectives:

- To observe the prevalence of infection with Mycoplasma pnemoniae, Chlamydia pneumoniae, Legionella pneumophilia in diagnosed patients of Acute Exacerbation of Bronchial Asthma, Chronic Obstructive Pulmonary Disease
- To subject sputum samples to sanger sequencing

Material and Methods: It was an observational study conducted at Government General & Chest Hospital, Erragadda, Hyderabad. Total 60 Patients with Acute exacerbation of Chronic Obstructive Pulmonary Disease, Acute exacerbation of Bronchial asthma with age 16 to 65 years and Patients presenting with symptoms and signs of fever, cough, dyspnoea and extrapulmonary symptoms were included in the study. No exacerbation in the month prior to enrolment (Bronchial Asthma & COPD). Bronchiectasis, Interstitial lung disease and Pulmonary Tuberculosis were excluded from the study.

Method:

Sputum collection: sputum sample were collected in 2 containers before the patient was started on antibiotics.

Aerosol induced sputum collected by allowing the patient to breathe aerosolized droplets, using an ultrasonic nebulizer containing 0.85% NaCl or until a strong cough reflex is initiated. Sputum is collected from the patients in separate sterile container under aseptic conditions and transported to laboratory immediately.

Sputum Processing

Macroscopic appearance: The nature of the sputum was observed – purulent, mucopurulent, mucoid, or blood stained.

Microscopic examination Gram stain: Smear was prepared from the sputum air dried, fixed and stained by grams method. Smear was examined and samples containing >25 polymorphonuclear leucocytes and <10 epithelial cells per low power field were subjected to culture

Culture: Sputum was inoculated onto 5% sheep Blood agar Chocolate agar and MacConkey agar. Plates were incubated for 18-24 hrs at 37^0Cfor 24 hrs. The samples which are showing no pathogenic organisms (NPO) were subjected to PCR for amplification of V3 region of 16srna followed by sanger sequencing.

Procedure: PCR for amplification of V3 region

The isolated DNA samples from sputum were used for PCR amplification of the conserved V3 region.

Analysis Of Sequencing Data: The obtained sequencing files were subjected to BLAST to identify the microorganism prevalent in the sputum sample.

Blood collection: The skin over the vein was cleaned with 70% alcohol and allowed to dry. Then povidone iodine was applied was allowed to dry for 1 minute. Blood sample was collected into a sterile bottle without anticoagulant. It was allowed to stand for formation of clot. Then it was centrifuged, and supernatant was taken and stored at - 20^0C.

Processing: All samples were subjected to ELISA to detect IgM antibodies for Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila. Test was performed according to manufacturer's instructions.

Anti-Mycoplasma pneumonia ELISA (IgM) EUROIMMUN

Principle: The ELISA test kit provides a semi quantitative in vitro assay for human antibodies of the IgM class against mycoplasma pneumoniae in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with mycoplasma pneumoniae antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate), catalyzing a colour reaction which is capable of promoting a colour reaction. The intensity of the formed color is proportional to the concentration of antibodies against Mycoplasma pneumoniaeantigens. The principle is similar for the following

Anti-Chlamydia pneumonia ELISA (IgM). EUROIMMUN

Anti-Legionella pneumophila ELISA (IgM). EUROIMMUN

Results:

The present study is done with 60 individuals among,30 having Acute Exacerbation of Bronchial Asthma and another 30 having Acute Exacerbation of chronic obstructive pulmonary disease attending the outpatient department /wards of Pulmonary Medicine in Government General and Chest Hospital, Erragadda, Hyderabad between March 2015 to March 2016.

Most (30%) Bronchial asthma cases were seen in younger age groups i.e, 16-26 years whereas later ages saw almost equal distribution was observed. On the contrary old ages i.e, 47 years and above acute exacerbation observed.

Majority 63.33% cases of males had Acute exacerbation of Bronchial Asthma. Only

males had Acute Exacerbation of COPD. Majority (83.3%) had mucoid sputum, 16.7% had Mucopurulent Sputum

Table 1: IgM antibodies detection of atypical pathogens by ELISA (n=30)

Pathogen	Acute exacerbation of bronchial asthma	Acute exacerbation of COPD
Mycoplasma pneumonia	1	0
Chlamydia pneumonia	0	2
Legionella pneumophila	0	4

Table 2: Detection of atypical pathogens by sanger sequencing (n=30)

Pathogen	Bronchial asthma	Acute exacerbation of COPD
Mycoplasma pneumoniae	1	0
Chlamydia pneumonia	0	1
Legionella pneumophila	0	2

Table 3: Bacteria detected by Sanger sequencing (In Acute Exacerbation of Bronchial Asthma)

	Frequency	Percentage
Mycoplasma pneumoniae	1	3%
Streptococcus pneumoniae	5	17%
Pseudomonas aeuroginosa	2	7%
Streptococcus Spp	11	36%
Chryseobacterium	2	7%
Prevotella	1	3%
Stenotrophomonas	2	7%
Uncultured	6	20%

Table 4: Bacteria detected by Sanger sequencing (In Acute Exacerbation of COPD)

	Frequency	Percentage
Chlamydia pneumoniae	1	3%
Legionella pneumophila	3	7%
Streptococcus pneumoniae	7	17%
Pseudomonas aeuroginosa	5	13%
Streptococcusparasanguinis	1	33%
Uncultured	9	27%

Discussion:

According to study by F. Blasi et al Acute exacerbation of Bronchial Asthma is caused by atypical bacterial infection. These patients are found to be infected 6% by Chlamydia pneumoniae and 2% by Mycoplasma pneumoniae[2].

According to Thumerelle C, et al study Serological tests for atypical bacteria were positive in 10% of patients (C. pneumoniae, 5%; M. pneumoniae, 5%) with Acute Exacerbation of Bronchial Asthma[4].

According to a serologically based prospective study by Lieberman et al, 18% of patients hospitalized for an acute

exacerbation of bronchial asthma, were found to have acute infection with M. pneumoniae[5].

According to Biscardi et al study, 20% (24/119) of the patients with previously diagnosed asthma were found to have acute M pneumoniae infection during the current exacerbation. C. pneumoniae infection was found in 4 (3.4%) of the patients during the current exacerbation[6].

In the present study IgM antibodies for M.pneumoniae has been noted in 1 (3.3%) cases of Acute Exacerbation of Bronchial Asthma. Mycoplasma alone is identified as causative pathogen for acute exacerbation of Bronchial Asthma in Lieberman et al study[5]. Remaining above studies identified Mycoplasma as well as Chlamydia is causing acute exacerbation of Bronchial Asthma. Because of small sample size present study differ from the above-mentioned previous studies.

According to the study by Nakou A, Papaparaskevas J, et al, the most common bacteria in Acute exacerbation of COPD were Haemophilus influenza and Pseudomonas aeruginosa (23.9 and 14.1%, respectively). Chlamydia pneumoniae or Mycoplasma pneumoniae infection was diagnosed in four and two (4.2% & 2.2%) patients, respectively[7].

Levent Erkan, et al study included 75 patients who had been diagnosed with acute exacerbation of COPD from a total of 156. An infectious agent was identified in 46 patients, either serologically or with sputum culture. Pathogens most demonstrated were: Haemophilus influenza (30%), Chlamydophila pneumoniae (17%), and Mycoplasma pneumoniae (9%). Mixed infections were diagnosed in 9 patients[8].

In the present study IgM antibodies for Chlamydophila pneumoniae has been noted in 2 cases and IgM antibodies for L. pneumophilia has been noted in 4 cases of acute exacerbation of COPD. Prevalence of Chlamydophila pneumoniae in our present study is like Nakou A, Papaparaskevas J, et al[7] study, but Mycoplasma pneumoniae is not identified as a causative pathogen in Acute exacerbation of COPD because of small sample size. Prevalence of L. pneumophilia in our present study is similar to Lazarovich Z, et al[9] study, but Mycoplasma pneumoniae is not identified as a causative pathogen in Acute exacerbation of COPD because of small sample size.

In our study cases have been selected based on high suspicion of atypical pneumonia and patients showing positive IgM for atypical bacteria were treated with macrolides and fluoroquinolones to which they responded and showed clearing of lung fields in follow up therefore clinical correlation helped clinch clinical diagnosis and proved. IgM detection as a useful tool in diagnosis.

Sanger sequencing in available data has been employed mainly as a research tool to identify strains in Legionella Spp, Mycoplasma Spp, Chlamydia species and has reported as highly specific[10].

Low sensitivity of Sanger sequencing in comparison with IgM detection could be due to low bacterial load at the time of collection of the samples. As a result, this method has identified the predominant bacteria in the samples.

Other bacteria detected by sanger sequencing in Acute exacerbation of Bronchial Asthma are streptococcus pneumonia (17%), Pseudomonas aeuroginosa (7%), streptococcus Spp (36%), Chryseobacterium (7%), Prevotella (3%), Stenotrophomonas (7%), Uncultured (20%).

Other bacteria detected by sanger sequencing in Acute exacerbation of COPD are streptococcus pneumonia(17%),

Pseudomonasaeuroginosa(13%), streptococcusPara sanguinis(33%), Uncultured(27%).

Conclusion:

This study shows that, acute exacerbations of Bronchial asthma and COPD are caused not only by common bacteria but also atypical pathogens.

The interplay between different etiologic factors (environment, viruses, atypical pathogens and bacteria) needs to be better understood to treat exacerbations Bronchial asthma, COPD, better by developing effective investigative and therapeutic strategies.

Clinically, the infection by atypical organisms is characterized by more systemic manifestations than the infections caused by common pathogens. Serological evidence of infection with atypical organisms helps in accurate diagnosis and specific treatment rather than starting an empirical treatment.

The need for designing an antimicrobial regimen which covers atypical pathogens imposes a significant challenge to clinicians. Macrolide antibiotics along with their antimicrobial activity confer an additional advantage in cases of COPD and bronchial asthma due to their added anti-inflammatory action.

We further conclude that Clinic-Radiological diagnosis supported by IgM ELISA is a reliable tool in identifying cases of atypical pneumonia caused by the three organisms L. pneumophila, M. pneumoniae, and C. pneumoniae.

Sanger sequencing can be used as a useful tool to substantiate the evidence of infection by atypical organisms when used along with IgM ELISA. Hence, it is useful in identification of bacteria at species and strain level and could be used when affordable

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