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A STUDY OF ASSOCIATION OF MICROBIAL PATTERN IN SPUTUM SAMPLE WITH SEVERITY STAGING IN PATIENTS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

Microbiology

Dr Soniya Saxena Assistant Professor, Department of Microbiology, LNMC & RC, Bhopal

Dr Nishant Srivastava* Associate Professor, Department of TB and Chest, GMC, Bhopal *Corresponding Author

Dr Vijay Kumar Ramnani Professor and head, Department of Microbiology, LNMC & RC, Bhopal

ABSTRACT

Introduction: Both the prevalence and mortality from COPD has been increasing worldwide. Recent studies have described a relationship between lung function decline and respiratory infections. Studies of sputum and bronchoscopy samples using standard culture and molecular techniques have clearly demonstrated that COPD is associated with a markedly increased prevalence of bacteria. Sputum cultures are still useful in researching the pathogenesis of COPD, as they provide the pathogens to be studied further . Aim: To study the association of microbial pattern with severity i.e. stages in patients of COPD, if any. Methods: 200 clinically diagnosed cases of AECOPD of age ≥45 years were recruited in study. Two sputum samples each were processed by conventional methods. Preparation of media, reagents, Gram staining, identification of culture isolates, antibiotic sensitivity tests and pulmonary function testing were carried out. Conclusion: Shifting from predominantly gram positive to gram negative isolates were observed with shifting of stages from mild to very severe stage (gram positive and gram negative bacteria were 90 % and 10% in mild stage ,12.77% and 31.60 % in moderate stage, 17.60 % and 82.40 % in severe stage and 7.45 % and 85.50 % in very severe stages of COPD).

KEYWORDS

COPD, severity staging, microbial pattern

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a common preventable and treatable disease, 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 [1]. Males are more often affected than females [2]. On average 5-15% of adults in industrialized countries have COPD [3]. It is both a rural and urban health problem, the prevalence varying from 1% in urban nonsmoker to 21% in rural smokers. Both the prevalence and mortality from this disease has been increasing worldwide [4].

According to B.R. Celli *et al* [5], bacterial pathogens have been associated with a more than two fold increase in the risk of exacerbation. More recent studies have described a relationship between lung function decline and respiratory infections. A decline in lung function in COPD have been shown to be related to Chronic bronchitis [6] respiratory infections [7,8] and sputum bacterial counts[9]. Bacteria can induce inflammation both in the context of exacerbations and in the stable baseline state. Antibiotics value in this context remains somehow uncertain [10]. Recent trials of the long-term use of macrolides have shown encouraging results [11].

Approximately 50% of exacerbations of COPD are associated with the isolation of bacteria from the lower respiratory tract [17,18]. The predominant bacterium are; *Hemophillus influenza , Streptococcus pneumoniae and Moxarella catarrhalis*. In severe and very severe disease *Pseudomonas aeruginosa* becomes more prevalent[18]. Studies of sputum and bronchoscopy samples using standard culture and molecular techniques have clearly demonstrated that COPD exacerbations are associated with a markedly increased prevalence of bacteria. Sethi and colleagues have concluded that the acquisition of a new strain of bacteria (particularly *H. influenza*) is associated with COPD exacerbations [19,20]. There are indirect evidences correlating the decline in lung function with airway infection and chronic bronchitis in COPD[21,22]. Role of bacteria in inflammation COPD is characterized by persistent inflammation that damages lung tissue, causing airflow obstruction[16].

Sputum cultures are still useful in researching the pathogenesis of exacerbations of COPD, as they provide the pathogens to be studied further [15].

Therefore this study was undertaken to isolate and identify the bacterial etiological agents and to evaluate if there is any association between deteriorating lung function and causative bacterial organism, or not .

AIM To study the association of microbial pattern with severity i.e. stages in patients of COPD, if any.

MATERIALS AND METHODS

Clearance and permission of institutional ethical committee was taken for this cross-sectional analytical study. 200 clinically diagnosed cases of AECOPD of age ≥45 years were recruited from clinics and wards of department of medicine and department of TB Chest of L N Medical College and research centre, Bhopal. The exclusion criteria were patients on maintenance treatment of oral steroids, Subjects with history of recent antibiotic therapy within 7 days, Patient having bronchial asthma, lung abscesses or lung cancer, known case of Pulmonary Koch's, diabetes mellitus or HIV and AECOPD Patient on ventilatory support. Deeply expectorated two sputum samples after an oral gargle with water was collected directly into a sterile and wide mouthed disposable universal container. After proper collection sputum samples were transported immediately to Microbiology laboratory for further processing by conventional methods. Murray and Washington's[12] grading system was followed for assessing the quality of sputum sample of stained smear. Appropriate sputum sample was inoculated on 5% Sheep Blood agar, Chocolate agar and MacConkey's agar .These inoculated plates were then incubated for a period of 18-24 hours after which they were examined for evidence of bacterial growth. A single well separated colony was identified. Preliminary tests like Grams staining of the colony, Hangingdrop preparation, Catalase test and Cytochrome oxidase test were done. Biochemical tests like Indole test, Methyl red test, Voges proskauer test, Citrate utilization test, Urease test, Triple sugar iron agar, Nitrate reduction test, Hugh-Leifson s oxidation fermentation test, coagulase production (for Staphylococci), Optochin Sensitivity (for *S. pneumoniae*)were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and Dulcitol, inositols were done to identify the isolate. These tests were performed according to standard methods. [13] Antibiotic sensitivity test of the isolates were performed by Kirby Bauer Disc Diffusion method [14]using Mueller Hinton agar and antibiotic discs, as described by Clinical Laboratory Standard Institute (CLSI) guidelines[15] was followed to perform antibiotic sensitivity test . Antibiogram was read, that is zones of inhibition were measured and sensitivities to various antibiotics were determined.

Patients with typical sign and symptoms of COPD with known risk factors were asked to perform spirometry, under ideal conditions and observation of an expert. Diagnosis and staging were ascertained as per GOLD (global initiative for obstructive lung diseases) guideline

for COPD, 2015 .Patients with value of FEV1 / FVC < 7 on spirometry were considered to have COPD. Staging was done as per GOLD guideline, which is based on the value of FEV_1 [28].

RESULTS

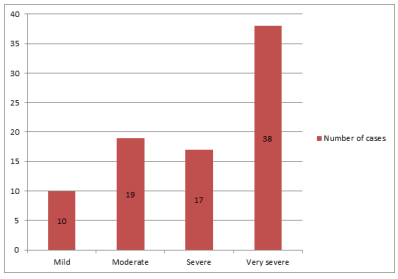
The individual bacterial isolates and spirometric pattern were also recorded in all two hundred (200) patients.

Cases distribution as per severity of stages of COPD was as following-

Table 01 : distribution of cases as per severity of COPD

Stages of severity	No. Of cases
Mild	10
Moderate	19
Severe	17
Very severe	38
Total	**84**

Graph 01 - distribution of cases as per severity of COPD



Out of 84 cases from which positive bacterial culture was obtained 10 / 84 (10.64%), 19/84(20.21%),17/84(18.08%) and 38/84(40.42%) belonged to mild, moderate, severe and very severe grade severity of obstruction, respectively. (Table 01/ Graph 01).

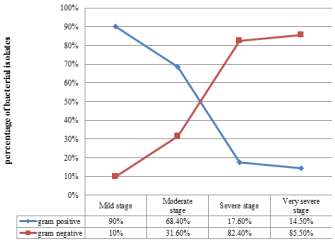
Association of microbial pattern with severity stages of COPD was as following-

TABLE 02 : distribution of isolated bacteria in different severity stages of COPD

	Gram negative bacilli				Gram positive cocci				
Stages of COPD	***K. Pneumoniae***	***P. aeruginosa***	***E.coli***	**Total Gram negative bacilli**	***S.aureus***	***CONS***	***S. pneumoniae***	**Total Gram positive cocci**	**Total**
MILD	1	0	0	1	8	1	0	9	10
MODERATE	5	0	1	6	11	1	1	13	19
SEVERE	11	1	2	14	3	0	0	3	17
VERY SEVERE	23	13	5	41	5	2	0	7	48
TOTAL	40	14	8	62	27	4	1	32	94
Chi Square test was applied, Chi-Square Value = 34.1, P Value = < 0.001									

Gram positive cocci were predominant in mild and moderate stages of COPD and Gram negative bacilli were predominant in severe and very severe stages of COPD COPD . Statistically significant (P Value = < 0.001) association was found between different stages of COPD and different bacterial strain.

Graph 2 - number of bacterial isolates in different severity stages of COPD



In Mild stage of COPD out of 10 isolates grown , 9(90%) were gram positive bacteria. *Staphylococcus aureus* (8/9) was most common organism. Single gram negative bacteria was isolated and it was *Klebsiella pneumoniae*. In moderate stage of COPD total 19 organism isolated. Out of them 13(68.4%) were gram positive bacteria. 6(31.6%) organism were gram negative organism. *Staphylococcus aureus* (11/13)was most common organism . In severe stage of COPD total 17 organism isolated. Out of them 3(17.6%) were gram positive bacteria. 14 (82.4%) organism were gram negative organism. *Klebsiella pneumoniae* (11/14)was most common organism . In very severe stage of COPD total 48 organism isolated. Out of them 7(14.5%) were gram positive bacteria. 41(85.5%) organism were gram negative organism. *Klebsiella pneumoniae* (14/41)was most common organism . Prevalence of Gram positive bacteria were 90%,68.40%,17.60% and 14.50% in mild, moderate, severe and very severe stages of COPD. Prevalence of Gram negative bacteria were 10%, 31.60%, 82.40% and 85.50% in mild, moderate, severe and very severe stages of COPD .

DISCUSSION

In present study bacteriological spectrum was analysed and spirometry was done in 200 AECOPD cases.

Out of 94 patients bacterial isolates 10.64% belonged to mild grade severity of obstruction , 20.21% belonged to moderate grade severity of obstruction , 18.09% belonged to severe grade severity of obstruction and 51.06% belonged to very severe grade severity of obstruction, as per GOLD criteria.

In our study total 10 organisms were isolated in mild stage group. Out of them 9 were gram positive bacteria. *Staphylococcus aureus* 88.88% was most common organism. Other gram positive bacteria isolated was *cougulase negative staphylococcus aureus* 11.11% . Single gram negative bacteria was isolated and it was *klebsiella pneumoniae*. In moderate stage total 19 organisms isolated. Out of them 68.4% were gram positive bacteria. *Staphylococcus aureus* 84.61% was most common organism. One isolate of *streptococcus pneumoniae* and *CONS* each isolated. 31.58% organisms were gram negative organism. 83.33% isolates were *Klebsiella pneumoiae* and 16.67% were *E.coli*. In severe stage 17.65% were gram positive bacteria and 82.35% organism were gram negative organism. *Klebsiella pneumoniae* 78.57% was most common organism . In very severe stage total 48 organisms isolated. Out of them 14.58% were gram positive and 85.41% were gram negative organism. *Klebsiella pneumoniae* 34.14% was most common organism. Similar observation was made by Niederman Michael S.*et al* [27] In their study, 112 patients were evaluated during exacerbation, and sputum culture results were evaluated in relation to lung function . it was observed that Gram negative bacteria were more common than any other class of bacteria during AECOPD, these organism were more common in the patients with the most severe lung dysfunction, where as the Gram positive bacteria predominated in the exacerbations of the patients with the mildest degree of lung function abnormalities[27]. Hallett wilbur Y [25] and Arora N. *et al* [26] also concluded that the frequency of isolating an etiological agent increased with severity stages of COPD. Yusuf Aydemir *et al* [29] revealed in is study that *Pseudomonas aeruginosa, Escherichia coli*, and *Acinetobacter baumannii* were isolated only from very severe stage COPD patients .

CONCLUSION

AECOPD have a major impact on the quality of life of patients with the condition. They are a major cause of hospital admission and health care utilization. Shifting from predominantly gram positive to gram negative isolates were observed with shifting of stages from mild to very severe stage (gram positive and gram negative bacteria were 90 % and 10% in mild stage ,12.77% and 31.60 % in moderate stage, 17.60 % and 82.40 % in severe stage and 7.45 % and 85.50 % in very severe stages of COPD). This knowledge can help physician to start empirical treatment. In future more elaborated similar studies are required, incorporating additional laboratory interpretations with personal, local socioeconomical and epidemiological markers.

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