Original Research Article

Clinical evaluation of bacterial colonization of bronchiectasis

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Abstract

Background: Bronchiectasis is a chronic pulmonary disease characterized by abnormal irreversible dilatation of one or more bronchi often with wall thickening. In Bronchiectasis initial colonization of the lower respiratory tract by different microorganisms as the first step leading to the inflammatory response. Persistence of microorganisms in the airways because of impairment in mucus clearance may lead to a vicious circle of events characterized by chronic bacterial colonization, persistent inflammatory reaction and progressive tissue damage and morbidity life.
Aim of the study: To evaluate the Bronchial inflammatory response and its relationship to bacterial colonization in Bronchiectasis.
Materials and methods: This study was done for a period of 7 months from February 2016 to August 2016 in Department of Thoracic Medicine, Government Villupuram Medical College and Hospital, Villupuram. The Bacterial flora from Lower Respiratory tract of Bronchiectasis patients who attended the Thoracic medicine outpatient department with diagnosis confirmed by a radiologist were studied. Bronchoalveolar lavage was done as an invasive procedure in 90 patients with bronchiectasis and from 6 patients admitted with chronic upper respiratory symptoms as laboratory control in Interleukin-8 estimation.
Results: Among 90 study population, analysis showed females were 58% and males were 42%. Cylindrical bronchiectasis 53%, followed by cystic bronchiectasis 36% in predominance: and positive culture growth rate for sputum samples were 68% and BAL samples were 77%. The microorganisms isolated predominantly were H. influenza, Pseudomonas, Streptococci, Staphylococci, and etc.

Conclusion: Increased incidence of bronchiectasis in females (58%). Cylindrical bronchiectasis was the commonest type followed by Cystic bronchiectasis regarding etiology of Bronchiectasis, 42% of bronchiectasis was Idiopathic followed by post infectious 21%. With the concordant value of 75% for sputum culture and bronchoalveolar lavage fluid culture, the Sputum culture is the non-invasive, alternative technique for bronchoalveolar lavage fluid culture.

Key words

Bronchiectasis, Sputum, Colonization, Bronchoalveolar Lavage Fluid Culture, Interleukin-8.

Introduction

Bronchiectasis is defined as abnormal permanent destruction and dilatation of one or more bronchi, often with wall thickening. Chronic bronchial sepsis has been used to describe the chronic bacterial infection of the impaired mucociliary action leads to microbial infection of the lower respiratory tract that leads to the release of inflammatory mediators [1]. Sepsis is the condition in which bacteremia occurs, whereas this is rare in bronchiectasis because an exuberant immune response confines the infections to the lung. In bronchiectasis, there is chronic inflammation in which lymphocytes predominate in the bronchial wall and Neutrophils in the lumen [2]. Mucus is poorly cleared from the bronchiectasis areas for several reasons. There is pooling in the abnormally dilated airways; ciliated cells are lost when the epithelium is damaged and mucus is less elastic more viscous and forms a vicious cycle [3]. Coles hypothesis explained the vicious cycle in bronchiectasis. In bronchiectasis, impairment of mucociliary clearance due to chronic inflammation and some congenital causes leads to microbial colonization of airways with poor elimination of microbes and secretions, leads to architectural damage leading to stagnation of secretion and this stagnation leads to microbial infections [4]. When chronic bronchial infection occurs with commensal or opportunistic bacteria it reflects the severity of impairment of lung defenses rather than the virulence of organisms. Following microorganisms were classified as PPM.

- Hemophilus influenza and parainfluenza
- Pseudomonas (various strains)
- Klebsiella species
- Staphylococcus aureus
- Moraxella catarrhalis
- Escherichia coli
- Streptococcus pneumoniae
- Proteus species
- Aspergillus species [5].

Materials and methods

This study was done for a period of 7 months from February 2016 to August 2016 in Department of Thoracic Medicine, Government Villupuram Medical College and Hospital, Villupuram. The Bacterial flora from Lower Respiratory tract of Bronchiectasis patients who attended the Thoracic medicine outpatient department with diagnosis confirmed by a radiologist was studied. Bronchoalveolar lavage was done as an invasive procedure in 90 patients with bronchiectasis and from 6 patients admitted with chronic upper respiratory symptoms as laboratory control in Interleukin-8 estimation. Proforma was designed and ethical committee clearance was obtained.

Inclusion criteria

- Patients admitted with HRCT Chest diagnosis of Bronchiectasis (non- cystic fibrosis).
- Age >14 years
- SpO2> 90% in Room air.

Exclusion criteria

- Patients with complications like Hemoptysis, Lung abscess and Amyloidosis.
- Patients with prior hospitalization within 2 months and had antibiotic within 4 weeks.

- Patients with cardiac illness and Recent Myocardial infection
- Patients with Renal failure.

The nature and purpose of the study was explained in detail to all the study Patients and written informed consent was obtained from all of them included in this study. Data collection was done as per the proforma.

Study procedure

Patients admitted with HRCT Chest taken within 3 months and confirmed bronchiectasis were studied with 1.Sputum for a)Modified Zhiel Neelson staining for Acid Fast Bacilli smear in RNTCP Lab b)Bacterial culture and sensitivity in Microbiological Lab c)Gram staining in Microbiology Lab 2.Spirometry to asses Pulmonary Function 3.Broncho alveolar lavage with Fiber optic bronchoscope. Patients admitted with HRCT Chest evidence of Bronchiectasis taken within 3 months duration were evaluated for study after inclusion and exclusion criteria analysis. Informed consent was obtained from all the patients and from parents of patients with age.

Statistical analysis

All the collected data were incorporated into Microsoft sheets, statistical analysis was done with the help of a professional statistician. Bronchoalveolar lavage fluid culture as the gold standard, the BAL fluid culture was analyzed with demographical factors. Fischer exact p-value = < 0.05 was considered as high significant > 0.05 was considered as weak significant.

Results

Among 90 patients, 58% of patients were females and 42% of patients were males 13.3% were alcohol consumers and 4.4% were past alcoholism history and 82 % were non-alcoholic. 12% were smokers and 6.7 were a past smoker and 73% were a nonsmoker. Sputum gram staining reported as the 29 patients sputum samples contained the normal throat commensals and 19% were contained gram-positive cocci and 47% were contained gram-negative bacilli and 3.3% samples were contained both gram-positive cocci and gram-negative bacilli.

Among the 90 patients in the study group starting from 16 years to 75 years; the patients with age 36-45 years were presented in the majority (32%) followed by age group in 26-35 years, followed by 56-65 years, followed by 46-55 years, followed by 16-25 years and lastly 66-75 years (**Table – 1**).

Table – 1: Age distribution.

Age in years	Frequency	Percent (%)
16-25 years	10	11.1
26-35 years	17	18.9
36-45 years	29	32.2
46-55 years	15	16.7
56-65 years	16	17.8
66-75 years	3	3.3
Total	90	100

Among the 90 patients in this study population 57.8% were females and 42.2% were males (**Table – 2**).

Table – 2: Sex distribution.

Sex	Frequency	Percent (%)
Male	38	42.2
Female	52	57.8
Total	90	100.0

Based on the duration of symptoms at the time of admission, a) 65% of patients had symptoms from 1 year to 5 years; and their BAL fluid culture reported as no growth in 25% of patients, 5.6% positive for Non-PPM growth and 69% were positive for PPM growth27% of patients had symptoms more than 5 years maximum up to 30 years; and their BAL fluid culture reported as 88% positive for PPM growth, 8% positive for Non-PPM growth and 4% were no growth.6.6% of patients had symptoms less than 1 year and 83.5% of their BAL fluid cultures were reported as no growth (**Table – 3**).

Among the 90 patients, the sputum gram staining reported as the 29 patients sputum

samples contained the normal throat commensals and 19% were contained gram-positive cocci and 47% were contained gram-negative bacilli and 3.3% samples were contained both gram-positive cocci and gram-negative bacilli (**Table – 4**).

Table - 3: Description of duration of symptoms with bronchoalveolar lavage fluid culture.

Symptom duration	No growth	Percent	Non PPM	Percent	PPM	Percent
< 1 year (N=6/6.6%)	5	83.5%	1	16.5%	0	0.0
1-5 years (N=59/65.4%)	15	25.4%	3	5.6%	41	69%
> 5 years (N=25/27%)	1	3.7%	2	8.3%	22	88%

Table - 4: Sputum gram staining results.

Normal Throat commensal	Gram positive cocci	%	Gram negative bacilli	%	Mixed-both gram +ve and gram -ve	%
29	16	19%	42	47%	3	3.3%

Table - 5: Etiological distribution.

Etiology	Frequency	%
Idiopathic	38	42.2
Post-infectious	19	21.1
Post Pulmonary TB	9	10.0
Congenital (undiagnosed)	7	7.8
MCTD	4	4.4
Rheumatoid arthritis	3	3.3
Kartagener syndrome	2	2.2
Sjogren syndrome	2	2.2
Young's syndrome	1	1.1
Cong. Hypogamaglobulinemia	1	1.1
CVID	1	1.1
Post aspiration	2	2.2
Occupational	1	1.1
Total	90	100

Among 90 patients in this study population, regarding etiology of Bronchiectasis Idiopathic bronchiectasis were 42%, Post infectious bronchiectasis were 21%, Congenital type in which undiagnosed etiology were 7.8% and secondary to connective tissue disease and kartagener syndrome, Common variable immunodeficiency 3% respectively (**Table – 5**).

Out of 90 patients in this study the microorganisms isolated in sputum culture were, Potentially pathogenic microorganisms (PPM) 61%, Non potentially pathogenic microorganisms (Non-PPM) were 6.6% and No growth in 32% of patients. In PPM (potentially pathogenic microorganisms) the following microorganisms were isolated: Hemophilus influenza were 14.4%, Pseudomonas aeruginosa 12%, Streptococcuspneumoniae 10%, Klebsiellapneumoniae 6.7%, Staphylococcusaureus 5.6%, Morexellacatarrhalis 4.4%, Acinetobacter species 3%, Klebsiella oxytacea 2%, Proteus mirabilis 1% and Escherichia coli 1%. The Non PPM isolated were Enterococcus 3.3%, Coagulase negative staphylococcus 2.2%, Streptococcus viridans 1% (**Table – 6**).

Table - 6: Microorganisms distribution in sputum culture.

Microorganism grown (>$10x^5$cfu)	Frequency	%
No Growth	29	32.2
Hemophilus influenza	13	14.4
Pseudomonas aeruginosa	11	12.2
Streptococcus pneumoniae	9	10.0
Klebsiella pneumoniae	6	6.7
Staphylococcus aureus	5	5.6
Moraxella catarrhalis	4	4.4
Acinetobacter species	3	3.3
Klebsiella oxytacea	2	2.2
Proteus mirabilis	1	1.1
Escherichia coli	1	1.1
Enterococcus	3	3.3
Streptococcus viridans	1	1.1
Coagulase negative staphylococcus aureus	2	2.2
Total	90	100

Out of 90 patients in this study, the microorganisms isolated in bronchoalveolar lavage fluid culture were, potentially pathogenic

microorganisms (PPM) 70%, Non potentially pathogenic microorganisms (Non-PPM) were 6.6% and No growth in 23%. In PPM, the following microorganisms were isolated. The Non PPM isolated were Enterococcus 2.2%, Coagulase negative staphylococcus 2.2%, Streptococcus viridans 2.2%. In 9% of patients, two different pathogenic bacteria were isolated i.e. Pseudomonas with Acinetobacter and Hemophilus influenza and Coagulase negative Staphylococcus aureus (**Table – 7**).

Table - 7: Distribution of the microorganisms in Bronchoalveolar lavage fluid ($10x^3$ cfu).

Microorganism has grown	Frequency	%
No Growth	21	23.3
Hemophilus influenza	15	16.7
Pseudomonas aeruginosa	14	15.6
Streptococcus pneumoniae	9	10.0
Klebsiella pneumoniae	4	4.4
Staphylococcus aureus	2	2.2
Moraxella catarrhalis	4	4.4
Acinetobacter species	2	2.2
Klebsiella oxytacea	3	3.3
Proteus mirabilis	1	1.1
Escherichia coli	1	1.1
Coagulase negative staphylococcus aureus	2	2.2
Enterococcus	2	2.2
Streptococcus viridans	2	2.2
Hemophilus parainfluenza + MRSA	1	1.1
MRSA + Acinetobacter species	2	2.2
H.influenza + Pseudomonas aeruginosa	1	1.1
Staph.aureus + Pseudomonas aeruginosa	2	2.2
CONS + Pseudomonas aeuruginosa	2	2.2
Total	90	100

Table - 8: Analysis of total cell counts in bronchoalveolar lavage fluid.

Growth pattern	No. of patients	Mean cell Count (x103)	SD
PPM	63	154.92	41.246
Non PPM	6	111.67	54.924
No growth	21	55.38	15.167
Total	90	128.81	56.400

Table - 9: Interleukin 8 levels in bronchoalveolar lavage fluid.

Growth type	No. of patients	IL-8 in Range pg/ml	Mean	SD
PPM	63	220 -835	556.95	133.604
Non PPM	6	220-770	651.67	222.478
No growth	21	81-320	155.81	83.816
Total	90	81-835	463.67	214.394
Control	6	2-7	5	

Difference between groups by ANOVA, p=0.0001. Out of 90 patients in this study, the Total cell counts calculated in bronchoalveolar lavage fluid were listed as below. In PPM patients: 90-220x10^3 cells in range and 120 x10^3 cells in the median; In Non-PPM patients were 50-220x10^3 cells in range and the median cell counts were 90x10^3 cells and in no growth patients were 40-180x10^3 cells and 50x10^3 cells in the median (**Table – 8**).

Out of 90 patients in this study, the interleukin 8 estimated were 1. In PPM patients 220-835pg/ml in range and 556 pg/ml in mean: in Non PPM patients were 220-770 pg/ml and 561pg/ml in mean and in No growth patients were 81-320pg/ml and 155pg/ml in mean. Difference between groups by ANOVA, p=0.0001 (**Table – 9**).

Discussion

The microorganisms from the airways of clinically stable bronchiectasis patients were isolated by sputum culture and bronchoalveolar lavage fluid culture. The BAL fluid culture (77% positive growth rate) was slightly higher than sputum positive culture (68%) [6]. The microorganisms isolated from sputum and BAL culture were H. influenza (17%), Pseudomonas species (16%), Streptococcus pneumoniae (10%), Moraxella, Klebsiella, and Staph aureus were 3-4% respectively as similar to previous studies [7]. By comparing the sputum and BAL fluid culture, among the 61 (68%) positive culture in sputum samples; 56 samples were positive for PPM growth and 5 samples were positive for Non-PPM growth in BAL fluid

culture. Among the 29 negative cultures in sputum samples - 7 samples were positive for PPM growth and 1sample was positive for Non-PPM growth and 21samples were found to be No growth in BAL fluid culture-with k coefficiency of 75% [8]. Hill SL, et al. study; the positive sputum culture was observed in 66% of patients, in BAL-positive culture was observed in 79% of patients; and both techniques agreed on the same positive growth in 30 patients /60 cases, and agreed on negative growth in 18 patients /60 cases with concordant value of 75% for both techniques; this observation in this study fully matched with these studies [9]. Hilton AM, et al. evaluated the increased colonization in age groups > 60 years, patients with smoking and alcoholism, in cystic bronchiectasis; and observed the inverse relationship between the airway colonization and pulmonary function more colonization leads to poor pulmonary function, and similar findings were evaluated in this study. By keeping the Bronchoalveolar lavage fluid culture as gold standard the following analysis was done [10]. The increased culture positive was observed in the age group 46-55 years (80%), 36-45 years (75%) and 26-35 years (65%). The people in the age group between 36-55 years were presented with long duration of symptom i.e. more than 5 years and up to 30 years duration -co-relating the long duration of symptoms, the microorganisms colonization was high. The females in this study were 52(58%) in comparison with males, the negative cultures observed in females were 12 /21 no growths, in contrast, males were 9/21 no growths [11]. Regarding the personal habits like smoking and alcoholism 17 patients (19%) were used to smoking and alcohol; the No growths observed in 18(25%) patients who were never smoker and never alcohol consumers and in 75% of smokers and alcoholics PPM and Non PPM growths were observed - co-relating that smoking and alcoholism increases the microorganisms colonization [12]. The total counts measured were $1086x10^3$ cells (maximum), and $100x10^3$-$1086x10^3$ cells (in range) with Neutrophilic predominance in 66% of patients and Interleukin-8 level measured were 120-5520 pg/ml (in range) and 195 pg/ml in median were measured in PPM positive group; whereas in the control group, total counts measured were $110x10^3$ cells with Neutrophilic pattern in 1% and the interleukin-8 measured were 0-32pg/ml (in range) - indicating the increased level of inflammation indirect proportion with microbial colonization [13]. Regarding the total count measured in BAL fluid; there was the increased amount of inflammatory cells in the airways of bronchiectasis in all the three groups i.e. in PPM, Non-PPM, and No growth patients. The mean cell counts observed were $120x10^3$ cells for PPM patients, $90x10^3$cells in Non-PPM patients and $50x10^3$ cells in No growth patients. The maximum cell counts measured were $220x10^3$ cells in PPM growth patients [14]. The least cell counts measured were $40x10^3$ cells in No growth patients. Regarding the differential cell count: Out of 90 patients in 44 patients (49%) the Neutrophils more than 50% of total cell count were measured, and in 40 patients (44%) the Lymphocytes more than 50% of total cell counts were measured and in 6 patients (7%) equal proportions of neutrophils and lymphocytes were measured [15]. In bronchiectasis airways more cellularity was measured and there was slight Neutrophilic pattern followed by lymphocytic pattern. The more number of neutrophils leads to the liberation of elastase, proteinase, tumor necrosis factor alpha, and cytokines predominantly interleukins-8, and this interleukin-8 causes neutrophilic chemotaxis in airways and the net result of more airway inflammation and more tissue damage. In this study, interleukin-8 was measured from 6 normal people as a control group and the values in range 2 - 7 pg/dl and 5pg/dl in the mean [16]. The mean values measured were 556.95pg/dl in PPM, 561.67 pg/dl in Non-PPM, and 155.81pg/dl in No growth patients respectively. The highest level of IL-8 835 pg/dl was measured in PPM group and the lowest value of IL-8, 81pg/dl was measured in patients with No growth sample [17]. The total range of IL-8 among the 90 patients was 81-835pg/dl. With this, the interleukin 8 was grossly raised in the airways of

bronchiectasis patients irrespective of the microorganisms colonization, but the peak of raise of IL-8 was higher in patients with microorganisms colonization especially in potentially pathogenic microorganisms colonization -indicating the earlier establishment of airway inflammation and the airway inflammation was worsened by the microorganisms colonization especially the potentially pathogenic microorganisms [18, 19, 20].

Conclusion

We found increased incidence of bronchiectasis in females (58%). Cylindrical bronchiectasis was the commonest type followed by Cystic bronchiectasis. Regarding etiology of Bronchiectasis, 42% of bronchiectasis was Idiopathic followed by post infectious 21%. With the concordant value of 75% for sputum culture and bronchoalveolar lavage fluid culture, the Sputum culture is the non-invasive, alternative technique for bronchoalveolar lavage fluid culture.

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