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Microbiological Pattern and Risk Factors of Bacterial Colonization in Patients with Clinically Stable Bronchiectasis

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ABSTRACT

Background: Bronchiectasis, dilatation of bronchi with diameter more than 2 mm is a septic and inflammatory process of the lung, caused by infections and systemic or local defense abnormalities of tracheobronchial tree that may lead to destruction of bronchial wall. Infections usually cause inflammatory reaction and destruction of bronchial wall, this further leads to more disturbance in local defense and a vicious cycle of inflammation and bacterial colonization occurs. These bacteria divided to Potentially Pathogenic Microorganism (PPM) or non-PPM. The purpose of this study was to find microbiologic pattern and associated (risk) factors in Iranian population and use of more narrow spectrum antibiotics.

Materials and Methods: Forty patients with proven diagnosis of bronchiectasis by HRCT in a clinically stable condition fulfilled the inclusion criteria. Fiberoptic bronchoscopy was performed just after spirometry and BAL sampling was achieved. Cut off point of 10000 CFU was considered for positivity of culture media.

Results: *S. pneumoniae* was the predominant pathogen. There was 85% rate of colonization by PPM. We found FEV1 < 80% and FVC < 80% as risk factors for bacterial colonization by PPM and *S. pneumoniae*. Age of diagnosis < 20 years was the additional risk factor for colonization of *S. pneumoniae*. Cystic bronchiectasis was predominant type of lesion and was more common in women.

Conclusion: We have found some differences regarding the rate of colonization, number of patients with airflow limitation, and the predominant pathogen as compared with Western societies. (*Tanaffos* 2004; 3(9): 53-59)

Key Words: Bronchiectasis, Bacterial colonization, Potentially Pathogenic Microorganism (PPM), BAL

INTRODUCTION

The lower respiratory tract of the healthy non-smoker is usually sterile, whereas in pulmonary disease such as Chronic Obstructive Pulmonary Disease (COPD) (up to 83 %), bronchogenic carcinoma (up to 42 %), and bronchiectasis (up to

88%) colonization by Potential Pathogenic Microorganisms (PPM) occurs (1). Some evidences suggest colonization by PPM or non-PPM may be harmful to patients with bronchiectasis (2). The phenomenon of chronic colonization, the ensuing inflammatory reaction and progressive bronchial wall damage is a vicious cycle. There is a mutual

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relationship between colonization by PPM and air flow limitation (i.e.; obstruction). In other words, the more severe air flow limitation the more chance of colonization and release of free radicals and proteolytic and inflammatory mediators of microorganism and non-immune defense system, thence more inflammation and air flow limitation (2). Bacterial colonization in bronchiectatic patients is divided into two groups: 1) Potentially pathogenic microorganisms (PPM) which include: *Hemophilus spp*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *staphylococcus aureus*, *Klebsiella spp*, *Moraxella*, *Nocardia spp.*, *Eschericia coli* and *Streptococcus pyogenes*. This group could produce symptomatic pulmonary infection. 2) Non-PPM including *Streptococcus viridans*, *Enterobacter*, *Corynebacterium*, *Neisseria spp* and *Staphylococcus* (coagulase negative). These organisms could not cause pulmonary infection in immunocompetent persons (1,2,3).

In order to obtain microbiological samples, some methods such as gram staining of sputum, Broncho-Alveolar Lavage (BAL) and Protected Specimen Brushing (PSB) have been developed. Sputum examination is limited by contamination of upper airway flora and lack of excellent negative predictive value (about 66%) (2). By cut off points of 1000 CFU and 100 CFU for BAL and PSB respectively, it appears that these methods are quite acceptable for microbiological studies of lower respiratory tract without contamination (2,3). In addition, BAL simultaneously assess inflammatory process in local milieu (3). *Hemophilus spp.*, *Pseudomonas spp.*, and *staphylococci* are the leading microorganisms according to the previous studies (1,2,3,4,5,6).

The purpose of the study was first to analyze bacterial flora by BAL to tailor antibiotic regimen, and then to analyze potential risk factors (associated factors) with colonization by PPM.

MATERIALS AND METHODS

This descriptive case series study was performed between November 2002 and November 2003 at Imam Khomeini Hospital in Sari, north of Iran. During this period, a total of fifty-five patients were diagnosed as bronchiectasis by HRCT criteria, including presence of cystic lesions with or without air fluid, tram lines, and signet sign or visibility of bronchi within 1 cm of periphery. Forty patients have met the inclusion criteria. HRCT was scored by scale of 0-3 (0= no, 1= involvement of one segment, 2= involvement of two segments, 3= involvement of entire lobe). Each lung was divided to three lobes. Clinically stable condition was defined by the absence of criteria of exacerbation according to O'Donnell criteria (Table 1)(7).

Table 1. Symptoms of acute exacerbation of bronchiectasis *

| |
|--|
| Change in sputum production |
| Increased dyspnea |
| Increased cough |
| Fever (temperature, > 38.0°C) |
| Increased wheezing |
| Malaise, fatigue, lethargy, or decreased exercise tolerance |
| Reduced pulmonary function |
| Radiographic changes consistent with a new pulmonary process |
| Changes in chest sounds |

* In a study by O'Donnell et al. A patient with four of these symptoms was defined as having an acute exacerbation.

Exclusion criteria were admission within the last two months and prior use of antibiotics within the last one month. An informed consent was completed for each patient. The study protocol was approved by the ethics committee of Mazandaran University of Medical Sciences. A questionnaire was completed and the patients underwent spirometry (Fukuda ST-90 Japan) just before fiberoptic bronchoscopy. After local sedation by lidocaine, a fiberoptic bronchoscope (FOB) was passed transnasally with minimal suctioning. After wedging FOB into the most involved segment, BAL was taken with three

aliquots of 50ml saline (for irrigation). BAL fluid was sent to the laboratory within 30 minutes and gram staining was achieved. Then BAL fluid was homogenized and undiluted as well as serial diluted specimens (1/10, 1/100, 1/1000), which plated on blood, chocolate, Wilkins-Chalgren, sabouraud agar and Lowenstein culture media. The specimen was streaked directly on the surface of a chocolate agar, then incubated aerobically in an atmosphere of 10% CO₂. Meanwhile, Mueller- Hinton agar and impregnated strips, containing factor X and V for identifying *H. Influenza* were used. The culture media were evaluated for growth after 24 and 48 hours. Negative culture for bacterial and fungal growth were discarded after 5 days and 4 weeks, respectively; however, negative Lowenstein media were discarded after 6 weeks. Susceptibility testing was performed using the broth microdilution or β -lactamase test classified as sensitive, intermediate or resistant according to the criteria (8). Cultures were considered positive with cut off point of 10000 CFU. This cut off point was chosen instead of 1000 CFU, because some references believed that, it is more precise and can eliminate any chances of contamination (1,2,3). The microorganisms were divided to PPM and non-PPM. Results were analyzed by Chi-square tests or Fisher exact tests appropriately (for categorical variables) and Mann- Whitney test (for continuous variable). All reported p-values were two tailed and the level of significance was 5%.

RESULTS

The study population included 26 females and 14 males, with the mean age (\pm standard deviation) of 44 \pm 16 years. of these, 32 (80%) have had bronchiectasis of cystic-varicose type. The demographic and baseline features of the studies patients are presented in table 2.

As shown in table 3 there was 85% rate of colonization by PPM. *Streptococcus pneumoniae* was the leading microorganism isolated in respiratory samples of the subjects, followed by *klebsiella*

pneumonia. Meanwhile, there was no positive culture for mycobacterium.

As table 4 shows FEV1 and FVC of PPM group are significantly lower than non-PPM group ($p=0.003$ and $p= 0.005$, respectively). While, FEV1/FVC and HRCT scores have shown no statistically significant difference. Meanwhile, cystic lesions on HRCT were more common in females than males (OR= 1.59, 95% CI= 0.72- 2.58, P= 0.014).

Table 5 shows risk factors associated with airway colonization by PPMs and *S. pneumoniae*. FEV1<80% and FVC< 80% are the common risk factors found with colonization of PPM and *S. Pneumoniae* by univariate analysis ($p=0.013$, $p=0.033$). Table 5 shows risk factors associated with colonization by *S. pneumoniae* which include age of diagnosis<20 years ($p= 0.004$, OR= 5.75, CI= 1.38-23.88), FEV1< 80% and FVC< 80% ($p= 0.033$).

Table 2. Baseline characteristics of 40 patients with clinically stable bronchiectasis.

| Characteristics | Values * | Range |
|--------------------------|-----------|----------|
| Mean (SD) age (years) | 44 (16) | 17-82 |
| Sex | | |
| Women | 26 (65%) | |
| Men | 14 (35%) | |
| Smoking history | | |
| Smoker | 4 (10%) | |
| Non-smoker | 36 (90%) | |
| Hemoptysis | 9(22.5%) | |
| Age of diagnosis | | |
| <20 years | 10 (25%) | |
| >20 years | 30 (75%) | |
| Mean (SD) HRCT score (%) | 19 (20) | 6-100 |
| Type of bronchiectasis | | |
| Cylindrical | 8 (20%) | |
| Cystic-varicose | 32 (80%) | |
| Forced spirometry | | |
| FEV1 (L) | 1.7 (0.7) | 0.96-4.0 |
| FEV 1(%) | 57 (17) | 27-90 |
| FVC (L) | 2.3 (0.7) | 1.1-4.5 |
| FVC (%) | 63 (16) | 30-90 |
| FEV1/FVC (%) | 76 (12) | 52-97 |

* Values are expressed as n(%) for qualitative variables and mean (SD) for quantitative variables.

HRCT= high resolution CT Scan; FEV1= forced expiratory volume in 1 Second; FVC = forced vital capacity.

Table 3. Microorganism isolated in respiratory samples of 40 patients with clinically stable bronchiectasis using BAL

| | N (%) |
|-------------------------|----------|
| PPMs * | 34 (85) |
| Streptococcus pneumonia | 14 (35) |
| Staphylococcus aureus | 2 (5) |
| Pseudomonas aeruginosa | 7(17.5) |
| Hemophilus influenza | 3 (7.5) |
| Klebsiella pneumonia | 9 (22.5) |
| Nocardia (spp) | 1 (2.5) |
| Non-PPMs | |
| Streptococcus Viridance | 3 (7.5) |
| Neisseria (spp) | 3 (7.5) |

PPM: Potential pathogenic microorganism

BAL: Broncho-alveolar Lavage

Table 4. Forced spirometric measurement and HRCT score in patients with and without PPMs airway colonization.

| | PPMs | non - PPMs | p-value |
|--------------|---------|------------|---------|
| FEV1 (%) | 54(16) | 82 (7) | 0.003 |
| FVC (%) | 60 (15) | 84 (7) | 0.005 |
| FEV1/FVC (%) | 75 (13) | 76 (11) | NS |
| HRCT score | 19 (10) | 19 (11) | NS |

NS= Not Significant.

Table 5. Risk factors associated with airway colonization by PPMs and *S. pneumonia*

| | PPMs | | <i>S. pneumonia</i> | |
|----------------------------|---------|-----------------|---------------------|----------------|
| | p-value | OR (95%CI) | p- value | OR (95%CI) |
| Univariate analysis | | | | |
| Smoking status | NS | | NS | |
| Hemoptysis | NS | | NS | |
| Age of onset disease | NS | | 0.004 | 5.7 (1.3-23.8) |
| Type of bronchiectasis | NS | | NS | |
| HRCT score | NS | | NS | |
| FEV1<80% predicted | 0.013 | 3.8(0.69-20.8) | 0.033 | 4(0.7-21.8) |
| FVC<80% predicated | 0.013 | 3.8 (0.69-20.8) | 0.033 | 4 (0.7-21.8) |
| FEV1/FVC < 70% predicated | NS | | NS | |

OR= odds ratio; CI= Confidence interval. OR is shown only in variables which were significant in univariate analysis.

DISCUSSION

This study shows a high rate (85%) of bacterial colonization by PPM in our patients. It is important to know that if we chose cut off point of 1000 CFU instead of 10000 CFU, this percent may be even higher. The rate of colonization by PPM was 85%, whereas in the study performed by Pang et al. was 81%, (4). Angrill showed the rates of 48% and 55% in two different studies (2,3). The predominant pathogens in several studies were *Hemophilus* (typable or non-typable) (29-42%) (2,3), *Pseudomonas aeruginosa* (13-31%) (4) and

S. pneumoniae (6-13%)(5,6). Our study showed *S. pneumoniae* was the predominant pathogen. The most common form of bronchiectasis according to HRCT findings was cylindrical in west Europe (73-76%)(2,3), whereas cystic type was the most common form in our study (80%), specially in female patients. Air flow limitation as defined by FEV1<80% of predicted values was present in 88% of our patients although 49% of patients in Angrill study had FEV1< 80% (2). This is consistent with higher rate of colonization in our population. Angrill

showed the results of BAL provides no additional information to PSB (2). It seems that 100 CFU and 1000 CFU by PSB and BAL respectively have equivalent efficacy for obtaining microbiological samples (2). In the studies carried out by Angrill, he found *Hemophilus* (non-typable) and *H.influenza*, whereas we selected 10000 CFU by BAL instead of 1000 CFU, for ruling out any contamination in the route of FOB. In 1989, Pang et al. used 10000 CFU by BAL, they found *Pseudomonas* as the predominant pathogen (4). The rate of colonization, dominant type of bronchiectasis, the number of patients with airflow limitation (i.e.; FEV1< 80%) and the predominant pathogen in study was different from those of Europe. Factors such as smoking habitus, previous use of antibiotics, the severity of airflow limitation and predominant type of bronchiectasis may explain these differences (1,2,9). Meanwhile, some believe that there is no correlation between the change in strain and either the occurrence of exacerbation or the use of antibiotic therapy (6). The acquisition and clearance of a strain is a complex, dynamic process involving host factors and receptor site on the organism that may help the ability of an organism to persist or damage airways (10). The clinical significance of non-PPM colonizations is controversial (2), although Angrill showed greater amounts of inflammatory mediators in BAL fluid of non-PPM colonizers in comparison to healthy group (3). Furthermore, he showed more severe inflammatory response in PPM than non-PPM.

Among these mediators are elastase, MPO, IL8, TNF α and increased numbers of neutrophils (3). Due to presence of much more severe inflammatory reaction in bronchi of patients colonized by PPM than non-PPM and the controversial role of non-PPM, it seems that routine use of antibiotics to eradicate them is not justified. It is not exactly known whether bacterial colonization specially with PPM is

associated with more rapid deterioration in air flow limitation or inflammatory reaction and airflow limitation leading to further impairment of mucociliary function and hence colonization by PPM (cause or effect?) or both of them. Colonizing bacteria seem to act as an inflammatory stimulus, since the greater the inflammatory response the more airflow limitation.

Increased rate of colonization in our study is partly due to predominance of cystic bronchiectasis, which represents the most severe form of injury (6), more inflammation and probably more bacterial load and increased numbers of patients with airflow limitation.

Angrill found age of diagnosis<14 years, type of bronchiectasis (cystic-varicose) and FEV1<80% as risk factors for colonization of bronchi with PPM (2). We only found FEV1<80% and FVC<80% as risk factors. There was not significant correlation with age of diagnosis <20 years and type of bronchiectasis with PPM colonization in our patients. This may be due to lower number of patients with age of diagnosis<20 years in our study (22.5%), whereas in Angrill's study age of diagnosis<14 makes 54% of population (2). Although some authors suggested more inflammatory reaction and airflow limitation in patients colonized with *Pseudomonas aeruginosa* (9,11), Angrill did not find any correlation (2). He attributed lack of any correlation with a specific microorganism to low number of cases of *Pseudomonas* colonization in his study (2). We found FEV1 <80%, FVC<80% and age of diagnosis<20 years as risk (associated) factors of bacterial colonization with *S.pneumoniae*. This is partly due to dominant numbers of the organism in our samples.

We have concluded that FEV1<80% and FVC<80% may be considered as the risk factors of colonization by PPM and *S. pneumoniae* as the predominant microorganism may be more colonized in patients in whom the disease was commenced before the age of 20 years. In addition cystic lesions are more common in our female patients.

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