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ASSESMENT OF AIRWAY INFLAMMATION IN BRONCHIECTASIS

Medicine

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ABSTRACT

INTRODUCTION: In Bronchiectasis initial colonization of the lower respiratory tract by different microorganisms as the first step leading to inflammatory response characterized by neutrophil migration within the airways and secondary secretion of variety of tissue damaging oxidants and enzymes such as neutrophil elastase, myeloperoxidase and cytokines [interleukins] . 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.

In order to evaluate the level of bronchial inflammation in relation with bacterial colonisation; we did bronchoalveolor lavage of patients admitted with HRCT Chest[taken within 3 months] evidence of bronchiectasis and fulfill with inclusion criteria of study. The obtained sample was centrifuged and stored in 2 to 8'C temperature for IL-8 estimation

METHOD OF STUDY: Continuous prospective study

RESULTS: Among 90 study population analysis showed females were 58% and males were 42%. Cylindrical bronchiectasis 53%, followed by cystic bronchiectasis36% in predominance: and positive culture growth rate for sputum samples were 68% and BAL samples were 77%. Regarding airway inflammation, the total counts of BAL fluid were increased in all the patients with slight higher level in positive culture growth patients with slight Neutrophilic predominance. [90-210X10*3 cells in range and 120x10*3 cells in median]. The IL-8 measured showed increased level in all three groups comparing control groups indicating earlier establishment of inflammation in bronchiectasis. [81-835pg/dl in range and 556pg/dl in median]

KEYWORDS

Bronchiectasis, Bacterial colonisation, Inflammation.

INTRODUCTION:

Bronchion-Greek word, means wind pipe ,ektasis-means stretching out.[1]. Bronchiectasis is a disease in which patients spends morbid life having dyspnoea and productive, often foul smelling sputum which produces social isolation and depressive states.In European respiratory journal april-2009 titled as mortality rate in bronchiectasis patients[2] , states the mortality rate of bronchiectasis in 12 year follow up period is 29.7% in the age group at 52 2 years. 70% cause of death in bronchiectasis is due to respiratory tract infection leading to respiratory failure[2,4]. The acute exacerbation of bronchiectasis is mainly due to growth of micro organisms and newer therapies like hypertonic solution like mannitol, Normal saline (up to maximum 7%) nebulisation and inhaled ciprofloxacin, gentamycin are under trial in reducing the exacerbation. By eliminating the micro organisms in bronchiectasis, deterioration of lung function can be reduced.

DEFINITION:

Bronchiectasis is defined as abnormal permanent destruction and dilatation of one or more bronchi, often with wall thickening [1,3,7]

PATHOPHYSIOLOGY:

Chronic bronchial sepsis has been used to describe the chronic bacterial infection of the impaired mucociliary action leads to microbial infection of lower respiratory tract that leads to release of inflammatory mediators. Sepsis is the condition in which bacteraemia occurs, where as this is rare in bronchiectasis because an exuberant immune response confines the infections to the lung.[3,8,9,10] In bronchiectasis, there is chronic inflammation in which lymphocytes predominate in the bronchial wall and Neutrophils in the lumen. Mucus is poorly cleared from the bronchiectatic areas for several reasons. There is pooling in the abnormal dilated airways; ciliated cells are lost when the epithelium is damaged and mucus is less elastic more viscous and forms a vicious cycle.[3,7,18,19] . 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 colonisation 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 [1].

AIRWAY INFLAMMATION IN BRONCHIECTASIS

In this study, inflammation of airway was studied mainly based on the following two studies.

1. Joaquim Angrill et al study in .ATS-volume 164.issue 9, Nov-1-2001
2. David A Bergin .Journal of inflammation research 2013:6 1-11

David A Bergin et al study did Interleukin[4, 10] and cell counts in 15 microlitre of BAL fluid at 405nm, from 45 bronchiectasis patients and the values were as below.

Total cell counts- median cell count was 23x103 cells and 10-1086x103 cells in range from Non colonising patients to potentially pathogenic micro organisms growth patients; and the Neutrophilic pattern was observed in 67(in median) 56-88 (in range) patients. Macrophage pattern was observed in 27(median) 8-38(in range)[11,12,20.]

Interleukin 4: 28.3pg/ml (in median) against the control 2.12pg/ml with p value =0.9. Interleukin 10: 151.3pg/ml (in median) against control 0.1pg/ml with p value =0.05.11,12,20

Jouquim Angrill et al study also evaluated the bronchial inflammation and the values were as below.

Total count: in control group- 1.1x105 cells in median in Non colonised group- 24.75x105 cells in median in PPM growth group - 22x105 cells in median[11,12,20]

Interleukin-8: in control - 0-31pg/ml in range. 195pg/ml in PPM group (median) and 0-5520pg/ml in range with p value =0.00111,12,20.

Above studies states that Non colonised patients showed a more intense bronchial inflammatory reaction than did control subjects. This inflammatory reaction was exaggerated in patients colonised by micro organisms with potential pathogenicity, with a clear relationship the bronchial bacterial load and showed that patients with bronchiectasis in a stable clinical condition present an active neutrophilic inflammation in the airways that is exaggerated by the presence of MPP, and the higher the bacterial load the more intense the inflammation.[23-26]

RISK FACTORS FOR BACTERIAL COLONISATION

J.Angrill et al study states that, the people with normal pulmonary function i.e. with FEV1> 80% were present in n=24 of the study population. FEV1< 65% was observed in n=11 of the study population and the later group had higher BAL level of interleukin 1beta (115pg/ml), TNF-alpha (41pg/ml), IL-10 (7.3pg/ml) suggesting the relationship between the level of inflammation and poor pulmonary function. A significant direct relationship between the bacterial load and the Neutrophils and Interleukin-8 was observed i.e. more bacterial

load leads to more inflammation[11,12,20,29,30]

C Agusti study et al states that following factors were associated with increased risk of microbial colonisation.

1. presence of chronic expectoration[long duration of symptoms]
2. evidence of cystic and varicose bronchiectasis in HRCT scan
3. diagnosis of bronchiectasis < 14 years of age
4. presence of sinusitis and lung function FEV1< 80%.

Smoking increases the bacterial infections of Respiratory Tract; by inhibition of Monocytes,Macrophages and Dendritic cells activity [innate immunity], in smokers immunoglobulin E will be increased with lowered level of immunoglobulin G[altered adaptive immunity]and Impairment of ciliary functions.[31]

INTERLEUKINS -8

Interleukin-8 (IL-8) is a member of a family of structurally-related low molecular weight proinflammatory factors known as chemokines. IL-8 is produced by stimulated monocytes but not by tissue macrophages and Tlymphocytes.[1,3,7,11]. IL-8 is a non-glycosylated protein of 8 kDa (72 amino acids). It is produced by processing of a precursor protein of 99 amino acids. Eventhough different interleukins were liberated into the site of inflammation, IL-8 which is mainly responsible for Neutrophilic chemotaxis in bronchiectasis.[1,3,7,11], Previous studies stated that Broncho alveolar lavage fluid level of IL-8 was significantly raised in Bronchiectasis in comparison with serum levels of IL-8; and it showed the inflammation was compartmentalised[11,12,20]

STUDY DESIGN This is prospective continuous study to evaluate 1.To assess the airway inflammation .Broncho alveolar lavage was done as invasive procedure in 90 patients with bronchiectasis. Patients were selected randomly

STUDY PERIOD This study was done for a period of 7 months from February 2014 to August 2014 STUDY POPULATION: 90 patients STUDY CENTRE This study was done in Rajiv Gandhi Govt General Hospital, Madras Medical College, Chennai-03

MATERIALS AND METHODS:

Proforma was designed and ethical committee clearance was obtained. 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

METHODOLOGY:

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

Pulmonary function test: Spirometry was performed using a computerized Easy one spirometer.Patient was made to sit or erect and asked to wear a nose clip and spirometry was performed fulfilling the acceptability and reproducibility criteria according to American thoracic society recommendation. The parameters measured in spirometry include Forced Vital Capacity (FVC), Forced expiratory volume in 1 second (FEV1), ratio of FEV1 to FVC (FEV1%). Lung function impairments were classified as 1.Normal spirometry: FEV1 and FVC>80% and FEV1/FVC>70% predicted. 2.Obstructive pattern: FEV170% predicted. Mixed pattern: FEV1 and FVC<80% and FEV1/FVC ratio <70%.

The Bronchoalveolar lavage was done on the next day of admission. Topical anaesthesia was achieved by nebulisation of 8ml of 5%Lignocaine for 15 minutes. Bronchoalveolar lavage was done with 150 ml of 0.9%Normal saline divided into three alliquots each consists of 50 ml. Broncho alveolar lavage was done with Pentax 18P Fiber optic bronchoscope [pentax india private ltd] from Bronchiectasis affected part of lung which was identified as per HRCT Chest. About 60-70% of administered fluid is aspirated. The obtained fluid was mixed well in a single sterile container and patients were managed with standard treatment protocol.Obtaied fluid was centrifuged and stored in Temperature 2 to 4 degree Celsius for IL-8 estimation. After obtaining 90 samples from Bronchiectasis patients and six samples from patients presented with upper respiratory tract symptoms [as laboratory control] ; all the centrifuged and stored samples were submitted for Interleukins estimation in National Institute of Research in Tuberculosis[NIRT] Chennai by Elisa method with RayBio Human IL-8 ELISA[Enzyme linked immunosorbent assay] Kit.

STATISTICALANALYSIS:

All the collected data's were incorporated into microsoft XL sheets, statistical analysis was done with the help of a professional statistician. Broncho alveolar lavage fluid cell count (total &differential) measured and values were analysed with demographical factors including Interleukin-8 with Fischer scale.

Fischer exact p value =< 0.05 is considered as high significant
> 0.05 is considered as weak significan

Study Analysis:

Among 90patients, 58% 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 past smoker and 73% were non smoker Regarding the pulmonary function measured by FEV1% in Spirometry; normal spirometry measured in 14% of patients, obstructive pattern measured in 64% of patients [with FEV1- 70-79%= 11% of patients, with FEV1 50-69%= 49.6% of patients and with FEV1 <50% =4.4% of patients]. Restrictive pattern was observed in 15% of patients [with FEV1<70 in 3 patients and with FEV1<50% in 11 patients] and Mixed pattern [both obstruction+restriction] was observed 7% of patients. In comparison of broncho alveolar lavage fluid culture with smoking history, in 72% of smokers &87% alcohol consumer PPM growths were observed increased cell counts and in 25% of non smokers&13% Non alcoholic no growth was observed with significantly less cell count Regarding Radiological types: Cylindrical types were 53%,(associated with 75% positive growth) : Cystic types were 35%, (associated with 84% positive growth) Varicose types were 4.4% , Traction bronchiectasis were 3% and 3% were mixed types i.e. Cystic plus cylindrical and Traction plus cylindrical Out of 90 patients in this study, the Total cell counts calculated in broncho alveolar lavage fluid were listed as below. In PPM patients: 90-220x103 cells in range and 120 x103 cells in median;In Non PPM patients were 50-220x103 cells in range and the median cell counts were 90x103 cells and in no growth patients were 40-180x103 cells and 50x103 cells in median Among the 90 patients in this study population the differential cell counts calculated in broncho alveolar lavage fluid were 1.Neutrophils >50% of total count in 44 patients , Lymphocytes >50% of total counts in 40 patients and in 6 patients both Neutrophils and Lymphocytes were in equal proportion. Out of 90 patients in this study the interleukin 8 estimated were 1.in PPM patients 220-835pg/ml in range and 556pg/ml in mean: in Non PPM patients were 220-770pg/ml and 561pg/ml in mean and in No growth patients were 81-320pg/ml and 155pg/ml in mean.

Regarding the micro organisms isolated in sputum and broncho alveolar lavage culture, Positive sputum culture growths were 68%, negative culture growths were 32%: In Broncho alveolar lavage fluid culture, positive culture growths were 77% and negative culture growths were 23%. Regarding inflammation of airways in bronchiectasis, the total counts were raised in potentially pathogenic micro organisms grown patients followed by nonpotentially

pathogenic micro organisms grown and lesser amounts in patients with no growth. The differential cell count Neutophils more than 50% of total count was observed in 44 patients ,Lymphocytes more than 50% were observed in 40 patients and both Neutrophils and Lymphocytes in equal portion in 6 patients indicating slight increase of Neutrophilic pattern followed by Lymphocytic pattern.

The interleukin-8 measured in BAL fluid showed, the increased level [range 81-835pg/ml , from no growth to PPM growth] was observed in all the three groups i,e in PPM , NonPPM and No Growth patients with comparing 6 controls ranged from 2-7pg/ml, indicating the earlier establishment of airway inflammation and the airway inflammation

was worsened by micro organisms colonisation.

CONCLUSION:

the micro organisms colonisation, exacerbates the airway inflammation :

1. Long duration of symptoms > 5 years
2. with habits of smoking and alcoholism
3. Cystic bronchiectasis and varicose bronchiectasis and

by eliminating the micro organism colonisation , the airway inflammation can be reduced .

Increased inflammation was observed in patients presented with

Table:1 & table : 2

Tota counts			Differential counts				
Growth type	No.of patients	Mean cell count(103)	Cell pattern	Growth type	No of patients	Mean cell count	Standard deviation
PPM	63	154.92	Neutrophil >50%	PPM	63	46.59	16.530
Non PPM	6	111.67		Non PPM	6	49.17	10.206
No growth	21	55.38		No growth	21	44.05	15.217
Total	90	128.81		Total	90	46.17	15.812
			Lymphocyte >50%	PPM	63	44.52	13.581
				Non PPM	6	45.00	8.367
				No growth	21	47.62	13.474
				Total	90	45.28	12.226

Table:1 showing cell counts distribution in BAL fluid
Difference between group (neutrophils) by ANOVA,p= 0.731
Difference between group (lymphocytes) by ANOVA,p= 0.654

Growth type	No.of patients	IL-8 in pg/dl	Mean	Std deviation
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
controle	6	2-7	5	

Table-2.showing IL-8 distribution in BAL fluid
Difference between groups by ANOVA,p=0.0001

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