Original Article

Pulmonary Nocardiosis

Pulmonary Infections caused by Nocardia Species in Patients with Chronic Obstructive Pulmonary Diseases

1. Muhammad Noman Rashid 2. Muhammad Asif Memon 3. Shazia Shakoor 4. Beenish Noman

1. Senior Lecturer of Physiology, Ziauddin University, Karachi 2 .Assoc. Prof. of Physiology, Ziauddin University, Karachi 3. Assoc. Prof. of Physiology, BM&DC, Bahria University, Karachi
3.Registrar, Dept. of Obst. & Gynae, Aga Khan University Hospital, Karachi.

ABSTRACT

Background: Pulmonary Nocardiosis (PN) is a severe and infrequent infection caused by Nocardia species, pathogen that can behave both as opportunistic as well as infectious with a high morbidity and mortality that mainly affects immunocompromised patients. In recent years, an increase in PN cases has been detected among patients with chronic obstructive pulmonary disease (COPD). The factors that are associated with its presence and determinants of its prognosis remain unknown. The aim of this study is to identify the frequency of nocardia in bronco alveolar lavage fluid (BALF) of patients suffering from chronic obstructive pulmonary disease.

Objective: Analyze the bronco alveolar lavage fluid (BALF) of patients suffering from COPD for presence of nocardia and determination of differential leukocyte count.

Study Design: Prospective study

Place and Duration of Study: This study was conducted at Tertiary Care Hospitals in Karachi from March 2012 till October 2012..

Materials and Methods: It is a prospective study of COPD patients. The study comprises of 140 patients with chronic obstructive pulmonary disease with or without treatment.

Results: All patients were divided in two groups A and B according to gender, and each group was further divided according to age i.e. above 40 years and below 40 years. Out of 140 patients 68 turned out to be positive for Nocardia.

Conclusion: Nocardiosis is considered to be uncommon in Pakistan, but our study suggests that prevalence of Nocardial infections in patients suffering from COPD is quite high and cases are not restricted to the classical immunocompromised host.

Key Words: Nocardia, COPD, Pulmonary Nocardiosis

INTRODUCTION

Pulmonary nocardiosis (PN) is an infrequent but severe infection that commonly presents as a sub acute, chronic suppurative lung disease, lung carcinoma or abscess[1]. Nocardia species are aerobic Gram-positive, catalase-positive, rod-shaped bacteria of the order Actinomycetales. It forms partially acid-fast beaded branching filaments. It has more than 85 species.[1,2] Some species are non-pathogenic found in oral micro flora, healthy gingiva as well as periodontal pockets while others are responsible for nocardiosis.[3] Nocardia are found worldwide in soil that is rich with organic matter. Most Nocardia infections are acquired by inhalation of the bacteria or through traumatic introduction.[3] In humans, N. asteroids complex is the predominant pathogen, but there are several other species, including: N. brasiliensis and N. otitidiscaviarum.[4,5] Pulmonary infection is usually produced by N. asteroides (85%), whereas N. brasiliensis causes cutaneous and subcutaneous abscesses.[6]

Nocardia most often enters through the respiratory tract, and is able to produce infection in a host compromised by systemic disease or anti-inflammatory therapy. However, it can also produce infection in patients with no significant medical history.[7]

In USA there are an estimated 500–1000 new cases each year. It is considered that the incidence of this infection has increased since 1960.[7,8] In Spain, one year mortality rate with Nocardia is 33%.[9,10] In India there is increasing incidence of Nocardia in obstructive lung diseases. The reason for this increasing incidence is unknown[11]. Nocardiosis has a high morbidity and mortality rate, which has been reported to be between 7 and 44% for disseminated nocardiosis[12]. The disease also has a marked tendency to recur. Immunocompetent patients usually develop localized cutaneous lesions, such as cellulitis, abscesses, or sporotrichoid forms[13]. Epidemiological studies showed that Nocardia infection have been found worldwide in all ages and ethnic groups[5,7]. It is two to three times more common in men, but there is no clear explanation for this gender predominance[14]. The organisms are readily aerosolized with dust, especially in dry areas[15]. Consequently, the

respiratory tract is the main portal of entry, with 50 to 70% of cases presenting with pulmonary involvement. Bronchiectasis and other structural lung abnormalities have been reported as an important risk factor for respiratory colonization by Nocardia species[4,5,12]. Organisms can also be acquired by direct inoculation, resulting in primary infections of the skin and subcutaneous tissues[16]. These infections can progress via lymphatic spread to regional nodes and, occasionally by direct spread to contiguous joints and bones[17]. Experience of pulmonary nocardiosis in the medical literature is limited to case reports, with few series.

MATERIALS AND METHODS

The study was conducted in Karachi, the largest city of Pakistan with a population of approximately 23 million[18]. belonging to different ethnicities. It is a prospective study conducted between March 2012 to October 2012 in which 140 patients were selected from different tertiary care hospitals, with history of chronic obstructive pulmonary diseases either with no treatment or poor compliance to treatment and with no history of any interventional therapeutic procedures.

A written informed consent and a detailed medical and surgical history was taken from all the patients. Pulmonary function test (FEV_1, FVC, and FEV_I/FVC) was performed to diagnose obstructive lung disease.

All patients have gone through fiber optic bronchoscopy in which 10 cc of bronchoalveolar lavage was collected. All the bronchoscopies were conducted by pulmonologist. Nocardial profile and differential leukocyte count was performed in Ziauddin University laboratory.

Hematology analyzer (Advia) was used to obtain WBC counts .This analyzer counts WBC in 2 ways, both of which use flow cytometry. In the first method called the peroxidase method, the white cells are stained with peroxidase and the cells are counted based on size and staining characteristics. This method also provides an automated differential cell count by separating the cells into clusters. The second method, called the basophiles method, involves stripping the cells of cytoplasm and counting nuclei.

For identification of nocardia 10 cc of bronchoalveolar lavage fluid was collected in sterile container then it was centrifuged at 2500 RPM for 15 min, after discarding the supernatant fluid, granules was taken on a slide crushed with another slide, it should be checked both slide has got the material, dry it at room temperature, then heat fixed, stained by Grams' stain, modified ZN stain and KOH mount preparation is made. Smears are examined for gram positive branching filaments and partially acid fast-branched mycelia and pigmented hyphae of nocardia.

RESULTS

All the subjects were divided in two groups. Group A and Group B according to gender and each group were further divided according to their age i.e. above 40 years and below 40 years. Most of the patients were belonging to low socio economic status, and males were mostly laborers in leather or hosiery factories with known addictions of smoking and gutka, all having on and off upper and lower respiratory tract infections which was treated with empirical antibiotic and steroid therapies without any proper investigation. (Table 1). All females were house wives using coal and kerosene stoves with similar past medical history. 34 females showed addiction towards huqa, in age group above 40 years. 37 females of age group less than 40 years showed addiction to gutka. (Table 1)

Differential cell count and nocardial analysis is shown in Table 2. In group A, above 40 years showed an average WBC count of 9.9 x 10^9/L, 73% were polymorphonuclear cells. Erythrocyte sedimentation rate 60. Lymphocytes of 65% and nocardia present in all samples. In group below 40 years age showed an average WBC count of 10.7 x 10^9/L, polymorphonuclear cells of 88%. Erythrocyte sedimentation rate 73. Lymphocytes of 58% and nocardia present in all samples except one sample. In group B, above 40 years showed an average WBC count of 11.0 x 10^9/L, polymorphonuclear cells of 90%. Erythrocyte sedimentation rate 80. Lymphocytes of 70% and nocardia present in all samples. In group below 40 years age group showed an average WBC count of 10.3 x 10^9/L, polymorphonuclear cells of 85%. Erythrocyte sedimentation rate77, Lymphocytes of 68% and nocardia was present in all samples.

All patients BALF run for differential cell count and nocardial analysis 68 patients sample showed nocardia species out of 140 patients, 21 males above the age of 40 turned out to be positive for Nocardia, 11 males below the age of 40 turned out to be positive for Nocardia, 22 females above the age of 40 turned out to be positive for Nocardia, 14 females under the age of 40 turned out to be positive for Nocardia. No gender variation was noted however it was observed that in both group A and B those patients having age more than 40 years have high presentation of Nocardia in BALF. Underlying pulmonary disorders, especially those associated with bronchopulmonary obstruction (viz. Bronchiectasis, emphysema, asthma, tuberculosis, and malignancy) may predispose Nocardia colonization of respiratory tract. All of the patients are of obstructive lung diseases with border line leucocytosis, raised ESR and mild to moderate dyspnea. All patients followed up regularly, none of the patients dropped out from the study.

Table No.1:

Gender	Age Group	Age	S/E Status	Occupation	Residence	Habits	Treatment History
Group A Males (n=70)	< 40	23	LSE	factory worker	Karachi	Smoker	Symptomatic treatment
	>40	55	LSE	factory worker	Sindh	Smoker	Symptomatic treatment
Group B Females (n=70)	<40	21	LSE	House wife	Karachi	Gutka (37)	Symptomatic treatment
	>40	49	LSE	House wife	Karachi	Huqa (34)	Symptomatic treatment

S/E: socio economic status, LSE: low socio economic status, A/B: antibiotics

Table No. 2:

Gender	Age	Average WBC (X 10^9/L)	Average PMN%	Average ESR mm/hr	Average Lymphocytes %	BALF samples +ve for Nocardia
Male	Above 40	9.9	73	60	65	21
	Below 40	10.7	88	73	58	11
Female	Above 40	11.0	90	80	70	22
	Below 40	10.3	85	77	68	14

WBC: white blood cell count; PMNs: Polymorphonuclear neutrophils; Lym: lymphocytes; ESR: erythrocyte sedimentation rate

DISCUSSION

Pakistan has a very high prevalence of pulmonary infections in both healthy and compromised hosts. Whereas nocardia infections are rare among the normal population, with most infections occurring in immuno compromised patients, showing pulmonary involvement[13]. Nocardial infection is a disorder affecting the lungs, brain, or skin.[19] It occurs mainly in people with weakened immune systems .Pulmonary nocardiosis is usually acquired by direct inhalation of Nocardia species from contaminated soil, and person-to-person transmission is rare.[20] Respiratory colonization can occur, and in a compromised host it can progress to tissue invasion and dissemination. Host resistance to infection with Nocardia species is thought to depend on functioning phagocytic cells. Neutrophils limit spread of infection in the early stage of tissue invasion. Activated macrophages and T-lymphocytes prevent dissemination and kill the bacteria.[21] The crucial role of cell-mediated immunity has been proved in experimental in vitro studies[22]; thus, it is not surprising that Nocardia species behaves as an opportunist microorganism in an immunocompromised host.[23] In our study 139 patients out of 140 showed nocardial colonization all the subjects colonized with nocardia had obstructive pulmonary lung disease , there were no patients of organ transplant or having any immunosuppressive disorders this gives an indication that nocardia is a common pathogen in patients with obstructive lung disease in Pakistani population as it mimics the symptoms of tuberculosis, study although does not have an extensive sample size to give incidence of pulmonary nocardiosis in Pakistani population but it throws a spot light on burden of disease.

The most important finding of this study is that pulmonary narcoidosis can affect any age group with history of obstructive lung disease with or without systemic steroid therapy which was not indicated in previous studies.[24]

Pulmonary narcoidosis have no specific type of symptoms or occurrence in advanced respiratory failure and having no specific radiological findings, these were the initial variables taken in previous studies[25,26] our study suggest that with changing environment nocardia species have changed their presentation which was not identified in previous studies[27]. There was no sex difference in the occurrence of nocardia, which was found to be male predominant in previous studies.[28]

Previous studies have used sputum for identification of nocardia[25,27] which is not considered as an ideal tool for identification of nocardia, in our study we used interventional technique, which is considered to be ideal technique for assessment of pathogen load.[30]

From clinical stand point it can be said that nocardia species are opportunistic pathogen of respiratory tract and it should be included in routine BALF analysis, it mimics pulmonary diseases so early bronco alveolar lavage should be done in pulmonary disease patients.

Although our study is an effort toward understanding pulmonary patho physiology of Pakistani population but it opens new horizons of research in obstructive pattern of respiratory diseases.

CONCLUSION

This study addresses risk factors, clinical presentation of Nocardia in chronic obstructive pulmonary disease

patients. Since the clinical and radiological manifestations are non-specific, and the microbiological diagnosis is often not considered, it seems likely that, in some patients, pulmonary nocardiosis will be mistaken for other infections, such as tuberculosis or bacterial pneumonia etc.

Nocardiosis is considered to be uncommon in Pakistan but our study suggests that prevalence of Nocardial infections in patients suffering from COPD is quite high and cases are not restricted to the classical immunocompromised host. A database is urgently needed to better evaluate the prevalence of the illness among the Pakistani population.

REFERENCES

1. Wilson JW. Nocardiosis: updates and clinical overview**.** Mayo Clin Prot 2012;87:403–407.
2. Menendez R. Pulmonary infection with Nocardia species: a report of 10 cases and review. Eur Respir J 2012;31;1542-6.
3. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clin Microbiol Rev 2012;15:194-222.
4. Leaker B, Hellyar A, Neild GH, Rudge C, Mansell M, Thompson FD. Identification of various pathogens in adult repiratory tract, Am J Physiol 2011;21:2103-2104.
5. Murray JF, Finegold M, Froman S, Will DW. The changing spectrum of nocardiosis. Am Rev Respir Dis 2011;83:315–330.
6. Filice GA. Inhibition of Nocardia asteroides by neutrophils. J Infect Dis 2011; 151: 47–56.
7. Limper AH, Overview of nocardia, Cecil Medicine. 24th ed. 2011.p.1455-75.
8. Torres A, Mason RJ, Broaddus CV, Martin TR, et al. Pyogenic Bacterial Pneumonia and Lung Abscess, Murray & Nadel's Textbook of Respiratory Medicine. 5th ed. 2011.p.477-96
9. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr: Clinical and laboratory features of the Nocardia species based on current molecular taxonomy. Clin Microbiol Rev 2011;19:259-282
10. Silva ACG, Martins EML, Marchiori E, Neto GT: Nocardiose pulmonar em paciente com síndrome da imunodeficiíncia adquirida**.** Radiol Bras 2011, 35**:**235-238.
11. Hui CH, Au VW, Rowland K, Slavotinek JP, Gordon DL. Pulmonary nocardiosis re-visited: experience of 35 patients at diagnosis**.** Respir Med 2011, 97**:**709-717.
12. Bocchino M, Paglia MG, Marruchella A, Contini S, Festa A, Saltini C: Molecular Diagnosis of Fatal Nocardia farcinica Pneumonia in an HIV-Negative Patient**.** Respiration 2008;75**:**461-65.
13. Sharma B, Ghosh G, Kamble U, Chaudhary K, Chauhan A, Lamba BM, et al. Allergic broncho pulmonary aspergillosis complicated by nocardiosis. Am J Respir and Crit Care Med 2012;87:65-75.
14. Garcia-Bellmunt L, Sibila O, Solanes I, Sanchez-Reus F, pulmonary nocardiosis in patients with COPD. Am J Respir Med 2012;48:280-85.
15. Al-Jahdali H, Baharoon S, Alothman S, Memish Z, Waness Nocardiosis in tertiary care hospital in Saudia Arabia A. J Glob Infect Dis 2011;3: 128-32
16. Sorrell TC, Mitchell DH, Iredell JR. Nocardia species. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious Diseases. 6th ed. 2005.p.856-66
17. Peterson BT, Idell S, MacArthur C, Gray LD, Cohen AB. A modified bronchoalveolar lavage procedure that allows measurement of lung epithelial lining fluid volume. Am Rev Respir Dis 2010;141:314-20.
18. Hasan A, Mohiburl M. Urban Slums Reports: The case of Karachi, Pakistan, World population matters oxford publishers 2013;147-58.
19. Pingleton SK, Harrison GF, Stechschulte DJ, Wesselius LJ, Kerby GR, Ruth WE. Effect of location, pH, and temperature of instillate in bronchoalveolar lavage in normal volunteers. Am Rev Respir Dis 2011;128:1035–37.
20. Rennard SI, Ghafouri M, Thompson AB, Linder J, Vaughan W, Jones K, et al. Fractional processing of sequential bronchoalveolar lavage to separate bronchial and alveolar samples. Am Rev Respir Dis 2010;141:208–17.
21. Hertz MI, Henke CA, Nakhleh RE, Harmon KR, Marinelli WA, Fox JM, et al. Obliterative bronchiolitis after lung transplantation: a fibroproliferative disorder associated with platelet-derived growth factor. Proc Natl Acad Sci USA 2010;89:10385–89.
22. Ferguson AC, Whitelaw M, Brown H. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. J Allergy Clin Immunol 2009;4: 609–13.
23. Chamberlain DW, Braude AC, Rebuck AS. A criticalevaluation of bronchoalveolar lavage. Criteria foridentifying unsatisfactory specimens. Acta Cytol 2009;31:599–605.
24. Boersma WG, Erjavec Z, van der Werf TS, et al. Bronchoscopic diagnosis of pulmonary infiltrates ingranulocytopenic patients with hematologic malignancies: BAL versus PSB and PBAL. Respiration 2007;101:317–25
25. Poletti V, Chilosi M, Olivieri D. Diagnostic invasiveprocedures in diffuse infiltrative lung diseases. Respiration 2004;71:107–19
26. De Brauwer EI, Ramsay G, et al. Detection ofnon-infectious conditions mimicking pneumonia in the intensive care setting: usefulness of

bronchoalveolar fluid cytology. Respir Med 2009; 93:571–78.
27. Rennard SI, Albera C, Carratu L, et al. Clinical guidelines and indications for bronchoalveolar lavage (BAL): pulmonary malignancies. Eur Respir J 2009;8:956–69
28. Tam M, Reichenberger TM, McGandy CE, et al. Diagnosis of pulmonary Kaposi's sarcoma by detection of human herpes virus 8 in bronchoalveolar lavage. Am J Respir Crit Care Med 2008;157:458–63.
29. Beskow CO, Drachenberg CB, Bourquin PM, et al. Diffuse alveolar damage: morphologic features in bronchoalveolar lavage fluid. Acta Cytol 2006;44:640–49
30. Brandt B, Thomas M, von Eiff M, Assmann G. Immunophenotyping of lymphocytes obtained by bronchoalveolar lavage: description of an all-purpose tricolor flow cytometric application. J Immunol Methods 2006;194:95–102.

Address for Corresponding Author:
Dr. Muhammad Noman Rashid,
Senior Lecturer of Physiology
Ziauddin University Clifton Karachi, Pakistan
Phone no: 92215862937-9 Ext 452
Mobile no: 923152074182
E-mail: MUHAMMAD.NOMAN@zu.edu.pk
dr.nomanrashid@hotmail.com