Study of Nocardia in HIV positive chest symptomatic individuals.

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Abstracts: Introduction: Pulmonary nocardiosis is a well-described infection in patients with neoplastic disease, human immunodeficiency virus (HIV) infection. The radiographic appearance of pulmonary nocardiosis is varied and nonspecific. Since the clinical and radiologic manifestations are non-specific, and the microbiological diagnosis is often difficult. In some patients, pulmonary nocardiosis will be mistaken for other infections, such as tuberculosis or bacterial pneumonia. Hence this study was undertaken to detect the prevalence of nocardiasis in HIV infected patients. Material and Methods: One hundred chest symptomatic patients screened for HIV infection and the samples of HIV positive patient processed for Nocardia using kinyoun's modification of zeihl Neelsen stain and culture. The CD_4 count of HIV patients with Nocardial infection and co-infection by mycobacterium tuberculosis studied. Observations: Out of 100 patients with cough and fever more than 2 weeks, 58 turned out to be HIV positive. Most of the patients included in the study were in their 3rd and 4th decades of life. The male to female ratio was 1:0.38. The open tuberculosis cases were 43.45% and sputum positive for acid fast bacilli. The nocardiosis was observed in 3.45% cases by modified Kinyoun's method of staining and culture. In 80.65% tuberculosis cases, CD_4 count less than 200/µl, while 19.35% had CD_4 count more than 200/µl. The cases with nocardiosis had CD_4 count less than 200/µl. Co-infection with tuberculosis and nocardiosis is not observed. The history of family contact for tuberculosis was found in 9.68% cases. Conclusion: The prevalence of nocardia in HIV positive individuals for north Maharashtra region is observed. The nocardia are easy to treat if diagnosed correctly and this will help in preventing morbidity in chest symptomatic patients. Unnecessary treatment with higher antibiotics can be avoided and cost effective treatment will be possible. Patients with CD_4+ count less than 200 cells/µl should be screened for Nocardia. [Ravindra K NJIRM 2016; 7(5):73-77]

Key Words: HIV, Nocardiasis, CD_4 count.

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Introduction: Pulmonary nocardiosis is a well-described infection in patients with neoplastic disease, human immunodeficiency virus (HIV) infection, and those receiving treatments with corticosteroids or various chemotherapeutic agents.[1] This disease is a subacute or chronic pneumonia caused by a species of the family Nocardiaceae. Seven species have been associated with human disease. N. asteroides is responsible for about 70% of infection caused by these organisms[2], and debilitated patients have a 45% mortality rate even with appropriate therapy. Mortality is increased in disseminated disease involving 2 or more organs. There is no age or race predilection. The patients may present with cough, fever, and breathing difficulties[3].

The radiographic appearance of pulmonary nocardiosis is varied and nonspecific. The most commonly described findings include localized consolidation, cavitations, and lobar infiltrative disease with characteristically thick-walled cavities. Computed tomography findings include consolidation with or without cavitation, multiple discrete pulmonary nodules, pleural effusion, and chest wall extension. Notably, AIDS patients diagnosed with pulmonary nocardiosis were found to have more irregular, spiculated nodules, and a higher incidence of cavitary masses[4,5].

The diverse radiological manifestations of pulmonary nocardiosis reflect its ability to cause both suppurative and granulomatous infection[6]. Since the clinical and radiologic manifestations are non-specific, and the microbiological diagnosis is often difficult. In some patients, pulmonary nocardiosis will be mistaken for other infections, such as tuberculosis or bacterial pneumonia. HIV-related nocardiosis usually appears in patients with advanced immunossupression. Previous studies have reported that between 57% and 68% of patients have AIDS-defining criteria at the time of diagnosis of nocardial infection[7].

There is no routine guideline for testing for nocardiosis. Hence true prevalence of this infection

is difficult to identify due to number of reasons. Nocardia are weakly acid fast actinomyecete and one can miss them with Ziehl Neelsen staining using 20% Sulfuric acid. Nocardia need longer incubation period than usual bacterial pathogen and we are likely to miss nocardia if plates are not incubated for 2 weeks. Nocardia are susceptible to sulphonamides and cotrimoxazole is frequently used drug is HIV patients[1]. Hence this study was undertaken to detect the prevalence of nocardiasis in chest symptomatic HIV infected patients and to find out the correlation between HIV, tuberculosis, nocardiosis and CD4 count.

Material and Methods: One hundred patients attending tuberculosis outpatient department with cough, breathlessness and fever more than 2 weeks are referred to Integrated Counseling and Testing Centre at SBH Govt. Medical College, Dhule after approval by institutional ethical committee. Blood sample is collected from all patients for HIV antibody detection test. The antibody detection is performed as per NACO guidelines and all samples are screened by Comb-Aids RS test (Span Diagnostic Ltd, India). The positive HIV antibody samples are retested by HIV Tridot test (J Mitra Co Ltd, India) and Parikshak Triline test (Bhat Diagnostic ltd, India) for confirmation of results. The CD_4 cell count performed on blood sample of HIV positive patients by Partec Cyflow ^{TM} machine.

The confirmed HIV positive patients with chest symptoms are subjected for sputum examination. The early morning sputum samples are collected in wide mouth sterile screw cap container on successive three days. Three smears will be prepared from each sample. One used for Gram's staining, second is subjected to Zeihl Neelsen Staining and third stained by modified Kinyoun's method[1].

Gram's stained smears are screened for gram positive branching filaments with ends breaking into bacilli. This is typical morphology of Nocardia. Second smear showed presence of Acid fast bacilli, mycobacteria which are commonly seen in HIV positive individuals. Third smear is specifically for Nocardia which we normally miss because this kinyoun's method is normally not used in Microbiology Laboratory. Samples which show presence of Nocardia are cultured on Thayer and Martins medium without antibiotics. Plates incubated for 15 days and colony characters are studied. Smears prepared from the colony and modified Kinyoun's method of staining is done to find out growth of same organism on culture medium as per direct smear finding[8].

Association of Mycobacteria and Nocardia is noted. CD4 count of persons showing presence of Nocardia is observed to find out correlation between infection by Nocardia and immunosuppression.

Result: The HIV testing is done in one hundred patients with cough and fever more than 2 weeks and 58 turned out positive. Most of the patients included in the study are in their 3rd and 4th decades. The male to female ratio was 1:0.38. Out of 58 HIV positive individuals, 31(43.45%) patients are open case of tuberculosis, detected to be AFB positive while nocardiosis was observed in 2(3.45%). Both nocardiosis cases are positive by modified Kinyoun's method of staining and culture. (Table 1). Among 31 tuberculosis patients only 3(9.68%) had history of contact in family.

Table-1: Prevalence of Tuberculosis and nocardiosis in HIV positive cases.

HIV Positive cases (n=58)	
Open Tuberculosis	31 (53.45%)
Nocardiosis	02 (3.45%)

Maximum number of the HIV positive patient who are AFB positive 25(80.65%) have the CD_4 count less than 200/µl, while 6(19.35%) AFB positive patients had CD_4 count more than 200/µL. Both cases with nocardiosis had CD4 count less than 200/µL. Co-infection with tuberculosis and nocardiosis is not observed. (Table 2)

Table-2: Co-relation of tuberculosis and nocardiosis with CD4 count in HIV positive cases.

HIV Positive cases (n=58)		
CD count	**CD4 count <200/µL**	**CD4 count >200/ µL**
Tuberculosis with AFB positive cases (n= 31)	25 (80.65%)	06 (19.35%)
Nocardiosis (n=2)	02 (100%)	00

Discussion: Pulmonary nocardiosis is an infrequent but severe infection that is most commonly found in immunocompromised patients. Common predisposing factors for nocardial infection include corticosteroid therapy, chemotherapy for neoplasm, and acquired immune deficiency syndrome (AIDS). Pulmonary nocardiosis is difficult to be diagnosed, and is often mistaken for other lung diseases[1].

The HIV epidemic has posed major, almost insurmountable, challenges to tuberculosis control efforts across the world. Piramanayagam etal estimated HIV seroprevalence among tuberculosis patients presenting to tertiary care centre in Delhi and 8.3% were found to be HIV-positive.[9] In our study, 58% were HIV positive patients. The prevalence of HIV-positive subjects in chest symptomatic cases observed in this study was substantially higher than that reported in other studies.

This study observed 43.45% patients of open tuberculosis among HIV positive individuals, and most of the patients included in the study were in their 3rd and 4th decades. The male to female ratio was 1:0.38. Ragini Ghiyal etal analyzed the prevalence and disease profile of HIV/AIDS coinfection in Vadodara, Gujarat, India. The HIV coinfection with tuberculosis was 49.2%. Among coinfected cases, 14.2% presented with demonstrable and documented tuberculosis whereas in 85.8% cases, tuberculosis was extemporaneously detected by actively screening the patients. Sixty nine percent of patients were males, while 10.5% of cases were below fifteen years of age.[10] Noeske et al. reported that 32% HIV-positive subjects were culture-positive for TB, of which 76% were AFB smear-positive.[11] In the study by Shailaja et al, M. tuberculosis was isolated in 42.89% HIV positive cases.[12]

HIV/AIDS pandemic is responsible for the resurgence of TB worldwide, resulting in increased morbidity and mortality. HIV and Mycobacterium tuberculosis have a synergistic interaction; each propagates progression of the other. Coinfection with HIV infection leads to difficulties in both the diagnosis and treatment of tuberculosis, increase risk of death, treatment failure and relapse.[13]

The present study showed that out of 58 HIV positive individuals, nocardiosis was observed in 3.45%. The cases were positive by modified Kinyoun's method of staining and culture. Uttamchandani etal identified 21 HIV-infected patients at the University of Miami who were diagnosed with pulmonary nocardiosis from 1983 to 1989.[14] However, in a retrospective study at St. Luke's/Roosevelt Hospital Center in New York, Kim et al. found that only 6 of 2,167 (0.28%) AIDS patients cared for at that institution from January 1980 through March 1989 were diagnosed with nocardiosis.[15] Alnaum HM etal determined the frequency of nocardiosis in HIV-positive and HIV-negative individuals clinically suspected of having tuberculosis (TB). Nocardia spp cause pulmonary infections (4.09%) in both immunocompetent (2.92%) as well as immunocompromised (1.17%) patients who attend chest clinics in Sudan[16]. In 1985, Holtz et al. described four parenteral drug abusers with AIDS complicated by actinomycete infections; three patients had N. asteroides infections, and one patient had lymphadenitis caused by a streptomycete.[17]

Unlike other opportunistic infections which occur at CD_4+ counts below 200/µl, active TB occurs throughout the course of HIV disease. Clinical presentation of TB in HIV-infected individuals depends on the level of immunosuppression resulting from HIV infection. In patients with relatively intact immune function (CD_4+ count > 200/µl), pulmonary TB is more frequently seen. Marchie etal assessed the effect of CD_4 T lymphocytes and features of pulmonary tuberculosis on HIV positive patients with co-existing tuberculosis attending clinic in university of Benin Teaching Hospital, Benin, Nigeria. The average CD_4 T lymphocyte count in the study group (HIV sero-positive) was 173.90 cells/µl and median of 172 cells/µl. 128(64%) subjects had CD_4 T lymphocyte counts less than 200cell/ µl.[18] Ramchandran etal reported a case of tuberculosis with the CD4 lymphocyte count was 86 cells/µl, (measured by flow cytometry using standard techniques on a Becton Dickenson FACSort)[19]. We observed maximum number of the HIV positive patient who were AFB positive (80.65%) have the CD_4 count less than 200/µl, while 19.35% AFB

positive patients had CD_4 count more than 200/µl.

HIV related nocardiosis usually appears in patients with advanced immunosupression. In the present study, 3.45% cases of nocardiosis had CD_4 count less than 200/µl. Previous studies have reported that between 57% and 68% of patients have AIDS-defining criteria at the time of diagnosis of nocardia infection, and that the CD4+ count is less than 200 cells/µl in 88-100% of these patients.[7]

The diagnosis of nocardiosis often is not considered in a patient with significant pulmonary infection, because the incidence of nocardia is relatively low compared with that of many other organisms. Moreover, nocardia is difficult to culture, and there is no reliable serologic test to detect its presence. Its marked radiographic pleomorphism also tends to exclude it from differential diagnosis of chest film abnormalities, since there are no characteristic findings that bring it to mind. For this reason, nocardiosis should be considered in the differential diagnosis of any chronic pneumonia.[3]

Conclusion: This study will help us to find out if nocardia are pulmonary pathogens in HIV positive individuals of our area of North Maharashtra Region. The pulmonary nocardiosis is varied and nonspecific. As nocardia are easy to treat if diagnosed correctly this will help in preventing morbidity in chest symptomatic patients. Unnecessary treatment with higher antibiotics can be avoided and cost effective treatment will be possible using simple staining method. Coexistence of tuberculosis with nocardiosis can be studied if present. Patients with lower CD4 count should be routinely screened for nocardia.

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