PULMONARY INFECTIONS DUE TO MYCOBACTERIUM TUBERCULOSIS & NON-TUBERCULOSIS MYCOBACTERIA IN HIV SEROPOSITIVE PATIENTS.

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ABSTRACT: Respiratory infections are the major cause of morbidity and mortality in persons with HIV infection. It is clear that with the progression of HIV infection, the function of pulmonary immunocompetent cells declines. There is severe reduction in concentration of pulmonary CD4 cells and impaired cytolytic activity¹. About 70% of HIV/AIDS patients with infection experience a pulmonary opportunistic infection in life time. Three most important and common pulmonary manifestations of HIV are bacterial pneumonia, tuberculosis and *Pneumocystis carinii* pneumonia. These comprise more than 90% of opportunistic infections worldwide².

The pandemic of AIDS and the evidence of an association with tuberculosis is now of serious concern. The life time risk of developing tuberculosis for people not infected with HIV is 5 - 10% and this rises to 50% if they are co-infected with HIV. Tubercular pulmonary involvement occurs in 74 to 100% of patients with HIV infection³. AIDS pandemic has reversed many of the hard won gains in the tuberculosis control in developed as well as developing countries like India ⁴.

Nontuberculous mycobacterial species (NTM) are common environmental organisms and occasional colonizers of the human respiratory system. The immunosuppressed individuals infected by human immunodeficiency virus (HIV) infection have become the most significant risk factor for disseminated NTM disease and of these, 95% are due to *Mycobacterium avium* complex. In developed countries, as the incidence of tuberculosis decreased, the occurrence of NTM in pulmonary diseases increased ⁵. In India infection with non-tuberculous mycobacteria (NTM) is reported to be low as *M. tuberculosis* is more prevalent and endemic. The infection with NTM is either overlooked by clinicians or in some places facilities are not available for the isolation of NTM.

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The present study was undertaken to determine the incidence of mycobacterial infections in lower respiratory tract in HIV seropositive patients with special emphasis on the use of paraffin slide culture for the isolation of non-tuberculous mycobacteria and *Nocardia*.

MATERIAL & METHODS: Present study was conducted in the Department of Microbiology, Government Medical College and Hospital, (GMCH), Nagpur. A total of 108 patients infected with Human Immunodeficiency Virus (HIV) presenting with signs and symptoms of lower respiratory tract infection were included in the study. A detailed history and clinical examination were conducted on each patient &. Investigations performed (Total leucocyte counts, CD4 counts) were recorded from the respective case sheet.

Direct or induced sputum was collected in all patients. The patients were advised to collect an early morning fresh sample. In patients having non-productive cough, the sputum was induced with 5% hypertonic saline which was given in the form of an aerosol using nebulizer.⁷ Each sputum sample was concentrated by Petroff's method.⁸

These sputum samples were used for.

- i) Zeihl Neelsen stain ⁽⁹⁾
- ii) Modified Ziehl-Neelsen Stain ^(10,11,12) Culture
- iii) For Acid fast bacilli. ⁽¹³⁾
- iv) For Non-tuberculous (atypical) Mycobacterium and Nocardia .⁽¹⁴⁾

(i) Culture of the sputum for acid fast bacilli (AFB)

A portion of concentrated sample was inoculated on two Lowenstein Jensen (LJ) slopes & incubated at 37°C. The slopes were examined twice a week. If growth appeared on LJ slopes, a smear was prepared and stained by ZN staining. It was further subjected to nitrate reduction test and para nitro benzoic acid (PNB) test to differentiate M.tuberculosis from Non-tuberculous mycobacteria (NTM).

(ii) Paraffin Slide Culture technique for NTM

A 0.5ml of concentrated sputum was added to 4.5ml of Czapek broth in sterile Mac Cartney bottle containing paraffin wax coated slide and then 0.1ml of PANTA plus was added (P-Polymyxin, A-Amphotericin, N-Nalidixic acid, T-Trimethoprim, A-Azlocillin)and incubated at 37°C for three weeks and checked daily for growth. If growth was observed on the slide either in the form of discrete colonies or confluent growth, the slides were taken out and stained by Kinyon's carbol fuchsin (cold carbol fuchsin) and seen under 100x (oil immersion) objective of the microscope.⁽¹⁵⁾ Acid alcohol fast staining procedure allowed differentiation between Nocardia species and NTM. ⁽⁵⁾

Control strain: Known strains of Mycobacterium avium complex (MAC), M. simian was used as positive controls and M. tuberculosis H₃₇ Rv was used as negative control. These strains were obtained from Department of Microbiology, M.G.I.M.S., Sewagram, Wardha. Each control strains were inoculated in 4.5 ml Czapek broth. Sterile paraffin coated slides were dipped into Czapek broth and it was incubated at 37°C and checked daily for growth.

RESULTS: A total of 108 HIV seropositive cases presenting with the signs and symptoms of involvement of lower respiratory tract were studied.

Demographic profile: The maximum number of cases (57) belonged to age group 36 years to 50 years followed by age group of 21 years to 35 years with 40 cases. A male preponderance was observed in the present study accounting for 85.18% cases.

Clinical presentation: Respiratory symptoms like cough with expectoration were present in all the patients. Hemoptysis was present in four patients. Other important clinical findings observed in these seropositive cases included oral thrush 46 (42.5%), dysphagia 19(17.5%), fever, significant weight loss 60 (55.55%) and diarrhea 45 (41.66%). Lymphadenopathy was observed in 57 (52.77%) cases. The history of blood transfusion was present in 10 cases (9.25%) and past history of tuberculosis was given by 21 cases. In as many as 95 cases, total leukocyte counts were below 4000/cmm.

Estimation of CD₄ counts was possible only in 27 cases. The maximum number of HIV seropositive patients with respiratory infections had CD4 counts between 50 and 200/cumm (55.5%). None of the patients with respiratory symptoms had counts > 350 /cumm.

Out of 108 seropositive cases, 13 sputum specimens were positive on both microscopic examination and culture, 5 were positive in ZN stained smears but negative in culture whereas 6 were positive on culture but negative in microscopy.

In 19 cases growth was obtained on LJ medium in 3-4 weeks. All these isolates were identified as M.tuberculosis by positive nitrate reduction test and absence of growth on LJ medium containing Para-nitrobenzoic (PNB) acid.

In Paraffin Slide Culture, in 1 specimen, yellowish coloured confluent growth was seen on part of Paraffin coated slide dipped in Czapek broth. Acid fast bacilli were demonstrated with Kinyoun's carbol fuchsin method,. Since Nocardia species are not stained with these method and M.tuberculosis does not grow on paraffin coated slides, the identity of the isolate was confirmed as NTM species.

Thus considering microscopy and culture together a total of 24(22.22%) cases were positive for acid fast bacilli.

Co-relation between CD4 counts and Mycobacterial infections:

Sputum positivity for AFB was again more in cases with counts between 50 and 350/cumm.

Sr. No.	CD4 count	Number of cases	Mycobacterial
1.	< 50	3	-
2.	50 - 200	15	2 (7.40%)
3.	200 - 350	9	3 (11.11%)
4.	> 350	-	-
	Total	27	5

DISCUSSION: Pulmonary complications involving airways, pleura and the lung parenchyma are the major source of morbidity and mortality in AIDS cases.⁽¹⁶⁾ In the present series, the maximum number (89.81%) of cases belonged to age group 21-50 years. HIV seropositive survey conducted have shown higher rate of HIV infections among people in 20 to 45 years of age. ⁽¹⁷⁾ A male predominance was observed in the present study accounting for nearly 85% of cases. In a study conducted by Sharma et al ⁽¹⁸⁾ on 135 patients, male contributes to age between 34 +/- 10 year and

female 17%. Similar finding has also been reported in the studies conducted elsewhere in India.^(19,20) Apart from the signs of respiratory system involvement, important clinical findings observed included fever, significant weight loss, diarrhoea, dysphagia, lymphadenopathy and oral thrush.

NACO⁽²¹⁾ reveals that the tuberculosis is the commonest infection in AIDS patients in India. A clinical study of HIV infected patients in South India.⁽²²⁾ found tuberculosis in 60% patients. Lanjewar and Duggal (2001) in their autopsy studies involving 143 HIV positive cases have noted that tuberculosis was the most frequently observed pulmonary infection accounting for incidence of 59%. Barnes et al⁽³⁾ have reported tubercular pulmonary involvement in 74 to 100% of patients with HIV infections. Attili et al⁽²³⁾ noted tuberculosis was the commonest opportunistic disease and found in 68% of cases.

For the isolation of non-tuberculous mycobacteria, paraffin slide culture technique was used⁽¹⁴⁾ in the present study. From one sputum specimen of HIV seropositive subject growth of NTM was obtained on paraffin coated slide. The parallel culture on L.J. medium was however negative. The identity of NTM was confirmed by Kinyoun's carbol fuchsin staining. Narang et al⁽⁵⁾ have reported that the paraffin slide culture had the advantage of in-situ staining for acid fast bacilli and lower contamination rate besides the high sensitivity for isolation of NTM. Three NTM were isolated from 42 sputum samples of HIV seropositive (2 *Mycobacterium avium* complex and one unspciated). The isolation rate of NTM from HIV seropositives was observed to be 7.14% by Nrang et al.⁽⁵⁾ The immunosuppressed individuals infected by HIV have become the most significant factor for disseminated NTM disease. In developing countries, little is known about NTM infections in AIDS. Shailaja et al⁽²⁴⁾ have reported incidence of 1% in AIDS patients.

Since *M.tuberculosis* does not grow on PSC, any growth on the paraffin slide is an immediate indication of NTM or *Nocardia* species. The *Nocardia* species are not acid alcohol fast using Kinyoun's method and are filamentous on PSC and hence can be easily differentiated from NTM.⁽⁵⁾

In the present study, paraffin slide culture technique was also used for recovery of *Nocardia* species from sputum samples, however we did not encounter even a single case of nocardiosis. Shailaja et al⁽²⁴⁾ have reported incidence of 1% pulmonary nocardiosis in HIV positive case.

In the present study estimation of CD4 counts could be done in only 27 cases as this facility was not available in this institute. In these cases, the incidence of bacterial infections was more in cases with counts between 50 and 350/cmm. The sputum positivity for AFB was also found to be more in this range of CD4 counts. Wallace (1993) observed that rates of PCP and bacterial pneumonias were significantly greater in cohort members with entry CD4 counts < 250.

CONCLUSION: In the present study, Culture positivity for *Mycobacterium tuberculosis* was found to be 17.59% in HIV reactive cases. Incidence of Non Tuberculous Mycobacteria was found to be 0.92% in this region in HIV seropositive cases. Paraffin slide culture was found to be better for primary isolation of NTM from sputum as compared to L.J. medium.

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APPENDIX:

Preparation of in house paraffin slide culture technique

Standard microscopic slide were cut longitudinally into two, so that they could fit within a Mac Cartney bottle & could be easily withdrawn. These cut slides were sterilized in hot air oven.

For coating of slides with paraffin, pieces of paraffin wax were melted in a beaker on bunsen burner and then sterilized in hot air oven at 160°C for 1 hour. With the help of a sterilized forceps, previously sterilized slides were dipped in beaker containing sterilized molten paraffin wax. The slides were kept for a few second, so that each slide was covered with a layer of paraffin wax. The coated slides were quickly transferred into a sterilized wide mouth bottle (Ollar et al 1990).