Short Communication

Mycobacterial co-infection in retroviral disease

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ABSTRACT

Atypical mycobacteria are increasingly afflicting pulmonary and extra pulmonary systems. Disease caused by these organisms are less common compared with tuberculosis caused by human strain of mycobacteria, but there has been a significant increase in pulmonary and extra pulmonary infections due to atypical mycobacteria especially in retroviral disease in the last two decades. 75 HIV positive patients who had pulmonary and/or extrapulmonary manifestations suggestive of Tuberculosis were included in our study. Of these,48 (64%) were males and 27(36%)were females. Majority of these patients were in economically productive and sexually active age group of 31-50 years. Of the 75 samples, 25 were culture positive for Acid Fast Bacilli whereas 50 samples showed no growth on culture at end of 8 weeks. Of the 25 who had positive cultures, 15 showed growth for Mycobacterium TB and 10 showed growth for Atypical mycobacteria. Of the 25 patients who were Culture positive, 16 i.e 64% had Sputum smears by Z-N stain positive for AFB, which was statistically significant. It was observed that Atypical mycobacterial infection was more common in CD4 count < 100 cells/cu mm. Infection with Mycobacterium TB was more common in CD4 count between 101-200 cells/cu mm.

Keywords: *Atypical mycobacteria, retroviral disease, CD4 count, mycobacterium tuberculosis, HIV positive*

Introduction

Atypical mycobacteria are opportuinistic pathogens increasingly recognized as causes of pulmonary and extra pulmonary diseases. [1] Atypical mycobacteria are widely distributed in nature and have been isolated from natural water, tap water, soil and surgical solutions. [2] Diseases caused by these organisms are uncommon compared with tuberculosis but there has been a significant increase in pulmonary and extra pulmonary infections due to atypical mycobacteria in the last two decades. [3] Although the pathogenic potential of atypical mycobacteria was reported throughout the 20th century, widespread appreciation of the clinical syndromes caused by atypical mycobacteria began during the association with the AIDS pandemic and the subsequent dramatic increase in disseminated Mycobacterium Avium Complex (MAC) infections. [4] These organisms can produce localized disease in the lungs, lyphnodes, skin, wounds or bone. Ocassionally they may produce disseminated disease. [5] In AIDS patients the manifestations may range from localized to disseminated disease. Today, owing to improved treatment regimens, prolonged survival of AIDS patients is becoming common, allowing MAC to infect most AIDS patients. Very low CD4 counts and defective cytokine

responses have been linked to severe infections due to M. avium complex from common sources like potable water. The rarity of Atypical mycobacterial infections renders good quality cinical trials difficult, but with growing recognition of Atypical mycobacteria as emerging pathogens we hope to see atypical mycobacteriosis as an increasing focus of research.

The impact of atypical mycobacterial infections on morbidity and mortality of AIDS patients has stimulated the initiation of studies of epidemiology, ecology, genetics, molecular biology and physiology of atypical mycobacteria.

Material and methods

The study was conducted at a tertiary care hospital from 2010 to 2013 after the approval of Ethics committee (Letter No.IEC/ Pharm/670/2010 dated 30/11/10 from Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai). Patients attending the outpatient department who were diagnosed to be HIV positive and who had symptoms suggestive of Tuberculosis (pulmonary as well as extra pulmonary) were included in the study after satisfying inclusion and exclusion criteria

Inclusion Criteria

- Both Genders
- Adults above 18 years
- Patients who were diagnosed to be positive for HIV by ELISA test

Exclusion Criteria

Patients who were not willing to get enrolled in the study

A detailed history was taken and thorough clinical examination was performed. All patients were subjected to Hemogram, Chest X ray and CD4 Counts. This study included isolation of Non Tuberculous mycobacteria from clinical specimens of these patients. Early morning well coughed out sputum was used in patients having respiratory complaints. Alternately, induced sputum, after nebulizing the patient with 3% hypertonic saline was collected in patients who were unable to produce the sputum. Bronchial washings were also obtained wherever indicated. Three sputum samples were collected. Biopsy and pus specimens in lymphadenitis, cerebrospinal fluid, pleural or ascitic fluid, urine were collected in cases of extrapulmonary tuberculosis. All specimens were collected with aseptic precautions in sterile leak proof containers and transported to the microbiology laboratory. The specimens were processed on the same day for microscopy and culture by standard procedures. Specimens like urine and sputum were decontaminated by petroff's method. Biopsy specimens were ground with sterile precautions and processed. Specimens in large volumes were centrifuged at 300 rpm for 30 minutes and the deposit was used as inoculum. Smears were made and stained with Ziehl Neelsen (Z-N) stain and specimens were inoculated onto Lowenstein Jensen (LJ) medium in duplicate and incubated at 37 degree C. The cultures were examined everyday for one week and thereafter once a week for 8 weeks. Smears were made from colonies on LJ medium and were stained by Z-N stain. When colony morphology and smear morphology were suggestive of M.Tuberculosis, they were further confirmed by niacin test. [6] Those suspected to be NTM were

subjected to biochemical tests for identification. Biochemical tests like catalase and aryl sulfatase tests were used for differentiation between typical and atypical mycobacteria. Atypical mycobacteria give a strongly positive aryl sulfatase test. Mycobacterium TB gives a positive peroxidase test.

Results

75 subjects were included in the study. Out of which 64% were male. Most of the patients were in economically productive age group i.e 31-50 yrs. (Figure.1)

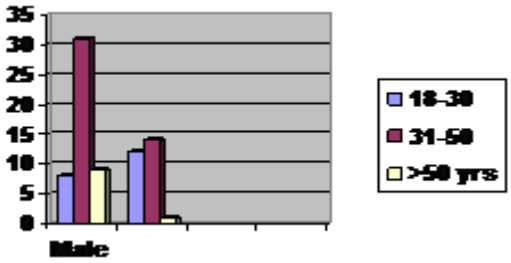

Figure 1 Distribution by Age and Gender

Of the 75 samples, 25 (33%) were culture positive for Acid Fast Bacilli whereas 50 (67%) samples showed no growth on culture at end of 8 weeks. Of these 25 who had positive cultures, 15 (60%) showed growth for Mycobacterium TB and 10 (10%) showed growth for Atypical mycobacteria. Of the 25 patients who were Culture positive, 16 i.e 64% had Sputum smears by Z-N stain positive for AFB, which was statistically significant. (Chi square statistic is 19.59, $p<0.01$) (Table 1) It was observed that Atypical mycobacterial infection was more common in CD4 count < 100 cells/cu mm. which was significant using Fishers exact test. (Table 2) Infection with Mycobacterium TB was more common in CD4 count between 101-200 cells/cu mm. which was significant using Fishers exact test. (Table 3) Most of the patients presented with more than one zone involvement. (Table 4)

Table 1: Relation between Sputum AFB smear by Z-N stain and Sputum Culture

	Culture Positive	Culture Negative	Total
Sputum AFB smear Positive	16 (64%)	7 (14%)	23
Sputum smear negative	9 (36%)	43 (86%)	52
Total	25	50	75

Table 2: Relationship between Culture for NTM and CD4 count

Culture for NTM	CD4 0-100	CD4 101-200	CD4 >200	Total
Positive for NTM	9	1	0	10
Negative for NTM	3	21	41	65
Total	12	22	41	75

Table: 3 Relationship between Culture for M.TB and CD4 count

Culture for M.TB	CD4 0-100	CD4 101-200	CD4 >200	Total
Positive for MTB	0	7	8	15
Negative for MTB	12	15	33	60
Total	12	22	41	75

Table: 4 Radiological Distribution

Zone Involved	Patients	Percentage
Right upper	11	**14.67**
Right mid	2	**2.67**
Right lower	2	**2.67**
Left upper	3	**4**
Left mid	4	**5.33**
Left lower	9	**12**
More than one zone unilateral	25	**33.33**
Bilateral	14	**18.66**
Pleural effusion	5	**6.67**
Total	**75**	**100**

Discussion

Non Tuberculous Mycobacteria (NTM), also known as atypical mycobacteria are saprophytes naturally distributed in soil, water and dust. Nevertheless, these organisms have been reported to cause a variety of infections, more so in immunocompromised individuals and to a much lesser extent in immunocompetent individuals. In developed countries, the incidence of tuberculosis has reduced but infections due to NTM are on the rise, whereas in developing countries like India, Tuberculosis is still a major health problem and NTM are also frequently reported as causative agents of human infections. [7] 75 HIV positive patients who had pulmonary and/or extrapulmonary manifestations suggestive of Tuberculosis were included in our study. Of these, 48 (64%) were males and 27(36%) were females. Majority of these patients were in economically productive age group of 31-50 years. These findings were consistent with study done by Pais P et al [8] and Sanjay Mehendale. [9] 25 (33.33%) samples showed growth on LJ media. Of these 25, 23(92%) were sputum smear positive by ZN stain. Of the 75 patients, 10 (13.33%) showed isolates of NTM on culture. Of the 10 Patients with atypical mycobacterial infections, 9 had CD4 counts of less than or equal to 100 cells/cu mm and 1 had CD4 count between 101-200 cells/cu mm On the contrary, in Mycobacterium TB infection, none of the patients had CD4 counts of < 100 cells/cu mm, 7 patients had CD4 count between 101-200 cells/cu mm and 8 patients had a CD4 count of> 200cells/cu mm. These findings were comparable to findings

obtained in a study by Levine and Unaison et al [10] who studied non tuberculous mycobacterial infections at John Hopkins Hospital in population of HIV positive subjects.

The management of NTM infections includes medical treatment with various antimicrobal agents based on susceptiblity patterns and surgical treatment as in cases of lymphadenitis, skin or soft tissue infections. Since most of these organisms are resistant to commonly used antimcrobials, drug susceptibility testing becomes mandatory for institution of an effective therapy.

Although there are many reports from India, the exact burden of NTM infections remains unclear. These infections are underdiagnosed mainly due the lack of facilities and expertise. Newer molecular methods like gene probes, PCR and DNA fingerprinting may be better diagnostic tools for NTM.

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