A STUDY OF MYCOBACTERIAL SPECIES CAUSING LYMPHADENITIS

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Abstract. This prospective study evaluated the non-tuberculous mycobacteria (NTM) cases of lymphadenitis. A total of 76 isolates of mycobacteria were obtained from 200 lymph node aspirates suspected of tuberculosis, 74 of which were *Mycobacterium tuberculosis,* one was *Mycobacterium fortuitum* and one *Mycobacterium kansasii*. These results highlight the importance of NTM in HIV-negative patients as a case of lymphadenitis, and indicates the re-emergence of NTM as potential lymph node pathogens in this part of the country. Further studies on a larger scale are needed to delineate the association between NTM infections in HIV positive and negative subjects.

INTRODUCTION

Non-tuberculous mycobacteria (NTM) were observed soon after Koch's discovery of *Mycobacterium tuberculosis,* though their clinical significance was not appreciated until the 1950s when they were classified as "atypical mycobacteria" (Henry *et al,* 2004). NTM comprised of over 95 species and are naturally seen as saprophytes (Katoch, 2004). However, distribution of NTM is not uniform and appears to be geographically or environmentally dependent, but this remains poorly defined (Daniel *et al*, 2000). There are many reports of NTM being associated with human infection involving lungs, lymph nodes, skin, and disseminated infections in immunocompromised individuals (Karak *et al*, 1996). Infection with the human immunodeficiency virus (HIV) is associated with an increasing frequency of mycobacterial infections in general and lymph node infections in particular (Rom and Gray, 2004).

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There are some reports of association between NTM and pulmonary tuberculosis (TB) mainly in immunodeficient persons (Ristola *et al*, 1999; Zumla and Grange, 2002) and sporadically in immunocompetent persons (Ramakrishnan, 1981; Paramasivan *et al*, 1985; Chakrabarti *et al*, 1990). Lymph nodes are the second commonest site for TB after the lungs. However, there is a paucity of information on NTM causing lymphadenitis, since much of the attention is focused on pulmonary tuberculosis. Hence the present study is aimed at identification and characterization of mycobacterial isolates obtained from lymph nodes.

MATERIALS AND METHODS

Two hundred fine needle aspirates collected from suspected cases of tuberculous lymphadenitis were studied. Patient details regarding relevant physical examination, history of previous episodes of tuberculosis (TB) and history of previous anti-TB treatment and HIV sero-positivity were recorded.

All aspirates were cultured for mycobacteria on two sterile Lowenstein-Jensen (LJ)

medium slopes and incubated for 8 weeks at 37ºC. The cultures were examined everyday for one week and thereafter once a week for 8 weeks. The time taken for positive cultures was recorded. Isolates were identified to species level by interpreting the results of standard biochemical tests as described below and properties, such as rate of growth and pigmentation.

Briefly, a standard suspension of each isolate was prepared by adding three loopfulls of colonies onto LJ slope (taken by 3 mm internal diameter, 29 SWG nichrome wire loop) to 0.2 ml of sterile distilled water in a 5 ml Bijou bottle containing 6 glass beads. The bottle was gently vortexed to produce a uniform suspension. Then 0.8 ml of sterile distilled water was added to the suspension and shaken well. One loopfull of the suspension was taken using a 27 SWG nichrome wire loop, for each individual test mentioned below (Sivasankari *et al,* 2006). Standard positive and negative controls were included for each test. Tests results were recorded per standard criteria for positive and negative tests (Table 1).

Table 1
Biochemical tests.

Biochemical test	Test result		Controls	
	Positive	Negative	Positive	Negative
Susceptibility to PNB	Growth	No growth	NTM	*M. tuberculosis*
Niacin accumulation test	Yellow	No color change	*M. tuberculosis*	*M. intracellulare*
Nitrate reduction test	Pink	No color change	*M. tuberculosis*	*M. intracellulare*
Catalase production at 68ºC	Bubbles	No bubbles	*M. fortuitum*/ *M. gordonae*	*M. tuberculosis*
Tween 80 hydrolysis	Pink	No color change	*M. kansasii*	*M. intracellulare*
Arylsulfatase test	Red	No color change	*M. fortuitum*	*M. intracellulare*
Tellurite reduction test	Black precipitate	Gray clumps	*M. avium*	*M. tuberculosis*
Growth on MacConkey agar	Growth	No growth	*M. fortuitum*	*M. tuberculosis*
Thiophene 2 carboxylic acid hydrazide susceptibility	No growth (*ie*, susceptible)	Growth (*ie*, resistance or >1% of colonies are resistant)	*M. bovis*	*M. tuberculosis*

PNB = para-nitrobenozic acid; NTM = Non-tuberculous Mycobacteria

Identification of mycobacteria.

Initially all isolates were confirmed as mycobacteria by Ziehl-Neelsen staining (ZN) and were inoculated onto LJ slope containing 0.5% para-nitrobenozic acid (PNB) (with a final concentration of 500 mg/l), to observe for PNB susceptibility. Growth on PNB medium broadly differentiates NTM from MTB complex (Kubica, 1973).

Identification of MTB complex

All susceptible isolates (suspected to be MTB complex) were subjected to niacin accumulation test and nitrate reduction test to differentiate between *Mycobacterium tuberculosis* and *M. bovis*.

Speciation of NTM

The isolates identified as NTMs were subjected to further speciation by a battery of biochemical tests: 1) assessment of photo

reactivity of mycobacteria, 2) ability to grow at 25º C and 44ºC, 3) aryl-sulphatase test, 4) tween-80 hydrolysis test, 5) catalase test, 6) tellurite reduction test, 7) growth on MacConkey agar and 8) resistance to thiophen-2-carbonic acid hydrazide, as described earlier (Kubica, 1973; Paramasivan *et al*, 1985; Laidlaw, 1989).

RESULTS

Seventy-six mycobacterial isolates were grown from 200 lymph node-aspirates obtained from 200 patients. The age of the patients ranged from 6 to 72 years, 90 were males and 110 were females. The majority of them were in the age group of 21-30 years (Table 2). One hundred seventy-nine specimens were from cervical lymph nodes and 21 from axillary lymph nodes. Thirty-four patients had a history of previous treatment for pulmonary tuberculosis. Twenty-eight patients were HIV positive (17 patients were in the age group of 21-30 years).

Mycobacteria were isolated from 76 (38%) of the total 200 aspirates. Two (2.6%) of 76 were resistant to PNB, indicating the presence of NTM. All the PNB susceptible isolates were identified as *Mycobacterium tuberculosis*.

On further speciation of NTM based on properties, such as rate of growth, pigmentation and biochemical tests, one specimen was identified as *M. fortuitum* (based on the rapid growing, positive arylsulfatase reaction and ability to grow on MacConkey agar), and another as *M. kansasii* (based on positive photo pigmentation, and positive tween-80 hydrolysis) (Tables 3, 4). From the 28 specimens from HIV patients, the fine needle aspirates on only 16 were positive on culture and all the isolates were identified as *M. tuberculosis.*

DISCUSSION

Historically *Mycobacterium tuberculosis*

Table 2
Age group distribution with HIV positivity and number of isolates of mycobacteria.

Age (yr)	Male	Female	HIV	No. of isolates (%)	Total
≤14	6	11	1	4 (23.5)	17
15-20	8	30	1	13 (34)	38
21-30	38	38	17	33 (43)	76
31-40	19	21	6	15 (37.5)	40
41-50	10	5	2	5 (33)	15
> 50	9	5	1	6 (43)	14
Total	90	110	28	76 (38)	200

Table 3
Rate of growth and pigmentation of mycobacteria.

Isolates (no. of isolates)	Optimum isolation temperature (degrees)	Time for growth (in days)	Light pigmentation	Dark pigmentation
M. tuberculosis (68)	37	21	No pigmentation	No pigmentation
M. kansasii (1)	37	18	Pigmentation	No pigmentation
M. fortuitum (1)	37	5	No pigmentation	No pigmentation

Table 4
Biochemical tests for speciation of NTMs.

Test / Biochemical tests	Clinical isolate number-1/result	Clinical isolate number-2/result
Growth on LJ and pigmentation	No pigmentation (pigmentation on exposure to light)	No pigmentation (no pigmentation on exposure to light)
Time taken	18 days	5 days
Temperature	37ºC	37ºC
PNB	Growth	Growth
Nitrate reduction test	Pink color observed	Pink color observed
Niacin utilization test	No color change	No color change
Catalase 68ºC	Bubbles observed	Bubbles observed
Tween 80	Pink color observed	No color observed
Arylsulfatase test	No color observed	Red color observed
Tellurite test	No black precipitate observed	No black precipitate observed
Growth on MacConkey agar	No growth found	Growth observed
Thiophene 2 carboxylic acid hydrazide	Growth observed	Growth observed
Species identified	*Mycobacterium kansasii*	*Mycobacterium fortuitum*

PNB = Para-nitrobenozic acid

and *Mycobacterium bovis* have caused the preponderance of human disease. NTM earlier thought to be rare in clinical specimens, is being increasingly reported, particularly in HIV-positive patients. The rise in the incidence of NTM disease has accelerated rapidly since the first reports of non-tuberculous mycobacterial disease in AIDS patients in 1982 (Falkinham, 1996). The actual frequency and type of mycobacterial disease varies from region to region and between ethnic groups.

Respiratory infections due to NTM are often associated with various predisposing lung conditions (*eg*, pneumoniosis) or amongst patients who were exposed to dust. NTM disease was not exclusively pulmonary, *Mycobacterium scrofulaceum* was found to be the causative agent of cervical lymphadenitis in children (Wolinsky, 1979), *Mycobacterium marinum* infections were found principally in the skin and were associated with cuts or abrasions and exposure to aquaria or swimming pools, or occupations in the fishing industry (Zeligman, 1972).

Pang (1992) reporting from Western Australia noted 118 children, under the age of 7, suffering from mycobacterial lymphadenitis; the disease was caused by *Mycobacterium tuberculosis* in five (4%), the *Mycobacterium avium* complex in 87 (74%) and *Mycobacterium scrofulaceum* in 23 (20%); whereas, in the 54 adults aged 15 years and over, they were caused by *Mycobacterium tuberculosis* in 48 (89%), *Mycobacterium avium* complex in 1 (1.9%) and *Mycobacterium scrofulaceum* in 2 (4%).

In developing countries the situation is different and very little is known regarding lymphadenitis due to NTM in India. *Mycobacterium tuberculosis* has always been found as the major cause of mycobacterial infection, and the proportion of NTM has varied from 1 to 8% (Pathak *et al*, 1973; Hardas and Jayaram, 1984; Paramasivan *et al*, 1986). Cultures with strict criteria are still not routinely practiced. Therefore, it would be difficult to comment on the exact magnitude of the problem due to NTM.

In this prospective study, data on 200 lymph node aspirates from tuberculosis sus-

pected patients showed the commonest age group affected was 21-30 years, followed by 31-40 years (Table 1). This was also noted by Subrahmanyam (1993). In the USA and the UK, the highest incidence of tuberculous lymphadenitis in patients occurs between 25 and 50 years of age (Monie *et al*, 1982; Alvarez and MeCabe, 1984). The ratio of males to females in this study was 1:1.3, which is similar to that found by Subrahmanyam (1:1.3), Dandapat *et al* (1:1.2), and Arora *et al* (1:1.3) (Subrahmanyam, 1993; Dandapat *et al*, 1990; Arora and Gupta, 2006). In the present study 28 patients with LNTB were co-infected with HIV and the majority of them were in the age group 21-30 years old.

Mycobacterium tuberculosis still appears to be the most common causative agent of tuberculous lymphadenitis. Two NTMs (one each of *Mycobacterium kansasii* and *Mycobacterium fortuitum)* and 74 *Mycobacterium tuberculosis* specimens were isolated.

Nataraj *et al* (2002) from Mumbai reported a NTM isolation rate of 3.8% and Jesudason and Gadstone (2005) from Vellore reported a NTM isolation rate of 3.9%. The prevalence of NTM observed in the present study was two out of 76 (2.6%). The detection of NTM is the main reason for culturing all specimens. The two NTM were isolated from HIV sero-negative patients. Isolation of these NTM strains shows that these strains were not only opportunistic infections but re-emerging as potential primary pathogens. This calls for increasing surveillance for NTM in this part of the country.

NTM is widely distributed in the environment, and it is believed that infections with NTM are on the rise in many developed and developing countries. Immunosuppression is considered a predisposing factor. However, in this study the tubercular lymphadenitis due to NTM was seen in a small number of cases. These NTM were isolated from non-immunosuppressed patients, while *Mycobacterium tuberculosis* was isolated from all immunosupressed cases. This raises the possibility of reemergence of NTM as primary pathogens. Further large scale studies are needed to clearly delineate the role of NTM in non-pulmonary infections amongst immunocompromised and immuno-competent patients.

The occurrence of lymphadenitis due to infection with NTM has been reported from other parts of the world where tuberculosis has declined and the relative frequency of NTM has risen, especially in immunocompromised individuals.

We conclude from the present study that lymph node infections due to NTM do occur in HIV-negative patients. Further studies on role of NTM in human disease should be undertaken in more detail and on a larger scale. Information is urgently needed in regard to the proper diagnostic procedures and possibilities for adequate treatment of NTM induced disease.

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