Prevalence of nontuberculous Mycobacterial infection in Non-HIV subjects of clinically presumed tuberculosis at a tertiary care center, Hyderabad

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Abstract

Introduction: Tuberculosis continues to be the leading cause of morbidity and mortality in developing nations. Off late infections due to mycobacterial species other than tubercle bacilli are being reported frequently. Nontuberculous mycobacteria are being increasingly recognized and isolated as pathogens from immunocompetent host too. They are found in both pulmonary and extrapulmonary form of disease. The prevalence of infections due to NTM is known to vary with place, host and climatic factors.

Aim: the present study is a retrospective cross-sectional observational study for a period of two years from Jan.2013- Dec.2014, at Princess Era Hospital,a teaching hospital of Deccan college of medical sciences at Hyderabad. Data from microbiology lab records was retrieved and analysed.

Materials and Methods: about 232 specimens from 229 patients with clinical symptoms of tuberculosis were analysed for laboratory diagnosis of nontuberculosis mycobacterial infection in mycobacteriology section of microbiology laboratory. Seventy nine samples were obtained from 78 patients with pulmonary form of disease and 153 samples were from 151 patients with extrapulmonary form of disease. Study was approved by ethical committee of the college and patient consent was obtained prior to sample collection. All the specimens received in the laboratory were processed by direct microscopy for acid fast bacilli using Ziehl Neelsen stain. Culture was performed on conventional LJ media and Middle Brook 7H12 media in MGIT 320 automated system. Identification of culture positive isolates was achieved by standard biochemical test and rapid immunochromatography test for detection of mycobacterial tuberculosis protein 64 antigen [MPT64].

Results: Direct microscopy was positive in 20.52%. and 4.25% of which were later identified as NTM. Culture yielded positive result in 22.27% of the subjects. NTM were isolated in 3.49% of the total subjects studied. *Mycobacterium tuberculosis* was the most common isolate as 84.31%, both in pulmonary and extrapulmonary form of disease as 76.92% and 92%. NTM were isolated in 15.68% of culture positive samples. Majority of the NTM's were from pulmonary specimens as 75% and rest from extrapulmonary specimens as 25%. In subjects above 50 years of age, NTM was more prevalent in men. The most common predisposing risk factor was chronic obstructive lung disease with bronchiectasis in majority and cavitary lesion in one. And pleural effusion in rest as 25%. In the present study only rapid growers were isolated and *M. chelonae* was the predominant species 87.5% followed by *M.fortuitum* in 12.5%. Sputum yielded maximum NTM's as 50%, BAL as 25% followed by pleural fluid and pleural biopsy as 12.5% each. MPT64 antigen test and Para Nitro Benzoic acid resistance at 500ug/ml concentration were found to be very useful presumptive test for identification of NTM. Samples in duplicate where ever feasible were obtained and processed for NTM; and all yielded the same microbe confirming the etiological role.

Conclusion: NTM infection in symptomatic subjects was seen in significant proportion of the culture positives. Therefore, laboratories performing mycobacterial culture should be equipped with facilities for identification and drug susceptibility test for NTM in order to provide proper diagnosis and targeted therapy. Failure to do so may increase the chances of them being misinterpreted and reported as MDR-TB.

Keywords: Mycobacteria tuberculosis, Nontuberculosis mycobacteria, MPT64 antigen, Para nitrobenzoic acid, Pulmonary form of disease, Extra pulmonary form of disease.

Introduction

Nontuberculous mycobacteria (NTM's) for longtime have been known to cause opportunistic infection in immunocompromised host, especially so in people with HIV/AIDS; but off late they are being increasingly recognized and isolated as pathogens of immunocompetent host too.[1] At present there are about 150 known species of NTM's with 42 documented as human pathogens by[2] They are reported from both pulmonary and extrapulmonary forms of disease in human beings with little variations in prevalence rates with respect to time, place and host.[2] In pulmonary disease they are said to produce symptoms similar to one caused by *M.tuberculosis* but in extrapulmonary form of disease the symptoms are varied and novel which are difficult to interpret.[1] Further, it has been established in several studies that they exhibit variation in geographic distribution, species spectrum, clinical presentation and antibiotic susceptibility test profile. Therefore, lack of proper identification and drug susceptibility testing facilities may increase the chances of them being misreported as multi drug resistant mycobacterium tuberculosis. Hence, at least level II & III laboratories besides facilities for isolation of mycobacteria, should be equipped with rapid & reliable tests for their identification and DST, in order to provide correct diagnosis and management of patients afflicted with NTM disease. Delayed plus improper diagnosis and treatment is often associated with increased morbidity and mortality.[3] The increase in the incidence and prevalence of NTM's has been attributed to several reasons like, advanced invasive,

diagnostic and therapeutic procedures, use of improperly sterilized and disinfected instruments during surgical and cosmetic procedures, use of contaminated irrigation fluids and in last but not least is the availability of newer diagnostic test for rapid diagnosis and identification of mycobacterial species.[3] Laboratory diagnosis of NTM's for long; has been dependent on isolation and identification of the species using conventional methods. The major drawback of conventional methods is the technique involved is tedious, requires chemicals which are difficult to obtain, need skilled personnel to perform and interpret the test results and finally compliance with infection control practices. Newer and rapid techniques based on nucleic acid detection & amplification, mycolic acid identification though available in market but requires sophisticated and expensive instruments and skilled personnel which limits their usage. Data on the incidence, prevalence, species specific geographic distribution and antibiotic profile of nontuberculous mycobacteria is scarce from this region of Telangana state, in south India. The present study makes an attempt to check the prevalence of NTM's, their species spectrum and clinical manifestations at a tertiary care hospital over a period of two years.

Aim

The present study is designed to know the prevalence of nontuberculous mycobacterial infection in non-HIV subjects of presumed tuberculosis at a tertiary care center in Hyderabad. And to know the species spectrum, demographic profile and the associated clinical manifestations.

Materials and Methods

A cross sectional retrospective observational study was designed during the period of two years from January 2013 - January 2015 at Princes Esra Hospital, a teaching hospital attached to Deccan college of medical sciences, Hyderabad, Telangana state of south India. To know the prevalence, species distribution and clinical presentation of NTM's infection in subjects with clinically presumed as tuberculosis. Data from clinical and microbiology laboratory records was retrieved for the study, analyzed and presented.

Inclusion and exclusion criteria – as per the American thoracic society [ATS] & Infectious disease society of America [IDSA], which combines clinical, radiological, histopathological and microbiological criteria for diagnosis of nontuberculous mycobacterial disease.

Materials

232 specimens from 229 patients registered with presumed clinical tuberculosis were involved in the study. Specimens from both pulmonary and extrapulmonary form of disease were included. Sputum samples were collected by DOTS [Directly observed treatment short course] staff by giving proper instructions to the patient on collection method. Whereas rest of the samples were physician provided; either by the surgeon's or pulmonologist as it involved invasive techniques. After collection, samples were analysed by smear microscopy and culture. Around 79[34.05%] of the total samples submitted for the laboratory diagnosis were from the pulmonary form of disease and another 153[65.94%] were from the extrapulmonary form of the disease. Samples in duplicate where ever feasible were subjected for isolation and identification of NTM's.

Methods

All the specimens after standard digestion and decontamination procedure where ever applicable were processed by direct smear microscopy (Ziehl Neelsen stain), culture and identification using various biochemical tests. Isolation of mycobacteria from clinical specimens was achieved using two different methods, conventional Lowenstein Jensen media and Middle Brook 7H12 in MGIT 320 system from BD USA. All the cultures were incubated in solid media for 8 weeks' time and in MGIT 320 SYSTEM as per the manufacturer guidelines. Samples with no growth at 8 weeks in solid media and in liquid media after 4 weeks were declared negative. Primary identification of culture positive isolates was done by noting the incubation period, colony morphology and pigmentation. Further, all the culture positive isolates were subjected to rapid identification test using MPT 64 antigen test from SD BIOLINE Germany for detection of mycobacterial protein antigen specific to *mycobacterium tuberculosis*. Basically, to distinguish the isolates provisionally as *Mycobacterium tuberculosis complex* [MTBC] and NTM's. Subsequently all MPT64 antigen negative isolates were further identified by a battery of conventional biochemical test like niacin accumulation, nitrate reduction, tellurite reduction, aryl sulfatase production, urease production, growth on Mac.Conkey agar without crystal violet, tolerance to 5% Nacl2, heat stable catalase production at 68^0c, resistance to para nitro benzoic acid (PNB) as mentioned in standard text book of diagnostic microbiology by Koneman EW and text book by C. Mahon.[4,5]

Screening for HIV 1 & 2 virus was done in integrated counselling & testing center [ICTC] for HIV/AIDS in Princess Esra Hospital. All the subjects were found to be nonreactive for both HIV 1 & 2 virus antibodies.

Ethical clearance – was obtained from ethical committee of the institute prior to the study and patient consent obtained before sample collection in all the subjects.

Quality Control

Following reference strains of mycobacteria from American type culture collection [ATCC] were used in the study as positive controls for identification of mycobacterial species, H37Rv ATCC 19977, for *Mycobacterium tuberculosis*, ATCC®35752 for *M.chelonae* and ATCC® 6841 for *M.fortuitum*.

Results

Of the 232 specimens from 229 patients received & processed for tuberculosis around 47 [20.52%] where positive by smear microscopy, 4.25% of which were later identified as NTM's. Culture yielded mycobacterial growth

for 51 specimens [22.27%], of which 26 [50.98%] were from pulmonary and 25 [49.01%] from extrapulmonary form of disease. While considering culture as the gold standard technique for diagnosis of mycobacterial disease the overall prevalence of tuberculosis was 43/229 [18.77%] and NTM as 8/231 [3.49%] as shown in Fig. 1.

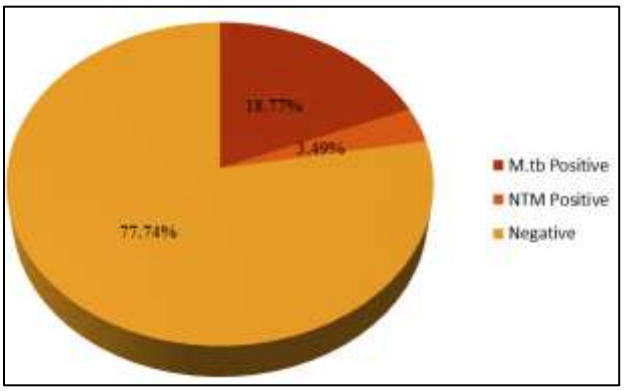

Fig. 1: Prevalence of Non tuberculosis mycobacterial infection

Majority of the samples received were from pleural fluid as 73/232 [31.46%] followed by sputum 56/232 [24.13%], lymph node 38/232 [16.37%], bronchoalveolar lavage fluid 21/232 [9.05%], gastrointestinal as 16/232 [6.89%] musculoskeletal as 11/232 [4.74%] pus as 5/232 [2.15%], miscellaneous as 5/232 [2.15%], genitourinary as 3/232 [1.29%], lung biopsy as 2/232 [0.86%], empyema and pleural biopsy as 1/232 [0.04%] each as shown in table 1 & 2 below.

Table 1: Shows distribution of pulmonary specimens and their culture findings

Clinical type of tuberculosis	Samples submitted	Culture positive for M.tb	Culture positive for NTM	Total Culture positive specimens	Total Culture negative specimens
Pulmonary	79	20	06	26	53
Sputum	56	18	04	22	34
BAL	21	02	02	04	17
Lung biopsy	02	00	00	00	02

Table 2: Shows distribution of extrapulmonary specimens and their culture findings

Clinical type of tuberculosis	Samples submitted	Culture positive for M.tb	Culture positive for NTM	Total Culture positive specimens	Total Culture negative specimens
Extrapulmonary	153	23	02	25	128
Pleural fluid	73	04	01	05	68
Pleural biopsy	01	00	01	01	00
Empyema	01	00	00	00	01
Lymph node	38	12	00	12	26
Gastrointestinal tract	16	01	00	01	15
Ascitic fluid	06	00	00	00	06
Peritoneal fluid	04	00	00	00	04
Pus from omentum	01	00	00	01	01
Pus from liver biopsy	04	00	00	00	04
Pus from abdominal cavity	01	01	00	01	00
Musculoskeletal	11	03	00	03	08
Synovial tissue	05	00	00	00	05
Fluid from greater trochanter of Humerus	01	00	00	00	01
Pus from psoas abscess	01	01	00	01	00
Pus from Sacroiliac joint	01	01	00	01	00
Potts spine	02	01	00	01	01
Bone marrow	01	00	00	00	01
Breast abscess	04	01	00	01	03
Pus	01	01	00	01	00
Urine	01	00	00	00	01
Endometrial curetting's	02	00	00	00	02
Miscellaneous	05	01	00	01	04

Table 3: Shows gender wise distribution of subjects studied and culture positive cases

Clinical type of tuberculosis	Male	Culture positive	M.tb Positive	NTM Positive	Female	Culture positives	M.tb Positive	NTM Positive	Total	Total culture positives
Pulmonary	36 [33.64%]	7 [19.44%]	04 [57.14%]	03 [42.85%]	42 [34.42%]	19 [45.23%]	16 [84.21%]	03 [15.78%]	78 [34.06%]	26 [50.90%]
Extra pulmonary	71 [66.35%]	8 [11.26%]	07 [87.5%]	01	80 [65.57%]	17 [21.25%]	16 [94.11%]	01 [5.88%]	151 [65.93%]	25 [49.01%]
Total	107 [46.72%]	15 [14.01%]	11 [10.28%]	04 [3.73%]	122 [53.27%]	36 [29.50%]	32 [26.22%]	04 [3.27%]	229 [100%]	51 [22.27%]

Table 4: Shows age wise distribution of symptomatic subjects and mycobacterial culture positive patients

Age group in years	Males			Females			Total culture positives for M.tb	Total culture positives for NTM species
	Total subjects studied	M.tb disease Positives	NTM disease Positives	Total subjects studied	M.tb disease Positives	NTM disease Positives		
1-10	03	00	00	01	00	00	00	00
11-20	13	03	00	23	12	00	15	00
21-30	15	02	00	34	10	02	12	02
31-40	09	00	01	11	03	01	03	02
41-50	14	03	01	17	03	00	06	01
51-60	15	02	00	11	02	00	04	00
61-70	22	01	01	11	00	01	01	02
71-80	12	00	01	08	01	00	01	01
81-90	04	00	00	05	00	00	00	00
91-100	00	00	00	01	01	00	01	00
Total	107	11	04	122	32	04	43	08

All the 51 isolates obtained in culture for mycobacteria were broadly & provisionally identified as MTBC and NTM's using MPT64 antigen tests which categorized them as 43/229 [18.77%] *Mycobacterium tuberculosis* complex and 8/229 [3.49%] non tuberculosis mycobacterial species. Among the 8 NTM's isolated six [75%] were from pulmonary form of disease and two [25%] from extrapulmonary form of disease. Of the six pulmonary cases which yielded nontuberculous mycobacterial growth, 4 [66.66%] samples were from sputum and two from bronchoalveolar lavage fluid [33.33%]. Of the two extrapulmonary samples which yielded growth one was pleural fluid [50%] and another pleural biopsy tissue [50%] as shown in table 1 & 2 above.

Further identification of the NTM isolates was done by growth rate and pigment production. For most of the NTM isolates growth appeared within one-two days of incubation in MGIT 320 system. And in solid media on LJ media most of the isolates grew within 5 days i.e. less than a week which excludes *M.tuberculosis* and slow growing NTM's belonging to group I,II & III of Runyon classification. All were non chromogenic both in light and dark. Therefore, we excluded Runyon group I & II mycobacteria as well. All were rapid growers belonging to Runyon group IV.

Later, other biochemical test were performed for RGM, like growth on Mac.Conkey agar without crystal violet, tolerance to 5% Nacl2, niacin accumulation, nitrate reduction, tellurite reduction, heat stable [at 68^0c] catalase production, urease production, aryl sulfatase production and resistance to Para Nitrobenzoic acid in 500ug/ml concentration for characterization of rapid growing mycobacteria. All the strains of NTM showed resistance to PNB in 500ug/ml concentration. Based on the results obtained for various biochemical test performed in the study mainly nitrate reduction, tolerance to 5% Nacl2 and rapid tellurite reduction and aryl sulfatase production within 3 days of incubation as mentioned in text book of diagnostic microbiology by[4,5] we found *M.chelonae* as the most predominant NTM species followed by *M.fortuitum*. *M.chelonae* was isolated from 7/8 as [87.5%] of the specimens, 6/7 [85.7%] from pulmonary and 1/7 as [14.28%] from the extra pulmonary disease. And 4/7 as [57.1%] from sputum, 2/7 as [28.57%] from BALs and 1/7 as [14.4%] from pleural fluid. *M.fortuitum* was isolated from 1/8 as [12.5%] extrapulmonary specimen i.e. pleural biopsy.

Demographic profile of the subjects studied revealed an overall female preponderance as 53% followed by males as 47%. Pulmonary and extrapulmonary manifestations were more common in females as 42/78 [53.81%] & 80/122 [65.57%] of the total subjects studied. Overall prevalence of disease for men was 15/107 as14.01% and for women 36/122 as 29.50% as shown in table.3 below. Culture also yielded maximum positive results in females as 19/26 [73.07%] in pulmonary form of disease and 17/25 [68%] from extra pulmonary disease. Hence in general whether pulmonary or extrapulmonary; mycobacterial disease was more common in females in our study.

Both in pulmonary and extrapulmonary form of disease M.tb was the predominant isolate in men and women. In culture positive pulmonary form of disease 26/78 studied. 19 were females and 7 males. Sixteen of 19 females as [84.21%] were having M.tb disease and 3 as [15.78%] were due to NTM infection. And of the seven males; 4/7 as

[57.14%] were suffering from M.tb infection & 3/7 [42.85%] were due to NTM infection. In case of 25 extrapulmonary culture positive cases 8/25 as [32%] were males and 7 of them as [87.5%] had M.tb disease and 1 [12.5%] was suffering from NTM disease. And of the 17 females as [68%] with extra pulmonary form of disease 16/17 as [94.11%] had M.tb infection and 1/17 [5.88%] had NTM infection [table 3]. However, overall prevalence of NTM infection in men was greater i.e. 3.73% when compared to women as 3.27%. With respect to gender and number of culture positive subjects; it was once again seen that NTM infection was more common in males as 4/15 [26.66%] than in females as 4/36 [11.11%].

The mean age of the subjects studied was 43.66 years. For males as 48.17 years and females as 39.45 years. Mean age of male subjects with clinical pulmonary form of disease was 51.60 years and for females as 36.07 years which is significant. In case of clinical EPTB disease the mean age for males was 42.26 years and for females as 41.13 years. As shown in table 4; Majority of the subjects in the study group were in the age group 11-40 years as 105/229 [45.85%] followed by 61-100 years as 63/229 [27.51%] and then in 41-60 years as 57/229 [24.89%] and least in the age group 1-10 years as 3/229 [1.3%] which is again significant. And almost similar pattern of distribution of age group was also seen in culture positive subjects with 34/51 as 66.66% in the age group 11-40 years and 11/51 as 21.56% in the age group 41- 60 years and least in 61-100 years age group as 6/51 [11.76%]. More so in females in the age group 11-40 years as 28/34 [82.35%] positives and 6/34 in males as [17.64%], then in the age group 41- 60 years it was noted higher in males as 6/11 [54.45%] than in females as 5/11 [45.45%]. In the age group 61-100 years it was observed equally in both genders as 3/6 [50%] each. NTM disease was more common in the age group less than 50 years as 5/8 [62.5%] of the total culture positive cases with 2/5 [40%] as males and 3/5 [60%] as females. In the age group above 50 years NTM's were seen in 3/8 [37.5%] of culture positives with more number of positive cases in males as 2/3 [66.66%] than in females as 1/3 [33.33%].

Clinical manifestations

It was observed that 75% of the culture positive cases of NTM disease had pulmonary symptoms of chronic obstructive pulmonary disease. Bronchiectasis as major finding followed by cavitation and bronchial solitary or multiple nodular disease on high resolution computerized tomography. Pleural effusion was seen in rest 25%. In our study we didn't had any cases of NTM infection arising due to invasive surgical or cosmetic procedures. Repeat samples in most of the cases yielded the same isolate confirming the a etiology.

Discussion

In the present study the overall prevalence of mycobacterial disease was 22.26% and one due to *Mycobacterium* tuberculosis complex as 18.77% & due to NTM disease as 3.49% which is in accordance with the report by V P Nayeedu et al as 26.33%, as the overall mycobacterial disease prevalence and 25.95% for M. tb and 0.38% for NTM infection.[6] M.tb was the predominant isolate in our study as 84.31% followed by NTM as 15.68% which is also seen in other studies by J Umrao et al as 70.9% and 29%, VP Nayeedu as 98.53% and 1.46%, MV. Jesudasan et al as 96% and 3.9%, Cleoni A et al as 83.1% & 16.9%, Kee Peng et al as 67.45% & 30.83%.S Premraj et al as 77.95 & 22.0%, Preeti C et al as 65.67% & 37.31%, AK Mourya et al 72.6% & 27.4% and Sarika J et al as 90.15 & 9.9%.[2,6-13]

According to various studies NTM disease prevalence varies with geographic, climatic & host predisposing factors. Highest prevalence is reported from United States both from North and South America as 17.9% & 16%, and Europe as 14%.[3,14] In Asia highest prevalence is noted from Taiwan and South Korea as 50% & 28.7%.[14] In India the prevalence has been reported to vary between 0.7% to 34%. Further within the various states of India prevalence is varied; from Chandigarh as 7.4%, Delhi as 8.3%, Kolkatta as 17.4%.[2] Across the world rapid growing mycobacteria [RGM] infections account for around 10-20% of all the NTM isolates.[14] In our study NTM infection due to RGM were prevalent in 15.68% of culture positive isolates, which is similar to one reported by Cleoni A et al as 16.9%, P Maureen as 17.8% and less when compared to reports of 27.4% by AK Mourya et al, J. Umrao as 29%, Kee Peng as 30.83% Preethi C et al as 36.23%.[2,8,9,11,12,15] Some authors have reported low prevalence rates like VP Nayeedu et al 0.38% and Sarika J et al as 9.9%, this could be due to reasons like geographic distribution, host preferences and isolation and identification facilities available.[6,13]

Direct microscopy was positive in 20.52% of the subjects with suspected tuberculosis which is in accordance with the international figures mentioned by WHO as the sensitivity of the smear is between 20-80%. In a study by AK Mourya, ZN was positive in 30.1% of the subjects, and one by J Umrao et al and Sarika J et al as 25.6%.[2,12-13] In our study for NTM disease direct smear was positive in only 25% of the culture positives. Hence it is always recommended to consider mycobacterial culture in first place than direct microscopy alone which may miss 75% of the cases. However, in absence of microbiological diagnosis even a combination of radiological findings and histopathological results are significant as per IDSA & ATS criteria.[1] It is also recommended that samples for NTM disease microbiological confirmation must be processed in duplicate where ever feasible to rule out mere contamination.[1]

Majority of the samples in laboratory confirmed NTM disease were from sputum around 57% followed by BAL as 28% then pleural fluid and biopsy as 14% which is similar to other reports, J. Umrao et al as 74.5% from sputum and from BAL as 7.2%, VP Nayeedu et al. reported an isolation rate from sputum as 36.66% in BAL as 8.3% and pleural fluid as 6.6%.[6] Anyhow some authors have reported NTM disease more in extrapulmonary tuberculosis like one by AK Mourya as 27.4%.[2,12]

NTM disease was found to be more prevalent in pulmonary form of disease as 6/76 [7.89%] than in extrapulmonary as 2/153 [1.30%] which is similar to the reports by P Maureen et al as 7.2% & 1.2%, VP Nayeedu as 0.28% & 0.12%.[6,15] In culture positive cases for NTM also 6/8 [75%] were from pulmonary disease and 2/8 [25%] were from extrapulmonary disease. Other authors too have reported a higher prevalence in pulmonary form of disease than extrapulmonary as 69.2% & 30.8% by Sarika J et al, Imran Ahmed as 88.40% & 11.53% and J Umrao as 79.4% & 18.2%.[2,13,16]

Majority of the subjects studied were females as 53.27% followed by males as 46.72% which is in contradiction to other studies where males predominated. Whether pulmonary or extra pulmonary form of disease culture was positive more in females 36/ 51[70.58%] than males as 15/51 as [29.41%]. With respect to gender also culture was positive more in females as 36/122 [29.50%] than in males as 15/107 14.07%. this is in contradiction to the report by Premraj S et al as pulmonary disease more common in males as 27.57% than in females as 19.88%, and extrapulmonary more common in females as 7.45% than in males as 2.65% in.[10] However, in our study NTM disease was more prevalent in males as 4/107 [3.73%] than in females as 4/122 [3.27%]. With respect to gender and culture positive cases as well, males predominated as 4/15 [26.66%] and females as 4/36 [11.11%]. Higher prevalence of NTM disease in males is also reported by other authors like J Umrao as 60.4%, Cleoni A as 64.8%, Preethi C et al 61.44%, Sarika J et al as 69.2% Claire A et al as 54.77%.[2,8,11,13,17]

The studied subjects where in the age group 9-96years. The mean age for NTM disease noticed was 46.12 years and for females as 37.25 years and for males as 55 years. Cleoni A et al reported mean age as 50 years.[8] Claire A et al reported as 60.2 years which is higher when compared to ours study.[17] NTM was isolated in 62.5% in the age group less than 50 years. This is in contradiction to other studies and the reason for it could be due host and environmental factors which might have predisposed to early onset of the disease manifestations. And in 37.5% of the cases it was seen in above 50 years of age. But most of the studies reported between 50-60 years as the most common age for NTM disease. In above 50 years it was more common in males as 66.66% indicating chronic lung disease as the predisposing factor.

The most common predisposing factors mentioned in several studies for pulmonary form of NTM disease is structural lung disease and impaired clearance of the organisms like in patients with cystic fibrosis, severe bronchiectasis, cavitary lung disease etc.[3] Seventy five percent NTM culture positive cases in our study were having COPD as the chief clinical manifestation, presenting with bronchiectasis [solitary or multiple nodular disease] on HRCT which is similar to the reports by Sarika as 54% having chronic lung disease with 32.9% as with COPD.[13] Claire A found it in 32.9% of the cases.[17] In others studies like by Koh WJ et al cavitary lung disease was most common sign as 42.05% followed by nodular bronchiectasis in 10%.[17,18] Pleural effusion was noticed in rest 25%.

In the present study rapid growing mycobacteria [RGM] were isolated from both pulmonary and extrapulmonary form of disease similar to the report by MV Jesudasan where RGM were reported as 87% of the total NTM isolates.[7] *M.chelonae* was the most predominant species as 75% followed by *M. fortuitum* as 25%. Similar findings are reported by MV Jesudasan et al as 46% & 41%.[7] AK Mourya reported *M.fortuitum* as most common in 27.8% of cases *M.chelonae* in 8%.[12] In J Umrao et al study most common isolate was *M.abscessus* as 31.3% followed by *M. fortuitum* in 22% & *M.chelonae* in 9%.[2] Cleoni A.et al found *M.abscessus* as most common as 32% *M. avium* in 12% and *M.fortuitum* in 9%.[8] Imran Ahmed et *al M.fortuitum* in 38.88%, Kee Peng et al found *M.fortuitum* in 45.7% *M. abscessus* in 26.2% *M.intracelulare* in 10.4%.[16] Koh WJ et al found *M.avium* in 48% *M. abscessus* in 33%[18] in the study by VP Nayeedu *M.simeae* was most common.[6] Sarika et al found *M.avium* as most common.[13] Hence, we reconfirm the findings of the study by V.Chilota[14] that species spectra varies according to geographical location.

The rapid antigen test for detection of *M. tuberculosis* has proofed a useful test and the results of which were in cent percent concordance with PNB resistance test. Other authors like B Sharma et al also have reported PNB test in 100% accordance with MPT64 antigen test.[19] AK Mourya, J Umrao and Preeti C also found MPT64 antigen test very useful in rapid presumptive identification of NTM.[2,11,12]

Conclusion in the present study NTM disease was found prevalent in significant proportion of culture positive cases. Hence it becomes crucial to have facilities for isolation and identification of mycobacterial species in laboratories were tuberculosis culture is performed. Further it is observed that NTM disease is more prevalent in males in the age group above 50 years and with predisposing factors like chronic lung disease. Therefore, it is recommended to screen male patients for tuberculosis with symptoms of chronic lung disease in this age group. *M.chelonae* is the most common isolate which reiterates the study by Violet C et al that there is variation in disease manifestation, species spectrum, age and gender predisposition from one geographic location to another. MPT 64 antigen test and PNB resistance test are good provisional test for differentiating mycobacterium tuberculosis from other species of mycobacteria.

Limitations

The present study identified non tuberculous mycobacterial species using conventional biochemical test, MPT64 antigen and PNB resistance test which are not confirmatory test. ATS/ IDSA recommends molecular assays for confirmation like Line probe assay [Genotype Mycobacteria CM/AS from Biomerieux], HPLC for detection of species specific mycolic acid, PCR for highly conserved regions like 16S rRNA, and gene coding for hsp65 protein or RFLP. However, in resource poor settings where these tests are not available or affordable conventional test can still be

continued for provisional diagnosis of NTM disease. Further we couldn't perform drug susceptibility test for the NTM species isolated, which is major drawback as NTM species vary in their DST profile and show false resistance to most of the first line drugs and few second line anti tuberculosis drugs which may be misinterpreted as MDR-TB.

Recommendations

As far as possible we recommend the clinical microbiologist where ever feasible to identify nontuberculous mycobacterial to the species level using confirmatory test and perform drug susceptibility for them due to reasons already mentioned in introduction part and elsewhere in the article.

Conflict of Interest: None.

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