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Mycobacterium tuberculosis Dominance over Nontuberculous *Mycobacteria* Irrespective of Immune Status: An Indian Scenario

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ABSTRACT

Introduction: In HIV-associated tuberculosis cases, identifying nontuberculous *Mycobacteria* (NTM) from clinical samples has become essential regarding patients' treatment and prognosis. This study aims to determine the prevalence of different *Mycobacteria* species from immunocompromised and immunocompetent patients with suspected tuberculosis attending a tertiary care hospital in Kolkata, India. **Methods*:*** Clinical samples from 112 suspected tuberculosis patients were examined by direct microscopy after Ziehl–Neelsen staining. After culture in BacT/ALERT 3D mycobacterial culture system, identification of the causative agents was performed using the GenoType MTCM system. **Results:** Culture positivity was higher in immunocompromised patients than in immunocompetent in both pulmonary and extrapulmonary samples. In smear-negative samples, culture positivity in immunocompromised patients (50%) was much higher than in immunocompetent patients (26.67%). Of 78 samples, four were NTMs (5.13%), including 1 *M. avium*, 1 *M. abscessus*, and 2 *M. fortuitum*, and 74 were *Mycobacterium tuberculosis* (MTBC). Two NTMs belonged to immunocompromised patients and two to immunocompetent individuals. The chance of Mycobacterial infection was higher in patients with a lower CD4 count. Both NTMs found in the immunocompromised group were from patients with a CD4 count<100. **Conclusion:** The present study showed that the MTBC is still the primary causative agent in pulmonary and extrapulmonary tuberculosis in this part of India.

INTRODUCTION

Currently, the genus *Mycobacterium* includes more than 100 recognized or proposed species. These organisms produce a spectrum of infections in humans and animals, ranging from localized lesions to disseminated diseases. Previously, nontuberculous *Mycobacteria* (NTM) were considered colonizers or harmless environmental contaminants. They are now increasingly recognized as important human pathogens in immunocompromised and immunocompetent populations [1]. Like TB, the lung is the most commonly involved organ in NTM-associated diseases. However, reports of surgical-site infections, post-injection abscesses, osteomyelitis, catheter-related bloodstream infections, and central nervous system infections are available [2]. NTB infections and associated diseases have significantly increased worldwide in recent years. The rates vary widely depending on the population and geographic location [3, 4].

Human immunodeficiency virus (HIV) patients possess an immunologic vulnerability due to defective T-cell-mediated immunity, making them particularly vulnerable to *Mycobacteria* pulmonary infection. In a retrospective analysis of HIV-related hospital admissions to a hospital in metropolitan Florida, USA, NTM was responsible for 11% of respiratory illnesses and associated with an extended hospitalization [5]. A study on hospitalized HIV-infected patients from 1989 and 2002 revealed three risk factors contributing to increased mortality: a) a single positive respiratory *Mycobacterium kansasii* culture, b) a positive sputum smear microscopy for NTM, and c) lack of an adequate mycobacterial treatment [6].

The exact burden of NTM disease is not known because it is not a reportable disease worldwide, including in India. Also, clinicians lack enough awareness of NTM, and laboratory capacities are unavailable to diagnose them [7]. Among few reports available, NTM isolation rates range

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from 0.5 to 8.6% in India [8]. A recent study from central India reported an increased NTM prevalence from 1.0% in 2005 to 3.5% in 2008, with 88.6% of the isolates being clinically relevant [8].

It is unclear whether the predictors and associated conditions of NTM disease described in non-HIV-infected populations are valid in HIV-infected patients with NTM. Categorized NTM culture positivity in individuals with HIV as infection vs. colonization concluded that NTM infection should be suspected in patients with longstanding symptoms, anemia, low CD4 count, and multiple positive sputum acid-fast bacilli (AFB) cultures [9]. Identifying NTM is vital from the clinical point of view; most of these bacteria are notably resistant or only partially susceptible to the standard anti-tubercular drugs. The treatment strategies and the duration of these infections differ from MTB.

Here, we investigated the prevalence of different *Mycobacteria* species from immunocompromised and immunocompetent patients with suspected tuberculosis.

MATERIAL AND METHODS

The study was conducted prospectively in a tertiary care hospital in Kolkata, India. One hundred twelve clinically suspected tuberculosis patients were included using a consecutive and convenience sampling method. Among them, 56 belonged to the immunocompetent individuals, and 56 to immunocompromised patients (HIV positive). Among the 112 samples, 75 were pulmonary, and 37 were extrapulmonary. Only HIV/AIDS patients were included under the immunocompromised group.

Patients who were already diagnosed and receiving anti-tubercular treatment and those who discontinued therapy without completion of the standard regime were excluded from the study. Written informed consent was obtained from all the participants, the Ethics Committee for the School of Tropical Medicine, Kolkata, West Bengal, India, approved the study (ethical code: CREC-STM/243).

Sample collection and laboratory processing. Clinical samples from 56 immunocompetent and 56 immunocompromised patients with suspected tuberculosis were examined by direct microscopy for AFB after Ziehl–Neelsen (ZN) staining (except for bone marrow samples).

Irrespective of smear positivity, all samples were decontaminated using the N-Acetyl-L-Cystine (NALC) – NaOH concentration method. All the sample processing was performed in a biosafety cabinet.

The concentrated and processed samples were transferred in a BacT/Alert 3D – 60 system to detect bacterial growth in MB/BacT liquid culture, containing Middlebrook 7H9 broth, a pancreatic digest of casein, Bovine serum albumin, and catalase in purified water. The instrument detected growing bacteria by monitoring CO_2 production using a colorimetric CO_2 sensor in each bottle.

DNA was extracted from the cultured bacteria using the GenoLyse kit (Hain Lifescience, Germany). The extracted DNAs were subjected to a multiplex PCR amplification, followed by identifying the causative agents by the GenoType MTCM system (Hain Lifescience, Germany). This system identifies *M. avium, M. intracellulare, M. chelonae, M. abscessus, M. fortuitum, M. gordonae, M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. peregrinum, M. marinum/M. ulcerans*, the *M. tuberculosis* complex (MTBC), and *M. xenopi*. This system performs reverse hybridization, i.e., probes (reaction zones or bands) on the nitrocellulose strips were used to target various *Mycobacterium* species DNA by detecting sequences complementary to the probes on strips.

RESULTS

A total of 75 pulmonary samples and 37 extrapulmonary samples were collected. In the pulmonary samples, 38 were from immunocompetent patients and 37 from HIV patients, with the majority (74.67%) originating from sputum. Among the extrapulmonary samples, 18 belonged to immunocompetent patients and 19 immunocompromised patients. The majority of the samples comprised pus. Forty-four samples (78.57%) from immunocompromised were culture positive, vs. 34 (60%) from immunocompetent patients. Among 31 culture-positive samples from immunocompromised patients, 30 were MTBC, and one was *M. avium*. Among 27 culture-positive samples from immunocompetent patients, 26 were MTBC, and one was *M. fortuitum*. Hence, of 58 culture-positive pulmonary samples, 56 were MTBC, and 2 were NTM. There was no significant difference in MTBC distribution between immunocompromised and immunocompetent individuals ($\boldsymbol{P}$ = 0.9416).

Among nine culture-positive samples from immunocompromised patients, eight were MTBC, and one was *M. fortuitum*. Among five culture-positive samples from immunocompetent patients, four were MTBC, and one was *M. abscessus*. Hence, of 14 culture-positive extrapulmonary samples, 12 were MTBC, and 2 were NTM. There was no significant difference in MTBC distribution among immunocompromised and immunocompetent individuals ($\boldsymbol{P}$ = 0.6392).

Study subjects were divided into eight groups according to the CD4 count status comprising the lowest (18 cells/μl) and the highest (702 cells/μl). Table 8 shows the genotyping of MTB and NTM concerning CD4 count. NTM bacteria were found only in patients with CD4 count <100. Patients with lower CD4 count were more culture-positive than patients with higher CD4 count.

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Table 1. Detected species according to patients' immune status

Immune Status	MTBC	NTM
Immunocompromised	42	2 (1 *M. fortuitum*, 1 *M. avium*)
Immunocompetent	32	2 (1 *M. fortuitum*, 1 *M. abcessus*)
Total	74	4

Table 2. Culture positivity and species in immunocompromised patients concerning CD4 count

CD4 count (cells/μl)	No. of patients	Culture +	Culture positivity (%)	Species
</= 100	10	9	90	7 MTBC, 1 *M. fortuirum*, 1 *M. abscessus*
101-200	14	11	78.57	
201-300	9	8	88.89	
301-400	8	6	75	
401-500	4	4	100	35 MTBC
501-600	5	4	80	
601-700	5	2	40	
>700	1	0	0	
Total	**56**	**44**		**42 MTBC, 2 NTM**

Final outcome. There were 78 positive cultures; 74 belonged to MTBC and 4 to NTM. Among the 4 NTM, 2 were *M. fortuitum,* 1 *M. avium,* and 1 *M. abscessus.*

DISCUSSION

This study investigated the prevalence of MTBC and NTM in immunocompromised and immunocompetent patients. We also determined the chance of mycobacterial infection in relation to CD4 count.

Regarding pulmonary specimens, culture positivity was higher in immunocompromised patients (83.78%) than in immunocompetent individuals (71.06%); but the difference was not statistically significant (***P*** = 0.0644). Similarly, in extrapulmonary cases, culture positivity was much higher in the immunocompromised group (68.42%) than in the immunocompetent group (38.89%), but the difference was not statistically significant (***P*** = 0.1411). High smear negativity among TB patients in a clinical setting with high TB and HIV prevalence implied that many HIV-associated pulmonary tuberculosis cases might be overlooked by microscopy and emphasize the need for new diagnostic methods [10]. Small *et al.* (1992) showed that HIV-positive patients were more likely to present with extrapulmonary or sputum smear-negative tuberculosis than HIV-negative patients. Like the present study, extensive pulmonary tuberculosis was considered an AIDS-defining criterion [11].

In our study, species identification from 78 DNA samples using line probe assay using the GenoType MTCM kit detected four (5.13%) NTM and 74 MTBC (94.87%). Jesudasan *et al*. (2005) at CMC, Vellore detected NTM in 3.9% of the samples, mainly comprising *M. chelonae* (46%) and *M.fortuitum* (41%) species [12]. Narang *et al*. (2004), using the paraffin baiting technique, for the first time, showed NTM including 4 *M. avium* complex and 2 *M. fortuitum* in the stool of HIV seropositive patients [13]. Further studies by the same authors in 2005 again demonstrated NTM mycobacteraemia in HIV seropositive patients for the first time. This was the first report demonstrating disseminated NTM infection in India where NTM were isolated from 6 HIV seropositive patients (8.4%), of which three were positive for MAC (4.23%) and another three for *M. simiae* (4.23%), a rare isolate to cause mycobacteraemia [14].

The chance of mycobacterial infection was more in patients with a lower CD4 count. Also, both NTMs in the immunocompromised group were from patients with a CD4 count<100. Interestingly, two NTM cases were in immunocompetent patients who had no definite relationship with CD4 count. The reason behind this phenomenon is poorly understood. The CD4 counts in such patients, as stated earlier, were less than 100 cells/μl, similar to a previous study that reported isolating 6 NTM using blood culture from HIV seropositive patients [15]. The rate of NTM bacteremia in AIDS patients coming to the Mahatma Gandhi Institute of Medical Sciencesin 2002 was 8.5%, but in 2006-2007 we recorded only 5.3% [16].

Clinically in AIDS patients, it is impossible to differentiate between *M. tuberculosis* and other mycobacteriosis. *M. tuberculosis* causes most pulmonary infections, and the risk is mainly increased if the CD4 count falls below 300 cells/μl [17]. In some cases, disseminated infections are also found. NTM, on the other hand, may colonize the gut or respiratory tract of HIV patients. However, once the CD4 counts fall <100 cells/μl, they start multiplying rapidly, enter the bloodstream and cause disseminated infections [15].

The present study reflects that the MTBC still plays the primary role in causing pulmonary and extrapulmonary tuberculosis in this part of our country. NTM-caused infections play a minor role in pathogenesis in both immunocompromised and immunocompetent patients.

There were a few limitations in the present study. NTM associated cases were significantly less during the study period. Besides, since it was a hospital-based study, it may not reflect an accurate picture of *Mycobacterium* species in immunocompromised and immunocompetent patients in a community.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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