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Emergence of Pleomorphic in pathogenic bacteria (*Mycobacterium tuberculosis)*

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Abstract

***Mycobacterium tuberculosis* still remains a substantial global threat due to ill defined mechanisms that enable this organism to persist and replicate. Although molecular methods have revolutionized the diagnostic techniques in tuberculosis, yet a conventional simple microscopy remains the most cheap, easy and simple method for its diagnosis. The only requirement for the same is a trained eye to identify different pleomorphic forms of acid fast bacilli from the clinical specimen. We present here a case report of tubercular lumbar abscess with an unusual morphology.**

Keywords: *Mycobacterium tuberculosis*, morphology, microscopy

INTRODUCTION

Mycobacterium tuberculosis, the agent of human tuberculosis, has been estimated to have infected one third of world population with 3 million deaths per year (Monteiro et al., 2003). The delay in making a correct diagnosis and identification are major difficulties that increase the problem of this disease.

M. tuberculosis generally gives distinctive colony morphology when grown on solid media but difficulty arises when only microscopy is available. This organism exhibits extreme pleomorphism in certain circumstances and can exist even as cell wall deficient or L- forms (Domingue and Woody, 1995). These L forms predominate and are crucial to survival of *mycobacteria*, whose cell wall deficient forms escape destruction by host defense system (Ratnam and Chandersekhar, 1976). We present here an unusual morphology of *M. tuberculosis* in direct microscopy smear which created an initial diagnostic dilemma between *M. tuberculosis* and *Nocardia.*

CASE REPORT

A 56 year old male presented to medical OPD with complaint of swelling in the right lumber region for the past 15 days. Patient also complained of nausea and decreased appetite since one month. Patient was a known case of type II diabetes mellitus for which he was on regular medication. He also gave history of hospitalization two years back for right lower zone pneumonitis.

On examination the patient was afebrile and appeared pale and debilitated. Pulse rate was 100/ min and boiling point was 100/70 mmHg. Chest auscultation revealed bilateral crepitation throughout the lung fields. Abdominal examination revealed a soft non tender mass in right lumbar region which was soft, 4 × 2 cm in size. X-ray lumbar spine and MRI lumbosacral region was performed which revealed left sided para spinal and gluteal abscesses extending from L_4 down to S_3 level with involvement of posterior spinal elements also. X-ray chest revealed a small cavity in the left upper zone with haziness in right lower zone. Left CP angle was clear. Bony thorax, heart and mediastinum appeared normal. FNAC was performed on the lumber areas and the pus sample was sent to Microbiology department for culture and microscopy. Three consecutive sputum samples were also sent due to past history of pneumonitis and a strong suspicion of tuberculosis.

Laboratory investigation showed hemoglobin (Hb) 9.1 gm/dl, Total leukocyte count (TLC) of 23.7×10^3 /µl with DLC N_{40}, L_{56}, M_2 and E_2), (Lymphocytosis) LFT were normal and ELISA for HIV was non reactive. IgG ELISA performed on patients serum for tuberculosis was strongly positive. Sputum was negative for acid fast bacilli by ZN stain and bacterial culture revealed normal flora. Pus sample from lumbar abscess revealed numerous beaded acid fast bacilli resembling *M. tuberculosis* with

unusual branching and filamentous forms. These filamentous branching forms seemed to resemble *Nocardia* more than *M. tuberculosis* (Figure I) and hence a modified ZN staining using 1% H_2SO_4 was performed (Forbes et al., 1998). This staining also revealed both morphological forms (typical *mycobacteria* and filamenttous forms), hence a provisional report of acid fast bacilli in the smear was given. Pus sample cultured on SDA (with and without chlorophenicol) at 25 and 37°C (to rule out *Nocardia*) showed no growth after two weeks of incubation. Bacterial culture of pus revealed no organism.

Based on clinical, laboratory and radiological investiation, a provisional diagnosis of tubercular lumbosacral abscess was made and the patient was put on complete course of ATT. Patient responded to treatment within two weeks and the size of swelling decreased. Our microscopic finding was confirmed by culture report which revealed growth of *M. tuberculosis* after four weeks from a reputed mycobacterial laboratory (Dr. Lal's Pathological Laboratory - NABL accredited Laboratory in New Delhi, India).

DISCUSSION

WHO declared TB a global emergency in 1993 due to increase in associated morbidity and mortality. India has been classified along with sub Saharan countries to be among these countries with high burden of disease (Group IV countries) (Brewer and Heymann, 2004). Moreover the incidence of TB has been increasing ever since the onset of HIV epidemic.

We had a case of tubercular Lumbar abscess which posed difficulty in making a diagnosis on microscopy alone. The pus smear on 20% ZN staining showed typical *Mycobacterium* showing acid fast bacilli with beaded appearance along with filamentous forms in the background resembling *Nocardia*. However, these branching forms were visible at 20% H_2SO_4 concentration and were present at 1% concentration also. Due to persistence at 20% concentration, the diagnosis of *M. tuberculosis* was made. The unusual forms could be because of cording occurring in pus sample *in vivo*. Property of cord formation can be utilized for presumptive identification of *M. tuberculosis* if facility for liquid/solid culture is available (Atttori et al., 2000). The distinct colony morphology of *mycobacteria* on solid medium also helps to characterize the species (Monteiro et al., 2003).

We did not have the benefit of all these identification features because of lack of culture facility. Thus the relevance of this case report lies in its diagnosis based only on microscopy and also with pleomorphic morphology. The morphological diversity within *mycobacteria* could also be due to heterogeneous environments in the different body site of patient/host (natural conditions). Moreover, *M. tuberculosis* is known to exhibit extreme pleomorphism in certain circumstances like existing as cell wall deficient form or L-forms (without rigid cell wall) (Michailova et al., 2005). The prevalence with L- form transformation state is that of drug resistance to several antimicrobial agents.

Thus we conclude that the morphological diversity of *Mycobacterium* still needs to be analyzed in depth and basic microbiology laboratory plays a pivotal role in control of Tb through rapid presumptive identification (Monteiro et al., 2003). Strong clinical suspicion supported by microscopic evidence is the bare minimum requirement to treat a patient of tuberculosis. This method could be used at regional laboratories to report or tentative identification before sending the culture to a reference centre for confirmation.

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