Chapter 1

Introduction
Tuberculosis is one of the most frequently occurring infectious diseases worldwide. According to the World Health Organization, approximately one third of the world population is infected with tubercle bacilli, while 8.8 million new cases of active disease develop each year and 1.4 million die from TB (Sudre et al. 1992, AK. Chakaraborthy et al. 2004, Rober Stainbrook MD- 2007, WHO-TB Report. 2012).

**WHO-Key fact of TB 2012.**

- Tuberculosis(TB) is second only to HIV/AIDS as the greatest killer worldwide due to single infectious agent.
- Over 95% of TB deaths occur in Low and Middle income countries, and it is among the top three causes of death for women aged 15 to 44.
- In 2009, there were about 10 million orphan children as a result of TB death among parents.
- TB is a leading killer of people living with HIV causing one quarter of all deaths. (WHO-TB report. 2012)

India with 1.8 million cases occurring annually, accounts for a fifth of the world’s new tuberculosis (TB) cases and 2/3rd of the cases in South-East Asia (fig 1). This makes India the highest TB burden country in the world. It has been estimated for the year 2000, that
there were about 3.8 million bacteriological positive TB cases in the country (AK. Chakraborty et al. 2004, WHO-TB Report. 2012).

The mortality and morbidity due to tuberculosis is increasing, particularly with advent of HIV epidemic. Although the highest priority under the Revised National Tuberculosis Control Program (RNTCP) is given to the new sputum smear-positive pulmonary tuberculosis cases, all types of tuberculosis are treated under this program.

1.2 Tuberculosis and HIV

India, with an estimated 5.1 million HIV (Human Immuno-deficiency Virus) infected persons, has the second highest HIV infected population in the world. Approximately two million Indians are estimated to be co-infected with TB and HIV.
HIV infection has a marked impact on the control of TB as the two diseases are closely linked. TB is the most common opportunistic disease that affects people infected with HIV. As HIV debilitates the immune system, vulnerability to TB is increased many fold. The lifetime risk of HIV negative people developing active tuberculosis is only 10% compared to over 60% in the case of people with HIV (Havlir & Barnes 1999).

HIV is also the most powerful risk factor for the progression of TB infection to the disease. In a reciprocal manner, TB accelerates the progression of HIV into AIDS, thus shortening the survival of patients with HIV infection.

TB is a curable disease even among the HIV infected people. The Directly Observed Treatment, Short-course (DOTS) in HIV infected TB patients is as effective as in HIV- negative TB patients. More than 90% of HIV infected TB patients who complete treatment can be cured of TB and can live longer healthier lives.

1.3. Impact of human immunodeficiency virus infection:

HIV infected persons are at increased risk of developing primary or reactivation tuberculosis (Havlir & Barnes 1999, Daley et al. 1992,
Selwyn et al. 1989, Corbett et al. 2003), and for second episodes of tuberculosis from exogenous re-infection (Small et al. 1993, Korenromp et al. 2003). CD4+ T-helper (Th) cells, upon antigenic challenge, are thought to differentiate along the separate pathways resulting in cell populations with different cytokine production profile termed Th1 and Th2 (Schluger & Rom 1998, Beutler & Cerami 1988). In murine models, Th1 cells that produce interferon –γ (IFN-γ) and interleukin-2 (IL-2) confer resistance to infection with mycobacteria (Lin et al. 1996). Th2 cells that produce interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6) and interleukin-10 (IL-10), do not contribute much to anti-mycobacterial immunity (Schluger & Rom 1998, Beutler & Cerami 1988, Sharma & Mohan 2001, Lin et al. 1996). It has been observed that when peripheral blood lymphocytes from HIV-positive patients with tuberculosis are exposed in vitro to *Mycobacterium tuberculosis* (*M.tb*), they produce less IFN-γ but similar amounts of IL-4 and IL-10 as compared with lymphocytes from tuberculosis patients who are HIV-negative (Lin et al. 1996). Thus, reduced Th1 response observed in HIV-infected patients is thought to increase their susceptibility to tuberculosis (Schluger & Rom 1998, Beutler & Cerami 1988, Sharma & Mohan 2001, Lin et al. 1996). The risk of tuberculosis increases as immunosuppression progresses (CTD 1998, Raviglione et al. 1992, Jones et al. 1993, Barnes & Barrows 1993).
1.4. Mycobacterium tuberculosis & Non tuberculosis mycobacteria

Tuberculosis due to mycobacteria other than Mycobacterium tuberculosis are almost certainly more common than tuberculosis in the developed nations such as United States. However, such has not always been the case. The recognition of these mycobacteria other than Mycobacterium tuberculosis (MOTT) or non tuberculosis mycobacteria (NTM) as pathogens is relatively recent, with reports of patients with chronic pulmonary disease due to NTM from the mid-1950s (Christianson & Dewlett 1960, Crow et al. 1957, Timpe & Runyon 1954). Recognition that these organisms, known initially as the yellow bacillus (Mycobacterium kansasii), the Battey bacillus (Mycobacterium avium complex, after the Battey Sanatorium in Georgia where it was first recognized), or collectively as anonymous mycobacteria (Wolinsky 1979), were true pathogens took significant time. Early scientists and clinicians such as Ernest Runyon (the Runyon classification system) (Timpe & Runyon 1954), William Shaffer (serotyping of Mycobacterium avium-intracellulare-scrofulaceum), John Chapman, Emanuel Wolinsky (the major treatise on these organisms as pathogens) (Wolinsky 1979), and Chai Ahn (diagnostic criteria and drug trials) (Ahn et al. 1982) were the major
participants in the effort to bring deserved recognition to these organisms.

Currently categorized as the nontuberculous mycobacteria (NTM), this group of organisms shares a number of features. Unlike *M. tuberculosis*, these organisms are normal inhabitants of the environment. Also unlike tuberculosis, pulmonary disease and other infections due to the NTM are not contagious so patients with these diseases are not generally isolated. Because these organisms frequent the environment, infections of the skin and the soft tissue following local trauma occur much more frequently. Many clinicians consider these organisms as opportunists rather than virulent pathogens, as some local or systemic immune impairment is required for them to cause disease. As with *M tuberculosis*, little is known of virulence genes or specific cellular products that results in tissue invasion and subsequent disease. No known toxins are produced by these species with the exception of *M ulcerans*. This lack of knowledge reflects the limited research and general lack of knowledge about the pathogenesis of mycobacterial infections, including leprosy and tuberculosis.
1.5. Pulmonary & Extra pulmonary tuberculosis

Tuberculosis can involve any organ system in the body and the clinical manifestations of tuberculosis are of two types: Pulmonary and Extra-pulmonary (EPTB). While pulmonary tuberculosis is the most common presentation, extra-pulmonary tuberculosis (EPTB) is also an important clinical problem. In extra-pulmonary tuberculosis, vascular areas such as lymph nodes, meninges, kidney, spine and growing ends of the bones are commonly affected (Fanning 1999, Iscmann 2000, Dutt et al, 1999).

The definition of extra-pulmonary tuberculosis under RNTCP follows the international classification which defines extra-pulmonary tuberculosis as tuberculosis of an organ other than the lungs, such as pleura, lymph node, abdomen, genito-urinary tract, skin, joints, bones, meninges etc (CTD 1997).

In the era before the human immunodeficiency virus (HIV) pandemic, and in studies involving immunocompetent adults, it has been observed that EPTB constituted about 15 to 20 per cent of all cases of TB (Fig.2a). In HIV-positive patients, EPTB accounts for more than 50 per cent of all cases of TB (Fig.2b). The diagnosis of EPTB, especially involving deeply located inaccessible areas is very difficult. Sparse literature is available regarding the relative contributions of
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pulmonary and extra pulmonary disease to the total number of tuberculosis cases from India as reliable epidemiological data are lacking (Mohan & Sharma 2001). Considering the stigma associated with and the reluctance to perform invasive procedures especially in HIV-positive patients in the Indian setting, even notified estimates of EPTB under the Revised National Tuberculosis Control Programme (RNTCP) are often based on presumptive diagnosis and are an overestimate of the problem (AIIMS-WHO 2002). Though it is estimated that EPTB constitutes 15 to 20 per cent of tuberculosis cases in general practice among HIV-negative adults in India (Mohan & Sharma 2001), a higher proportion of EPTB cases have been documented in tertiary care centers.

Lymph node / Cervical lymph node TB (LNTB) is the most common manifestation of extra-pulmonary form of TB. However, neurological, pleural, pericardial, abdominal involvement has been described and virtually every site in the body can be involved in HIV-positive patients (Raviglione et al. 1992, Jones et al. 1993, Barnes & Barrows 1993). In studies reported from India, EPTB constituted to 45 to 56 per cent of all the cases of tuberculosis in persons with AIDS (Sharma et al. 1997, Kumar et al. 2002). Lymph node is considered to be the local manifestations of a systemic disease. In HIV-negative patients, isolated cervical lymphadenopathy is seen in two-thirds of
the patients. In HIV–positive patients, multifocal involvement, intra-thoracic and intra-abdominal lymphadenopathy and associated pulmonary disease are common (fig 1.2 & 1.3).

The major pitfalls in the diagnosis of lymph node tuberculosis and other extra-pulmonary tuberculosis are atypical clinical presentations simulating other inflammatory and neoplastic conditions, resulting in delay or deprivation of treatment. Therefore a high index of suspicion is necessary to make an early diagnosis and with advent of HIV infections these *Mycobacterium tuberculosis* and non tuberculosis mycobacterial (NTM) infections have been increasingly recognized in both pulmonary and extra-pulmonary tuberculosis.

**Fig 1.2.** Distribution of tuberculosis cases by anatomical site in HIV-negative patients. (Data derived from references of Dutt el al., 1999, Report 1987, MRC 1982, Snider 1995, CDC 2000). PTB, pulmonary tuberculosis;

1.6. LYMPH NODE TUBERCULOSIS

1.6.1. INTRODUCTION

Historically, lymph node tuberculosis (LNTB) has been called the “King’s evil” referring to the divine benediction, which was presumed to be the treatment for it. It was also referred to as “scrofula” meaning “glandular swelling” (Latin) and “full necked sow” (French) (Kumar 2001) (fig 1.4).
Diagnosis and treatment have varied widely over the centuries. Whereas Herodotus suggested the isolation of these afflicted souls (Appling & Miller 1981, Wong & Jafex 1974), the European kings of the middle ages imparted the royal touch to cure the “King’s evil,” to which mycobacterial lymphadenitis was referred to Cantrell et al. 1975 (fig 1.5).

In tuberculosis, peripheral lymph nodes are most often affected and cervical involvement is the commonest among them (Kumar 2001, Appling & Miller 1981, Thompson et al. 1992). In India and other developing countries LNTB continues to be the most common form of EPTB, lymphadenitis due to non-tuberculous mycobacteria (NTM) is seldom seen (Dandapat et al. 1990, Subrahmanym 1993, Chen et al. 1992).
1.6.2. Anatomy of Lymph node

Lymph nodes are discrete structures surrounded by a capsule composed of connective tissue and a few elastic fibrils. The capsule is perforated by multiple afferent lymphatic that empty into a fenestrated sub capsular peripheral sinus. Lymph extravasates from this sinus and slowly percolates through the node, eventually collecting in medullary sinusoids and exiting through a single efferent lymphatic
vessel in the hilus, which also serves as the point of penetration by a single small artery and vein. Situated in the cortex subjacent to the peripheral sinus and spherical aggregates of lymphoid cells, the so-called primary follicles, that represents the B-lymphocyte areas. Between the primary follicles is the Para cortex, a region rich in T lymphocytes.

1.6.3. Pathogenesis

Lymph node tuberculosis (LNTB) is considered to be the local manifestation of a systemic disease whereas NTM lymphadenitis is thought to be a truly localized disease. *M. tuberculosis* gains entry into the body via the respiratory tract and undergoes haematogenous and lymphatic dissemination. Hilar and mediastinal lymph nodes are initially involved. This may occur at the time of primary infection or may occur later in life due to reinfection or reactivation of previous infection. Sometimes, tonsil is an important portal of entry. The infection then spreads via the lymphatic to the draining cervical lymph nodes. Initially, the nodes are discrete. Periadenitis results in matting and fixation of the lymph nodes. The lymph nodes coalesce and break down due to formation of caseous pus. This may perforate the deep fascia and present as a collar-stud abscess. Overlying skin becomes indurated and breaks down, resulting in sinus formation, which may
remain, unhealed for years. Healing may occur from each of the stages with calcification and scarring.

**1.6.4. Clinical presentation**

LNTB often affects children and young adults. Patients usually present with slowly enlarging lymph nodes and may otherwise be asymptomatic. In HIV-negative patients, isolated cervical lymphadenopathy is most often seen in about two-thirds of the patients (Thompson et al. 1992, Dandapat et al. 1990, Subrahmanyam 1993, Fain O et al. 1999). Bem et al (Bem et al. 1997) observed that among HIV-negative as well as HIV-positive patients, cervical lymph nodes were most commonly affected followed by axillary and inguinal lymph nodes. Multifocal involvement was observed in 39 and 90 per cent among HIV-negative and HIV-positive patients respectively.

In HIV positive patients, multifocal involvement, intrathoracic and intraabdominal lymphadenopathy and associated pulmonary disease are more common (Thompson et al. 1992, Dandapat et al. 1990, Subrahmanyam 1993, Bem et al. 1997, Powell et al. 1999). Some patients with LNTB may manifest systemic symptoms and these include fever, weight loss, fatigue and occasionally night sweats. Patients with mediastinal lymphadenopathy may present with cough and dysphagia (Thompson et al. 1992, Dandapat et al. 1990,
Subrahmanyam 1993, Bem 1997, Powel 1999, Mert et al. 2002, Geldmacher et al. 2002, Abba et al. 2002). With wider availability of computerised tomographic (CT) scan, it is expected that more cases of intrathoracic, intraabdominal lymphadenopathy and other associated lesions may be reported.

Peripheral tuberculous lymphadenopathy has been classified into five stages. These include:

(i) Stage 1 → enlarged, firm mobile discrete nodes showing non-specific reactive hyperplasia;

(ii) Stage 2 → large rubbery nodes fixed to surrounding tissue owing to periadenitis;

(iii) Stage 3 → central softening due to abscess formation;

(iv) Stage 4 → collar-stud abscess formation; and

(v) Stage 5 → sinus tract formation.

Physical findings depend upon the stage of the disease. The enlarged lymph nodes may be of varying size, usually firm and may be discrete or matted. If necrosis and abscess formation have taken place they may become cystic in consistency. The lymph nodes are usually not tender unless secondary bacterial infection has occurred. Physical examination may be unremarkable but for palpable lymphadenopathy. Occasionally, lymph node abscess may burst leading to a chronic non-
healing sinus and ulcer formation. Classically, tuberculous sinuses have thin, bluish, undermined edges with scanty watery discharge. Uncommon manifestations observed in patients with mediastinal lymph node involvement include dysphagia (Jons & Campbell 1962, Singh et al. 1996), oesophagomediastinal fistula (Gupta et al. 1992, Ohtake et al. 1996, Adkins et al. 1990), and tracheo-oesophageal fistula (Im JG et al. 1990). Upper abdominal and mediastinal lymph nodes may cause thoracic duct obstruction and chylothorax, chylous ascites or chyluria (Vennera et al. 1983, Kohn & Atlman 1973). Rarely, biliary obstruction due to enlarged lymph nodes can result in obstructive jaundice (Paredes et al. 1990).

1.6.5. Diagnosis of Lymph node tuberculosis.

Lymph node tuberculosis is an entity with an extensive differential diagnosis. In developing nations where tuberculosis remains common, tuberculous lymphadenitis continues to be one of the most frequent causes of lymphadenopathy (30% to 52%) (Gupta et al. 1992). In contrast, in developed nations, tuberculosis is clearly found less frequently to be the cause of lymphadenopathy as low as 1.6% in one series (Gupta et al. 1992, Dandapat & Patra 1987, Klines 1981). Mycobacterial lymphadenitis can mimic a variety of ailments, including sarcoidosis, carcinoma, lymphoma or sarcoma, viral or bacterial adenitis, fungal diseases, toxoplasmosis, catscratch fever,
collagen vascular diseases, and diseases of the reticuloendothelial system. Traditionally, excisional biopsy was required to diagnose tuberculous lymphadenitis. Diagnosis is established by visualizing mycobacteria either by histopathology or by smears subjected to acid-fast stains or mycobacterial cultures. Histopathology revealing caseating granulomas is highly suggestive of mycobacterial infection, but other factors may produce similar histology. Over the past decade, the role of fine needle aspiration (FNA) has become increasingly important in the evaluation of peripheral lymphadenopathy. Its high sensitivity and specificity for diagnosing the cervical malignancies have been demonstrated (Feldman et al. 1983, Young et al. 1981). Several authors promoted FNA as the initial technique in the diagnosis of mycobacterial lymphadenitis. Specimens are routinely evaluated by cytology, acid-fast bacillus (AFB) smear, and AFB culture. The cytological criteria for diagnosis have been well described. The diagnostic findings on cytology are epithelioid cell granulomas with or without multinucleate giant cells and caseation necrosis (Finfer et al. 1991, Gupta et al. 1992, Gupta et al. 1993, Lau et al. 1990).

Revised National TB control standard diagnostic algorithm for LNTB in India, recommends fine needle aspiration cytology (FNAC) and Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB) in clinically
suspected patients (CTD 2005). However, cytology mimics other granulomatous infections such as sarcoidosis, cat scratch fever etc. whereas, Ziehl-Neelsen staining is specific, though, less sensitive (Pagel et al. 1964, Verenkar et al. 1996).

Hence, more sensitive and specific tests such as molecular diagnostic methods like polymerase chain reaction (PCR) and culture may be appropriate for a definitive diagnosis of LNTB. However, molecular methods may not be feasible in all the settings especially in the field, owing to the requirement of special facility and expertise. Fine needle aspirate culture which is not being emphasized as a diagnostic modality in the standard guidelines is the key method in the isolation and identification of mycobacterial species for both epidemiological information and to know their drug susceptibility pattern.

With the above background, the present study was carried out to study the mycobacterial species causing lymph node tuberculosis in both HIV positive and negative patients and also to assess the bacteriological role in the diagnosis of lymph node tuberculosis with fine needle aspiration cytology, and to know the drug susceptibility pattern of mycobacterium tuberculosis isolates from lymph node tuberculosis and the species characterization.