 review of literature

"There is a dread disease which so prepares its victim, as it were, for death... a dread disease, in which the struggle between soul and body is so gradual, quiet and solemn, and the results so sure, that day by day, and grain by grain, the mortal part wastes and withers away, so that the spirit grows light.... a disease in which life and death are so strangely blended that death takes the glow and hue of life and life the gaunt and grisly born of death.... a disease which medicine never cured, wealth warded off, or poverty could boast exemption from which sometimes moves in giant strides and sometimes a tardy sluggish pace, but, slow or quick is, ever sure and certain."

Charles Dickens & Nicholas Nickle by, 1870

“I have no business to live this life if I cannot eradicate this horrible scourge from the mankind.”
Robert Koch, delivering a lecture at Berlin University on his discovery of tuberculosis bacilli, 1882.

It has been centuries into its ‘known’ existence, Mycobacterium tuberculosis (MTB) continues to haunt the mankind and tuberculosis (TB) the disease caused by it remains the leading cause of preventable death worldwide.

Since Robert Koch’s initial cultivation of the acid fast tubercular bacillus more than a century ago, tremendous research has been done about tuberculosis and its causative agent Mycobacterium tuberculosis. Despite the availability of effective antitubercular chemotherapy for more than fifty years and major advances in the biology and epidemiology of Mycobacterium tuberculosis, pulmonary and extrapulmonary tuberculosis remains leading cause of mortality and morbidity both in adults and children; it claims approximately two million individuals annually (Palmino Leao R, 2007).

Clinically tuberculosis is divided into pulmonary and extra pulmonary tuberculosis. While pulmonary tuberculosis is the major form, extra pulmonary cases are rising now (Palmino Leao R, 2007).
HISTORY

Tuberculosis (TB) has a long history. It was present before the beginning of recorded history and has left its mark on human creativity, music, art, and literature; and has influenced the advance of biomedical sciences and healthcare. Its causative agent, Mycobacterium tuberculosis, may have killed more persons than any other microbial pathogen (Daniel 2006).

The term phthisis (meaning consumption, to waste away) appeared first in Greek literature. Around 460 BC, Hippocrates identified phthisis as the most widespread disease of the times. It most commonly occurred between 18 and 35 years of age, and was almost always fatal. Although Aristotle (384-322 BC) considered the disease to be contagious, most Greek authors believed it to be hereditary, and a result, at least in part, of the individual's mental and moral weaknesses. Clarissimus Galen (131-201 AD), the most eminent Greek physician after Hippocrates, defined phthisis as an ulceration of the lungs, chest or throat, accompanied by coughs, low fever, and wasting away of the body because of pus. He also described it as a disease of malnutrition (Pease, 1940).

In the past tuberculosis was referred to as the “white plague” and by John Bunyan as “the captain of all these men of death”. Accounts of the disease appeared in the Vedas and other ancient Hindu texts, in which it was termed “rajyakshma”- the king of disease (Narayanan S et al., 2002). Hippocrates (460-370 BC) called this disease as pthisis sylvias meaning wasting away and Herdatus (484 - 425BC) described the exclusion of those afflicted with leprous or scrofulous lesions from general population (Zink A et al., 2003).

Precise pathological and anatomical descriptions of the disease began to appear in the 17th century. Franciscus Sylvius de la Böe of Amsterdam (1614-1672) was the first to identify the presence of actual tubercles as a consistent and characteristic change in the lungs and other areas of consumptive patients. The English physician Richard Morton (1637-1698) confirmed that tubercles were always present in TB of the lungs (Palomino Leao R, 2007).
There is also evidence of the presence of the disease in pre-historic Asia, but it was only toward the end of the 19th century that peaks in incidence were observed in India and China. In the 18th century, TB was sometimes regarded as vampirism.

Gaspard Laurent Bayle (1774-1816) definitely proved that tubercles were not products, or results, but the very cause of the illness. The name 'tuberculosis' appeared in the medical language at that time in connection with Bayle's theory. More precisely, the name 'tuberculosis' was coined in 1839 by the German professor of Medicine Johann Lukas Schönlein (1793-1864), to describe diseases with tubercles; but he considered scrofula and phthisis to be separate entities.

Laemaec and Bayle (1789-1826) performed hundreds of postmortems and found nodules in the lung which they called tubercles and thus decided to call the disease “Tuberculosis” (Dale JW et al., 2003). The transmissible nature of tuberculosis was established by inoculating rabbits with tubercular material from humans and cattle scrofula and pulmonary tuberculosis were manifestations of the same disease processes (Waddington K, 2004).

On the evening of March 24, 1882, in Berlin, before a skeptical audience composed of Germany's most prominent men of science from the Physiological Society, Robert Koch (1843-1910) made his famous presentation Die Aetiologie der Tuberculose. Using solid media made of potato and agar, Koch invented new methods of obtaining pure cultures of bacteria. His colleague Julius Richard Petri (1852-1921) developed special flat dishes (Petri dishes), which are still in common use, to keep the cultures. Koch also developed new methods for staining bacteria, based on methylene blue, a dye developed by Paul Ehrlich (1854-1915) and counterstained with vesuvin. "Under the microscope the structures of the animal tissues, such as the nucleus and its breakdown products are brown, while the tubercle bacteria are a beautiful blue", he wrote in the paper that followed his dramatic presentation that March evening (Palmino Leao R, 2007).

Robert Koch discovered a staining technique that enabled him to see Mycobacterium tuberculosis. What excited the world was not so much the
scientific brilliance of Koch's discovery, but the accompanying certainty that now the fight against humanity's deadliest enemy could really begin (Dormandy & Thomas, 2000).

Another important development was provided by the French bacteriologist Calmette, who, together with Guerin used specific culture media to lower the virulence of the bovine TB bacterium, creating the basis for the BCG vaccine still in widespread use today. Then, in the middle of World War II, came the final breakthrough, the greatest challenge to the bacterium that had threatened humanity for thousands of years – chemotherapy (Waksman S A. 1964 & Zink A et al., 2003).

Smith (1898) differentiated human tubercle bacilli from bovine type. As research progressed, certain cases of pulmonary tuberculosis turned out to be caused by organisms which were mycobacteria like but appeared to be different and distinct from *Mycobacterium tuberculosis* or other well characterized mycobacterial species.

Pinner (1935) labelled these organisms as “atypical mycobacteria.” Later Runyon (1959) called these organisms as “anonymous” and American Thoracic Society (1963) proposed the name – unclassified mycobacteria (Cheng VC et al., 2004).

**MYCOBACTERIUM**

The mycobacteria are rod shaped, acid fast, aerobic or micro-aerophilic, non-spore forming, non motile, non-capsulated and lipid rich bacteria Most of them grow slowly on solid Lowenstein - Jensen (LJ) medium, are resistant to drying, disinfectants and remain viable in clinical samples for a long period of time (Evans, 1998). The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*).

Since MTB has a cell wall but lacks phospholipids in their outer membrane, it is classified as a gram-positive bacterium. However, if a Gram stain is performed, MTB either stains very weakly gram-positive or does not retain dye due to the high lipid and mycolic acid content of its cell wall. MTB is
a small rod-like bacillus that can withstand weak disinfectants and survive in a dry state for weeks. In nature, the bacterium can grow only within the cells of a host organism, but *M. tuberculosis* can be cultured in vitro.

Since MTB retains certain stains after being treated with acidic solution, it is classified as an acid-fast bacillus (AFB). The most common acid-fast staining technique, the Ziehl-Neelsen stain, dyes AFB’s a bright red that stands out clearly against a blue background. Other ways to visualize AFB’s include an auramine-rhodamine stain and fluorescent microscopy.

The *Mycobacterium tuberculosis complex* (MTBC) members are causative agents of human and animal tuberculosis. Species in this complex include: *M. tuberculosis*, the major cause of human tuberculosis, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. canetti*, *M. caprae*, *M. microti* and *M. pinnipedii*. *M. africanum* is not widespread, but in parts of Africa it is a significant cause of tuberculosis. *M. bovis* was once a common cause of tuberculosis, but the introduction of pasteurized milk has largely eliminated this as a public health problem in developed countries. *M. microti* is mostly seen in immune deficient people, although it is possible that the prevalence of this pathogen has been underestimated. Other known pathogenic mycobacterium includes *Mycobacterium leprae*, *M. avium* and *M. kansasii*. The last two are part of the nontuberculous mycobacteria (NTM) group. Nontuberculous mycobacteria cause neither TB nor leprosy, but they do cause pulmonary diseases resembling TB (Runyon E H, 1960).

**A global perspective of tuberculosis**

**Problem statement**

In the year 2009, it is estimated that globally there were 9.4 million incident TB cases and there were 1.3 million TB deaths. The incidence rate, prevalence rate and mortality rate due to TB is highest in the WHO Africa region. However, in terms of the absolute number of incident and prevalence cases, South-East Asia Region has the highest TB burden globally contributing 35% to the global TB incidence.
In 2009, 5.8 million cases of TB (new cases and relapse cases) were notified to NTPs, including 2.6 million new cases of sputum smear-positive pulmonary TB, 2.0 million new cases of sputum smear-negative pulmonary TB (including cases for which smear status was unknown), 0.9 million new cases of extrapulmonary TB and 0.3 million relapse cases (WHO, 2010).

World Health Organization estimated TB mortality in India as 280,000 (23/100,000 population) in 2009. With RNTCP implementation, death due to TB has come down to half in the country. It was estimated that the TB mortality was over 5 million annually at the beginning of the revised national TB control programme (RNTCP). In the year 2009, the prevalence of TB in India was estimated to be 249 per 100,000 populations, and the mortality due to TB is 23 per 100,000 populations (WHO, 2010).

A global perspective of extrapulmonary tuberculosis

**Epidemiology**

Extrapulmonary tuberculosis (EPTB) comprises 9.7% - 46% of all cases of tuberculosis (TB) (Rieder HL, 1990; Cowie RL, 1997; Huang J, 2001; Md. Wassim H, 2004; Palmino Leao R, 2007; Sreeramareddy CT, 2008; Sharma SK, 2009 & Sandhu GK, 2011). In the era before the human immunodeficiency virus (HIV) pandemic, and in studies involving immune competent adults, it has been observed that EPTB constituted about 15% to 20% of all cases of TB (Snider DE Jr, 1992; Snider DE, 1995; Fanning A, 1999; CDC, 2000; ATS, 2000; Sharma SK, 2001; Palmino LR, 2007 & Sharma SK, 2009). In HIV-positive patients, EPTB accounts for more than 50% of all cases of TB (Theuer CP, 1990; Shafer RW, 1991; Raviglione MC, 1992; Antonucci G, 1992; Jones BE, 1993; Haas DW, 1994; Lado Lado FL, 1999; Lee MP, 2000; Poprawski D, 2000; Huang J, 2001; Md. Wassim H, 2004; Palmino LR, 2007; Sharma SK, 2009 & Sandhu GK, 2011). The diagnosis of EPTB, especially involving a deeply located inaccessible area is very difficult. Sparse literature is available regarding the relative contributions of pulmonary and extrapulmonary disease to the total number of tuberculosis cases from India as reliable epidemiological data are lacking (Sharma SK, 2009).
In addition, about 20% of patients with EP disease may have a positive sputum culture and 40% of them a positive sputum smear, even in the presence of normal or non-suspicious chest X-ray findings (Angels orcau, 2011).

Several studies from European, North American and Asian countries in the last decade indicate that between 12% and 53% of TB patients present with a major form of EP TB. The variability of EPTB depends not only on the prevalence of well-characterized risk factors among the population, but also on the definitions (Angel’s orcau, 2011).

**INDIA**

Though India is the second-most populous country in the world, India has more new TB cases annually than any other country. In 2009, out of the estimated global annual incidence of 9.4 million TB cases, 2 million were estimated to have occurred in India, thus contributing to a fifth (21%) of the global burden of TB (WHO, 2010). It is estimated that about 40% of Indian population is infected with TB bacillus. The national Annual Risk of Tuberculosis infection (ARTI) being 1.5%, the incidence of new smear positive TB cases in the country is estimated as 75 new smear positive cases per 100,000 populations. The prevalence of TB has been estimated at 3.8 million bacillary cases for the year 2000, by an expert group of Govt. of India. However the recent estimate by WHO gives a prevalence of 3 million (WHO, 2010).

There are 22 high burden countries which account for 80% of all estimated incident cases worldwide. The five countries that rank first to fifth in terms of number of incident cases in 2009 are India (2 million), China (1.3 million), South Africa (0.49 million), Nigeria (0.46 million) and Indonesia (0.43 million). India and China alone account for an estimated 35% of TB cases worldwide (WHO, 2010).

Though it is estimated that EPTB constitutes 15% to 20 % of tuberculosis cases in general practice among HIV-negative adults in India (Md. Wassim H, 2004 & Sharma SK, 2009), a higher proportion of EPTB
cases have been documented in tertiary care centers. For example, at the Tuberculosis Clinic at the All India Institute of Medical Sciences, (AIIMS), New Delhi (n=1137) and the Sri Venkateshwara Institute of Medical Sciences (SVIMS), Tirupati (n=612), patients with EPTB constituted 53% and 30.4% respectively during the period 1994-2002 (Md. Wassim H, 2004 & Sharma SK, 2009).

**PATHOGENESIS**

About 90% of those infected with *Mycobacterium tuberculosis* have asymptomatic, latent TB infection (sometimes called LTBI) with only a 10% lifetime chance that a latent infection will progress to TB disease. However, if untreated, the death rate for these active TB cases is more than 50%.

The lung is the main entrance gate of the tuberculous bacillus, which causes a focal infection in the site where it is deposited after inhalation. If the infection cannot be contained at the local level, bacilli dissemination is produced initially by hematogenic route, probably inside phagocytic cells, towards different organs and, eventually, to the contiguous pleura. It reaches hilar lymph nodes via the lymphatic route, and from there, a second systemic dissemination can occur, through the thoracic duct and superior vena cava, with the development of local foci in the lungs. Extrapulmonary foci can also be produced by hematogenic and lymphatic dissemination. The clinical manifestations of TB depend on the local organic defenses on the sites of bacilli multiplication (Palmino Leao R, 2007).

TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within the endosomes of alveolar macrophages. The primary site of infection in the lungs is called the Ghon focus, and is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe. Bacteria are picked up by dendritic cells, which do not allow replication, although these cells can transport the bacilli to local (mediastinal) lymph nodes. Further spread is through the bloodstream to other tissues and organs where secondary TB lesions can develop in other parts of the lung (particularly the apex of the upper lobes) peripheral lymph nodes,
kidneys, brain and bone. All parts of the body can be affected by the disease, though it rarely affects the heart, skeletal muscles, pancreas and thyroid.

Tuberculosis is classified as one of the granulomatous inflammatory conditions. Macrophages, T lymphocytes, B lymphocytes and fibroblasts are among the cells that aggregate to form a granuloma, with lymphocytes surrounding the infected macrophages. The granuloma functions not only to prevent dissemination of the mycobacteria, but also provides a local environment for communication of cells of the immune system. Within the granuloma, T lymphocytes (CD4+) secrete cytokines such as interferon gamma, which activates macrophages to destroy the bacteria with which they are infected. T lymphocytes (CD8+) can also directly kill infected cells.

Importantly, bacteria are not always eliminated within the granuloma, but can become dormant, resulting in a latent infection. Another feature of the granulomas of human tuberculosis is the development of cell death, also called necrosis, in the center of tubercles. To the naked eye this has the texture of soft white cheese and was termed caseous necrosis.

If TB bacteria gain entry to the bloodstream from an area of damaged tissue they spread through the body and set up many foci of infection, all appearing as tiny white tubercles in the tissues. This severe form of TB disease is most common in infants and the elderly and is called miliary tuberculosis. Patients with this disseminated TB have a fatality rate of approximately 20%, even with intensive treatment.

In many patients the infection waxes and wanes. Tissue destruction and necrosis are balanced by healing and fibrosis. Affected tissue is replaced by scarring and cavities filled with cheese-like white necrotic material. During active disease, some of these cavities are joined to the air passages bronchi and this material can be coughed up. It contains living bacteria and can therefore pass on infection. Treatment with appropriate antibiotics kills bacteria and allows healing to take place. Upon cure, affected areas are eventually replaced by scar tissue.

Tuberculous Lymphadenitis is considered to be the local manifestation of a systemic disease whereas nontuberculous mycobacteria (NTM)
lymphadenitis is thought to be a truly localized disease. *M. tuberculosis* gains entry into the body via the respiratory tract and undergoes hematogenous 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 lymphatics 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. In contrast NTM, gain entry into the lymph nodes directly via oropharyngeal mucosa, salivary glands, tonsils, gingiva or conjunctiva (Olson RN, 1981 & Manolidis S, 1993) and lymph node involvement represents a localized disease.

**Types of tuberculosis**

Tuberculosis may occur in any part of the body. On the basis of site of tuberculosis it is mainly divided into two categories: pulmonary and extrapulmonary.

**Extrapulmonary tuberculosis**

Although the predominant form of tuberculosis is pulmonary disease, infection with *M. tuberculosis* may be seen in any organ system. Extrapulmonary tuberculosis mainly results from hematogenous dissemination or lymphogenous spread from a primary, usually a pulmonary, focus. This hematogenous spread may occur years before the onset of progressive tuberculosis, as foci of latent infection may lie dormant before reactivation occurs (Vanhoenacker FM, 2004). The precise incidence of extrapulmonary tuberculosis has not been determined, but an increasing incidence has been noted both in developing countries and in developed countries since the mid-

In these patients multiple extrapulmonary sites are often involved (Van den Brande P, 2003). Other factors that have contributed to the increased prevalence of extrapulmonary tuberculosis are the development of drug-resistant strains of *M. tuberculosis*, and aging of the population (Engin G, 2000 & Van den Brande P, 2003). Finally the more widespread use of cross sectional imaging modalities may also explain why extrapulmonary tuberculosis is more commonly been seen. The most common sites of extrapulmonary tuberculosis consist of lymphatic, genitourinary, bone and joint, and central nervous system involvement followed by peritoneal and other abdominal organ involvement.

The majority of the extrapulmonary forms of TB affect organs with suboptimal conditions for bacillary growth. For this reason, the extrapulmonary disease generally has an insidious presentation, a slow evolution and paucibacillary lesions and/or fluids. In the immunocompetent patient, the tuberculin skin test (TST) response is usually positive (induration ≥ 10 mm) (Palomino Leao R, 2007).

In immunocompetent patients, the extrapulmonary forms only occasionally coexist with active pulmonary TB. Nevertheless, the chest X-ray is mandatory for the evaluation of evidence of primary infection lesions, which provide a good verification to support the diagnosis (Rottenberg, 1996)

*Mycobacterial lymphadenitis*

Peripheral lymph node involvement is the commonest form of extrapulmonary mycobacterial disease and cervical is the most frequent site (Appling D, 1981; Dandapat MC, 1990; Thompson MM, 1992; Gangane N, 2008; Lakmini, 2008; Sharma, 2009 & Verma P, 2011). In the present era Mycobacterial tuberculosis is the most common cause of mycobacterial lymphadenitis and lymphadenitis due to nontuberculous mycobacteria (NTM) is also being increasingly encountered.
Etiology agent

The genus Mycobacterium includes members of the *Mycobacterium tuberculosis* complex, which contain medically important species (Shinnick and Good, 1994). The species found in the complex are *M. tuberculosis* (the most widely spread bacterium responsible for tuberculosis), *M. bovis* (worldwide but limited by pasteurization of milk and it has the broadest host range of species in the complex), *M. africanum* (mainly found in Africa), *M. microti* and *M. canetti* (limited to small rodents) (Sales et al., 2001). *M. tuberculosis, M. bovis, M. africanum* and non-tuberculous mycobacteria can cause lymphadenitis, but lymphadenitis caused by *M. tuberculosis* complex is more chronic as compared with lymphadenitis caused by other mycobacteria, which has a more rapid course (Singh et al., 2000).

In a study conducted by Jindal N, 2003 on 190 children of chronic cervical lymphadenitis showed tuberculous etiology on histopathological examination in 92 (48.4%) and bacteriological evidence of mycobacterial infection (smear and/or culture) in 42 (22.1%). He reported that the positive culture for mycobacteria was obtained in 40, of which 30 (75%) were typical *M. tuberculosis* and 10 (25%) were atypical mycobacteria.

In India, *Mycobacterium tuberculosis* still appears to be the most common causative agent of tuberculous lymphadenitis, it was reported that 90% - 98% of mycobacterial lymphadenitis in adults is due to *M. tuberculosis* and 2.6% due to the NTM, It was also reported that 70 % - 89 % mycobacterial lymphadenitis in children is caused by atypical mycobacteria Reddy VS, 2008. Nataraj et al., 2002 from Mumbai reported a NTM isolation rate of 3.8% and Jesudason & Gadstone, 2005 from Vellore reported a NTM isolation rate of 3.9% (*M. avium – intracellular, M. fortuitum and M. kansasii*) (Nataraj et al 2002; Jesudason and Gadstone, 2005 & Reddy VS, 2008).

Epidemiology

In 2009, 5.8 million cases of TB (new cases and relapse cases) were notified to NTPs, including 2.6 million new cases of sputum smear-positive
pulmonary TB, 2.0 million new cases of sputum smear-negative pulmonary TB (including cases for which smear status was unknown), 0.9 million new cases of extrapulmonary TB and 0.3 million relapse cases (WHO, 2010). The incidence of mycobacterial lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide.

A study conducted in Iran on 31 patients with TBLN depicted that cervical lymph nodes were the most frequently involved sites and comprised 50% of the cases (Velayati, et al., 1998). Approximately 75% of the infection is associated with cervical lymph nodes (Singh et al., 2000). In developing countries where the incidence of tuberculosis is high, TBLN is a cause for 30% - 64% of lymphadenopathy, responsible for about 80% of all exclusively EPTB cases and more than 6% of all tuberculosis cases (Brainard, 2001). In another study done in Texas, USA, from a total of 1,878 TB patients, 538 (28.6%) were identified EPTB cases among African – Americans. Of these EPTB cases, the most common sites of infection were lymph nodes 43% (Gonzalez et al., 2003).

In India and other developing countries LNTB continues to be the most common form of EPTB and lymphadenitis due to non-tuberculous mycobacteria (NTM) is seldom seen (Dandapat MC, 1990; Jawahar MS, 1990 & Subrahmanyam M, 1993). On the other hand, NTM are the most common cause of lymphadenopathy in the developed world (Roba-Kiewicz M, 1974 & White MP, 1986). In patients with mycobacterial lymphadenitis in the USA, M. tuberculosis has been the most common pathogen among adults whereas NTM were the most common pathogens among children (Manolidis S, 1993). In England, there has been a decline in LNTB and a rise in NTM lymphadenitis (Yates MD, 1992), and persons of Indian ethnic origin were more often affected than the local residents (Thompson MM, 1992 & Yates MD, 1992).

Similar results have been reported among native Americans and in persons originating from south east Asia and Africa (Pang SC, 1992 & Lee KC, 1992). Asians and Hispanic patients and African American also seem to
have a high predilection for developing mycobacterial lymphadenitis (Pang SC, 1992 & Lee KC, 1992).

In India, tuberculous lymphadenitis is one of the most common type of lymphadenopathy encountered in clinical practice (Bhaskran CS, 1990; Gupta AK, 1992 Prasad RR, 1993; Goel MM, 2001; Paul PC, 2004 & Sharma SK, 2009) whereas it is in sharp contrast to very low frequency of 1.6% in western studies (Kline TS, 1984; Paul PC, 2004 & Sharma SK, 2009).

**Clinical presentation:**

Lymphadenitis is the most common clinical presentation of extrapulmonary tuberculosis. Tuberculous lymphadenitis can be local manifestation of the systemic disease. Tuberculous lymphadenitis most frequently involves the cervical lymph nodes followed in frequency by mediastinal, axillary, mesenteric, hepatic portal, perihepatic and inguinal lymph nodes (Thompson MM, 1992; Brizi MG, 1998 & Geldmacher H, 2002).

Mycobacterial infection should be considered in the differential diagnosis of a cervical swelling, especially in endemic areas. The duration of symptoms before diagnosis may range from few weeks to several months (Pang SC, 1992 & Thompson MM, 1992). It may present as a unilateral single or multiple painless slow growing mass or masses developing over weeks to months, mostly located in the posterior cervical and less commonly in supraclavicular region (Penfold CN, 1996 & Kanlikama M, 2000). A single node especially in the jugulodigastric triangle is likely to be non-tuberculous lymphadenitis. There is a history of tuberculous contact in 21.8%, and tuberculous infection in 16.1% of the cases (Kanlikama M, 2000).

The prevalence rate of pulmonary TB in association with lymph node TB has a wide variation. In primary pulmonary tuberculosis lymph node enlargement is easily identified in a large majority i.e. 87% (Sharma SK, 2001 & 2009). Active pulmonary tuberculosis was seen in 22% cases of tuberculous lymphadenitis (Jha BC, 2001).
Different studies showed wide variations in the prevalence rate of pulmonary TB in association with lymph node tuberculosis which is 5% to 42% (Dandapat MC, 1990; Thompson MM, 1993; Subrahmanyam M, 1993; Chen Y-M, 1994; Jeena PM, 2000; Lt Col MS Barthwal, 2005; Pahwa R, 2005 & Lakmini KB, 2008). The frequency of pulmonary involvement in reported series of patients with tuberculous lymphadenitis is quite variable, ranging from approximately 5% to 70% (American Thoracic Society, 2000; Pahwa R, 2005; Lakmini KB, 2008 & Mohapatra PR, 2009).

Fistula formation was seen in nearly 10% of the mycobacterial cervical lymphadenitis (Konishi K, 1998 & Kanlikama M, 2000). Cervical nodes in the submandibular region are most commonly affected in children (Dhooge I, 1993 & Danielides V, 2002). Young children significantly more often present with only one lesion and the referring physician more frequently suspects a neoplasm, bacterial adenitis or reactive adenopathy (Kvaerner KJ, 2002).

In *M. tuberculosis* lymphadenitis systemic symptoms are common. Classically patients present with low grade fever, weight loss and fatigue and some what less frequently with night sweats (Dandapat MC, 1990; Lee KC, 1992 & Kvaerner KJ, 2001). Cough is not a prominent feature of tuberculous lymphadenitis (Dandapat MC, 1990 & Kvaerner KJ, 2001). Upto 57% of patients have no systemic symptoms (Kvaerner KJ, 2001). Studies have also shown that systemic symptoms are present in 43% patients. Multiplicity, matting and caseation are three important findings of tuberculous lymphadenitis.

Jones and Campbell (Jones and Cambell, 1993) classified peripheral tuberculous lymph nodes into following five stages.

1. stage 1, enlarged, firm, mobile, discrete nodes showing non-specific reactive hyperplasia;
2. stage 2, large rubbery nodes fixed to surrounding tissue owing to periadenitis;
3. stage 3, central softening due to abscess formation;
4. stage 4, collar-stud abscess formation; and
5. stage 5, sinus tract formation.

Clinical features depend upon the stage of the disease. The lymph nodes are usually not tender unless (i) secondary bacterial infection, (ii) rapidly enlarging nodes or (iii) co-existing HIV infection are evident. The lymph node abscess may burst infrequently leading to a chronic non-healing sinus and ulcer formation. Classically, tuberculous sinuses have thin, bluish, undermined edges with scanty watery discharge. Scrofuloderma is a mycobacterial infection of the skin caused by direct extension of tuberculosis into the skin from underlying structures or by contact exposure to tuberculosis.


Nontuberculous mycobacterial lymphadenitis:

Mycobacteria other than Mycobacterium tuberculosis complex (MOTT) mainly exist in the environment as saprophytes. First such mycobacterium was recognised as a cause of human disease in 1908 (Duvall C, 1908). The emergence of HIV and AIDS has significantly increased the risk of TB and diseases due to MOTT (American Thoracic Society, 1990; Narain JP, 1992 & Doloin PJ, 1994). The infection due to nontuberculous mycobacteria has been observed to be major cause of morbidity and mortality in western countries (American Thoracic Society, 1990). Of the 121 known species of
mycobacteria so far, 45 species have been found to associate with disease in man (Katoch VM, 2004 & Euzéby JP, 2008).

Very little is known regarding lymphadenitis due to NTM from India. In the western literature, NTM lymphadenitis has often been described in children. Both sexes are equally affected (Jones PG, 1962; Olson RN, 1981; Stanley BR, 1983 & Pang SC, 1992). Constitutional symptoms seldom develop and the disease generally remains localized to the upper cervical area. If untreated, the nodes often progress to softening, rupture, sinus formation, healing with fibrosis and calcification (Jones PG, 1962; Olson RN, 1981; Stanley BR, 1983 & Pang SC, 1992). Recent statistics for NTM indicate an increase in prevalence and isolation of cervical lymphadenopathy caused by NTM. Because NTM is not generally reportable, the true incidence is difficult to determine. In nontuberculous adenitis, Mycobacterium avium-intracellulare complex is the most common causative agent (Katoch VM, 2004 & Mohapatra PR, 2009).

Nataraj et al. 2002 from Mumbai reported a NTM isolation rate of 3.8% and Jesudason & Gadstone, 2005 from Vellore reported a NTM isolation rate of 3.9% in the cases of mycobacterial lymphadenitis (M. avium – intracellular, M. fortuitum and M. kansasii). In India, it was reported that 2.6% mycobacterial lymphadenitis in adults was caused by NTM. It was also reported that 70% - 89% mycobacterial lymphadenitis in children is caused by atypical mycobacteria by Reddy VS, 2008. (Nataraj et al., 2002; Jesudason and Gadstone, 2005 & Reddy VS, 2008).

Differential Diagnosis

There are numerous causes of peripheral lymphadenopathy. The diagnosis of tuberculous lymphadenitis depends on the physician considering the possibility of TB in patients at risk and submitting material for mycobacterial culture and pathological examination. Multiplicity, matting and caseation are three features which help in diagnosis of tuberculous lymphadenitis.
The definitive diagnosis of tuberculosis depends on the isolation and identification of the etiological agents responsible for the infection. Identification and treatment of affected patients is the primary strategy for the control of tuberculosis (Frieden et al., 2003). The low sensitivity of conventional methods in detecting tubercle bacilli in clinical specimens makes the diagnosis of tuberculosis in general and extra pulmonary tuberculosis in particular, a major challenge in developing countries (Banavaliker, et al., 1997). It is estimated that only 50% - 60% of all patients with tuberculosis worldwide are actually diagnosed. A high index of suspicion is needed for the diagnosis of mycobacterial cervical lymphadenitis (Sharma SK, 2009).

A thorough history and physical examination, tuberculin test, staining for acid-fast bacilli, radiologic examination, and fine-needle aspiration cytology (FNAC) will help to arrive at an early diagnosis of mycobacterial lymphadenitis which will allow early institution of treatment before a final diagnosis can be made by biopsy and culture (Ibekwe AO, 1997; Albright JT, 2003& Mohapatra PR, 2009). For effective diagnosis of tuberculosis, the combination of conventional methods and molecular techniques has been recommended (Kurabachew et al., 2004 & Sharma SK, 2009).

Apart from focussed history and detailed clinical examination, several other studies are required for confirming the diagnosis of lymph node tuberculosis.

Direct smear Microscopy (Ziehl-Neelsen Staining)

The detection of acid-fast bacilli using Ziehl-Neelsen staining is the primary method for the diagnosis of tuberculosis (Nataraj et al., 2002; Palmino LR, 2007 & Sandhu GK, 2011). Although specific and rapid, the technique has low sensitivity in the detection of tubercle bacilli in various clinical specimens (Bouakline et al., 2003). The specificity of direct smear examination is considered to be 98% in areas where the prevalence of non-tuberculosis bacteria is low (Yates and Grange, 1992 & Palmino LR, 2007).

Smears can be obtained either from a draining sinus or by FNA. Ziehl-Neelsen staining of the smears may reveal mycobacteria in the fresh
specimens. Chance of finding AFB is higher in patients with cold abscess. The sensitivity and specificity of FNA cytology in the diagnosis of tuberculous lymphadenitis are 88% and 96%, respectively (Chao SS, 2002 & Mohapatra PR, 2009).

However, the rate of acid-fast bacilli (AFB) positivity in FNA smears of TBLN is in the ranges of 41% - 56.4 % (Ergete and Bekele, 2000 & Mohapatra 2009). Previous studies conducted on FNA revealed a wide variation in the frequency of AFB positivity. AFB positivity is low in epithelioid granuloma without necrosis (5.8% - 30%) but significantly higher in epithelioid granuloma with necrosis (32% - 65%) (Rajwanshi et al., 1989, Mohapatra PR, 2009 & Sharma SK, 2009).

Singh HB, 2006, used two PCR systems targeting IS6110 and MPB 64 for confirmation of diagnosis of tuberculous lymphadenitis on 80 samples of fine needle aspirate; he reported that, the positivity with AFB was 28.4% (Singh HB, 2006). Purohit MR, 2008, studied the air-dried fine needle aspirates of cervical lymph nodes from 98 patients, reported that mycobacteria was detected by Ziehl-Neelsen stain in 15 (15.3%) (Purohit MR, 2008).

In an study conducted by Reddy VS, 2008, to evaluate the diagnostic utility of mycobacterial culture of fine needle aspirate (FNA), in comparison with the cytological examination and acid fast staining on 157 aspirates, reported 18% positivity by ZN smear ( Reddy VS, 2008).

In an study conducted by Marrakchi, 2010, to evaluate the contribution of microbiology, cytology, and anatomopathology in 50 cases of peripheral tuberculous lymphadenitis, reported, the Ziehl-Neelsen stains positivity in 29.7% cases (Marrakchi, 2010)

In another study conducted by Nigussie M, 2010, to investigate the detection rate of AFB in FNAC specimens using the Ziehl-Neelsen staining method and see if bacillary detection depends on cytomorphologic features of inflammation, reported that only 52 (21.9%) aspirated samples were found to be positive for AFB (Nigussie M, 2010).
Sezai M, 2010, in the study, to evaluate 694 tuberculous lymphadenitis cases reported in Turkey, said and reported that tuberculous lymphadenitis was identified in 10.6% (51/479) of the cases by direct microscopical examination (Sezai M, 2010). In another study conducted by Mittal P, 2011 to evaluate and compare the role of FNAC, mycobacterial culture, and PCR in diagnosis of tuberculous lymphadenitis, reported that tuberculous lymphadenitis was correctly diagnosed by acid-fast bacilli (AFB) smear in 26 cases and overall sensitivity of AFB smear was 76.47% (Mittal P, 2011).

In a study conducted by Joshi P, 2012 to evaluate the efficacy of auto fluorescence in the diagnosis of tuberculous lymphadenitis from fine-needle aspiration biopsies reported, ZN staining positivity in 37.5% (30/80) of cases (Joshi P, 2012). In another study conducted by Uma H, 2012, on the patients of nodal tuberculosis, reported the 76.47% sensitivity of AFB on aspirates smear from lymph nodes (Uma H, 2012). Derese Y, 2012, in a study, on 134 FNA samples, to compare PCR with standard culture of fine needle aspiration samples, reported 11.8% positivity by direct smear (Derese Y, 2012). James K, 2012, in his study with the aim to evaluate the diagnostic value of biopsy using each of the component diagnostic modalities of FNA (microscopy, cytology and culture) in the diagnosis of tuberculous lymphadenitis, reported that among specimens obtained by FNA, sensitivity of microscopy was found to be 18% (James K, 2012).

Smear negative and culture positive results can occur since a significant number of bacilli must be present in the given specimen to be detected by acid fast smear examination. For instance, 5,000 -10,000 acid-fast bacilli per ml of sputum must be present to permit detection by acid fast staining (Daniel, 1989).

Cytology

Historical perspective

It was in the Europe that fine needle aspiration cytology (FNAC), as the technique was usually called, began to flourish in the 1950’s and 1960’s. Soderstrom (Soderstrom N, 1966) and Franzen (Franzen S, 1960) in Sweden,
lopes Cardozo in Holland (Lopes Cardozo P, 1954 & 1978) (all clinician/hematologist by training), Zajdela (Zadela A, 1963) in France and others became major proponents, studying thousands of cases each year. Zajicek (Zajicek J, 1974 & 1979) among the first of pathologist to embrace FNAC in collaboration with Franzen at the Karolinska Hospital, applied the requisite scientific rigor to define precise diagnostic criteria and to determine diagnostic accuracy in a variety of conditions. Disciples of these pioneers have spread the gospel to Europe, the Americas, Asia and Australia and the technique is now part of the service of all sophisticated departments of pathology.

Fine needle aspirate cytology (FNAC) was initially conceived as a means to confirm a clinical suspicion of local recurrence or metastasis of known cancer without subjecting the patient to further surgical intervention. The clinical value of FNAC is not limited to neoplastic conditions. It is also valuable in the diagnosis of inflammatory, infectious and degenerative conditions, in which samples can be used for microbiological and biochemical analysis in addition to cytological preparations.

Fine needle aspirate cytology (FNAC), a relatively non-invasive, pain free, outpatient procedure with no morbidity appeared on the scene about two decades ago. Over a period of time, it has established itself as a safe, cheap and reliable procedure for the diagnosis of peripheral lymphadenopathy (Finfer M, 1991 & Gupta AK, 1992).

Fine needle aspirate cytology (FNAC) is a good diagnostic approach in the diagnosis of tuberculous lymphadenitis or extra pulmonary tuberculosis (Dandapat, et al., 1992 & Abedi AJ, 2008). FNA cytology is a useful diagnostic method in areas where it is difficult to perform molecular techniques. Diagnostic criteria for tuberculous lymphadenitis include: Clinical signs such as enlarged, discrete or matted, usually unilateral non-tender lymph nodes with or without draining sinuses. Thick yellowish appearance of fine needle aspirates, cytological finding showing epithelioid granulomas with caseous necrosis (Singh et al., 2000; Ergete & Bekele, 2000 & Mohapatra PR, 2009).
In addition to epithelioid cells, the smear may contain clumps of amorphous acellular debris or caseous necrotic materials. Lymphocytes, Langhan giant cells and neutrophils may be present or; epithelioid granulomas without caseous necrosis. Groups of epithelioid cells found along with a variable number of lymphoid cells or; necrotic materials without epithelioid granuloma and clumps of amorphous acellular material (Sharma SK, 2004).

The merits of FNAC described by Dandapat et.al 1992 (Dandapat et al., 1992):

(i) It is a time saving procedure for both the patient and the clinician because; a report can be obtained in a few hours.

(ii) The equipment and technique are simple. No anaesthesia is required.

(iii) It is safe and has a high patient acceptance. It can be carried out in the O.P.D. just after clinical examination when the patient attends for the first time.

(iv) It is relatively less expensive.

(v) This procedure is almost free of complications.

(vi) In experienced hands, it has a high diagnostic accuracy.

The role of fine needle aspirate cytology (FNAC) in the evaluation of lymphadenopathies is well known (Das, 1991; Reid et al., 1998; Kline et al., 2000; Gita & Chew, 2000 & Reddy K et al., 2009). When compared with conventional lymph node biopsy, the diagnostic yield of FNAC gives a better sensitivity and specificity as an initial procedure in the diagnosis of lymphadenopathy (Sunpaweravong et al., 1999; Reddy K et al., 2008 & Lakmini KB, 2011).

Instead of excision of the lymph node for histopathologic and microbiologic examination to confirm the diagnosis of tuberculous lymphadenitis, fine needle aspiration cytology (FNAC) provides an alternative and easy procedure for collection of material for cytomorphiclogic and bacteriologic examination (Metro & Jayaram, 1987; Radhika et al., 1989; Gupta et al., 1993; Radhika et al., 1993; Bezabih et al., 2002 & Reddy k et al., 2009).
There are number of studies reporting the sensitivity and specificity of FNAC in the diagnosis of lymphnode tuberculosis to be 77% and 93%, 88% and 96%, 52.9% and 100% respectively (Radhika et al., 1989; Lau et al, 1990; Dandapat MC, 1990; Baek CH, 2000; Chao SS, 2002; Yassin Md. A, 2003 & Lakmini KB, 2011).

The use of FNAC in the diagnosis of tuberculous lymphadenitis is being described increasingly and cytologic criteria for its diagnosis have been well established as described in several papers (Rajwanshi et al., 1987; Kardos et al., 1989; Finfer et al., 1991; Nayak S, 2004; Abedi AJ, 2008 & Sharma M, 2011). The diagnostic findings are epithelioid cell granuloma as with or without multinucleated giant cells and caseation necrosis (Jayaram, 1985; Shariff & Thomas, 1991; Bezabih M, 2002; Nayak S, 2004 & Sharma, 2009).

In a study conducted by Khan R, 2009, on 1827 patients with cervical lymphadenopathy over a three-year period, reported that fine needle aspiration cytology constituted the main diagnostic tool, with a positive yield in 90% of patients (Khan R, 2009).

However, in cases of presenting with a cold abscess, well-formed epithelioid cell granulomas may not be seen; likewise, a similar granulomatous inflammation may be seen in non-tuberculous conditions as well. Combination of FNA with culture or a Mantoux test further increases the diagnostic yield in mycobacterial cervical lymphadenitis (Lau SK, 1991; Tunkel DE, 1999 & Ellison E, 1999). FNAC is a sensitive, specific and cost-effective way to diagnose mycobacterial cervical lymphadenitis (Lau SK, 1988; Nayak S, 2004 & Sharma SK, 2009), especially in children presenting with a suspicious neck swelling (Liu ES, 2001). If cytological findings are inconclusive repeatedly, tissue biopsy by surgery is advisable (Dandapat MC, 1990; Lau SK, 1990; Pang SC, 1992; Chao SS, 2001 & Mohapatra PR, 2009).

**Culture**

The identification of tubercle bacilli by culture is required for the ultimate proof of mycobacterial infection. However, due to unavailability of
laboratory equipment and safety procedures, the method is not practiced in resource poor settings (Hung and Sun, 2000). Culture is often used as a reference method due to its high sensitivity (as high as 89%) and specificity (greater than 98%). However, in significant proportion of clinical samples, low numbers and slow growth rate of tubercle bacilli limit the sensitivity of culture (Velayati, et al., 1998). The probability of finding bacilli is greater by culture than by microscopy when specimens contain only small number of mycobacteria. Many different culture media have been devised for growing the tubercle bacillus.

These may be divided into three groups:
1. Egg based media
2. Agar based media
3. Liquid media

Egg based media various egg based media and their constituents

<table>
<thead>
<tr>
<th>Medium</th>
<th>Components</th>
<th>Inhibitory agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowenstein-Jensen medium</td>
<td>Coagulated whole eggs, defined salts, glycerol and potato flour</td>
<td>Malachite green 0.025gm/100ml</td>
</tr>
<tr>
<td>Petragnini medium</td>
<td>Coagulated whole eggs, glycerol, milk and potato flour</td>
<td>Malachite green 0.052gm/100ml</td>
</tr>
<tr>
<td>American thoracic society medium</td>
<td>Coagulated egg yolk, glycerol and potato flour</td>
<td>Malachite green 0.02gm/100ml</td>
</tr>
<tr>
<td>LJ- GRUFT modification</td>
<td>Same as LJ medium</td>
<td>Penicillin, Nalidixic acid and Malachite green</td>
</tr>
<tr>
<td>Mycobactosel (LJ medium)</td>
<td>Same as LJ medium</td>
<td>Lincomycin, Cycloheximide, Nalidixic acid and Malachite green</td>
</tr>
<tr>
<td>IUAT version of Lowenstein-Jensen medium</td>
<td>Defined salts, whole eggs, glycerol and asparagines</td>
<td>Malachite green 0.025gm/100ml</td>
</tr>
<tr>
<td>Dorset’s egg medium</td>
<td>Beated eggs and sterile broth</td>
<td>Malachite green 0.025gm/100ml</td>
</tr>
</tbody>
</table>
Historically, the egg based media are among the best known solid media used for isolation of *Mycobacterium tuberculosis* (Sobero R and Peabody J, 2006).

**Characteristics**

- These media are solidified by heating to 85°C - 90°C for 30-45 minutes (inspissation) for three days. These are very rich media and contain phospholipids and proteins that tend to bind or neutralize toxic products in clinical specimens.
- Tend to yield high number of positives from direct clinical specimens because it is less inhibitory to the mycobacteria.
- Not useful for research purposes because of being very complex, not reproducible, variation in quality of ingredients and effects of heat.
- Colonies are rough and beige to brown color and show up well on the green background.
- Chromogenic studies and biochemical tests are more accurate when performed on subculture from LJ medium.

**Agar based media** (Roberts C.D et al., 1995).

Various agar based media and their constituents
These media are usually prepared from semisynthetic basal media enriched with supplements (SOPM Thailand, 1994).

**Characteristics**

- These are transparent media and offer better opportunity to study colonial morphology microscopically after just 10 days of inoculation.
- These offer better defined components than egg based media.
- These allow a more rapid recovery of growth (within 2-4 weeks).
- Addition of 0.1% casein hydrolysate to Middle brook 7H11 medium improves the recovery rate and enhances the growth of INH resistant mycobacteria.
- Usually reserved for identification, sensitivity tests and research purposes.

**Liquid media**

Liquid media are useful when the specimens contain very small numbers of bacilli, as positive cultures could be more often obtained in these media using a large inoculum.
A list of liquid media used commonly is given below:

- Kirchner-Herman medium
- Dubo’s medium
- Sula’s medium
- Youmen and Karlsons
- Proskauer and Beck medium
- 7H9 synthetic medium
- 7H9 broth + PANTA → Biphasic medium → Septi-check AFB system

Middlebrook 7H9 broth medium is most commonly used for sub culturing mycobacteria and preparing inocula for antimicrobial susceptibility and biochemical testing.

Disadvantages

- The growth of tubercle bacilli in the liquid media is difficult to follow, since it can't be measured optically.
- Sample plating is not accurate because of severe clumping of the bacilli.

Mycobacterial growth observed on culture media should be quantified in some way.

The following is the widely used scale which is recommended by the American Thoracic Society:

<table>
<thead>
<tr>
<th>No colonies - Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fewer than 50 colonies</td>
</tr>
<tr>
<td>50-100 colonies</td>
</tr>
<tr>
<td>100-200 colonies</td>
</tr>
<tr>
<td>200-500 colonies (almost confluent)</td>
</tr>
<tr>
<td>&gt;500 colonies (confluent)</td>
</tr>
</tbody>
</table>

In a study conducted by Jindal N, 2003 on 190 children of chronic cervical lymphadenitis showed tuberculous etiology on histopathological
examination in 92 (48.4%) and bacteriological evidence of mycobacterial infection (smear and/or culture) in 42 (22.1%). He reported that the positive culture for mycobacteria was obtained in 40, of which 30 (75%) were typical M. tuberculosis and 10 (25%) were atypical mycobacteria (Jindal N, 2003).

A study done on 1,817 bacteriological cultures that were received from 1,817 patients with tuberculous lymphadenitis in South east England, from 1981 to 1989, showed that 1,677 (92.3%) of the cultures were found to be positive for M. tuberculosis, 25(1.34%) were M.bovis, 21(1.16%) M.africanum and 94 (5.17%) other environmental mycobacteria (Yates & Grange, 1992). In the United States, it was reported that 95% - 98% of mycobacterial lymphadenitis in adults is due to M. tuberculosis and 86% - 92% mycobacterial lymphadenitis in children is caused by atypical mycobacteria (M. scrofulaceum, M. avium - intracelluar and M. kansasii) (Brainard, 2001).

However, a negative culture result should not exclude the diagnosis of mycobacterial cervical lymphadenitis. Studies also demonstrated that the cultures are positive in 10% – 69% of the cases of tubercular lymphadenitis (Kanlikama M, 1993; Gupta SK, 1993; Kanlikama M, 2000 & Sharma M, 2011).

Several authors had reported the culture positivity between 10 % to 76% (Singh HB, 2006; Purohit MR, 2008; Lakmini KB, 2008; Reddy VS, 2008; Marrakchi, 2010; Sezai M, 2010; Sharma M, 2010; Asimacopoulos EP, 2010; Mittal P,2011 & James K, 2012). Singh HB, 2006, used two PCR systems targeting IS6110 and MPB 64 for confirmation of diagnosis of tuberculous lymphadenitis on 80 samples of fine needle aspirate; he reported that, the positivity with M. tuberculosis culture in 13.6% (Singh HB, 2006). Purohit MR, 2008, studied the air-dried fine needle aspirates of cervical lymph nodes from 98 patients, reported that the mycobacteria was detected by culture in 24 (24.4%) cases (Purohit MR, 2008.). In another study conducted by Lakmini KB, 2008 on 43 aspirates found out only 13 aspirates were positive by culture (Lakmini KB, 2008).

In a study conducted by Reddy VS, 2008, to evaluate the diagnostic utility of mycobacterial culture of fine needle aspirate (FNA), in comparison
with the cytological examination and acid fast staining on 157 aspirates, reported 45% positivity by culture (Reddy VS, 2008).

In an study conducted by Marrakchi, 2010, to evaluate the contribution of microbiology, cytology, and anatomicopathology in 50 cases of peripheral tuberculous lymphadenitis, reported, the culture positivity in 10.8% of cases (Marrakchi, 2010)

Sezai M, 2010, in the study, to evaluate 694 tuberculous lymphadenitis cases reported in Turkey, said and reported that tuberculous lymphadenitis was identified in 15.9% (65/408) by culture. (Sezai M, 2010).

The studies conducted by Sharma M, 2010 on 150 suspected TBL patients found out that 20 were culture positive among them i.e 20/150 (Sharma M, 2010). In another study conducted by Asimacopoulus EP, 2010 on 97 suspected TBL patients, 67% were positive by the culture (Asimacopoulus EP, 2010).

In another study conducted by Mittal P, 2011 to evaluate and compare the role of FNAC, mycobacterial culture, and PCR in diagnosis of TBL, reported that TBLAP was reported that TBLAP was correctly diagnosed by culture in 30 cases and overall sensitivity of culture was 88.23% (Mittal P, 2011).

In another study conducted by Derese Y, 2012 on 139 FNA samples found out that the, 38% of the samples were positive for TB by culture (Yohannes Derese, 2012). James K, 2012, in his study with the aim to evaluate the diagnostic value of biopsy using each of the component diagnostic modalities of FNA (microscopy, cytology and culture) in the diagnosis of tuberculous lymphadenitis, he reported that sensitivity of culture performed on FNA specimens was 86% (James K, 2012).

There have been only a few studies on the correlation of cytomorphology with smears and culture examination of FNAC material in tuberculous lymphadenitis. Some studies demonstrated the accuracy of the conventional bacteriologic methods is less than 50% (Gupta et al., 1990; Radhika et al., 1993; Sharma SK, 2004 & Mohapatra PR, 2009).
Mostly specimen submitted for culture of mycobacteria contains many other organisms which grow in one or two days and within a week they would overgrow the entire surface of the medium and probably digest it before the mycobacteria start to grow. Such material must be treated in an attempt to destroy them but to preserve the mycobacterial.

**BACTEC culture**

In 1969, Deland and Wanger developed a technique for automated detection of the metabolism of bacteria by measuring the $^{14}$CO$_2$ liberated during decarboxylation of $^{14}$C-labeled substrates present in the medium. This technique has been applied successfully to blood culturing, detection of antibiotic effect on bacterial growth, *Nesseria* spp. differentiation by substrate metabolism and serum assay of aminoglycoside antibiotics. (Siddiqi SH *et al.*, 1996)

Cummings and co-workers in 1975 carried out preliminary work that showed the same principle could be applied to detect growth of *Mycobacterium tuberculosis*. Middlebrook further developed the technique and introduced 7H12 liquid medium containing a $^{14}$C-labelled substrate specific for mycobacterial growth. He reported a significant time saving in the primary isolation of mycobacteria from clinical specimens using the new radiometric medium (Siddiqi SH *et al.*, 1996 & Gatongi D K *et al.*, 2005).

**Principle**

The BACTEC TB medium (12B) is an enriched Middlebrook 7H9 broth base. Mycobacteria utilize a $^{14}$C labelled substrate (fatty acid) present in the medium and release $^{14}$CO$_2$ into the atmosphere above the medium, when the vials are tested on the BACTEC 460 TB System instrument, the gas is aspirated from the vial and the $^{14}$CO$_2$ radioactivity is determined quantitatively in terms of numbers on a scale from 0 to 999. These numbers are designated as the Growth Index (GI). The GI numbers are displayed by the BACTEC 460TB System instrument and are also printed along with the identifying rack and bottle numbers (100 GI units are approximately equal to
0.025 μCi). The daily increase in the GI is directly proportional to the rate and amount of growth in the medium (Siddiqi SH et al, 1996).

The BACTEC instrument also introduces fresh 5% to 10% CO₂ into the medium head space every time a vial is tested. This enhances the growth of mycobacteria. The instrument automatically tests 60 vials at the rate of approximately one vial every 82 seconds and stops at the end of the run. If an inhibitory agent is introduced into the medium, inhibition of metabolism is indicated by reduced production of ¹⁴ CO₂ when compared to a control having no inhibitory agent. This basic principle is applied for drug susceptibility testing and in differentiating TB from other mycobacteria (Siddiqi SH et al, 1996).

The BACTEC 460 TB System instrument must be used with a special TB hood when employed for mycobacteriology. The TB hood provides HEPA filtered exhaust air and negative pressure in the test area. In addition, the TB hood is equipped an ultraviolet light source in the test area. The unit is designed for automatic testing of vials and must not be used for inoculation or sub culturing in place of biological cabinet.

The development of 7H12 medium (BACTEC12A) led to several studies which reported excellent recovery of mycobacteria from sputum as well as extrapulmonary specimens. These studies used an inoculum volume of 0.1 ml per 2.0 ml of medium. Subsequent studies showed that improved recovery occurred if 0.4 ml of medium per vial was inoculated with 0.5 ml of inoculum. A modified 7H12 was then introduced and designated as BACTEC 12B medium (Siddiqi SH et al, 1996).

Since the BACTEC system utilizes a liquid medium, it is important to add an antimicrobial supplement to suppress growth of contaminating microorganisms which may survive decontamination process. Initially, a modification of Mitchison’s antimicrobial mixture which contained polymixin B, amphotericin B, carbenicillin and trimethoprim (PACT) was added to 12A medium. With increased inoculum size in 12B medium, it was found that the contamination rate higher. Siddiqi et al, 1996 reported another antimicrobial mixture (PANTA) which suppressed contamination significantly better than
PACT. PANTA contains polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin.

Although BACTEC TB medium supports rapid growth of most mycobacteria, occasional strains of *Mycobacterium tuberculosis*, such as isolates from treated chronic cases, may grow poorly. Recently, a growth promoting substance, polyoxyethylene stearate has been reported by Siddiqi *et al.*, 1996, when added to the BACTEC TB medium, this substance enhanced growth of those strains which grew slowly or poorly. This growth-promoting substance has been incorporated in the PANTA reconstituting fluid (RF), which should be used to reconstitute lyophilized PANTA supplement.

The BACTEC 460 TB system offers a simple automated technique with significant convenience and time saving. It also offers the opportunity to bring much needed standardization into TB bacteriology, allowing laboratory results to be compared throughout the country. The BACTEC 460 TB system offers techniques and media which are well established through numerous clinical trials and comparative studies (Davies P D O *et al.*, 2006).

Studies conducted by Negi SS *et al*, 2002 & 2005 on pulmonary and extrapulmonary TB cases and Jalesh N. Panikar, 2011 on TBM cases reported that there is an increase in the yield of the positive cultures from clinical samples and lessening the time to detection is achieved with use of the BACTEC system when compared with conventional solid media. The detection time for *M.tuberculosis* averages 9-14 days: it may be less than 7 days for some strains of mycobacteria other than *M.tuberculosis*. (Negi SS *et al*, 2002; 2005 & Jalesh N.P, 2011)

The value of the BACTEC in detection of mycobacteria from sputum, blood and other clinical specimens has been demonstrated in several fields’ trials and clinical correlation studies (Banks J, 1985; Heifets L, 1989; Burns DN, 1991& Gotto M, 1991). They reported that the recovery of M. tuberculosis from clinical specimens was accomplished by the BACTEC system in 14 days.

In a study conducted by Margie A, 1983, in which the BACTEC system and three conventional media (Middlebrook 7H10, selective Middlebrook
7H11) and Lowenstein-Jensen (LJ) were compared for their mean recovery times and recovery rates of mycobacteria from acid-fast, smear-negative clinical specimens. He reported that of the 71 smear-negative, culture positive specimens recovered from 2,165 submitted smear-negative cultures, the BACTEC system detected 71.8%, compared with 88.7% for the conventional three-medium system. The mean detection time for the BACTEC system was less than that by conventional methods for the seven species of mycobacteria recovered. Detection times for Mycobacterium tuberculosis on the BACTEC system and conventional cultural systems were 13.7 and 26.3 days, respectively (Margie A, 1983).

In a study conducted by Roberts GD, 1983, in which a total of 463 respiratory specimens, all smear positive for acid-fast bacteria, were inoculated onto routine solid media and into BACTEC 7H12 Middlebrook medium for detection of mycobacterial growth. He reported that the average detection times for BACTEC-positive cultures were 8.3 days for *Mycobacterium tuberculosis* and 5.2 days for mycobacteria other than tuberculosis; by conventional methods, they were 19.4 and 17.8 days, respectively. An average of 18 days was required by the BACTEC method for complete recovery and drug susceptibility testing of *M. tuberculosis*, as compared with 38.5 days for the conventional methods (Roberts GD, 1983).

Peter Anargyros in year 1990, conducted 4-month trial involving 2,563 routine clinical specimens, to compare the improved BACTEC TB system (12B medium) with the conventional Lowenstein-Jensen (LJ) media for the isolation, identification, and susceptibility testing of mycobacteria. In the study one hundred sixty-two mycobacterial isolates were recovered, 147 (91%) with BACTEC and 118 (73%) with LJ media. Of these, 62 were Mycobacterium tuberculosis complex strains, 59 (95%) of which were isolated with BACTEC and 54 (87%) of which were isolated with LJ media. Of the remaining 100 isolates, which were mycobacteria other than tuberculosis (MOTT), BACTEC and LJ media detected 88 and 64%, respectively. The mean isolation time for
M. tuberculosis complex with BACTEC was 15.5 days, compared with 25.6 days with LJ (Peter Anargyros, 1990)

In a study conducted by SS Negi, 2005, to evaluate the performance of 65 kDa antigen based PCR assay in clinical samples obtained from pulmonary and extrapulmonary cases of tuberculosis. One hundred and fifty six samples were processed for detection of *Mycobacterium tuberculosis* by ZN smear examination, LJ medium culture, and BACTEC radiometric culture and PCR tests. He reported that a significant difference was seen in the sensitivities of different tests, 55.8% for BACTEC culture (P<0.05). The mean detection time for *M.tuberculosis* was 24.03 days by LJ medium culture, 12.89 days by BACTEC culture (SS Negi, 2005)

Lakshmi V, 2006 had reported the experience with the BACTEC 460 TB system and its performance characteristics and its advantages over the conventional LJ medium for mycobacterial culture. Clinical specimens (3597) from patients suspected to have tuberculosis were submitted for mycobacterial culture between May 2000 and August 2005 and were processed using the BACTEC 460 TB system. Pulmonary samples were 1568 while the extra pulmonary samples were 2029. BACTEC achieved detection of 681 (18.93%) *M. tuberculosis* cases (499- pulmonary, 182 extrapulmonary) with a recovery time shorter by 13.2 days compared to conventional method, and concluded that automated systems can have a great impact and thrust on an early diagnosis of tuberculosis allowing an early and appropriate management of the patient and thereby a better disease outcome. (Lakshmi V, 2006)

In a study conducted by, C S Rodrigues, 2007, to evaluate, the efficacy of BACTEC 460 TB system for the diagnosis of tuberculosis in a tertiary care hospital in Mumbai, India, compared 12,726 clinical specimens using BACTEC 460 TB system and conventional method for detection of *Mycobacterium tuberculosis* over a period of six years. He reported that the overall recovery rate was 39% by BACTEC technique and 29% using Lowenstein-Jensen (LJ) medium. An average detection time for Bactec 460
TB system was found to be 13.3 days and 15.3 days as against 31.2 days and 35.3 days by LJ method for respiratory and nonrespiratory specimens respectively. The average reporting times for drug susceptibility results ranged from 6-10 days for the BACTEC 460 TB system, and concluded that the BACTEC system is a good system for level II laboratories, especially in the diagnosis of extrapulmonary and smear negative tuberculosis (C S Rodrigues, 2007).

**Tuberculin Skin Test**

This intradermal test (Mantoux test) is used to show delayed type hypersensitivity reactions against mycobacterial antigen, in which the reagent is mostly protein purified derivative (PPD). The test becomes positive 2–10 weeks after the Mycobacterial infection. Positive reactions (>10-mm induration) can occur in M. tuberculosis infections after 48-72 hours. Intermediate reactions (5- to 9-mm induration) can occur after BCG vaccination, M. tuberculosis infection or nontuberculous mycobacterial infections. Negative reactions (< 4-mm induration) represent a lack of tuberculin sensitization. False-negative reactions can occur in about 20% of all persons with active tuberculosis. The test may be positive in different conditions, like other infections, metabolic disease, malnutrition, live virus vaccination, malignancy, immunosuppressive drugs, newborns, elderly people, stress, sarcoidosis and inadequate test application.

The tuberculin test is considered diagnostic in Mycobacterial infections, though its value in diagnosing disease is debated (Lau SK, 1991; Evans MJ, 1998 & Sharma 2009). Children with atypical mycobacterial adenitis have a decreasing tuberculin response to repeated testing, while children with tuberculous adenitis have a stable response (Schuit KE, 1978). In Mycobacterial cervical lymphadenitis cases the test may be positive (49.4%), intermediate (35.6%) or negative (15%) (Kanlikama M, 2000 & Mohapatra PR, 2009).

The presence of a reaction to the purified protein derivative in patients with tuberculous lymphadenitis is generally reported as 74% to 98%
(Dandapat MC, 1990; Manolidis S, 1993 & Geldmacher H, 2002) while it is often non reactive in patients with NTM lymphadenitis (Wright JE, 1996; Bayazit YA, 2004 & Lazarus AA, 2007). However a negative tuberculin test does not rule out the possibility of tuberculosis.

Radiology and imaging

Chest radiograph, ultrasound, CT and MRI of the neck can be performed in mycobacterial lymphadenitis. Associated chest lesions as seen on chest radiography are very common in children but less common in adults, evident nearly 15% cases (Dandapat MC, 1990; Thompson MM, 1992; Jha BC, 2001 & Gupta KB, 2007).

In the patients with tuberculous lymphadenitis, abnormalities are often discernable on the chest radiography. There is a wide variation in the reported incidence of chest radiographic abnormalities which ranges from 5 % to 44% (Jha BC, 2001; Sharma M, 2009; Mohapatra PR, 2009; Chandir S, 2010 & Asimacopoulus EP, 2010). CT scan of the chest is required for accurate evaluation of the thoracic lymph nodes if the chest radiography shows any evidence of mediastinal or hilar lymphadenopathy (Sharma SK, 2009).

Ultrasound of the neck can demonstrate singular or multiple hypoechoic and multiloculated cystic lesions that are surrounded with thick capsule. On CT, the presence of conglomerated nodal masses with central luscency, a thick irregular rim of contrast enhancement and inner nodularity, a varying degree of homogeneous enhancement in smaller nodes, dermal and subcutaneous manifestations of inflammation, such as thickening of the overlying skin, engorgement of the lymphatics and thickening of the adjacent muscles and a diffusely effaced fascial plane may suggest mycobacterial cervical lymphadenitis (Kim YJ, 1993; Nadel DM, 1996; Sharma SK, 2009 & Mohapatra PR, 2009).

USG and CT modalities are complementary in diagnosis and management of tuberculous lymphadenitis presenting with complications. USG helps in better localization of site for biopsy/FNAC procedure and drainage of pus with high diagnostic yield. While CT helps in better anatomical
localization of lymph nodes not usually detected on clinical examination (Gupta KB, 2007). USG and CT are known to be effective in detection, localization and delineation of enlarged lymph node of neck (Hajec PC, 1986 & Gupta KB, 2007).

USG has a high sensitivity when compared with clinical examination (92 % and 70 % respectively) (Bruneton JN, 1984 & Mohapatra PR, 2009) and high specificity when combined with FNAC (95%) (Baatenburg RJ, 1991 & Gupta KB, 2007). Similarly, CT provides knowledge not only of the site and extent of cervical tuberculous lymphadenitis but also status of affected lymph node. However, these findings may also be seen in other diseases like lymphoma and metastatic lymphadenopathy (Kim YJ, 1993).

MRI may reveal discrete, matted and confluent masses. Necrotic foci, when present, are more frequently peripheral rather than central, and this together with the soft tissue edema may be of value in differentiating mycobacterial cervical lymphadenitis from metastatic nodes (King AD, 1999). If the cervical mass is necrotic, there will be low and high signal intensity in the center of the mass in T1- and T2-weighted images, respectively (K.B. Gupta, 2007 & Sharma SK, 2009).

Molecular diagnostic

During the past 10 years, several molecular methods have been developed for direct detection, identification, and susceptibility testing of mycobacteria. These methods can potentially reduce the diagnostic time from weeks to days.

Polymerase Chain Reaction

Early detection is of major importance in the control of tuberculosis, an infectious disease which worldwide kills more people than any other single infectious disease. Conventional diagnosis of tuberculosis is time-consuming, because culturing of Mycobacterium tuberculosis can take 4 to 8 weeks. Direct staining and microscopy of clinical specimens lack sensitivity and specificity.
The polymerase chain reaction (PCR) is a method for amplifying specific nucleic acid sequences by use of repeated cycles DNA synthesis. The principle of the PCR is simple, requiring a three step process:

1. Denaturation of double stranded DNA.
2. Annealing of primers.
3. Primer extension.

This powerful technique offers several advantages over existing DNA technologies:

1. PCR can amplify specific DNA sequences from as few as 25 base pairs up to 10,000 base pair in length using only the primer specified target sequence rather than the entire genome.
2. It is more sensitive than direct hybridization and requires only a single target DNA molecule that need not be highly purified.
3. It is fast, copying a single DNA sequence over a billion times within 3 hours.

The limitations of PCR should not be underestimated. False negative reactions can result from an inadequate number of primers and false positive reactions can result from the amplification of contaminating DNA. Potentially, PCR represents a direct application of biomolecular research techniques from the bench to bedside and in the recognition of the impact and promise of this technique, its developer, Kary Mullis, was awarded the Nobel Prize in Chemistry in 1993.

The amplification of specific DNA sequences by polymerase chain reaction (PCR) is a novel tool for the detection of different infectious organisms and has already been applied to detect mycobacterial DNA sequences in several materials (Pao et al., 1990; Plikaytis et al., 1991; Sritharan et al., 1991; Ghossein et al., 1992; Saboor et al, 1992 & Popper et al., 1994). There are various studies conducted on PCR, by several authors: In a study conducted by Chung HB, 2000 on 29 suspected cases of TBL and found out 13 (76.4%) were positive by PCR, the sensitivity and specificity was
found out to be 76.4% and 100% respectively (Chung HB, 2000). In another study conducted by Negi SS, 2005 on 156 suspected cases of PTB and EPTB, found out that the sensitivity and specificity of PCR was 74.4% and 100% respectively (Negi SS, 2005).

The study conducted by Allio, OA, 2011 was aimed at using Polymerase Chain Reaction for detection of Mycobacterium tuberculosis complex from clinical samples using universal sample processing methodology. 200 clinical samples were processed by universal sample processing methodology for PCR; smear microscopy was carried out on sputum samples by Ziehl-Neelsen staining technique; and cultured on Middlebrook agar medium containing oleic acid albumin dextrose complex supplement after decontamination of samples. They reported Ninety six (48%) samples were detected positive for *M. tuberculosis* complex by polymerase chain reaction using the combination of boiling and vortexing and microscopy detected 72 (36%) samples positive for acid fast bacilli. Using culture method as gold standard, it was found that polymerase chain reaction assay was more sensitive (75.5%) and specific (94.8%) in detecting *M. tuberculosis complex* from clinical samples (Allio, OA, 2011).

In another study conducted by Hanif F, 2012, to evaluate the yield of polymerase chain reaction (PCR) for detection of *Mycobacterium tuberculosis* from different clinical specimens. Different clinical specimens received for *Mycobacterium tuberculosis* PCR were dealt during the study. They reported that out of a total 4620 samples for PCR, 299 were positive for *Mycobacterium tuberculosis* (6.5%). Yield for different clinical samples for PCR was 63/471 for sputum (13.4%), 3/29 for endobronchial washings (10.3%), 59/851 for body fluids (6.9%) and 24/400 for urine (6%). Positive yield from blood was the lowest (101/2156, 4.7%). They concluded that PCR for *Mycobacterium tuberculosis* is a rapid and reliable method for the diagnosis of both pulmonary and extrapulmonary tuberculosis (Hanif F, 2012).

In another study conducted by Nogueira CL, 2012, to improve polymerase chain reaction (PCR) sensitivity in detecting
Mycobacterium tuberculosis in sputum samples. The study comprised 120 sputum samples, 60 of which were treated with the routine protocol, while 60 were treated with the modified protocol using glass beads. They reported that the samples treated with the routine protocol had a sensitivity of 56.7% (95%CI 44.1-69.2) in 16S rRNA PCR and 81.7% (95%CI 71.9-91.5) in insertion sequence (IS) 6110 PCR, compared with culture. Samples treated with the modified protocol had a sensitivity of 73.3% (95%CI 62.1-84.5) in 16S rRNA PCR and 100% in IS6110 PCR (Nogueira CL, 2012).

The study conducted by Zakham F, 2012 was aimed to evaluate polymerase chain reaction (PCR) as a rapid and direct molecular method for the diagnosis of Mycobacterium tuberculosis (MTB) in 70 clinical specimens (62 sputum samples, six cerebrospinal fluids, and two biopsies) using heat shock protein (hsp65) as the gene target. They reported that the sensitivity of PCR was 81.13%, with specificity of 88.24%; the positive and negative predictive values were 95.56% and 60%, respectively (Zakham F, 2012).

In another study conducted by Barani R, 2012, the study was aimed to evaluate a dual target polymerase chain reaction (PCR) diagnostic assay to detect Mycobacterium tuberculosis from pulmonary and extra-pulmonary samples at a tertiary care centre in South India. The Samples were collected from patients with a low index of suspicion of TB. Acid-fast smears were performed by Auramine O fluorescent microscopy and PCR was performed by using two site-specific primer pairs targeting IS6110 by nested PCR and TRC4 by conventional PCR. Amplified products for IS6110 and/or TRC4 were indicative of M. tuberculosis. They reported that among 114 (19 pulmonary and 95 extra-pulmonary) samples tested by PCR assay, 12 (11%) were positive for both IS6110 and TRC4, of which 11 (10%) were non-respiratory and one was (1%) respiratory in origin. PCR for TRC4 alone was positive for eight (7%) non-respiratory and two (2%) respiratory samples, while IS6110 alone tested positive for six (5%) non-respiratory samples and one (1%) respiratory sample. Of a total of 29 PCR positive samples, 17 (15 %) were acid-fast smear positive (Barani R, 2012).
Derese Y, 2012 study on 139 FNA samples found out that the, 23.4% samples were positive by PCR. The sensitivity and specificity of PCR was 42.2% and 89.2% respectively (Derese Y, 2012).

**PCR in Tuberculous Lymphadenitis**

Polymerase chain reaction (PCR) is a fast and useful technique for the demonstration of mycobacterial DNA fragments in patients with clinically suspected mycobacterial lymphadenitis (April MM, 1996 & Manitchotpisit B, 1999 &). The presence of few dead or live microorganisms is enough for PCR positivity. The PCR can be applied on the materials obtained by FNA or biopsy, and can reduce the necessity for open biopsy (Baek CH, 2000 & Singh KK, 2000). Its sensitivity ranges between 43% and 84%, and its specificity between 75% and 100% (Manitchotpisit B, 1999 & Hirunwiwatkul P, 2002). PCR can be applied when smears and cultures are negative (Kwon KS, 2000). PCR is a confirmatory and sensitive technique for the diagnosis of mycobacterial cervical lymphadenitis.

In a study conducted by Singh HB, 2006, he used two PCR systems targeting IS6110 and MPB 64 for confirmation of diagnosis of tuberculous lymphadenitis on 80 samples, reported that, PCR targeting IS6110 , 56 of 81 (69.1%) samples showed positive results as compared to PCR targeting MPB64 by which 39 of 81 (48.2 %) samples were positives (Singh HB, 2006).

In an another study conducted by Khan R, 2009, on 1827 patients with cervical lymphadenopathy over a three-year period, reported that fine needle aspiration cytology constituted the main diagnostic tool, with a positive yield in 90 % of patients and Polymerase chain reaction analysis was performed in 126 patients, and reported 63 % the sensitivity of PCR (Khan R, 2009). Sharma M, 2010, studied 150 patients with lymphadenopathy, found out that 57 patients were positive by PCR, the sensitivity and specificity was found out to be 100% and 92.1% respectively (Sharma M, 2010). In another study conducted by Mittal P, 2011 to evaluate and compare the role of FNAC, mycobacterial culture, and PCR in diagnosis of TBL, reported that TBLAP was
correctly diagnosed by PCR in 30 cases and the overall sensitivity of PCR was 88.23% (Mittal P, 2011).

Derese Y, 2012 study on 139 FNA samples found out that the, 23.4% samples was positive by PCR. The sensitivity and specificity of PCR was 42.2% and 89.2% respectively (Derese Y, 2012).

PCR is a confirmatory and sensitive technique for the diagnosis of mycobacterial cervical lymphadenitis. It can also differentiate between lymphadenitis caused by *Mycobacterium tuberculosis* and that caused by NTM. PCR is used as an adjunct to conventional techniques in the diagnosis of mycobacterial infections (Singh KK, 2000 & Hirunwiwatkul P, 2002).

A PCR assay has been reported for diagnosing *Mycobacterium tuberculosis* by targeting HupB gene. This gene codes for a protein called as the histone-like protein. This allows differentiation of two closely related species, namely *M. tuberculosis* and *M. bovis* of the MTB complex. The N and S primer-generated PCR amplicons differed in *M. tuberculosis* and *M. bovis*, these amplicons were determined to be 645 bp and 618 bp respectively.

A study done in Ethiopia, PCR sensitivity was found to be 83% and specificity to be 100% in diagnosing tuberculous lymphadenitis with fine needle aspirates cytology (Dawit Kidane, 2002).

Detection of an NTM helps ensure that the negative result for *M. tuberculosis* is a true negative which is very important and can be achieved with the help of Polymerase chain reaction (PCR) and Restriction fragment length polymorphism (RFLP).

In these years, peripheral blood mononuclear cells-PCR (PBMC-PCR) is being employed for the detection of *Mycobacterium tuberculosis* in clinically suspected tuberculous lymphadenitis cases which showed a positivity rate of 65.2%, as compared to culture, (Mirza S, 2003). In another study conducted in Karnal peripheral blood mononuclear cells-PCR (PBMC-PCR) also showed a positivity of 43.75 % (Ahmed N, 1998).

In a study conducted by Sharafeldin GS, 2007, to determine whether *Mycobacterium tuberculosis* infection spreads through the blood to different lymph-node groups in patients with tuberculous lymphadenitis, reported that,
**M. tuberculosis** DNA were detected in the blood samples of 30/39 (77%) patients with tuberculous lymphadenitis (Sharafeldin GS, 2007).

In another study conducted by Khosla R, 2009; on 38 suspected extrapulmonary TB cases and 89 non-tuberculous subjects, found out that overall sensitivity was 60.53 and negative predictive value (NPV) was 76.95 which was found to be superior to the gold standard of mycobacteria culture (10.53% and 72.36%) (Khosla R, 2009).

Despite peripheral blood mononuclear cells (PBMC-PCR) showed high sensitivity and specificity over conventional methods and cytology a lot of work has to be done for its establishment. Despite its usefulness in the diagnosis of tuberculous lymphadenitis, fine needle aspiration cytology (FNAC) faces several limitations, and its sensitivity and specificity are not well established (A. S. Aljafari, 2004).

Since the ‘90s, a series of in-house assays have been described. Each laboratory uses its own protocol for pre-treatment, DNA extraction and detection of amplification products. The insertion element IS 6110 and the 16S rDNA are the most common targets used (Thierry et al., 1990; Kox et al., 1995; Springer et al., 1996; Kirschner et al., 1996; Garcia-Quintanilla et al., 2002 & Tortoli, 2003). Other regions used for amplification include the rpoB gene encoding the beta-subunit of the RNA polymerase (Kim et al., 1999; Chaves et al., 2000 & Adekambi et al., 2003), and the 16S-23S rRNA internal transcriber spacer (Roth et al., 1998).

**Polymerase chain reaction – Restriction fragment length polymorphism (PRA) Analysis**

Mycobacterial isolates have traditionally been identified to the species level based on their reactions in a series of phenotypic and biochemical tests. However, the biochemical reactions of isolates of the same species may vary from each other and from time to time, and in many cases no definitive identification is obtained. Because biochemical testing is slow, cumbersome, and may yield ambiguous results, laboratories are increasingly using molecular methods for species identification.
Telenti et al. (1993) developed a rapid method, based on the amplification of the gene encoding the 65-kDa heat shock protein, followed by restriction-fragment-length polymorphism, using two restriction enzymes BstEII and HaeIII. Isolates from both solid and liquid cultures can be used. The fragments of the restriction enzyme digestion were analyzed by agarose gel electrophoresis and compared.

The 65-kDa protein contains epitopes that are unique as well as are common to various species of mycobacteria. The *M. avium - M. intracellulare complex* can be discriminated to *M. avium* and *M. intracellulare* species. The *M. fortuitum complex* can also be separated to the subspecies level. PRA is going to be performed only to identify maximum and accurate species Telenti et al. 1993.

In a study conducted by Taylor TB, 1997 evaluated the usefulness of PCR RFLP in routine identification of mycobacteria growing in Bactec 12 B, and found out that 100 of 103 isolates were identified by the method (Taylor TB, 1997).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is increasing used as a method to in rapid differentiation and identification of mycobacteria to the species level (Wong et al., 2003; da Silva T et al., 2005; Leao et al., 2005; Martin A, 2007; Neonakis IK, 2008; Kim S, 2008; Shin JH, 2009; Ong CS, 2010; Whang J, 2011 & Campos CE, 2012).

In a study conducted by Whang J, 2011 reported that the rpoB-PRA using Smal or MspI and HaeIII restriction of the rpoB gene is a simple, convenient, and reliable confirmatory assay for simultaneous identification of MAP and other MAC members.

In a study conducted by Roth et al., 2000 also evaluated the usefulness of PCR RFLP using 16-23Sr RNA, testing 811 strains and found out that this method was useful in identification of mycobacteria to subspecies level and can be used by reference and routine laboratories.

In TB-endemic countries, the molecular methods like in-house PCR and RFLP may improve the diagnostic and epidemiologic research. Since
Restriction fragment length polymorphism (RFLP) developed, it is being used for the detection of mycobacterial species. By the use of 16S-23Sr RNA, *M. lentiflavum-M. triplex, M. bohemicum* (which is closely related to *M. avium*), *Mycobacterium interjectum- Mycobacterium intermedium, M. farcinogenes*, or *Mycobacterium obuense* can be easily differentiated and identify (Roth *et al.*, 2000).

In a study conducted by Lee H, 2000, used the novel region of the rpo B gene for the identification of mycobacteria, a total 50 reference mycobacterial strains were used, they found out that this gene was more simple, rapid and accurate than the conventional procedures for differentiating the species. Bum-Joon Kim, 1999 and Lee H, 2000 also found that pathogenic *M. kansasii* is easily differentiated from nonpathogenic *M. gastri* (93.1% similarity): Sau3AI (restriction enzyme), could be used to differentiate *M. africanum* from other members of *M. tuberculosis* complex (Bum-Joon Kim, 1999 and Lee H, 2000).

In the study conducted in Ethiopia, the author enrolled 72 patients of tuberculous lymphadenitis, they used the PCR technique to identify the causative agent in tubercular lymphadenitis, they used three primer sets to identify the causative agent at the genus (antigen 85 complex), complex (*IS6110* insertion sequence), and species (*pncA* gene and allelic variation) levels. Positive signals at *Mycobacterium* genus and *M. tuberculosis* complex levels were obtained in FNAs from 35 (87.5%) of 40 patients with a clinical and cytological diagnosis of TBLN.

The study states that Polymerase chain reaction (PCR) is a powerful tool for the diagnosis of tuberculous lymphadenitis at the species level, provided that appropriate steps are followed to recognize and overcome pitfalls such as contamination, low target amplification, and inhibition. It also states that PCR is a powerful technique for diagnosis and speciation of mycobacterial infection in Fine needle aspirates cytology from the patients (Dawit Kidane, 2002)
PCR-based sequencing has become the gold standard for identification of mycobacterial species. The method consists of PCR amplification of mycobacterial DNA with genus-specific primers and sequencing of the amplicons. The organism is identified by comparison of the nucleotide sequence with reference sequences. As commonly practiced, only one sequencing reaction is needed for a definitive identification. This method also allows for direct detection of mycobacterial species that cannot be grown on conventional laboratory culture media, and several previously unrecognized species have been identified (Springer B, 1993 & Springer B, 1995).

The target most commonly used is the gene coding for the 16S rRNA. This gene is present in all bacterial species and contains both conserved and variable regions, making it an ideal target for taxonomic purposes.

Several other target genes have been characterized for this purpose. The genes coding for the 32-kDa protein (Soini H, 1994), the 65-kDa heat shock protein (Kapur V, 1995), and the 16S-23S rRNA internal transcribed spacer (Roth A, 1998) contain enough sequence diversity to distinguish all clinically important mycobacteria except for the members of the M. tuberculosis complex. In addition, because of the intraspecies variation observed in the 65-kDa protein gene, this target can also be used for distinguishing clones of certain mycobacterial species (Pai S, 1997). The system offers the ability to mycobacteriology laboratories to identify many of the recently described mycobacteria. Furthermore, the technology is simple and easy to implement in most of the laboratories (Hanna S, 2001).

The DNA probe technology for identification of bacteria is one of the most successful molecular methods. The AccuProbe (Gen-Probe, San Diego, CA, USA) is the assay based on this technology that is used by the majority of clinical mycobacterial laboratories worldwide. It has the ability to identify a series of clinically important mycobacteria. These are M. tuberculosis complex, M. avium complex, M. avium, M. kansasii, and M. gordonae. The DNA probes are single-stranded DNA oligonucleotides labelled with acridinium ester that are complementary to the target, which is the rRNA. After
sonication, the probes are added to the broken mycobacterial cells, to form a stable DNA-RNA complex. Following separation of the labelled complex from unhybridized DNA, the hybridization is detected by light emission in a luminometer. The AccuProbe can be used for both solid and liquid cultures. The method is easy to perform and only a sonicator and luminometer are required as equipment. The method has been widely evaluated with good results. The AccuProbe kits are rapid, highly sensitive and specific. The procedure can be completed in less than two hours (Neonakis IK, 2008).

The line probe technology includes PCR (with biotinylated primers), reverse hybridization with different specific DNA probes, immobilized in parallel lines on a strip and colorimetric detection in an automated instrument. The banding pattern is indicative of the species of the isolate. The turnaround time is approximately five hours. Two systems of line probe assay are commercially available: (a) the Inno LiPA Mycobacteria v2 and (b) the GenoType Mycobacterium: Inno LiPA Mycobacteria v2, (Innogenetics, Ghent, Belgium) This assay is based on the amplification of the mycobacterial spacer region 16S-23S rRNA for the simultaneous identification, in just one strip test, of the 17 most frequently isolated mycobacterial species: \textit{M. tuberculosis complex}, \textit{M. avium}, \textit{M. intracellulare}, \textit{M. scrofulaceum}, \textit{M. kansasii}, \textit{M. xenopi}, \textit{M. chelonae}, \textit{M. gordonae}, \textit{M. fortuitum complex}, \textit{M. malmoense}, \textit{M. genavense}, \textit{M. simiae}, \textit{M. smegmatis}, \textit{M. haemophilum}, \textit{M. marinum}/\textit{M. ulcerans} and \textit{M. celatum}. Moreover, it has the ability to discriminate subgroups within \textit{M. kansasii} and \textit{M. chelonae} on the same strip. Mixed populations are easily identified. Mijs \textit{et al.} 2000 evaluated the test, comparing the results of the assay with the results obtained using biochemical and molecular tests. The accuracy of the assay was 99.2% after discrepancy analysis (636/641 mycobacterial isolates) and both sensitivity and specificity were 100% (all 641 mycobacterial isolates reacted with the Mycobacterium species probe and all 27 non-Mycobacterium species scored negative). This technology is revolutionizing the laboratory identification of slow growing microorganisms such as mycobacteria.
GenoType Mycobacterium (Hain Lifescience, Nehren, Germany): The procedure includes a multiplex PCR, followed by reverse hybridization and line probe technology. There are three kits: (a) the GenoType MTBC for distinguishing members of the *M. tuberculosis complex*, and (b) the GenoType Mycobacterium CM (Common Mycobacteria), and GenoType Mycobacterium AS (Additional Species) for NTM. The GenoType MTBC is based on the gyrB gene polymorphism (Richter et al., 2003). The AS and CM assays use 23S rDNA as their target, thus the amplicon generated in the CM assay can be used for the AS assay without the need to perform a second PCR. The combined use of CM and AS can distinguish almost 30 different NTM including the following species: *M. avium, M. chelonae, M. abscessus, M. fortuitum, M. gordonae, M. intracellulare, M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. marinum, M. ulcerans, M. peregrinum, M. xenopi, M. simiae, M. mucogenicum, M. goodii, M. celatum, M. smegmatis, M. genovense, M. lentisavum, M. heckeshormense, M. szulgai, M. phlei, M. hemophilum, M. gastr, M. asiaticum and M. shimoidei*. The GenoType assays are rapid, easy-to-perform and easy-to-interpret (Gitti et al., 2006). They have allowed clinical mycobacteriology laboratories to detect infrequent mycobacterial species, without the need of sophisticated techniques (Neonakis et al., 2007).

Richter et al. (2003) evaluated the ability of the GenoType MTBC to differentiate *M. tuberculosis complex* species and demonstrated that all the *M. tuberculosis complex* species can be unambiguously identified, with the exception of *M. tuberculosis, M. africanum subtype II and M. canetti* that have identical hybridization patterns. Neonakis et al., 2007 analyzed 120 clinical *M. tuberculosis complex* isolates with GenoType MTBC assay and found full agreement with previous identification with gene probes and biochemical analysis. According to Russo et al., 2006 the sensitivity and the specificity of the GenoType Mycobacterium, compared with 16S rRNA gene sequencing, were 97.9% and 92.4% for CM and 99.3% and 99.4% for AS, respectively. Richter et al. 2006 found 92.6% and 89.9% concordant results with CM and AS assays respectively, when they were compared with sequencing data.
Pyrosequencing (Biotage, Uppsala, Sweden) technology is a novel method of nucleic acid sequencing-by-synthesis that is based on the detection of released pyrophosphate (PPi) during DNA synthesis (Ronaghi, 2001 & Tuohy et al., 2005). The cascade of enzymatic reactions generates visible light. The generated light is proportional to the number of incorporated nucleotides. The method is optimal for determining short sequences (typically 20–30 bases of a DNA) rapidly and in a semi-automated format (Ronaghi, 2001). The ability of this technology to accurately characterize common strains of mycobacteria was evaluated by Tuohy et al., 2005. It provided an acceptable identification for 179 of 189 (94.7%) isolates tested. Although the short sequences generated by Pyrosequencing are not as discriminating as the 300 to 500-bp sequences that can be generated by traditional sequencing, the authors demonstrated that abundant clinically useful information may be obtained.

DNA microarrays (DNA chips): The method is based on hybridization of fluorescently labelled PCR amplicons of an unknown strain to a DNA array, containing nucleotide probes for 16S ribosomal RNA gene. The hybridization pattern and intensity is determined by scanning the chip using laser confocal fluorescence microscopy. The process of generating the target, its hybridization and reading on the chip requires approximately two hours. It allows the identification of a large number of strains in one reaction (Gingeras et al., 1998). Gingeras et al. (1998) designed an array to determine the specific nucleotide sequence of 705 bp of the rpoB gene of M. tuberculosis. It accurately detected RMP resistance associated with mutations of 44 clinical isolates of M. tuberculosis (Gingeras, 1998). Troesch et al., 1999) investigated this probe array strategy focusing on mycobacterial diseases (Affymetrix, Gene Chip technology, Santa Clara, Calif). Sequences of regions from the 16S rRNA and rpoB loci had been developed. Unique hybridization patterns allowed for the identification of Mycobacterium species and the RMP-resistant alleles (Troesch et al., 1999). Seventy mycobacterial isolates from 27 different species and 15 RMP-resistant M. tuberculosis strains were tested. A total of 26 of 27 species were correctly identified as well as all of the rpoB
mutants alleles (Troesch et al., 1999). A great disadvantage is, however, the current high cost of the required equipment (Neonakis IK, 2008).