“You can’t go back and you can’t stand still, if the thunders don’t get you, then the lightning will…”

“If the number of victims which a disease claims is the measure of its significance, the all diseases, particularly the most dreaded infectious diseases, such as bubonic plague, Asiatic cholera etc., must be ranked far behind tuberculosis.”

Robert Koch, 1882.

One Indian dies of tuberculosis every minute and over a thousand Indians dies of tuberculosis every day (RNTCP, 2011).

Tuberculosis is India’s worst scourge bordering on the silent genocide. Despite the fact that Bacillus Calmette-Guerin (BCG) vaccination is given at birth all over the country, tuberculosis remains the biggest killer disease in India (Gursimrat KS, 2011).

In 2010, there were 8.8 million (range, 8.5–9.2 million) incident cases of TB, 1.1 million (range, 0.9–1.2 million) deaths from TB among HIV-negative people and an additional 0.35 million (range, 0.32–0.39 million) deaths from HIV-associated TB (WHO, 2011). In 2010, there were 5.7 million notifications of new and recurrent cases of TB, equivalent to 65% (range 63–68%) of the estimated number of incident cases in 2010. India alone accounted for an estimated one quarter (26%) of all TB cases worldwide.

Tuberculosis is worldwide in distribution, but is particularly more prevalent in Asia and Africa. According to a 2008 World Health Organization (WHO) report, 9.2 million cases were detected and 1.7 million people lost their lives due to TB the world over. India, China, Indonesia, South Africa and Nigeria rank first to fifth respectively in terms of absolute numbers of cases (WHO, 2008). India has the highest TB burden accounting for one fifth of the global incidence. According to a report issued by the government of India, nearly 40% of the Indian population is infected with the TB bacillus (RNTCP, 2009).
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).

Although the predominant form of tuberculosis is pulmonary disease, infection with \textit{M. tuberculosis} may be seen in any organ system. Extrapulmonary tuberculosis (EPTB) is defined as the isolated occurrence of TB in any part of the body other than lungs. Extrapulmonary tuberculosis mainly results from haematogenous dissemination or lymphogenous spread from a primary focus, usually a pulmonary lesion. This haematogenous 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-1980’s (Mehta J.B, 1991 & Mohapatra PR, 2009). Lupatkin H, 1992, reported an increased prevalence of extrapulmonary tuberculosis especially in patients infected with HIV. In these patients multiple extrapulmonary sites are often involved (Van den Brande P, 2003).

Tuberculous lymphadenitis in the cervical region is known as scrofula, a term derived from the Latin for “glandular swelling. The microbiological cause of scrofula was first appreciated by Bollinger, May and Demme in the mid to late 19th century when they noted that \textit{Mycobacterium bovis} from cows was the cause of this ailment. Lymphadenitis is the most common clinical presentation of extrapulmonary tuberculosis. Tuberculous lymphadenitis can be local manifestation of the systemic disease, which 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).
Cervical lymph nodes are the most common site of involvement and reported in 60% to 90% patients with or without involvement of other lymphoid tissue (Sharma SK, 2009 & Mohapatra PR, 2009). Cervical lymphadenitis, which is also referred to as scrofula, may be manifestation of a systemic tuberculous disease or a unique clinical entity localized to neck. Mycobacterium tuberculosis is the most common causative agent in India (ICMR, 1955-58; Dandapat MC, 1990; Jawahar MS, 1990; Sharma SK, 2009 & Mohapatra PR, 2009).

In Nontuberculous lymphadenitis, Mycobacterium avium-intracellulare complex is the most common causative agent. Mycobacterial lymphadenitis most frequently affects patients in their second decade but may afflict patients of any age (Enarson DA, 1980).

Early confirmation of the diagnosis of tuberculosis is a challenging problem especially in case of paucibacillary and extra-pulmonary forms. Conventional methods available for diagnosis namely, tuberculin test, radiological examination and other imaging methods and sputum smear microscopy have their own limitations. Histopathology is characteristic but there could be problems to get representative specimen, and non specific features.

In tubercular lymphadenitis cases, a rapid, sensitive and specific diagnosis is needed, owing to the limitations of the traditional microbiological methods, paucibacillary nature of the specimen and the extensive differential diagnosis (Singh KK et al., 2000).

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, showing epithelioid granulomas with caseous necrosis (Singh KK et al., 2000 & Ergete & Bekele, 2000). Laboratory diagnosis of tuberculosis is based on the traditional methods of Ziehl-Neelsen acid-fast stain and laboratory culture of the causative organism, M. tuberculosis. The Ziehl-Neelsen stain, although rapid and inexpensive, lacks sensitivity in clinical specimens.
Histology and cytology are useful in diagnosis having sensitivity of approximately 32% – 59% (Singh KK, 2000 & Mirza S, 2003). 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 (Aljafari AS, 2004).

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.

Culture of mycobacterium is diagnostic for mycobacterial cervical lymphadenitis. However, a negative culture result should not exclude the diagnosis of mycobacterial cervical lymphadenitis. Culture on Lowenstein-Jensen (LJ) medium is the gold standard, but takes 8 to 12 weeks, requires 10–100 organisms per sample. Different studies revealed 9% and 70% positivity in the clinically suspected tubercular lymphadenitis cases on fine needle aspirates or biopsy specimens (Huhti E, 1975; Kim SS, 1996; Singh KK et al, 2000; Gong G, 2002; Mirza S, 2003 & Sharma SK, 2009). Some studies also demonstrated that the cultures are positive in 10% – 69% of the cases Kanlikama M, 1993; Gupta SK, 1993; Kanlikama M, 2000; Mirza S, 2003; Sharma SK, 2009; Verma P, 2010 & Sharma M, 2011).

The laboratory culture of M. tuberculosis requires a long culture period and therefore, clinical and therapeutic decisions have to be made before the laboratory diagnosis becomes available. In these cases the rapidity of diagnosis is the key for successful management.

Automated and radiometric methods for culturing and for antimicrobial susceptibility testing have been in general use in diagnostic bacteriology for more than a decade; so much so, that this technology is now of critical importance to many segments of microbiology. Despite the fact that the protracted doubling time of Mycobacterium tuberculosis should have made it a prime target, the development of rapid diagnostic procedures in mycobacteriology has only occurred very recently.

In recent years, radiometric method (BACTEC) is being used for early diagnosis of tuberculosis. In this method the sample is incubated in a medium
containing palmitic acid, which has radiolabelled carbon. It detects the radiolabelled carbon dioxide released by the mycobacteria and converts proportionally to quantitative growth index on a scale of 0 to 999, which takes lesser time i.e. 12 days.

Data from various studies indicates that BACTEC offers promise as a method for the rapid detection of mycobacterial growth. At this time, however, this system should be used concurrently with conventional culturing methods. This system has been widely used for drug susceptibility testing and is currently used as a comparative standard (Venkataraman P, 1998 & Bemer P, 2002).

Introduction of Polymerase Chain Reaction (PCR) has enhanced the diagnostic predictability of the disease especially in the extrapulmonary, paucibacillary samples. High specificity and sensitivity have been reported in different samples.

PCR allows sequences of DNA present in only a few copies of mycobacteria to be amplified \textit{in vitro} such that the amount of amplified DNA can be visualized and identified. If appropriate sequences specific for \textit{Mycobacterium tuberculosis} are selected, 10 -1000 organisms can be readily identified. The PCR methodology is rapid; results are available within a day of DNA extraction from the sample.

A variety of PCR methods have been developed for detection of specific sequences of \textit{M. tuberculosis} and other mycobacteria (Katoch VM, 1997). Developments in this area have been very rapid and a large number of PCR assays targeting different gene stretches of \textit{M. tuberculosis} have been described (Brisson-Noel A, 1989; Hermans PW, 1990; Sjobring U, 1990; Shankar P, 1991; Sritharan V, 1991; Eisenach KD, 1991; Reddi PP, 1993; Verma A, 1995; Baevis KG, 1995; Kadiyal GV, 1996; Ahmed N, 1998; Singh, 1999; Narayanan S, 2001; Negi SS, 2005; Lakmini KB, 2008 & Sharma M, 2011). Indian laboratories have been active in development of PCR methods for detection of \textit{M. tuberculosis}. Different Indian investigators have used separate gene targets like MPB 64 (Shankar P, 1991 & Pahwa R, 2005), repetitive sequences (Reddi PP, 1993 & Khandekar P, 1994), GC repeats
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The positivity rates varying from 40% - 90 % have been reported by PCR in case of tubercular lymphadenitis (Back CH, 2000; Narayanan S, 2000; Kwon KS, 2000; Singh KK, 2000; Vatoch VM, 2004; Lakmini KB, 2008 ;Verma P, 2011 & Sharma M, 2011 ).

Presently available evidence suggests that PCR is very helpful in establishing the etiological diagnosis in some patients with granulomatous lymphadenitis in whom the conventional methods of FNAC, histopathology and mycobacterial culture are inconclusive. A PCR assay system for tuberculosis, which is commercially available (Baevis KG, 1995) has been found reported to be reproducible, sensitive as well as specific (Soini H, 1996).

An alternative approach has been implemented in the detection of *M. tuberculosis* or *M. avium* DNA with a PCR in peripheral blood mononuclear cells (PBMC-PCR) of patients with pulmonary TB (Schluger NW, 1994; Kulski JK, 1995; Condos R, 1996; De Francesco MA, 1996; Del Prete R, 1997 & Honore S, 2001). The hypothesis is that patients with active infection harbor mycobacterial DNA in peripheral scavenging cells such as macrophages (Schluger NW, 1994 & Del Prete R, 1997). In TB patients co infected with human immunodeficiency virus (HIV), the PCR has sensitivities of 95%–100% and specificities of 89%–100% (Schluger NW, 1994; Kulski JK, 1995; Condos R, 1996 & De Francesco MA, 1996).

In case series with immunocompetent and immunosuppressed patients, the performance of the assay is highly variable, ranging from 33% to 87% depending on the study (Rolfs A, 1995; Aguado JM, 1996; Del Prete R, 1997; Ahmed N, 1998 & Honore S, 2001). The use of the PBMC-PCR for the diagnosis of extrapulmonary TB is still incipient but holds promise (Honore S, 2001 & Tzoanopoulos D, 2001).

In the recent years, peripheral blood mononuclear cells-PCR (PBMC-PCR) is being employed for the detection of *Mycobacterium tuberculosis* in

Since Restriction fragment length polymorphism (RFLP) developed in, 1993, it is being used for the detection of mycobacterial species. PCR-restriction fragment length polymorphism analysis (PRA) is simple to perform, rapid, and economical, features that make it highly attractive for routine clinical laboratories.

Polymerase chain reaction-restriction fragment length polymorphism analysis (PRA) techniques have been developed for several mycobacterial genes, such as hsp65, 16SrRNA gene (Kox LF, 1997 & Kirschner P, 1998), the 23S rRNA gene (Padilla E, 2004 & Makinen J, 2006), the 16S-23S rRNA spacer, and rpoB (Telenti A, 1993; Lee H, 2000 & Roth A, 2000).

PCR based sequencing is considered the "gold" standard for identification of mycobacteria. Initially, PCR amplification takes place followed by sequencing of the amplicons in an automatic sequencer. The identification of an unknown strain is completed by comparison of the nucleotide sequence with a library of known sequences. The databases for this purpose are available in the internet. Such databases are the GenBank (Gen Bank, 2008), the Ribosomal Differentiation of Medical Microsystems database (RIDOM) (Harmsen et al., 2002) and that of the European Molecular Biology Laboratory (EMBL) (EMBL, 2008).

Several target genes have been used for the procedure like rpo B, Hsp 65, 16-23Sr RNA spacer (Telenti A, 1993; Lee H, 2000 & Roth A, 2000). 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.

In the present study, we have used various diagnostic techniques i.e. cytology, hematology, culture and Polymerase chain reaction (PCR) to diagnose clinically suspected cases of tuberculous lymphadenitis. We have also used the blood specimen and evaluated its importance in the diagnosis of tuberculosis, so that it can be fully explored as an alternative sample in diagnosis and management of the disease.