EXTRAPULMONARY TUBERCULOSIS OF PATIENT VISITING TRIBHUVAN UNIVERSITY TEACHING HOSPITAL (TUTH) KATHMANDU, NEPAL

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MASTER OF SCIENCE IN MICROBIOLOGY
(ENVIRONMENT AND PUBLIC HEALTH)

BY
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Recommendation

This is to certify that **Miss Meera Pudasaini** has completed this dissertation work entitled **"EXTRAPULMONARY TUBERCULOSIS ON PATIENT VISITING TRIBHUVAN UNIVERSITY TEACHING HOSPITAL KATHMANDU NEPAL"** as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. To our knowledge, this work has not been submitted for any other degree.

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On the recommendation of **Prof. Dr. Basista Rijal** and **Mr. Binod Lekhak,** this dissertation work of **Miss Meera Pudasaini** entitled **"Extrapulmonary tuberculosis on patients visiting Tribhuvan University Teaching Hospital"** has been approved for the examination and is submitted to the Tribhuvan University in the Partial fulfillment of the requirements for **Master of Science Degree in Microbiology**.**(Envioronment and Public Health)**

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Abstract

Tuberculosis is one of the giant killer disease which accounts for massive death all over the world. Extrapulmonary tuberculosis becomes a serious problem throughout the globe including Nepal.The samples were collected and decontaminated and AFB staining as well as culture on Ogawa medium was done. The isolates were identified on the basis of biochemical tests.

Within the study period from June 2006 to Feb 2007, a total of 150 samples were collected from the patients visited to the Tribhuvan University, Teaching Hospital (TUTH) suspected of Extrapulmonary Tuberculosis. A varieties of Extrapulmonary specimens were brought to the Mycobacteriology laboratory, that includes Urine, Endometrial biopsy, Pleural fluid, peritoneal fluid, Cerebrospinal fluid, Blood, Ascitic fluid and Pus. The samples were collected , decontaminated , AFB stained and cultured on Ogawa medium. The isolates were identified on the basis of biochemical tests.

Of the total 150 samples, 40 were from male patients and 110 were from female patients. The highest percentage of suspected patients (54.67%) was found in the age group 20-30 followed by 30-40 years. The highest number of female patients 67 (60.91%) were found in the age group 21-30 years followed by 12 (10.91%) in 30-40 years. Similarly, the highest number of male patients 15 (37.50%) were found in the age group 20-30 years followed by 10 (25.00%) in 30-40 years.The highest number of samples 65 (43.33%) was endometrial biopsy followed by urine 30 (20%). The least number of samples were blood 2 (1.33%).Out of 150 cases of suspected extra pulmonary T.B, only 18 (12%) cases were found to be positive.

Among 150 samples, 10 (6.67%) showed positive result from direct smear, 13 (8.67%), showed positive result from culture and only 5 (3.33%) were found to be positive from both direct smear and culture. The highest percentage of positive cases 9 (49.99%) was found in the age group 20-30 followed by 5 (27.77%) in 10-20 years. The highest number of female patients 7 (38.88%) were found in the age group 20-30 years followed by 2 (11.11%) in 10-20 years. Similarly, the highest number of male patients 3 (16.66%) were found in the age group 10-20 years followed by 2 (11.11%) in 20-30 years.

The highest number of positive cases in both the direct smear examination and culture was obtained from pus sample followed by pleural fluid and urine samples .All the isolated culture tested for Niacin and Nitrate reduction test were found to be positive and negative for heat labile catalase test. Thus, the isolates were confirmed as *Mycobacterium tuberculosis*

.Key Words:Extrapulmonary tuberculosis, Ogawa medium, direct smear, Culture, Mycobacterium tuberculosis.

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LIST OF ABBREVIATIONS

AFB : Acid Fast Bacilli

AIDS : Acquired Immune Deficiency Syndrome

ART : Anti-Retroviral Therapy

CDC : Centre for Disease Control and Prevention

CSF : Cerebro Spinal Fluid

CT : Computed Tomography

DOTS : Directly Observed Treatment Short course

EPTB : Extra Pulmonary Tuberculosis

FNAC : Fine Needle Aspiration Cytology

GUTB : Genito Urinary Tuberculosis

INH : Isoniazid Hydrochloride

IVU : Intra Venous Urogram

MAC : Mycobacterium Avium Complex

NTC : National Tuberculosis Centre

NTP : National Tuberculosis Program

PCR : Polymerase Chain Reaction

SAARC : South Asian Association for Regional Co-operation

TB : Tubercle Bacilli

TNF : Tumour Necrosis Factor

TUTH : Tribhuvan University Teaching Hospital

US : United States

WHO : World Health Organization

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CHAPTER -I

1. INTRODUCTION

Tuberculosis is a specific infectious disease caused by *Mycobacterium tuberculosis.* The disease primarily affects lungs and causes pulmonary tuberculosis. It can also affect intestine, meninges, bones and joints, lymph glands, skin, and other tissues of the body resulting extrapulmonary tuberculosis. The disease is usually chronic with varying manifestations. The disease also affects animals like cattle; this is known as "bovine tuberculosis,"which may sometimes be communicated to man (Park, 2000).

Tuberculosis (TB) constitute a major public health problem in most developing countries of the world. It accounts for the largest burden of mortality due to any infectious agent worldwide. The incidence of TB rose so rapidly over a number of years that World Health Organisation (WHO) declare it a global emergency in April 1993, the first declaration of this sort ever (WHO,1993).Tuberculosis in man is caused predominantly by *Mycobacterium tuberculosis* and occasionally by *Mycobacterium bovis* and *Mycobacterium africanum*. These organisms are also known as tubercle bacilli because they cause lesions called tubercle and as acid fast bacilli (AFB) as once stained by hot carbol fuchsin, they resist decolorisation by dilute mineral acids and are therefore referred to as acid fast bacilli.Tuberculosis as a killer disease has probably been recognized since the Stone Age. Traces of tuberculous lesion have been found in the lungs of 3000 year old Egyptian mummies. Today the co-epidemic of tuberculosis and HIV is a major problem in the world. HIV increases the risk of getting tuberculosis 30-50 times (STC, 2000).Tuberculosis remains a world wide public health problem despite the fact that the and vaccines are available making tuberculosis a preventable and curable disease. According to conservative estimates there are 15-20 million cases of infectious tuberculosis in the world. The "infectious pool" is maintained by the occurrence of 7.25 million new cases and 3 million deaths each year (WHO, 1998).

Tuberculosis is a socio medical problem (STC, 2001). Diagnosis is performed clinically, radiologically and bacteriologically. Demonstration of bacillus in the lesions

by direct microscopy either by Ziehl-Neelsen staining or by fluorochrome staining allows highly accurate diagnosis. Examination by bacteriological culture provided the definitive diagnosis (WHO, 1998). Different biochemical tests such as niacin test, nitrate reduction test, catalase test etc, confirms the identification in culture(kent et al,1985) Culture also provides the necessary material for drug susceptibility testing. Besides, there are also various molecular techniques which help in identification of mycobacteria, such as Polymerase chain reaction(PCR), Ligase chain reaction (LCR), Gas liquid chromatography (GLC), High performance liquid chromatography (HPLC) etc.(Forbes et al, 2002)

Technologically advanced countries have achieved spectacular results in the control of tuberculosis. (WHO, 1982). But the problem of tuberculosis is acute in developing countries. Tuberculosis is one of the major public health problems in Nepal. About 45% of the total population is infected with tuberculosis; out of which 60% are in the productive age group (DOHS, 2002). Every year 40,000 people develop active tuberculosis, of which 20,000 have infectious pulmonary diseases **(**DOHS, 2003**).**Nepal presenting an exemplary scenario has about 45% of its total population infected with Tuberculosis, out of which 60% are in economically productive age group of 15-49 years. Even after implementation of highly cost effective and extremely successful treatment strategy of DOTS (Directly Observed Treatment Short Course) as a part of National Tuberculosis Program (NTP) in April 1996, around 44,000 Nepalese develop active tuberculosis every year, of whom 20,000 have the infectious pulmonary form claiming 6,000 to 7,000 lives annually (NTP, 2003).

Tuberculosis can occur in any part of the body and most common site of infection is lung causing pulmonary tuberculosis. Eighty percent of tuberculosis occurs in the lungs called “Pulmonary tuberculosis.”. But, TB bacteria can attack any part of the body such as the Kidney, Spine, and Brain etc (Extra-Pulmonary Tuberculosis). If not treated properly, TB disease can be fatal. Extra pulmonary tuberculosis is much less common than pulmonary, which occurs more frequently among persons with HIV.The site of the

disease in the body defines the type of tuberculosis which is caused by *Mycobacterium tuberculosis* complex and *Mycobacterium species*.

Though tuberculosis is one of the most prevalent disease condition in our country, it is usually not diagnosed in early stages or rather misdiagnosed due to delay in seeking medical help, most of the cases goes unnoticed with propagation of cases to extra pulmonary tuberculosis. This study will be helpful for tracing out the existence of extrapulmonary cases in our country.

This study aims to find out the status of extrapulmonary tuberculosis among the patients visiting Tribhuvan University Teaching Hospital (TUTH), Nepal. The study also conducted to establish the best technique to be used for the diagnosis of extrapulmonary TB.

CHAPTER-II

2. OBJECTIVES

General objective:

- To study the occurance of Extra pulmonary Tuberculosis in patients visiting TUTH, Kathmandu.

Specific objectives:

- To find out the commonest extrapulmonary tuberculosis among the suspected patients.
- To confirm *Mycobacterium tuberculosis* biochemically.
- To compare the Acid Fast Bacilli (AFB) staining technique and culture for the diagnosis of Extra-pulmonary tuberculosis.

CHAPTER-III

3 LITERATURE REVIEW

3.1 INTRODUCTION

3.1.1 Definition

Tuberculosis is a chronic bacterial infection caused mainly by *Mycobacterium tuberculosis* and *Mycobacterium tuberculosis* complex characterized by the formation of granuloma in infected tissue as a result of cell-mediated immunity. In majority of the cases it affects the lungs causing pulmonary tuberculosis. But may also have extra-pulmonary extension affecting the lymph nodes, intestine, meninges, bones and joints, skin and other parts of the body with varying clinical manifestations such as, evening rise of fever, decreased appetite and weight loss, hemoptysis and progressive weakening of the body (WHO, 2004).

Tuberculosis can involve any organ system in the body. While pulmonary tuberculosis is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical problem. The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung. However, when an extrapulmonary focus is evident in a patient with pulmonary tuberculosis,such patients have been categorized under pulmonary tuberculosis as per the guidelines of the World Health Organization (WHO). Since tuberculosis can virtually involve any organ system and a detailed description regarding EPTB at each of these sites is too exhaustive **(**Sharma and Mohan, 2004**).**

Tuberculosis (TB), one of the oldest known human diseases, is still is one of the major causes of mortality, since two million people die each year from this malady. TB has many manifestations, affecting bone, the central nervous system, and many other organ systems, but it is primarily a pulmonary disease that is initiated by the deposition of

Mycobacterium tuberculosis, contained in aerosol droplets, onto lung alveolar surfaces. From this point, the progression of the disease can have several outcomes, determined largely by the response of the host immune system. The efficacy of this response is affected by intrinsic factors such as the genetics of the immune system as well as extrinsic factors, *e.g.*, insults to the immune system and the nutritional and physiological state of the host. In addition, the pathogen may play a role in disease progression since some M. tuberculosis strains are reportedly more virulent than others, as defined by increased transmissibility as well as being associated with higher morbidity and mortality in infected individuals **(**Smith, 1996**).**

Tuberculosis (TB) is a contagious bacterial infection caused by the bacterium Mycobacterium tuberculosis. It is "disseminated" if it has spread from the lungs to other organs of the body by the blood or lymph system. The Alternative names are Milliary tuberculosis; Tuberculosis-disseminated; Extra pulmonary tuberculosis **(**ummc.org**)** Extrapulmonary involvement can occur in isolation or along with a pulmonary focus as in the case of patients with disseminated tuberculosis (TB). The recent human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) pandemic has resulted in changing epidemiology and has once again brought extrapulmonary tuberculosis (EPTB) into focus. EPTB constitutes about 15 to 20 per cent of all cases of tuberculosis in immunocompetent patients and accounts for more than 50 per cent of the cases in HIV-positive individuals. Lymph nodes are the most common site of involvement followed by pleural effusion and virtually every site of the body can be affected. Since the clinical presentation of EPTB is atypical, tissue samples for the confirmation of diagnostic can sometimes be difficult to procure, and the conventional diagnostic methods have a poor yield, the diagnosis is often delayed.Availabiity of computerized tomographic scan, magnetic resosnance imaging laparoscopy, endoscopy have tremendously helped in anatomical localisation of EPTB.

The disease usually responds to standard antituberculosis drug treatment. Biopsy and/or surgery is required to procure tissue samples for diagnosis and for managing complications. Further research is required for evolving the most suitable treatment

regimens, optimal duration of treatment and safety when used with highly active antiretroviral treatment (HAART) (Sharma and Mohan, 2004).

3.1.2 Historical Background

Tuberculosis was present in Egypt from early dynasty times, perhaps as early as 3700 B.C. (Morse et.al., 1964). Certainly, tuberculosis was well recognized by the time of Hippocrates (377-400B.C) who gave an excellent clinical description of the disease. The Dutch physician, franciscus sylvius (1614-1672) deduced from autopsies that tuberculosis characterizes by the formation of nodules, which he named “tubercles”

During the course of time, the modern concept of tuberculosis started from the work of Rene Theoclore Laennec (1781-1826), a French clinician, who himself was a consumptive and succumded to the disease. In 1819, he invented the Stethoscope and accurate description of tuberculosis lesions; he described follicular (Milliary) and exudative forms of tuberculosis. Later, Robert koch, announced to discovery of the tubercle bacillus in 24th March 1882 and succeeded in culturing it on inspissated serum.

Tuberculosis is a disease of great antiquity having been identified in mummies from the fourth million B.C. when and where the battle with the disease began remains a matter of conjecture but there is evidence that *Mycobacterium tuberculosis* must have preceded the recorded history.

The clinical features of both Pulmonary and spinal tuberculosis were well described by Hippocrates in about 400 B.C. Accounts of the diseases appeared in the Vedas and other ancient Hindu texts, in which it was sometimes termed Rajyachhyama (meaning the king of Maladies in Sanskrit), the king of diseases and it afflicted Neolithic man and pre Columbian Amerindians (Grange,1990). It is the Hippocratic collection that describes the first authentic account of clinical tuberculosis. Scattered throughout the volumes are numerous references to Pthisis the term which Greek word which mean” to consume spit” and “to waste away” (Grange, 1996).

The transmissible nature of tuberculosis was clearly established by Jean-Antoine Villemin, a French military Doctor. In 1868, Villemin published the result of a series of studies in which he convincingly demonstrated that tuberculosis could be produced in rabbit by inoculating them with tuberculous material from man or cattle. The disease could be passed from animal to animal and differences in virulence were observed between human and bovine material. In addition Villemin established that Scrofula (tuberculous cervical lymphadenitis) and pulmonary tuberculosis were different manifestations of the same disease **.** Villemin's prediction that the causative agent of tuberculosis could be isolated was realized in 1882 when Robert Koch succeeded in culturing the bacilli in inspissated serum. In addition to culturing the causative organism. Koch succeeded in staining it by treatment with alkaline solution of methylene blue for 24 hours. The technique was subsequently improved by Ehrlich by using a hot solution of the aryl methane dye fuschin and it is this technique, slightly modified by Ziehl and Neelsen whose name it bears, that is still used today **(**Grange, 1990**).**

Though the disease has been identified earlier, the modern era of tuberculosis treatment began only in 1946 with the advent of streptomycin and in 1952 A.D. with development of Isoniazid Hydrochloride (INH).Since than the modalities of treatment regimens were constantly revised and updated. At present, giving short course chemotherapy of six months duration treats tuberculosis.All members belonging to Mycobacterium Tuberculosis Complex (MTC) cause tuberculosi infections. *Mycobacterium tuberculosis* is the cause of most cases of human tuberculosis, particularly in developed countries. An estimated 2 billion persons, one- third of the world's population are infected with *Mycobacterium tuberculosis* Of great concern is the emergence of epidemic multidrug resistance strains of *Mycobacterium* tuberculosis (Forbes et al, 2002)

3.1.3 Brief Microbiology

3.1.3.1 Etiological agents

The genus Mycobacterium is the only genus in the family Mycobacteriaceae. Currently there are 71 recognised or proposed species in the genus Mycobacterium (Forbes et.al, 2002). It is convenient to divide mycobacteria of clinical interest into MTC and mycobacteria other than tuberculosis bacilli (MOTT). MTC is associated with other human disease. Several other collective names given to MOTT are atypical, anonymous, non-tuberculous, tuberculoid, opportunistic, envioronmental bacilli (Collee et al, 1996)

Most human TB is caused by *M.tuberculosis* but some cases are due to the *M.bovis* which is principal cause of tuberculosis in the cattle and many other animals. But *M.bovis* rarely causes diseases in the area of the world where animal husbandry includes TB screening and milk pasteurization. *M.microti* is a pathogen of voles and other small animals. *M.africanum* believed to represent a transitional organism between *M.bovis* and *M.tuberculosis* and also cause human tuberculosis and mainly found in equatorial Africa (Chakraborty, 2003)

MOTT exists as saprophyte of soil and water and occasionally causes opportunistic disease in human. Infection caused by this organism is known as mycobacterioses. Four main types of diseases are caused by MOTT. They are skin lesions followed by traumatic inoculation of bacteria, localized lymphadenitis, and tuberculosis like pulmonary lesions and disseminated diseases (Chakraborty, 2003)

Three main types of skin lesions are caused by MOTT bacteria. Post injection abscesses are caused by rapid growing pathogens mainly *M.chelonei* and *M.fortitum,* swimming pool granuloma also called fish tank granuloma0 caused by *M.marinum* and buruli ulcers caused by *M.ulcerans.* Pulmonary diseases is most frequently caused by *M.kamsasii.* Most cases of disseminated diseases are caused by *M.aviumintracellularae*

And this species is now a well recognized cause of secondary disease in AIDS victims. It is clear that the mycobacteria are the cause of very important group of infectious diseases of man (Grange, 1998)

3.1.3.2 General characteristics and morphology

Organisms belonging to the genus mycobacterium are very thin,slender, complex, unicellular organisms with a wide range of antigenic determinants (Collee et. al 1996).Size varies from 0.2to 0.4×2 to 10µm (Forbes et.al., 2002). Mycobacteriaare acid and alcohol fast, non-motile, non-sporing, weakly gram positive, aerobic or microaerophilic, straight or slightly curved rod shaped bacteria. Some mycobacteria display cocco-bacillary, filamentous or branched forms and some produce yellow to orange pigment in the dark or after exposure to light (Good et al, 1998)

The typical cell morphology of *M.tuberculosis* as seen in acid fast stains is a thin, straight or slightly curved (bacillus) rods measuring of about 0.3 to 0.6 by 1 to 4um deeply stained(strongly acid fast), with a distinct beaded appearance. They are non-motile, non-sporing and non-capsulated. They are gram positive but many species stains poorly with this stain even after prolonged staining, because of the characteristics of the cell wall, which is rich in chemically diverse lipids (upto 60% of the cell wall). The thickness of the cell wall is due to the presence of long chain fatty acids (mycolic acids) which form a thick palisade. It is B-hydroxy fatty acid linked covalently to murein. Hence the slow growth results from inability to transport nutrients rapidly across the wax layer (Collee et al, 1996)

Tubercle bacilli are obligate aerobes and will not grow in absence of oxygen, even a moderate reduction in the oxygen tension results in an appreciable decrease in the metabolism of the bacilli. The bacilli grow slowly, the generation time in vitro being 14-15 hrs colonies appear only in about two weeks and sometimes may be delayed up to 6 to 8 weeks. Optimum pH is 6.4 to 7.0 and grows only in especially enriched media containing egg, asparagines, potatoes, serum and meat extract (Forbes et al, 2002)

3.1.3.3 Classification of Mycobacteria:

I. Strict pathogens

A) *M.tuberculosis* complex- human type

M.tuberculosis- human type

M.bovis-bovine type

M.microti- muaine type

B) Lepra bacilli

M.leprae – causing leprosy in human

M.lepraemurium - causing rat leprosy

C) Other animal pathogens

M.microti –murein type

M.paratuberculosis- Johne's bacillus

M.ulcerans

M.balnei

II. Atypical mycobacteria

Runyon Group I- Photochromogens

Runyon Group II- Scotochromogens

Runyon Group III- Non-chromogens

Runyon Group IV- Rapid growers

III. Saprophytic mycobacteria (non- pathogenic)

M.smegmatis-present in smegmas, thermophiles grow at 52ºC

M.pheli-present in grass

M.stercoris-present in dung

M. thermoresistible

3.1.3.4 Pathogenesis

Tuberculosis is caused by bacteria of the complex of *Mycobacterium tuberculosis*. Transmission is usually by airborne spread of bacteria from individuals with infectious pulmonary tuberculosis. Crowding in poorly ventilated rooms is one of the most important factors for transmission of tuberculosis. Patients whose sputum contains

bacterial particles visible by microscopy play the greatest role in the spread of infection, while patients with extrapulmonary tuberculosis are usually noninfectious.

The clinical manifestation and eventual outcome of TB as of all infectious processes depend on the virulence of the pathogen and the nature of the host's immune responses (Grange, 1998)

While the risk of acquiring tuberculosis infection depends largely on external factors, the likelihood of developing disease after being infected depends more on endogenous factors like the individual's innate defenses against disease. A prime factor for developing tuberculosis in infected individuals is co-infection with HIV which suppresses cellular immunity. Other conditions known to increase the risk of developing active disease include lymphoma, leukemia, hemophilia, chronic renal failure, insulin dependent diabetes mellitus and conditions associated with malnutrition like gastrectomy and jejunal bypass surgery. (Collee et al, 1996)

After droplets containing tuberculi microorganisms are inhaled, a majority of the organisms are trapped in the upper airways and are expelled by ciliated mucosal cells. The small number of bacilli which reach the alveoli are ingested by macrophages. Dependent upon how strong the host's defense is to the invading bacteria, the macrophages may halt the multiplication of the bacteria by producing proteolytic enzymes or the bacteria by begin to multiply, killing the macrophages by lysis in the process. After two to four weeks of infection, additional host defenses against the bacteria are initiated including a tissue-damaging response, which destroys non-activated macrophages containing multiplying bacteria, and a macrophage-activating response, which results in the activation of macrophages capable of killing and digesting tubercle bacilli. It is the balance between the tissue-damaging response and the macrophage-activating response which determines whether active tuberculosis will develop or whether the infection will be resolved. (Golden and Vikram, 2005)

Granulomatous lesions are formed with the accumulation of large numbers of activated macrophages at the alveoli. The tissue-damaging response not only destroys

macrophages but also produces a caseating necrosis in the center of the lesion. The necrotic material resembles soft cheese and therefore the process is called caseous necrosis. The tubercle bacilli can survive in the necrotic environment, but their growth is limited by the low oxygen and low pH. Some of the granulomatous lesions heal by fibrosis and calcification while others undergo further development. Even after healing takes place, viable bacilli may remain dormant within macrophages or within the necrotic material. In a small number of cases, the macrophage-activating response is weak and the microbial growth can only be attempted to be inhibited by tissue-damaging responses. In this case, the lesion enlarges, the caseous material at its center liquefies, bronchial walls and blood vessels are invaded by bacteria and destroyed and cavities are formed in the lungs. Within the cavities, bacilli multiply and spread into the airways through expectorated sputum.

In the early stages of the infection, bacilli are usually transported by macrophages to regional lymph nodes from which they can be disseminated throughout the body. Lesions may then form in various organs in the body which resemble those which form in the lungs. Although such lesions usually tend to heal, in patients with poor natural immunity, dissemination may result in fatal miliary tuberculosis or tuberculous meningitis. (Golden and Vikram, 2005)

3.1.3.5 Clinical Manifestations

In primary pulmonary tuberculosis, the lesion is often localized to the middle and lower lung zones. Usually, the lesion heals spontaneously and may later be evident as a small, calcified nodule called a Ghon lesion.

Primary pulmonary tuberculosis may progress rapidly to clinical illness in children and patients with impaired immunity. The primary lesion enlarges rapidly, its central portion undergoes caseasting necrosis and caviatation develops to result in progressive primary tuberculosis. It is usually accompanied by hilar or mediastinal lymphadenopathy due to the spread of bacilli from the lung to lymphatic vessels. The enlarged lymph nodes may cause bronchial obstruction and resultant lobar collapse.

Partial obstruction may cause obstructive emphysema and bronchiectasis (dilated bronchi and bronchioles). With hematogenous spread of the bacilli, miliary tuberculosis and fatal tuberculous meningitis may develop in immunocompromised individuals.

Post-primary disease which is also called adult-type, reactivation or secondary tuberculosis results from reactivation of latent infection. It is usually localized to the apical and posterior segments of the upper lobes where bacterial growth is favored by the high oxygen concentration. The amount of lung tissue involved varies widely from small lesions to extensive cavitation. Massive involvement of pulmonary segments with coalescence of lesions results in tuberculous pneumonia. Some untreated patients soon die due to severe pulmonary tuberculosis while others undergo spontaneous remission or progress along a chronic, progressively debilitating course called "consumption." Individuals with chronic disease continue to expel bacilli into the environment and can infect others.

Hematogenous spread of bacilli can result in extrapulmonary tuberculosis. Lymph node tuberculosis is the most common extrapulmonary presentation. It presents as painless swelling of the lymph nodes, especially the cervical and supraclavicular nodes. Pleural tuberculosis is also common, resulting when a few bacilli invade the pleural space. The effusion may be small and resolve spontaneously or be large enough to cause fever, chest pain and dyspnea. Tuberculosis of the upper airways, which may involve the larynx, pharynx and epiglottis, is almost always another complication of advanced cavitary pulmonary tuberculosis. Symptoms include hoarseness of voice and dysphagia.

EPTB (Extra pulmonary tuberculosis) is invariably a post primary manifestation of infection with TB. Following the initial bacillemia during the primary infection, multiple sites are seeded with the mycobacteria The local immune response controlled by macrophages and T lymphocytes contains the bacilli within these sites but is not effective enough to render these sites sterile These sites are generally those with a rich blood supply, facilitating both the delivery of the mycobacteria and their growth in an oxygen rich environment(Golden and vikram, 2005)

EPTB usually develop in the setting of recent infections with symptoms manifesting rapidly among children and adolescents, whereas in older people it is a feature of its late reactivation. Clinical manifestations generally fall into two presenting situations- low grade systemic symptoms, often as pyrexia of undetermined origin or a biopsy finding of a granulomatous inflammation in an organ. Both these situations call for considering an extensive differential diagnosis.

The difficulties and costs of diagnosing extrapulmonary TB add to the burdens faced by national TB programmes, particularly in the case of India where there are more people with TB than in any other country. According to India's country profile in WHO latest Global Tuberculosis Control report, 1,296,000 new or relapsed cases were notified during 2007; 17% of new notified cases were of extrapulmonary TB. The rise of drug-resistant forms of TB is amongst the programme's other challenges (WHO, 2004).

3.2 TYPES OF EXTRA PULMONARY TUBERCULOSIS

3.2.1 Tuberculous Lymphadenitis

Lymphadenitis is the most commonly occurring form of extrapulmonary tuberculosis. Cervical adenopathy is most common, but inguinal, axillary, mesenteric, mediastinal, and intramammary involvement all have been described. Although previously considered a disease of childhood, lymphadenitis has a peak age of onset of 20 to 40 years, and in the United States it is most common in women and immigrants**.** (Golden and Vikram ,2005)

3.2.2 Pleural Tuberculosis

In the United States, pleural tuberculosis accounts for about 5 percent of all tuberculosis cases. Tuberculous effusions can follow early post primary, chronic pulmonary, or miliary tuberculosis. Pleural tuberculosis often is an acute illness with cough, pleuritic chest pain, fever, or dyspnea. AFB smears of pleural fluid are seldom positive (5 % of

cases) unless the patient has tuberculous empyema. Pleural fluid cultures for *M. tuberculosis* are positive in less than 40 % of cases.

3.2.3 Genitourinary Tuberculosis (GUTB)

Genitourinary tuberculosis is another extrapulmonary manifestation. urinary frequency, dysuria, hematuria and flank pain are common presentations. Patients may however be asymptomatic and the disease discovered only after severe destructive lesions of the kidneys have developed.

Renal disease may be the result of direct infection of the kidney and lower urinary tract or may present as secondary amyloidosis. Patients present with dysuria, hematuria, or flank pain. More than 90 % of asymptomatic patients have sterile pyuria with or without microscopic hematuria. In renal tuberculosis, bacillary shedding may be intermittent. Hence, it is advisable to test 3-6 consecutive morning samples of urine. Mycobacterial culture of three morning urine specimens establishes the diagnosis in 90 % of patients **(**(Golden and Vikram,2005). Ureteral TB is an extension of the disease from the kidneys, generally to the ureterovesical junction. It only rarely affects the middle third of the ureter. Ureteral TB often causes ureteral strictures and, sometimes, hydronephrosis. Occasionally, severe cases can cause stricture of virtually the entire ureter. Ureteral TB develops in about half of all patients with renal TB**.)**

Bladder TB is secondary to renal TB and usually starts at the ureteral orifice.. Epididymis TB is always hematogenous in origin and not a direct extension from the bladder. The formation of a draining sinus is not common in developed countries, but epididymal induration and beading of the vas are common. Involvement of the testis is usually due to direct extension from the epididymis. Infertility may result from bilateral vasal obstruction.. Orchitis and the resulting testicular swelling can be difficult to differentiate from other mass lesions of the testes. Prostate TB is also spread hematogenously, but involvement is rare. Genitourinary tuberculosis is another extrapulmonary manifestation. Urinary frequency, dysuria, hematuria and flank pain are common presentations. Patient’s may, however, be asymptomatic and the disease

discovered only after severe destructive lesions of the kidneys have developed **(**Khan et al, 2008**).**

GUTB in developing countries accounts approximately 15-20% of extrapulmonary cases of TB, with most common age group between 30-45 years.It is rare but also is documented cases in the 5 to 12 years old age group have been reported. The male to female ratio is 5:3. (Golden and Vikram, 2005)

3.2.4 Skeletal Tuberculosis

Bone and joint tuberculosis may account for up to 35 percent of cases of extrapulmonary tuberculosis and, overall, for almost 2 percent of all cases of TB. Skeletal tuberculosis most often involves the spine, followed by tuberculous arthritis in weight-bearing joints and extraspinal tuberculous osteomyelitis.. Infection begins in the anteroinferior aspect of the vertebral body with destruction of the intervertebral disc and adjacent vertebrae .The resulting anterior wedging and angulation of adjacent vertebral bodies with disc space obliteration are responsible for the palpable spinal prominence (gibbus) and a classic radiographic appearance. Paraspinal and psoas abscesses can develop, with extensions to the surface or adjacent tissues. Patients present with local pain, constitutional symptoms, or paraplegia secondary to cord compression. (Golden and Vikram, 2005)

Musculoskeletal tuberculosis involves the spine in approximately one-half of patients. The next most common syndrome is tuberculous arthritis, followed in frequency by extraspinal tuberculous osteomyelitis .Spinal TB (Pott's disease) most often affects the lumbar and lower thoracic region; upper thoracic and cervical disease is less common but potentially more disabling .Tuberculous abscess, a complication of spinal TB, is frequently bilateral. Tuberculous arthritis tends to occur in the weight-bearing joints, the hip and the knee, and is usually monoarticular. However, multifocal lesions are reported in 10 to 15 percent of cases in developing countries

3.2.5 Central Nervous System Tuberculosis

Central nervous system tuberculosis includes tuberculous meningitis (the most common presentation), intracranial tuberculomas, and spinal tuberculous arachnoiditis. One of the most serious extrapulmonary forms of tuberculosis is tuberculous meningitis. It occurs most often in children, but can also occur in adults, especially those with HIV. It results from hematogenous spread or the rupture of a tubercle into the subarachnoid space. The disease may present with nonspecific symptoms including headache and mental changes or acutely with confusion, lethargy, altered senses and neck rigidity. Paresis of cranial nerves and hydrocephalus is common. Tuberculous meningitis can be seen near the basal part of the brain in a CT scan. If unrecognized, tuberculous meningitis will be fatal. (Golden and Vikram ,2005)

3.2.6 Gastrointestinal Tuberculosis

Tuberculous enteritis can result from swallowing of infected sputum, ingestion of contaminated food, hematogenous spread, and direct extension from adjacent organs. The intestinal lesions can be ulcerative (most common), hypertrophic, or ulcero-hypertrophic. Symptoms include abdominal pain, diarrhea, weight loss, and fever. Melena, rectal bleeding, and abdominal tenderness also can be present. A mass in the right lower quadrant is palpable in 25 to 50 percent of patients. (Golden and Vikram, 2005)

3.2.7 Tuberculosis Peritonitis

Tuberculous peritonitis results from reactivation of latent foci in the peritoneum. Patients present with the insidious onset of ascites, abdominal pain, and fever. The risk of tuberculous peritoninis is greater in patients with HIV infection or cirrhosis and im those undergoing continuous ambulatory peritoneal dialysis.

3.2.8 Miliary Tuberculosis

The term miliary tuberculosis refers to any progressive, disseminated form of tuberculosis; the disease can occur during primary dissemination or after years of untreated tuberculosis. Miliary disease is seen in 10% of patients who have AIDS and pulmonary tuberculosis, and in 38% of those who have AIDS and extrapulmonary tuberculosis. Miliary or disseminated tuberculosis is due to the hematogenous spread of tubercle bacilli. In children it is usually due to a recent infection or due to the reactivation of old, disseminated foci. The lesions are small, yellowish circular granulomas resembling millet seeds. Symptoms are nonspecific and depend upon the primary site of infection.Examination of the sputum, bronchoalveolar lavage, gastric washing, CSF, blood culture, or biopsies of liver and bone marrow may be necessary for diagnosis. (Golden and Vikram , 2005)

3.2.9 Tuberculous Pericarditis

Pericardial tuberculosis may result from direct progression of a primary focus within the pericardium, reactivation of a latent focus or rupture of an adjacent lymph node.Pericardial involvement in tuberculosis may result in acute pericarditis, In India, TB accounts for nearly two-thirds of the cases of constrictive pericarditis. TB has been reported to be the cause of acute pericarditis in four per cent of patients in the developed world and 60 to 80 per cent of the patients in the developing world. TB pericarditis has been estimated to occur in one to eight per cent patients with pulmonary tuberculosis. In industrialized countries TB pericarditis is not so common except in patients with HIV infection and AIDS. Pericardial tuberculosis may result from direct progression of a primary focus within the pericardium, reactivation of a latent focus or rupture of an adjacent lymph node. (Sharma and Mohan, 2004)

3.2.10 TNF-alpha Inhibitor-Associated Tuberculosis

Two recent reports describe several cases of active tuberculosis occurring after treatment with the TNF-alpha inhibitors infliximab(Remicade) and etanercept(Enbrel),

mainly in patients with rheumatoid arthritis or Crohn's disease.Tuberculosis was diagnosed sooner after initiation of infliximab than after initiation of etanercept (median of 12 weeks versus 12 months) TNF-alpha inhibitor-associated tuberculosis accounted for 52-57 % of cases. As a result, it is now recommended that patients be screened for latent tuberculosis infection or active disease before initiation of therapy with a TNF-alpha inhibitor.

3.3 LABORATORY DIAGNOSIS

Microbiology laboratory contributes to the diagnosis and management of TB (Rattan, 2001) in:

1. Detection and isolation of Mycobacteria
2. Identification of species of the isolate
3. Antibiotic susceptibility testing of the isolate

Microscopical Examination

The detection of acid-fast bacilli in stained smears is the easiest and most rapid procedure for evaluating a clinical specimen. A recommended method for examining the smears is to make three longitudinal sweeps of the stained area, parallel to the length of the slide. A report from the laboratory should provide an estimate of number of acid-fast bacilli detected.Microscopical examination is relatively quick, easy and inexpensive and must be performed on cases suspected of having TB. Smear microscopy is also used to monitor treatment progress and control program outcome.

Culture

Cultural techniques are more sensitive than microscopy and may detect as few as 10-100 organisms per ml of specimen. Lowenstein- Jensen medium, a solid egg based medium containing glycerol is widely used for the isolation of *M.tuberculosis* and similar media containing pyruvic acid in place of glycerol are used for the isolation of *M.bovis.* Liquid media are used in the radiometric methods. Biphasic systems

containing broth and slides coated with solid agar based media are commercially available. These permits bacterial growth to be detected more rapidly than by conventional methods but not as rapidly as radiometric techniques (Collee et al, 1999).

Inoculated culture media are usually incubated for at least 8 weeks and inspected weekly for growth. Most strains of the *M.tuberculosis* complex produce visible within 4 weeks, but growth may be delayed if the patients has received antituberculosis drugs. Culture should be incubated at 35ºC. Colonies appearing on the medium are shown to be mycobacteria by means of Ziehl-Neelsen staining and are usually identified by simple cultural and biochemical tests.Thus, members of the *M.tuberculosis* complex are clearly identifiable by their slow growth rate, lack of pigment, failure to grow at 25ºC and sensitivity to p-nitrobenzoic.

3.4 GLOBAL SCENARIO

The number of reported cases of tuberculosis in the United States began to increase in the late 1980's and early 1990's due to a number of factors including HIV infection, emergence of bacterial strains resistant to antibiotic drugs and social factors like poverty, immigration from countries with a high prevalence and drug abuse. Tuberculosis is uncommon in young adults of European descent while infection is relatively high among elderly Caucasians. Tuberculosis infection is prominent among young adults who are infected with HIV or are part of disadvantaged populations.

Murray et al, 1990 estimated a case rate of 229 per 1,00,000 population death in Sub Saharan Africa due to tuberculosis. Where as, Kochi (1991) estimated a case rate of 272 per 1,00,000 population, in African region.Similarly,Murray et al, (1990) estimated that there were 2.5 million deaths from all forms of tuberculosis in developing countries . Kochi (1991) estimated that worldwide, tuberculosis caused 2.9 million deaths in 1990. Both estimate showed tuberculosis to be the largest cause of death from a single pathogen in world, out of which, 1.7 to 1.8 million deaths occurring in Asia only.

It is estimated that 1.7 billion individuals are infected with *M.tuberculosis*. Every year 8 million people get infected. 95 % of the cases are in developing countries and approximately 3 million patients die each year. South Asian Association for Regional Cooperation (SAARC) region bears 22 % of the global population and 29 % of the global burden of tuberculosis. It is estimated that SAARC region with population of 23 million and it gets 201 tuberculosis cases per 100,000 populations of which 90/100.000 is smear positive tuberculosis.

The largest annual numbers of cases are from South-East Asia, accounting for almost half of the total cases in the world. However, the incidence rate is estimated to be highest in Africa and lowest in industrialized countries. It was reported that nearly 95% of tuberculosis death were in developing countries **(**Rieder et al, 1998**).**

The World Health Organization (WHO) estimates that one-third of the world's population is infected with *Mycobacterium tuberculosis*, resulting in an estimated 8 million new cases of tuberculosis and nearly 2 million deaths each year. Approximately 10 million people are estimated to be coinfected with *M tuberculosis* and HIV, and over 90% of these dually infected individuals reside in developing nations.

In some areas of sub-Saharan Africa, the rates of co-infection exceed 1,000 per 100,000 population .Worldwide; tuberculosis is the most common cause of death among patients with AIDS, killing 1 of every 3 patients. After decades of steady decline, tuberculosis cases increased in 1986 in the United States. Between 1985 and 1990, tuberculosis cases were increased by 20%, resulting in 28,040 excess cases of tuberculosis. The U.S. Centers for Disease Control and Prevention (CDC) estimates that AIDS-related tuberculosis accounted for a minimum of 30% of these excess cases. Fortunately, tuberculosis cases have been declining in the United States since 1992. Between 1992 and 1999, tuberculosis cases were decreased by nearly 34%. HIV-related cases have also declined. From 1993 to 1998, the proportion of HIV-related cases has decreased from 29% to 20% in the 25- to 44-year-old age group.

The decline in HIV-related tuberculosis in the United States and other industrialized countries has paralleled an overall decline in tuberculosis cases. Whether or not the use of effective antiretroviral therapy (ART) has hastened this decline is not clear. Two cohort studies have described the frequency of tuberculosis during the current era of treatment.In the Frankfurt AIDS cohort study of 1,000 HIV-infected homosexual men with CD4 T-lymphocyte counts <200 cells/mm^3, the overall incidence of AIDS-defining conditions decreased by >70% between 1992 and 1996. However, the rate of tuberculosis, although low, remained stable over the study period. In contrast, the EuroSIDA cohort study of 7,000 HIV-infected patients reported dramatic declines in the rate of tuberculosis and disseminated *Mycobacterium avium* complex (MAC) from the period before 1993 to the period after 1997, coinciding with the introduction of potent ART. Studies are needed to assess the impact of antiretroviral therapy on the rates of tuberculosis in different populations (Gooze et al, 2002**).**

According to a study, in India, the number of Tuberculosis patients is increasing at the rate of 1.5 million per year, and a quarter of these are sputum positive.Thus,about 40% of all Indians are infected with *Mycobacterium tuberculosis.*

Despite an overall decrease in numbers of tuberculosis (TB) cases in the US, the proportion of extrapulmonary TB cases has increased. The study objective was to determine the most important predictors of all-cause mortality among patients with extrapulmonary TB. A retrospective chart review of adult extrapulmonary TB cases registered between 01/1995 and 12/2001 at Grady Memorial Hospital (a 1,000 bed, public inner-city hospital in Atlanta) was performed. Risk factors for death within 12 months after diagnosis of extrapulmonary TB were identified in multivariate analysis using log-binomial regression model. A total of 212 cases of extrapulmonary TB were identified; 100 (47%) were HIV-infected. The majorities of patients were male (68%) and African-American (84%); mean age was 40 years. The most common sites of extrapulmonary TB were: lymph node (26%), pleural (21%), disseminated (20%), and central nervous system (CNS) or meningeal (16%). All-cause mortality rate in patients with extrapulmonary TB was 15% (21% among HIV-seropositive and 9% among HIV-

uninfected patients, p = 0.02). In multivariate analysis, independent predictors of mortality included disseminated disease (PR = 4.66, 95% CI 1.93-11.24) and CNS/meningeal extrapulmonary TB (PR = 4.29, 95% CI 1.78-10.33), controlling for HIV infection. Extrapulmonary TB continues to be a persistent problem in the inner city and is associated with high mortality rates, especially among HIV-infected. Disseminated disease and the presence of CNS/meningeal TB are associated with poor prognosis. (Golden and Vikram, 2005)

Accurate data on the incidence of disease is difficult to find except in countries where good national data is available. There is wide variation between series depending on the region studied and the ethnic groups. In the UK the White population present with an extrapulmonary site in 15% of cases but those of Bangladeshi, Pakistani or Indian ethnic origin present with an extrapulmonarysite in up to 50% of cases. Patients with HIV positive disease present with more than 50% extrapulmonary disease In order to know extrapulmonary tuberculosis the results of all samples submitted for culture of mycobacteria to the Microbiology Department, Fundacion Jimenez Diaz, from 1980 to 1993 were analyzed. During this period 290 cases of extrapulmonary cases were diagnosed, 101 from 1980 to 1985 and 189 from 1986 to 1993. The most common site of infection before 1985 was in genitourinary tract (42.6%); in contrast, from 1986 onwards the more common sites of infection were pleural (22.8%), genitourinary tract and lymphatic glands (22.2% in both sites). When EPT was compared in the two periods of time the observations made were a relative decrease of genitourinary infections (p = 0.00004) and increase in disseminated (p = 0.015) and pleural tuberculosis (p = 0.011) from 1986 compared with previous years. From 1986 a greater proportion of disseminated form was observed (p < 0.0001) in positive-HIV patients and of genitourinary (p = 0.011) and pleural (p = 0.076) forms in negative HIV-patients. In conclusion, extrapulmonary tuberculosis has increased in our environment during the period 1980-1993, and this increase is not attributable only to positive-HIV patients. The distribution of clinical forms of this disease was different in the two studied periods and among positive and negative HIV patients. (Golden and Vikram, 2005)

In USA, of all newly detected tuberculosis cases, 5% are those of tuberculosis cervical lymphadenitis.90% tuberculosis cervical lymphadenitis is unilateral and 90% involve one node group. Any of the cervical nodes may be involved, but the most common are the nodes of the deep jugular chain, followed by those of the sub-mandibular region and then of the posterior triangle. Lymph nodes other than in the cervical region are less commonly involved in tuberculosis and account for 35% of tuberculosis adenitis (Baskota et al, 2001**).**

Tuberculosis continues to be a major health problem in India. Nearly one third of global tuberculosis burden is contributed by India alone. Renal tuberculosis is the most common site of extra-pulmonary tuberculosis. This infection can result in caseation and destruction of renal mass and healing can lead to strictures, obstruction and infection causing renal functional loss and failure. 63 patients of renal tuberculosis were seen from July 1998 to August 2000 in the department of nephrology in a tertiary care hospital in the valley of Kashmir. Detailed history, thorough clinical examination, urine examination and tubercle bacillus cultures, imaging studies and cystoscopy and histological examinations were conducted to arrive at the diagnosis. 27 (43%) of our patients had urine cultures positive for tubercle bacilli. All except 8 intravenous urograms (IVU's) were abnormal and revealed hydronephrosis in 10(20%), hydro-ureter in 6(12%), calcification in 7(14%), pyelonephritic changes in 5(9%), non-visualized kidneys in 4(8%), vesicu-ureteric reflux and nephrolithiasis in 3(6%) each and distortion, cavitation and scarring in 21(42%), thimble bladder in 3(6%) and renal abscess in 3(6%) of our patients. 7(11%) of the patients had caseating granulomas on histology of bladder mucosa.Renal tuberculosis should not be a difficult diagnosis to make in patients with urinary symptoms plus abnormal urine analysis that should be screened for tuberculosis after routine urine cultures have been found to be negative. The urine culture positivity rate for tuberculosis has been less in our patients as against western reports, but radiological features in our patients were more prominent and advanced. Late presentation and advanced disease could be an explanation for the lower culture positivity and more prominent radiological abnormalities**.** (Najar et al, 2005)

Tuberculosis of the knee joint can occur in any age group and it is the common site for osteoarticular tuberculosis. It accounts for nearly 10% of all skeletal lesions. The initial lesion is quite frequently synovial in type, but may also start in tie subchondral bone or in juxtra-articular area. The synovial lesion may remain for many months as tubercular synovitis . In cases where the disease starts as an osseous lesion, there may be tubercular abscess in the sub chondral bone, epiphyseal bones or in the metaphyseal region leading to various degree of destruction of the bone. It was found from a study thatBones and Joints are next common site of tuberculosis in the body after lungs and Lymphnodes. Bone and Joint tuberculosis is always secondary to active disease in other parts usually the lymph glands-a point that must never be forgotten in the treatment. The spine is the commonest site of bone and joint tuberculosis, constituting about 50% of the total number of cases. The hip, the knee and the elbow are the next in order of frequency.

An estimated 10-15 million Americans are currently infected with dormant tuberculosis (TB), and more than 17,000 cases of active disease were reported to the CDC in 1999. TB rates in Los Angeles are high compared to most U.S. cities because of the large population of people in high-risk categories for harboring the disease, than 17,000 cases of active disease were reported to the CDC in 1999. TB rates in Los Angeles are high compared to most U.S. cities because of the large population of people in high-risk categories for harboring the disease.

Isolated epididymo-orchitis is an uncommon presentation of Tuberculosis. It was reported that a case of left sided epididymo-orchitis and scrotal involvement due to tuberculosis in a young male patient. The diagnosis was suspected on clinical examination of scrotum and confirmed by Fine Needle aspiration cytology (FNAC) of scrotum and testis. Patient improved after taking anti tubercular treatment.

Cutaneous tuberculosis (TB) is essentially an invasion of the skin by Mycobacterium tuberculosis, the same bacteria that cause TB of the lungs (pulmonary TB). Cutaneous TB is a relatively uncommon form of extra pulmonary TB (TB infection of other organs

and tissues). Even in countries such as India and China where TB still commonly occurs, Cutaneous outbreaks are rare (<0.1%) (Golden and Vikram, 2005).

Tuberculosis has been a serious public health problem for a long time. In the 1800s, the disease caused more than 30% of all deaths in Europe. With the advent of antituberculosis antibiotics in the late 1940s, the battle against tuberculosis seemed to be won. However—because of factors such as inadequate public health resources, reduced immune response due to AIDS, the development of drug resistance, and extreme poverty in many parts of the world—tuberculosis continues to be a deadly disease worldwide, as the following statistics from 2006 show:

- There were 9.2 million new cases of symptomatic tuberculosis and 3 million deaths from the disease. The number of new cases varies widely by country, age, race, sex, and socioeconomic status.

- Of the 9.2 million new cases, about 3 million occurred in Africa, 3 million in Southeast Asia, and about 2 million in the Western Pacific region.
- India and China reported the largest total number of new cases, but South Africa had the highest rate of new cases in the world, with 940 new cases per 100,000 people.

In total, about one third of all the people in the world are thought to have a dormant (latent) tuberculosis infection, although only about 5 to 10% of these progress to active tuberculosis.

In the United States, the rate of new cases has decreased 10-fold since 1953 (when national reporting for tuberculosis first began). In 2007, 13,293 cases (about 4.4 cases per 100,000 people) were reported. However, there is a wide range of incidence, from 10.2 per 100,000 people in Washington, DC to 0.4 per 100,000 in Wyoming. Over half of new cases occurred in people born outside the United States in areas where tuberculosis is relatively common (such as Africa, Southeast Asia, or Latin America). In the US, US-born blacks, the homeless, people in jails and prisons, and other

disenfranchised minorities are much more likely to be infected. The rate of new cases among these high-risk groups is likely to be almost as high as that in areas of the world where tuberculosis is relatively common.

In the United States and other developed countries, tuberculosis has traditionally been more common among older people. In developing countries, it is a disease of young adults. More cases have occurred among older people because they were more likely to have acquired the infection in an era when tuberculosis was more common. Moreover, the body's immune system weakens as people age, allowing inactive (dormant) bacteria to become reactivated. However, the incidence of tuberculosis among older people is declining because fewer people in each generation entering old age have inactive (latent) infection. Because the number of new cases among people born outside the United States is increasing, the age profile of tuberculosis infection in the US is getting younger **(**Edward and Nardell, 2008**).**

Pulmonary TB has been variously described as consumption and phthisis, both terms indicating the severe wasting and the coughing of blood associated with later stages of the disease. Pott's disease or spinal tuberculosis, marked by spinal deformity and other bone defects, was named after an 18th-century English physician, but Hippocrates thought there was a great similarity between this bone disease and pulmonary tuberculosis and possibly a common origin. Scrofula, or cervical lymphadenitis, was a common disease in the middle ages that presented with swelling of lymph nodes in the neck. It was also called "The King's Evil" because of the myth that it could be cured by the touch of a reigning monarch. Villemin (mentioned above) showed in the 1860s that scrofula and pulmonary TB had an identical cause. Tuberculosis also can develop in the central nervous system, in which case meningitis is the predominant form of the disease, and also in the urogenital tract, the digestive system, and cutaneously in the form named lupus vulgaris. The incidence of these various extrapulmonary forms of tuberculosis varies from country to country, such that on the average between 1964 and 1989, 20% of the 20,000 new cases of TB in the United States were extrapulmonary while 5 to 10% of the approximately seven million new cases each year in the developing countries

were extrapulmonary . This distribution also can be affected by origin of the individuals within a country. In one study of TB patients in England, 20% of patients of European origin had extrapulmonary TB, of which lymph node, bone and joint, and genitourinary involvement accounted for almost 90%. Of patients whose origin was on the Indian subcontinent, 45% had extrapulmonary tuberculosis, and 60% of these sites of infection were in lymph nodes and in bones and joints. Autopsies of deceased human immunodeficiency virus (HIV)-negative TB patients in another study in New York City showed that 68% had extrapulmonary TB whose lesions were widely and randomly distributed throughout the body with no apparent predilection for a limited number of sites as noted in the English study**(** Edward and Nardell, 2008**).**Accurate data on the incidence of disease is difficult to find except in countries where good national data is available. There is wide variation between series depending on the region studied and the ethnic groups. In the UK the White population present with an extrapulmonary site in 15% of cases but those of Bangladeshi, Pakistani or Indian ethnic origin present with an extrapulmonary site in up to 50% of cases. Patients with HIV positive disease present with more than 50% extrapulmonary disease (Kumar et al, 1998).

3.4 NEPALESE SCENARIO

Tuberculosis is an immense problem in Nepal, causing great suffering and death. Recent estimates suggest that about 45%of the total populations are infected with tubercle bacilli and each year about 50,000 people develop tuberculosis, over 20,000 of who have infectious sputum smear positive disease. According to the recent estimates, about 80,000 to 90,000 people in Nepal have active tuberculosis and annual death is about 8,000 to 11,000(NTP, 2004).

A survey was carried out by National Tuberculosis Centre (NTC) of Nepal in cooperation with Japanese expert team in 2000 which showed the annual risk is 1.8% and 45% of total population are infected. Likewise, 80-90 thousand people have active tuberculosis, 44 thousand new cases occur annually and 20 thousand new positive

(infection) cases detected per year and 3 deaths occur in every 2 hours by tuberculosis (Baskota, 2004).

In Nepal five years ago, it was estimated that about 16,000 people were dying from tuberculosis every year. Current estimates have shown a profound decline in the number of deaths to about 8,000 per year. This decline in death rate is due to improvements in program performances to control tuberculosis. (NTP, 2002).

Majority of cases are in rural areas where more than 90% of population resides. The annual rate of infection is estimated at about 3%.In hilly area it is about 1.5%, in Terai area it is about 2.5%, in urban area it is about 4% and in mountain area is less than 1% .Although numbers of pulmonary cases are more than extrapulmonary tuberculosis,42.72% in Dhankuta, 38.89% in Terhathum and 25.45% in Taplejung were the extrapulmonary infection. In one study it was found that out of 349 cases diagnosed histopathologically, lymphnode tuberculosis was found to be the commonest (66.3%) Humans are very susceptible to the Tuberculosis infection, but are remarkably resistant to the Tuberculosis disease; which is dependent largely on the state of the hosts immune system. Of all the Mycobacterial species *Mycobacterium tuberculosis* remains the most common cause of pulmonary tuberculosis and remains the most virulent of all the Mycobacterial species.

The disease, as now well known, is highly contagious. Although the disease involves all susceptible individuals, the incidence is higher amongst disadvantaged minorities. Industrialization; increased crowded housing and nutritional deprivation have influenced the spread. With the emergence of HIV and resultant immunocompromise, TB has emerged as a major killer not only in the third world countries but is also resurging in the western world. According to World Health Organization (WHO) reports, each year an estimated eight million new cases of Tuberculosis occur, leading to three million deaths; and almost a third of the world's population is infected by the causative organisms, *Mycobacterium tuberculosis*. (WHO, 2004)

According to a study, in India, the number of Tuberculosis patients is increasing at the rate of 1.5 million per year, and a quarter of these are sputum positive.Thus, about 40%

of all Indians are infected with *Mycobacterium tuberculosis* With the emergence of multiple drug resistant strains due to poorly administered therapeutic measures and patient non-compliance, *Mycobacterium tuberculosis* is challenging its containment, on the basis of empirical treatment alone

CHAPTER-IV

4. MATERIALS AND METHODS

A list of materials, chemicals, equipments, media and reagents required for the study is presented in Appendix I.

4.1 Site of the study

This study was conducted in Tribhuvan University teaching Hospital (TUTH) Kathmandu, Nepal from June 2006 to November 2006 .Standard method for Acid-Fast Bacilli (AFB) staining and culture was followed.

4.2 Specimen collection and Transport

Samples from the sterile sites such as endometrium, peritoneal fluid, pleural fluid, peritoneal fluid, synovial fluid, lymph node aspirate, and bone marrow were collected by physician in a sterile glass container.After receiving, samples were centrifuged at 3000 rpm for 15 minutes, and then supernatant was discarded and deposit was inoculated in 2 tubes of 3% Ogawa medium. Lymph node, endometrial tissue or aspirate and other surgically resected tissue were cut into small piece with sterile scalpel or scissors. The specimen was homogenized in a sterile scalpel or scissors. The specimen was homogenized in a sterile mortar using 0.5 to 1 ml sterile saline and mixed well and centrifuged at 3000 rpm for 15 minutes, then the supernatant was discarded and 0.1ml of sediment was inoculated in each 2% Ogawa medium in duplicate.

4.2.1. Urine Specimens

Early-morning voided urine specimens in sterile containers was submitted daily for at least 3 days. Twenty-four-hour urine specimens were undesirable because of excessive dilution, higher contamination, and difficulty in concentrating.

4.2.2. Fecal specimen

Feces were collected in a clean, dry, wax-free container without preservative or diluent. Contamination with urine should be avoided.

4.2.3. Tissue and Body Fluid Specimens

For recovery of mycobacteria from CSF, at least 10ml of CSF was recommended. Similarly, as much as possible(10 to 15 ml minimum)of other body fluids such as pleural, peritoneal, and pericardial fluids, was collected in a sterile container or syringe with a Leur tip cap.

4.2.4. Blood Specimens

Blood for culture of mycobacteria was collected in a manner as for routine blood cultures.

4.2.5. Wounds, skin lesions, and Aspirates

If attempting to culture a skin lesion or wound, an aspirate is the best type of specimen to collect. The skin was cleansed with alcohol before aspiration of the material into a syringe. If the volume is insufficient for aspiration, pus and exudates may be obtained on a swab and then placed in transport medium, such as Amie's or Stuart's transport medium(dry swabs are unacceptable). However, a negative culture of a specimen obtained on a swab is not considered reliable, and this should be noted in the culture report.

4.3 Specimen processing

4.3.1 Specimen requiring decontamination

The contaminated specimens were processed by commonly used digestion-decontamination methods are the sodium hydroxide (NaOH) method (Petroff's

Method). In NaOH digestion method, the samples was mixed with 4ml of NaOH and vortexed for 30minutes.Following mixing, the container was allowed to stand for 15 minutes before opening, to prevent the dispersion of fine aerosols generated during mixing. All of these procedures were carried out in Biological safety cabinet (BSC). Following digestion and decontamination, specimens were concentrated by centrifugation at around 3000rpm.

4.3.2. Specimens Not Requiring Decontamination

Tissues or body fluids collected aseptically usually do not require the digestion and decontamination methods used with contaminated specimens. Cerebrospinal fluid was handled aseptically and centrifuged for 30 minutes at 3000rpm to concentrate the bacteria. The supernatant was decanted, and the sediment was vortexed thoroughly before preparing the smear and inoculating media. If insufficient quantity of spinal fluid is received, the specimen should be used directly for smear and culture because recovery of acid fast bacilli from CSF is difficult. Pleural fluid was collected in sterile anticoagulant containing container. If the fluid becomes clotted, it should be liquefied with an equal volume of sputolysin and vigorously mixed.

4.4. Sample processing

1. Standard Ziehl-Neelsen Technique for AFB Staining.
2. Culture in Ogawa Medium.
3. Biochemical tests for identification of the Mycobacterium isolates.

4.4.1 Standard Ziehl-Neelsen Technique for AFB Staining.

ZN Staining was performed according to WHO guideline. The method is:

4.4.1.1Smear Preparation:

Smear was prepared on a new, clean, and unscratched slide at one end with the patient’s number. An appropriate portion of sample was transferred to the slide with the help of broken end of a wooden stick. The sample was smeared on the slide over an area of

approximately 2.0 by 1.0 cm and made it thin enough to be able to read through it. The smear was allowed to air dry for 15 minutes without heating. Thereafter, the smear was heat fixed passing the slide through a flame 3 to 4 times with the smear uppermost and allowed to cool before staining. These steps were performed inside a safety cabinet.

4.4.1.2 Staining:

1% Carbol fuchsin was poured to cover the entire surface of the slides. The slides were heated underneath until vapour start rising. The slides were allowed to stand for 5 minutes. The slides were then rinsed with tap water and excess water was drained off. The slides were decolorized with 3% acid alcohol for 3 minutes. The slides were rinsed thoroughly with tap water and excess water was drained off. The slides were flooded with 0.05%malachite green and let to stand for 1 minute. The slides were gently rinsed with tap water and excess water was rinsed off from the slides. The slides were allowed to air dry and then examined under microscope in 100x oil immersion.

Expected Results

Mycobacterium tuberculosis will appear as red, beaded appearance where as non mycobacteria will appear blue. (Recording and Reporting was done according to WHO, 1998) given in Appendix IV.

4.4.2 Culture in 2% ogawa medium.

The specimens were cultured in 2 % Ogawa medium and detail procedure for the culture is given in Appendix II**.**

4.4.3 Biochemical tests for identification of Mycobacterium isolates

4.4.3.1 Niacin Test for identification of *M.tuberculosis*

Procedure

One ml of sterile water was added to the culture slant. If growth is confluent, puncture the medium with a Pasteur pipette to allow contact of the water with the medium.

The tube was horizontally placed so the fluid covers the entire surface of the medium. It was allowed to stand for 30 minutes for the extraction of niacin. The extraction time may be longer if the culture has few colonies. The slant was raised upright for 5 minutes to allow the fluid to drain to the bottom. Then 0.5ml of the fluid extract was removeto a clean screw cap tube. 0.5ml of the 4% aniline solution and 0.5ml of 10% Cyanogen bromide was added sequentially. The tubes were closed and observed for the formation of a yellow colour (=positive result) within 5 minutes. The yellow colour appears as a ring at the interface of the two reagents, or if the tube is shaken, as a yellow column of liquid. 2-3ml of 4% NaOH solution was added to each tube and discarded.

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4.4.3.2 Nitrate Reduction Test

M.tuberculosis is one of the strongest reducers of nitrate among the mycobacteria, which allows for this test to be used in combination with the niacin test in differentiating *M.tuberculosis* from the other mycobacteria. Culture to be testes for nitrate reduction should be four weeks old and have abundant growth .

Procedure

Initially 0.2ml of sterile saline was added to a screw cap tube.Then two loopfuls or spadefuls of a 4 week old culture in the saline was emulsified using sterile loop or spade. Then 2ml of $NaNO_3$ was added to it.It was shaked well and incubated upright in

a 37 °C water bath for 3 hours and the tubes were removed. After that1drop diluted HCl was added and shaked well. Then 2 drops 0.2% sulphanilamide was added to it. Finally 2 drops 0.1% N-napthylethylene-diaminewas added. Then It was examined immediately for development of pink colour to red colour and compared to colour standard and the results were interpretated as given in Appendix VI.

4.4.3.3 Catalase Test

Procedure

First of all with a sterile pipette, 0.5ml of 0.067M phosphate buffer (pH 7.0) was aseptically added to 16x125mm screw cap tubes.Then several loopfulls of test cultures was suspended in the buffer solution using sterile loops.The tubes containing the emulsified cultures were placed in previously heated water bath at 68ºC for 20 minutes. (Time and temperature are critical).The tubes were removed from heat and allowed to cool at room temperature.Then 0.5ml of freshly prepared Tween-peroxide mixture was added to each tube and caps were replaced loosely. The formation of bubbles appearing on the surface of the liquid was observed. (Do not shake the tubes because Tween 80 also may form bubbles when shaken, resulting in false positive results).Hold negative tubes for 20 minutes before discarding.

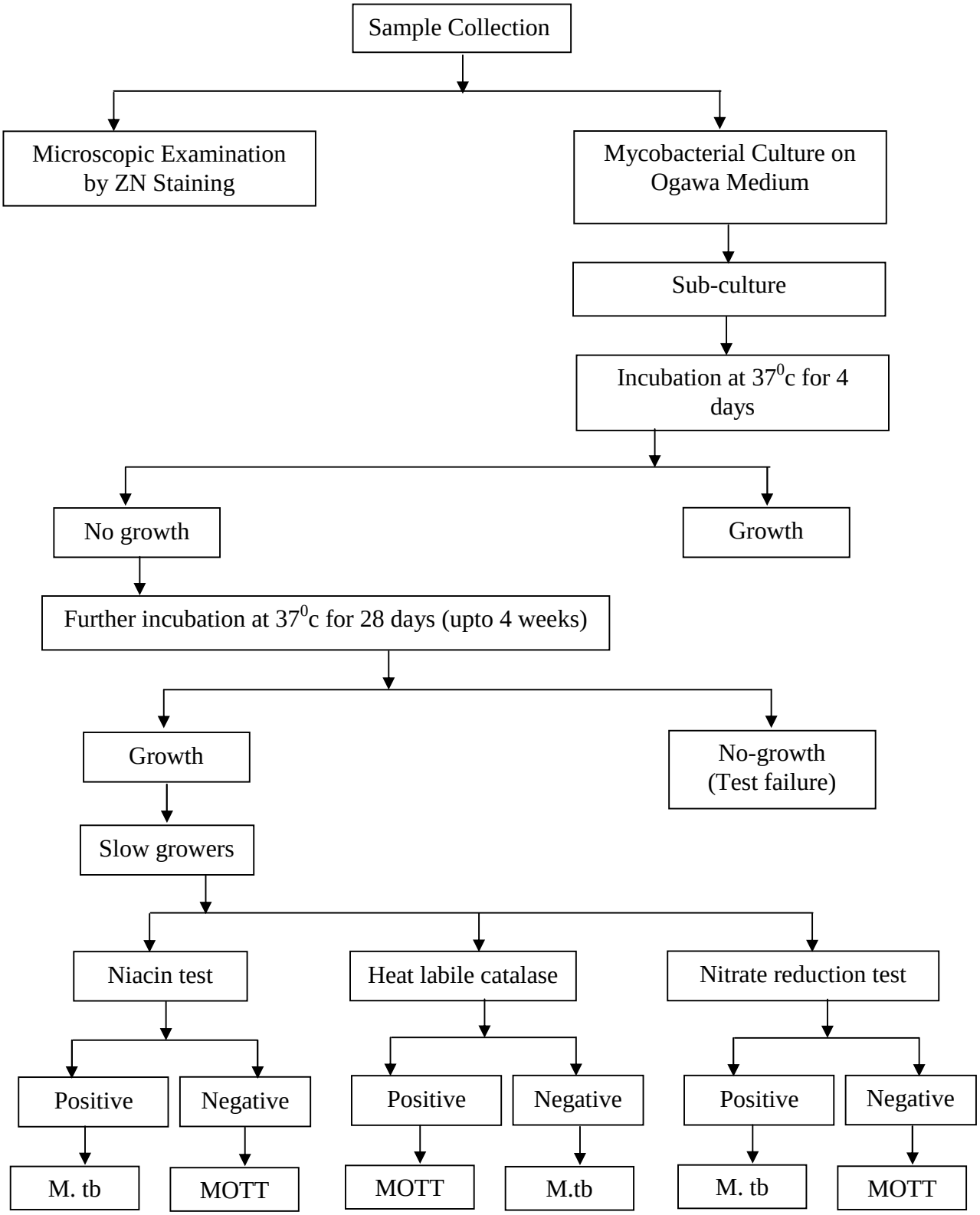

Figure : Research Design

CHAPTER-V

5. RESULTS

A total of 150 samples were collected from the patients visited to the Tribhuvan University Teaching Hospital (TUTH) suspected of Extrapulmonary Tuberculosis during the study period. A varieties of Extrapulmonary specimens were brought to the Mycobacteriology laboratory, that including Urine, Endometrial biopsy, Pleural fluid, peritoneal fluid, Cerebrospinal fluid, Blood, Ascitic fluid and Pus.

Table 1 Age and gender wise distribution of the patients visiting TUTH.

Age group	**Male**		**Female**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
10-20	3	7.50	9	8.18	12	8.00
20-30	15	37.50	67	60.91	82	54.67
30-40	10	25.00	12	10.91	22	14.67
40-50	4	10.00	9	8.18	13	8.67
50-60	3	7.50	6	5.45	9	6.00
60-70	3	7.50	6	5.45	9	6.00
70-80	1	2.50	1	0.91	2	1.33
>80	1	2.50	0	0	1	0.66
Total	**40**		**110**		**150**	

The result showed that of the total 150 samples, 40 were from male patients and 110 were from female patients.The highest percentage of suspected patients 82 (54.67%) was found in the age group 20-30 followed by 22 in age group 30-40 years. The highest number of female patients 67 (60.91%) were found in the age group 20-30 years followed by 12 (10.91%) in 30-40 years. Similarly, the highest number of male patients 15 (37.50%) were found in the age group 20-30 years followed by 10 (25.00%) in 30-40 years. (Table 1)

Table 2 Distribution of samples according to the origin of sample

S.N	Origin of sample	No. of specimen	Percentage
1.	Endometrial biopsy	65	43.33
2.	Urine	30	20.00
3.	Pleural fluid	12	8.00
4.	Ascitic fluid	9	6.00
5.	CSF	3	2.00
6.	Pus	15	10.00
7.	Tissue	10	6.67
8.	Peritoneal fluid	4	2.67
9.	Blood	2	1.33
	Total	**150**	**100.00**

The result showed that highest number of samples i.e. 65 (43.33%) was endometrial biopsy followed by urine which was 30 (20%). (Table 2)

Table 3 Age and gender wise distribution of positive cases

Age group	Male		Female		Total	
	No. of positive cases	%	No. of positive cases	%	No. of positive cases	%
10-20	3	16.66	2	11.11	5	27.77
20-30	2	11.11	7	38.88	9	49.99
30-40	0	0	0	0	0	0
40-50	0	0	1	5.56	1	5.56
60-70	1	5.56	1	5.56	2	11.12
70-80	1	5.56	0	0	1	5.56
>80	0	0	0	0	0	0
Total	**7**	**38.89**	**11**	**61.11**	**18**	**100**

The highest percentage of positive cases 9 (49.99%) was found in the age group 20-30 followed by 5 (27.77%) in 10-20 years. The highest number of positive cases in female patients 7 (38.88%) was found in the age group 20-30 years followed by 2 (11.11%) in 10-20 years. Similarly, the highest number of male patients 3 (16.66%) were found in the age group 10-20 years followed by 2 (11.11%) in 20-30 years. (Table 3)

Table 4 Distribution of positive cases according to the origin of sample

S.N.	Origin of sample	No. of sample	Direct smear		Culture	
			No. of positive cases	%	No. of positive cases	%
1.	Endometrial biopsy	65	0	0	0	0
2.	Urine	30	2	6.67	4	13.33
3.	Pleural fluid	12	1	8.33	3	25.00
4.	Ascitic fluid	9	0	0	0	0
5.	CSF	3	0	0	0	0
6.	Pus	15	7	46.67	6	40.00
7.	Tissue	10	0	0	0	0
8.	Peritoneal fluid	4	0	0	0	0
9.	Blood	2	0	0	0	0
	Total	**150**	**10**		**13**	

Out of 150 samples, highest number of positive cases in direct smear examination was obtained from pus sample (7; 46.67%) followed by pleural fluid (1; 8.33%) and urine samples (2; 6.67%). Similarly, in culture highest number of positive cases was obtained from pus sample (6; 40.00%) followed by pleural fluid (3; 25.00%) and urine samples (4; 13.33%). (Table 4)

All the tested isolates were Niacin and Nitrate reduction test positive and negative for heat labile catalase test. Thus, the isolates were confirmed to be *Mycobacterium tuberculosis*. (Table 5)

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Tuberculosis is an immense problem of the world, causing great suffering and death. It remains as the most significant cause of morbidity and mortality due to a single infectious agent in the world. In Nepal, each year 40,000 people develop active disease. Despite of the implementation of the DOTS strategy by national Tuberculosis Programme (NTP) 5,000 to 7,000 people still die from TB each year (NTC, 2007)

In recent years, the decrease is reported in tuberculosis which is entirely due to a drop in number of cases of pulmonary disease. There has been little change in the average number of Extra-pulmonary cases reported. A study on extrapulmonary tuberculosis on Myanmar has shown that it differs from pulmonary tuberculosis with regard to sex and race distribution, diagnosing physian's speciality and proportion of cases according to specific anatomic site with regard to the above characteristics as well as age distribution. These epidemiologic differences in tuberculosis of different sites are unexplained. (Forssbohm et al., 2007)

The study is mainly based upon sample collection, sample processing by direct smear examination using ziehl-Neelsen staining procedure and cuture in Ogawa medium that is followed by identification of the culture isolates using different biochemical tests such as Niacin test, Nitrate reduction test, Catalase tests etc. For the identification of isolates, the buff coloured and rough colonies appearing break crumbs or cauliflower (rough, tough, buff colonies), no growth than one week (slow glowers), no pigment production and their inability to grow to grow at 25^{o}c were used as preliminary identification criteria. The confirmatory tests were done by Niacin production , Nitrate reduction and Catalase test to confirm the isolates as *M.tuberculosis.*

The most common types of extrapulmonay TB are, in descending order of frequency, pleural, lymphatic, bone and joint, genitourinary, military disease, meningitis and peritonitis. The classical symptoms of pulmonary TB are well known and fairly easily recognized: persistent cough with or without sputum and/or blood, fever, sweats, chills, anorexia, weight loss, and malaise. Extrapulmonary TB may be associated with many symptoms, often very mild and indolent, such as microscopic hematuria, malaise, or back discomfort.The direct extension of infection into pleural, pericardial or peritoneal spaces can occur, as can seeding of the gastrointestinal tract by swallowing infected secretions.(Brooks et al, 2004)

EPTB is more common in AIDS patients and can be very serious and life threatening. The most common methods of spread is by hematogenous dissemination at the time of primary infection or less commonly from pulmonary or other foci.

EPTB is considered diagnostic criteria in the case definition of Acquired immuno deficiency syndrome (AIDS). In AIDS patients unlike others, concurrent pulmonary and extrapulmonary forms of tuberculosis are common. Studies on EPTB in developed countries is very common, but the studies in the high burden counries like Nepal is lacking, therefore we carryout this study to find out or just to trace out the status of Extrapulmonary tuberculosis in Nepal. But the result that we obtained may not be similar throughout the country as the study just included one hospital of Kathmandu valley within limited time period.

The study was carried out among the patients visiting the Tribhuvan University Teaching Hospital (TUTH), suspected of EPTB from June 2006 to Nov 2006. Though is the EPTB sample flow compared to the pulmonary sample is lower, for every 20 pulmonary sample there is only one or two Extra pulmonary sample per day. Different varieties of extrapulmonary specimen were brought to the Mycobacteriology laboratory. Altogether within the study period, a total of 150 samples were collected and processed in the laboratory.

EPTB refers to TB outside the lungs. Mycobacteria may spread through lymphatic or hematogenous dissemination to any tract or through coughing and swallowing to the Gastrointestinal (GI) tract.Bacteria may remain dormant for years at a particular sites before causing disease. Since EPTB can affect virtually all organs; it has a wide variety of clinical manifestations, which causes difficulty and delay in diagnosis. Obtaining materials for culture confirmation of EPTB is much more difficult than that for pulmonary tuberculosis.

It is established that certain forms of tuberculosis such as intrthoracic lymphatic tuberculosis, have predilection for the young ages where as genitourinary tuberculosis is rarely found in children, such differences may partially be explained by maturation factors and development of the cellular immunity. Furthermore, the manifestation of tuberculosis is linked to time elapsed since infection was acquired, making it difficult to separate the effects of age and time since infection. (Rieder et al, 2007)

EPTB is mostly diagnosed in women and young patients which are also accordance to our study, the predominance of the suspected samples were from the female patients and the positive result also proves the finding. As observed in other studies, female tuberculosis patients were, with exception of those with pleural tuberculosis. Considerably more likely to present with an extrapulmonary manifestation than male patients. The reason for this predominance is not clearly known . The increased likelihood of female with tuberculosis presenting with an extrapulmonary disease manifestation was particularly pronounced among those aged 45-64 years. An explanation for this finding remain elusive but it suggests that endocrine factor might play role (Rieder et al, 1998).

Altogether 150 samples were collected and processed in the laboratory. The study revealed the most predominant specimen to be the endometrial biopsy sample (43.33%) followed by Urine (20%) and least specimen that of Blood (1.33%). Urogenital infection show little evidence of decreasing, whereas 2-3% of patients with Pulmonary tuberculosis exhibit urinary tract involvement, 30-40% of patients with Genitourinary

disease have TB of some other sites. Though the predominant sample was endometrial biopsy sample, the positivity of which was found to be nil in this study, the reason behind this may be to rule out the case of Primary and secondary subfertility and infertility, one of the screening test may be sending the endometrial biopsy sample to mycobacteriology laboratory for tuberculosis, as many studies showed that infertility is also the one of the cause of tuberculosis. Though the female genital tract is the uncommon site for EPTB (Golden and Vikram, 2005), but in this study the predominant suspected specimen was endometrial biopsy sample, it might be because those with clinical manifestations with primary subfertility as well as secondary subfertility, this also falls as an screening test for the EPTB after other routine fertility tests have been found to be negative. (Najar et al, 2005)

The patients present with clinical manifestations of urinary TB including frequency of urination(most common), dysuria, hematuria, and flank pain. So, the urine sample for the suspicion of renal tuberculosis is common.Similarly Renal tuberculosis shouldn't be a difficult diagnosis to make in patient with all these urinary symptoms plus abnormal urine analysis that should be screened for TB after routine urine cultures have been found Negative **(**Forbes et al, 1998)

The test study was conducted on one of the hospital where no specialized examinations regarding tuberculosis was done, so the in and out patients visiting this hospital suspected of having extrapulmonary tuberculosis following their examinations were just screened out, so , the rate of positivity as well as flow of sample were found to be low.

The diagnosis of extrapulmonary tuberculosis is challenging for a number of reasons the lack of adequate sample amounts or volumes; the apportioning of the sample for various diagnostic tests (histology/cytology, biochemical analysis, microbiology, and PCR), resulting in nonuniform distribution of microorganisms; the paucibacillary nature of the specimens; the presence of inhibitors that undermine the performance of nucleic acid amplification-based techniques; and the lack of an efficient sample processing technique universally applicable on all types of extrapulmonary samples. The poor

performance of conventional microbiological techniques in extrapulmonary specimens has stimulated the increased use of PCR tests in the laboratory diagnosis of tuberculosis. The exact diagnostic role of PCR assay for *M. tuberculosis* in high-prevalence areas for tuberculosis has to be assessed in appropriate control groups, particularly in the case of extrapulmonary tuberculosis. In contemporary practice, clinicians neither start nor stop treatment for this condition based solely on PCR results. Keeping in mind the relatively low yield of organisms by AFB smear and culture in extrapulmonary specimens, the test resultshould be better evaluated the test results against histology, cytology, and microbiology.

6.2 CONCLUSION

Extrapulmonary tuberculosis accounts for 12% of total cases visiting TUTH, Kathmandu, Nepal. Since the cultural technique showed more positivity than AFB staining, the prior is regarded as the best method of detection.

CHAPTER- VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

During the study period of from June 2006 to November 2006, a total of 150 clinically suspected samles were examined by ZN staining and cultured in Ogawa medium. Primary culture samples were subcultured. The subcultures were observed for their cultural characteristics for up to 4-6 weeks and then subjected for different biochemical tests for their confirmation as *M. tuberculosis*. Out of 150 cases of suspected extra pulmonary T.B, only 18 (12%) cases were found to be positive giving the biochemical tests positive for *M.tuberculosis*. Niacin tests, Nitrate reduction tests and Catalase test were performed for the confirmation.

7.2 RECOMMENDATIONS

Based on the findings and experience of the study, the following recommendations have been made:

1. Neither the conventional methods nor the newer alternative methods alone satisfy all the requirements of definitive identification, rapid results, and cost effectiveness. Also, even if *M.tuberculosis* is identified by an alternative method such as nucleic acid amplification, the organism must still be cultured for susceptibility testing. For these reasons, a combination of alternate and conventional method is recommended for the proper identification and management of the disease.
2. As the test was carried out in TUTH, it doesn't necessarily reveal the total picture of the whole country, therefore this type of study should be carried out in hospitals and laboratories as well as in communities of different parts of our country in order to obtain information regarding geographical, ethnic and gender wise variation of extrapulmonary tuberculosis so that the exact reporting of the

cases and management of disease would be easier.For that all the hospitals and private laboratories in our country should have the facility for testing extrapulmonary tuberculosis.

3. The method of specimen processing allowed us to carry out different diagnostic tests, namely, smear microscopy, culture, cyto and histopathology conveniently on a single specimen without dividing it into portions for the individual tests, which might have resulted in nonuniform distribution of the tubercle bacilli or cross contamination during tube-to-tube transfers. The use of a single specimen-processing platform enabled us to analyze with confidence the results of different tests leading to a more definitive diagnosis. . A clinicopathological correlation with microbiological and molecular tests results was observed to be an ideal approach to diagnose these forms of extrapulmonary tuberculosis.

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APPENDIX-I

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

1. **Bacteriological Media**
   2%Ogawa Medium

2. **Reagents/Chemicals (QUALIGENS)**
   Absolute ethanol
   Acid-alcohol
   Buffer
   Distilled water
   Eggs
   Glycerol
   Lysol
   Malachite green
   Methylene blue
   Potassium dihydrogen phosphate, anhydrous
   Sodium hydroxide
   Sulphuric acid
   Tris HCl

3. **Glasswares (BOROSIL)**
   Beaker
   Culture bottles
   Petriplate
   Pipettes
   Slides
   Test tubes
   Conical flask
   Glass rod
   Measuring cylinder

4. **Equipments**
   Biological Safety Cabinet, class IIA
   Autoclave
   Coagulator
   Centrifuge
   Distilling apparatus
   Incubator
   Microscope
   Refrigerator
   Sterilizer

5. **Miscellaneous**
   Bacteriological loop, Bunsen burner, Cotton, Forceps, Gloves, Labelling stickers, Staining rack, Spirit lamp, Soaps, Tube holder, Tissue paper.

APPENDIX-II

A. COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA

Ogawa Medium

- **Ingredients**

A. Mineral Salt solution

Potassium dihydrogen Phosphate anhydrous (KH_2PO_4)	3.0g
Sodium glutamate	3.0g
Distilled water	300ml

Dissolve the ingredients in distilled water by heating in an autoclave at 121oC for 30 minutes to sterilise. Cool to room temperature. This solution keeps indefinitely and may be stored in suitable amounts in the refrigerator.

B. Malachite green solution, 2%

Malachite green dye	2.0g
Sterile distilled water	100ml

Using aseptic techniques dissolve the dye in sterile distilled water by placing the solution in the incubator for 1-2 hours. This solution will not store indefinitely and may precipitate or change to a less-deeply coloured solution. In either case discard and prepare a fresh solution.

C. Homogenised whole eggs

Fresh hen's eggs, not more than seven days old, are cleaned by scrubbing thoroughly with a hand brush in warm water and a plain alkaline soap. Let the eggs soak for 30 minutes in the soap solution. Rinse eggs thoroughly in running water and soak them in70% ethanol for 15 minutes. Before handling the clean dry eggs scrub the hands and wash them. Crack the eggs with a aseptically into a sterile flask and beat them with a sterile egg whisk or in a sterile blender.

- **Preparation of complete medium.**

The following ingredients are aseptically pooled in a large, sterile flask and mixed well:

Mineral salt solution	300ml
Malachite green solution	18ml
Whole hen's eggs (12-16 eggs, depending on size)	600ml
Glycerol	18ml

The resulting pH of the medium is 6.8. The medium is mixed well and distributed in 6-8ml volumes in sterile test tubes.

- **Coagulation of medium**

Before loading, heat the inspissator to 80 ºC to quicken the build-up of the temperature. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 80- 85 ºC (since the medium has been preoared with sterile precautions this heating is to solidify the medium, not to sterilise it).Heating for a second or third time has a detrimental effect on the quality of the medium.

The quality of egg medium deteriorates when coagulation is done at too high a temperature or for too long. Discoloration of the coagulated medium may be due to excessive temperature. The appearance of little holes or bubbles on the surface of the medium also indicates faulty coagulation procedures.

- **Sterility check**

After inspissation, the whole media batch or a represented sample of culture bottles should be incubated at 37 ºC for 24 hours as a sterility testing.

- **Storage**

The medium should be dated and stored in refrigerator and can keep for several weeks if the caps are tightly closed to prevent drying out.

B. COMPOSITION AND PREPARATION OF DIFFERENT STAINING AND TESTS REAGENTS

Preparation of Ziehl-Neelsen Staining reagent

1. Stock Carbol Fuchsin.

Basic fuchsin	3g
Ethanol (95%)	100ml

Dissolve the basic fuchsin in ethanol.

2. 5% Phenol solution

Phenol melted	5ml
Distilled water	95ml

Gently warm the bottle with pure phenol crystals to liquefy and measure it with a pipette. (Be careful that the phenol is corrosive so never suck phenol by mouth).Add the melted phenol slowly to distilled water while stirring.

3. Ziehl's solution (working Carbol fuchsin solution)

Stock alcoholic Fuchsin	10ml
5% phenol solution	90ml

Mix the stock alcoholic fuchsin with 5% phenol while stirring. Filter the solution before use for removing fuchsin crystal or particles.

4. Decoloriser (3%Acid alcohol)

95% Ethanol	97ml
Conc HCl	3ml

Add HCl to ethanol slowly in a chemical fume hood.

5. Counter Stain . (0.5% Malachite green)

Malachite green	0.5g
Distilled water	100ml.

APPENDIX-III

ZIEHL-NEELSON (ZN) STAINING PROCEDURE

Principle

Heating the slide allows greater penetration of carbolfuchsin into the cell wall. Mycolic acids and waxes complex the basic dye, which then fails to wash out with mild acid decolorization.

Method

1. The completely air dried smear was heat fixed by passing it gently through the flame 2-3 times.
2. The smear was flooded with carbolfuchsin stain reagent and the slides was gently steamed for 1 minute by flaming from below the rack with a gas burner or spirit lamp, the slides was not allowed to boil or dry out.
3. The stain was allowed to remain on the slides for an additional 4-5 minutes without heat.
4. The slide was then rinsed with deionised water and excess water was rinsed off.
5. Then the slide was decolorized with 3%Acid alcohol (95% ethanol and 3%acid alcohol) for 2 minutes. And the slide was rinsed with deionised water and excess water is drained off.
6. The slide was then counter stained with 0.05% malachite green or 0.1% methylene blue for 4-5 minutes.
7. The slide was again rinsed with deionised water and allowed to air dry.
8. Finally the slide was examined under oil immersion (100X) for the presence of acid fast bacilli.

Expected Results

Mycobacterium spp will appear red or have a red-blue, beaded appearance, whereas non-mycobacterial will appear blue.

APPENDIX-IV

RECORDING AND REPORTING

(According to WHO/1998)

Observation	Report
No bacilli seen (300 fields)	AFB not found.
1-9AFB/100 fields	AFB found (Record the exact number).
10-100 AFB/100fields	AFB found (+).
1-10 AFB/fields	AFB found (++).
(Observed at least 50 visual fields)	
> 10AFB/fields	AFB found (+++).
(Observed 20 visual fields)	

APPENDIX-V

PROCEDURE FOR THE CULTURE OF SPECIMEN IN 2% OGAWA MEDIA

1. According to the type of sample received, whether or not decontamination should be carried or not was estimated accordingly.
2. Two freshly prepared 2% Ogawa media tubes were taken for one specimen.
3. The properly processed specimen in measured volume of 400ul was added to each of the two media tubes and spread thoroughly to the entire slant surface.
4. Then the Tubes were incubated for over night in the slanting position.
5. After overnight incubation at the inclined position the tubes were incubated upright position at 37 °C for 6 to 8 weeks.
6. Then the growth was observed daily for the growth of organism.
7. After 8 weeks only the culture tubes were discarded if no growth was observed throughout the incubation period.
8. Observation was made properly and then from the positive culture tubes the different Biochemical tests were performed for the complete identification of organisms.

APPENDIX-VI

RESULTS AND INTERPRETATION OF NITRATE REDUCTION TEST

Negative: No colour. If no colour develops, the test is either negative or the reduction has proceeded beyond nitrite. Add a small amount of powdered zinc to all negative tests by tipping the end of a slightly moistened applicator stick into dry zinc and shaking into the liquid.

a) If nitrate is still present, it will be catalysed by zinc and a red colour will develop, indicating a true negative

b) If no colour develops the original reaction was positive but the nitrate was reduced beyond nitrite. Repeat the test to confirm the observation.

Positive: Red colour, which vary from pink to very deep red-crimson

Faint pink = +/-

Clear pink = 1+

Deep pink = 2+

Red = 3+

Deep red = 4+

Purplish red = 5+

Only 3+ to 5+ is considered positive.

APPENDIX-VII

LABORATORY REQUEST FORM:

Name of patient: ……
Age: …… **Sex:** Male ·
Female ·
Complete Address:……..
Patient's register No:……...
Source of specimen:

- Pulmonary
- Extrapulmonary site …….

Reason for diagnosis:

- Diagnosis
- Follow-up of chemotherapy

Culture results: Preliminary report

Laboratory serial no. …….
Date of specimen received……
Culture results:
Culture methods……..

No growth	·	3+	·
1-19 colonies	·	4+	·
1+	·	contaminated	·
2+	·		

Cultivation yield……..growth of Mycobacterium with the characteristic of tubercle bacilli.

Culture Results: Final Report

Laboratory serial no……
Date of specimen received……..
Microscopy results:
Staining methods

- Ziehl-Neelsen,
- Fluorochrome

Culture method ……
Culture results:

No growth	·
Contaminated	·
Not done	·
1-19 colonies	·
1+	·
2+	·
3+	·
4+	·