CHAPTER-I

1. INTRODUCTION

Tuberculosis (TB) is the world's most serious public health problem in spite of the availability of effective diagnosis and treatment measures. It is a disease of great antiquity and contributing to more morbidity and mortality than any other bacterial infection (Grange, 1998). TB remains one of the deadliest threats to public health. Every year, two million people die of the disease, which is caused by the microorganism, *Mycobacterium tuberculosis* that spreads to others via aerosol route. Roughly, one third of the world's population is infected and more and more bacterial strains have developed resistant to drugs.

TB primarily attacks the most economically productive group of the society (people aged 15-45 years); the community injury that it causes extends far beyond individual disease and death (Sbarbaro, 2001). TB is a disease of poverty; virtually all TB deaths occur in the developing world, affecting mostly the vulnerable such as the poorest and malnourished. TB, if not treated each person with active TB infects an average 10 to 15 people every year. According to recent data from WHO, almost 9 million new TB cases occurred in 2004, 80% of them in 22 countries (WHO, 2006).

Ten percent of the infected people (who are immunosuppressive e.g:- with HIV/AIDS) develop active TB disease as HIV weakens the cellular immunity. *M. tuberculosis* owes its virulence due to its ability to survive with in the macrophage rather than the production of toxic substance (Grange, 1998). Nepal, one of the countries of SAARC region, has an elevated risk of TB infection, estimated to be 2.0% in rural and 4.5% in urban areas (WHO, 1997). It is estimated that 6 out of 10 adults in Nepal are infected with TB and 80,000 Nepalese populations have active TB disease. Every year, 40,000 people develop active TB, of whom 20,000 have infectious pulmonary disease. These 20,000 are able to spread the disease to others. Introduction of treatment by Directly Observed Treatment Short

Course (DOTS) has already reduced the number of deaths; however, 5,000-7,000 people continue to die every year from this disease (DoHS, 2004/2005).

DOTS is the strategy for improving treatment outcome to control tuberculosis by giving drugs to the patients under direct observation of health workers. DOTS has been found 100 % effective to cure tuberculosis. The treatment success rate in DOTS is now 88%. Nationally, this year over 34,000 TB patients have been registered and are being treated under the NTP (DoHS, 2004/2005).

Acquired Immune Deficiency Syndrome (AIDS) is a fatal disease state caused by Human Immuno-deficiency virus (HIV-1and HIV-2), belonging to the Retroviridiae family. Since the first case of AIDS was reported in 1981, infection with HIV-1 virus has become a global medical crisis. The current situation of HIV in Nepal is different from when the first case was diagnosed in 1988. Nepal is low prevalence country for HIV and AIDS (0.5%). However, some of the groups show evidence of a concentrated HIV epidemic e.g: -sex workers (19.5%), migrant population (4-10%) and intravenous drug users (IDUs) both in rural and urban areas (68%) (DoHS, 2004/2005).

The cumulative HIV/AIDS cases from 1988 to December 2004 are 4,593. However, using mathematical models it has been estimated that there were more than 62,000 people living with HIV/AIDS in Nepal at the end of 2004 (NCASC, 2004). HIV infection in Nepal mainly occurs among the age groups of 15-19 to 40-49. Most of the infected people are in the age group of 20 to 39 years reflecting the highest reported number of HIV infection at the age group of 30-39 years. By the end of the first decade of the 21st century, the HIV may become number one killer of Nepalese in the 15-49 age group (NCASC, DoHS, 2004).

Today, TB and HIV/AIDS are two of the world's major pandemics. HIV has a dramatic impact on TB control in countries with a high burden of TB/HIV. At the same time, tuberculosis is not only the leading cause of death among people with AIDS, but also the most common curable infectious disease among people living with HIV/AIDS (PHA). TB

is the most important life threatening opportunistic infection associated with HIV. In Nepal 75% of AIDS patients have had pulmonary tuberculosis (WHO Regional office for South East Asia, 2004). In case of Nepal, estimated prevalence of HIV in new adult TB cases is 2.9% and estimated incidence of TB in HIV positive adults aged 15-49 years is 0.9(thousands).

Worldwide, TB remains a problem of enormous proportions with the interaction of TB and HIV causing huge and increasing burden of illness and death in much developing world. Moreover, people with HIV/TB are 30 to 50 times more likely to develop active TB making TB the biggest AIDS related killer in the world today. To make matter worse, global rates of MDR-TB are also on rise. MDR-TB is very complicated, difficult and very expensive to treat and often fatal. Evidence also suggests that HIV can promote the emergence of multi drug resistance strains of *M. tuberculosis* (Pizzo and Wilfert, 1998).

Rationale

TB and HIV; Deadly Symbiosis

HIV and TB form a lethal combination. HIV positive individuals do not have the internal immune-system resources to keep the mycobacterium TB in check. As a result, they succumb to the disease at an alarming rate. Currently, over 12 million people are co-infected and rising. HIV fuels the TB epidemic in several ways. HIV promotes progression to active TB both in people with recently acquired and with latent *M. tuberculosis* infections. HIV is the most powerful known risk factor for reactivation of latent TB infection to active TB.

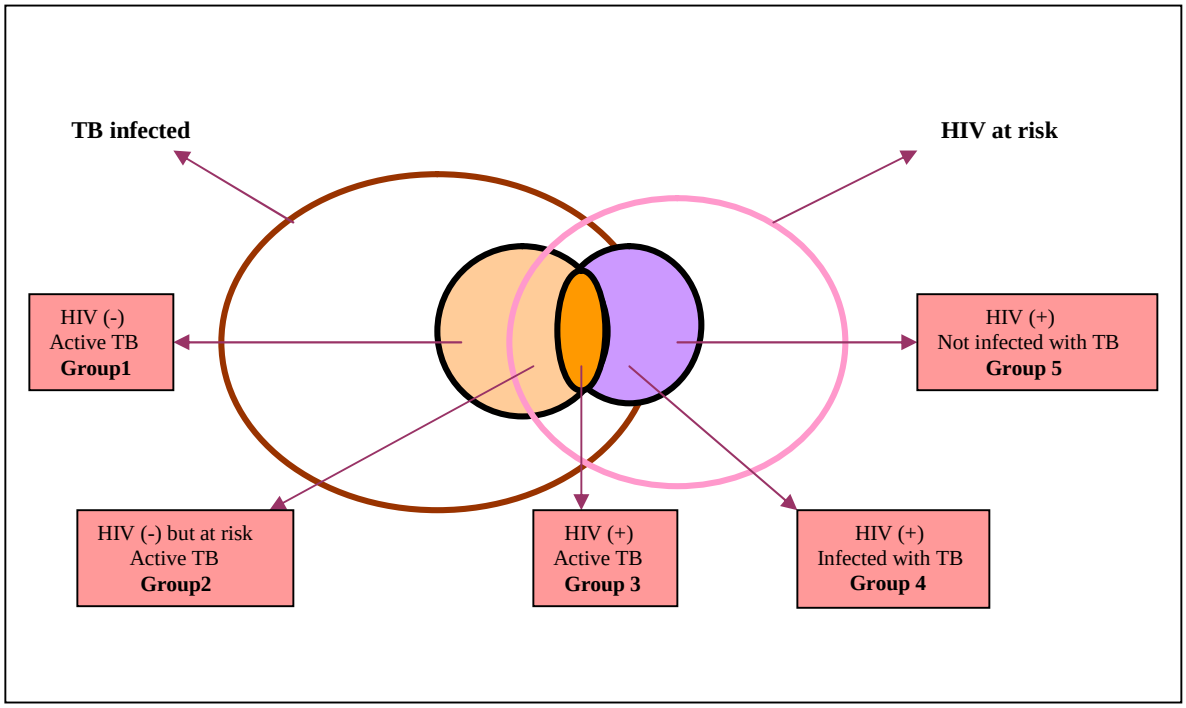


Figure 1: Epidemiological classification of TB/HIV

CHAPTER-II

2. OBJEVTIVES

General objective

- To determine TB/HIV co-infection cases visiting DOTS center and Research center.

Specific objectives

- To compare prevalence of HIV in suspected TB patients visiting the DOTS center and Research center.
- To identify tuberculosis (AFB) by Z-N staining technique.
- To perform the AFB culture to identify tuberculosis.
- To identify HIV sero-positive patients by ELISA method.

CHAPTER-III

3. LITERATURE REVIEW

3.1 TUBERCULOSIS

Tuberculosis, or TB, is an infectious bacterial disease. It is a chronic granulomatous disease that has become a major public health concern worldwide as it is recognized as the leading cause of death among the infectious diseases. It is the disease, which most commonly affects the lungs. Because of the serious health threat posed by tuberculosis, the WHO declared it a global emergency' in 1993 (Cheesbrough, 2003). It is a particularly insidious problem to those who have AIDS. HIV infection is the strongest risk factor for TB infection becoming active TB disease, speeding the progression from latent or recently acquired infection to active clinical disease (Bam and Rahman, 2002). In these patients, the T-lymphocytes that normally mount a response to *Mycobacterium tuberculosis* are also destroyed and patient cannot respond bacterial infection. However, in healthy people, infection with *M. tuberculosis* often causes no symptoms, since the person's immune system acts to “wall off” the bacteria.

3.1.1 History

Mycobacteria were among the first bacteria to be ascribed to specific diseases. In 1874, Armauer Hansen identified a rod-shaped bacillus (*Bacillus leprae)* in a tissue biopsy from a lepromatous leprosy patient and suggested that it was the aetiological agent of leprosy (Hansen, 1880). Eight years later, Robert Koch identified a rod-shaped bacillus (*Bacterium tuberculosis*) as the causative agent of tuberculosis and formulated Koch's postulates for establishing a causal relationship between a suspected pathogen and a given disease (Koch 1882, Zopf 1883). These species were subsequently renamed *Mycobacterium leprae* and *Mycobacterium tuberculosis*, respectively, and placed in the genus *Mycobacterium* ('fungus bacterium', named to reflect the mould-like pellicle formed by *M. tuberculosis* on liquid medium) (Lehmann and Neumann, 1896).

In the past tuberculosis has been referred to as the "white plague" and by John Bunyan as "the captain of all these men of death". In Ancient Hindu texts, tuberculosis is referred to as Rograj and Rajayakshma meaning **The King of disease** (Grange, 1998). Certainly, tuberculosis was well recognized by the time of Hippocrates (377-400 BC), who gave an excellent clinical description of the disease, called "pthisis", a Greek word that mean, "to consume to spit" and "to waste away" (Grange, 1998; Miller and Schieffelbein, 1998). The Dutch Physician, Franciscus Sylvius (1614-1672) deduced from autopsies that tuberculosis characterized by the formation of nodules, which he named "tubercles".

Robert Koch discovered the *M. tuberculosis* organism in 24 March 1882 and succeeded in culturing it on inspissated serum. The transmissible nature of tuberculosis was clearly established by Jean-Antoine-Villemin, a French military doctor in 1868 (Webb, 1936).

The word "tuberculosis" means "a small clump". Several names have been used to refer to tuberculosis in the year gone by; acute progressive tuberculosis has been referred "tabes pulmonali". The acid fast nature of the organism was discovered by Ehrlich in 1885 and the present method of acid-fast staining was developed by Ziehl and subsequently modified by Neelsen and hence the named Ziehl Neelsen staining technique (Dhungana, 2004).

At the beginning of this 21st century, *M. tuberculosis* was the only species of Mycobacterium routinely isolated from, and associated with, human disease. As other species of Mycobacterium were recognized as causes of human disease, they were often simply categorized as non- tuberculosis mycobacterium without further speciation.

3.1.2 Etiological agent: Mycobacteria

The genus Mycobacterium is the only genus in the family Mycobacteriaceae. The name Mycobacterium (Greek Mykes, fungus; bacterium, small rod), meaning ' Fungus – like bacterium' is derived from the mould – like appearance of *M. tuberculosis* when growing in liquid media (Watt *et al*., 1996). The genus Mycobacterium consists of more than 55 well defined species, including the causative agents of TB, leprosy, and chronic hypertrophic

enteritis (John's disease) of cattle (Hasleton, 1996). Of these, 14 are known to cause disease in humans (Forbes and Sahm, 2002).

Mycobacteria are rod- shaped, aerobic bacteria that do not form spores. Mycobacteria are acid and alcohol fast, meaning that once stained by an aniline dye, such as carbol fuchsin, they resist decolorization with acid and alcohol. Therefore mycobacteria are often called “acid-fast bacilli” (AFB). The acid and alcohol fastness is due to the presence of thick, complex, lipid rich, and waxy cell wall component called mycolic acid. The degree of acid fastness is different for different species due to variation of lipid percent (40%-60%) in the species. In addition to mycolic acid (Principle constituent) layer, mycobacterium possess peptidoglycan (innermost) layer, Arabinogalactan (external to peptidoglycan) layer and mycosides layer (forming species or strain species surface lipid). The organisms are poor gram positive and either straight or slightly curved rods but coccobacillary, filamentous and branched forms may also occur. They usually measure 1-4μm by 0.3-0.6μm. The morphology varies from species to species (Hasleton, 1996). Mycobacteria have a cell wall with high lipid content that includes waxes having characteristics mycolic acid with long branched chains.

Mycobacteria of clinical interest are divided into those associated with TB- the *M. tuberculosis* complex or MTC (*M. tuberculosis*, *M. bovis*, BCG, *M. africanum* and *M. micro*ti- and other mycobacteria that may be associated with human disease or "atypical" 'anonymous', 'non-tuberculous', tuberculoid, opportunist and mycobacteria other than tuberculosis (MOTT). Many MOTTs are found in the environment but they can colonize in man and cause clinical infection (Watt *et al*., 1996).

3.1.2.1 *Mycobacterium tuberculosis*

3.1.2.1.1 Morphology and colony characteristics

Tuberculosis is a chronic granulomatous disease affecting humans and many other mammals. It is caused by four very closely related species: *M. tuberculosis* (the human

tubercle bacillus), *M. bovis* (the bovine tubercle bacillus), *M. microti* (the vole tubercle bacillus) and *M. africanum* (Grange, 1998).

Koch first described the tubercle bacillus in 1882 now known as *M. tuberculosis*. Most human tuberculosis is caused by *M. tuberculosis*, but some cases are due to *M. bovis*, which is the principal cause of tuberculosis in cattle and many other mammals *M. tuberculosis*, the cause of TB, which is one of 54 recognized species of mycobacterium, is classified in the family Mycobactericeae of the order Actinomycetales. *M. tuberculosis* is a small (1 to 4um long), slender, slightly curved, rod shaped bacterium of about 3 X 0.3 μm in size. It is a non-spore forming, non-encapsulated, non-motile, slow growing, and obligate aerobe, with a generation time 15 to 20 hours. In sputum and other clinical specimens they mainly occur singly or in small clumps on microscopic examination and in liquid cultures, human tubercle bacilli often grow as twisted rope like colonies termed serpentine cords.

3.1.2.1.2 Cultural characteristics

Mycobacteria are obligate aerobes and slow growers (average generation time 18 hours). Colonies of *M. tuberculosis* in culture are rough and characteristically buff colored (non-pigmented) i.e. gives luxuriant growth i.e. eugonic growth on glycerol pyruvate medium. Colonies of human tubercle bacilli (*M. tuberculosis*) generally appear on egg media i.e. Lowenstein-Jensen (L-J) medium after 2-3 weeks at 35-37^{0}C. Most disease associated mycobacteria require up to 8 weeks on complex media enriched with eggs. Colonies first appears as small 1 to 3 mm, dry, friable colonies that are rough, warty, granular and off-white (buff) colored. After several weeks, these increase in size (as 5-8mm) typical colonies have a flat irregular margin and a 'cauliflower' center. Colonies are easily detached from the medium's surface but are difficult to emulsify. In clinical microbiology laboratory, specialized culture media such as Lowenstein Jensen (L-J) media or Middle brook 7H10 or 7H11 agar are used for the mycobacterial culture because *M. tuberculosis* will grow slowly, do not produce yellow pigment and fail to grow on egg media containing p-nitro benzoic acid (500 mg/l). They even fail to grow at 25 and 41^{0}C (Grange, 1998). In biochemical test, *M. tuberculosis* are niacin positive and nitrate reduction is also positive (Forbes and Sahm,

2002). Characteristically, strains of *M. tuberculosis* form cords when growing on solid medium. When colonies are suspended in liquid or when they are grown in liquid culture, the cording characteristic can be seen clearly in stained preparations (Good and Shinnick, 1998).

3.1.2.1.3 Susceptibility to chemical and physical agents

Mycobacteria are as susceptible as other non-spore-forming bacteria to heat and to some other physical and chemical agents, although some early work on the heat susceptibility of mycobacteria may have suggested otherwise (Corper and Cohn, 1937). Mycobacteria are generally resistant to acids and alkalis, and this feature is used to advantage in isolation procedures. As much as 2% sodium hydroxide, 2% sulphuric acid or 2.5% oxalic acid can be used to kill contaminants in specimens prior to culture. However, killing activity of acids and alkalis increases with increasing temperature of exposure, and resistance varies greatly among different species. Turbercle bacilli are also resistant to quaternary ammonium compounds and, indeed, cetylpyridinium chloride has been used for decontamination of clinical specimens prior to culture (Kent and Kubica, 1985).

With respect to chemical disinfectants, mycobacteria are suspectible to a variety of chemical agents including: alcohols (ethyl and isopropyl although the latter is not as active as the former), chlorine, glutaraldehyde, iodophores, phenolic compounds, ethylene oxide, formaldehyde and hydrogen peroxide. Derivatives of phenol, in which a functional group replaces one of the hydrogen atoms on the aromatic ring, are effective and safe to use (Marsik and Denys, 1995).

Mycobacteria are resistant to drying and can survive for long periods on inanimate objects if protected from ultraviolet (UV) light, which is highly tuberculocidal. Tubercle bacilli can remain suspended as a stable aerosol in air for many hours. The persistence of tubercle bacilli on surfaces and in air prompted development of methods for removal including treatment of the air and surfaces with UV light (Riley 1957, 1961).

3.1.2.1.4 Mycobacteria other than tuberculosis (MOTT)

Human disease can also be caused by species of mycobacteria other than *M. tuberculosis* (MOTT), also known as atypical mycobacteria. These organisms are widespread in nature and have been frequently found in environment habitants that may colonize and occasionally cause infection in humans and animals. MOTT have been isolated from a variety of sources, including soil, dust, water, milk, animals and birds. Infection caused by these organisms is called mycobacteriosis. They are becoming more prevalent with the increasing prevalence of immunocompromised hosts, particularly in relation to the AIDS pandemic and in patients with preexisting lung disease (Haslett *et al.*, 1999). MOTT are still a rare cause of disease in sub-Saharan Africa. The large majority of patients in Africa who are diagnosed and treated for TB, even those infected with HIV, have disease caused by *M. tuberculosis* (Jamison *et al*., 2006).

The most commonly encountered MOTT that is isolated in clinical specimens and rare cause of disease are *Mycobacterium kansasii, Mycobacterium xenopi, Mycobacterium malmosense,* MAIS complex-*Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium scrofulaceum.*

3.1.2.2 Pathogenesis

M. tuberculosis is the classical representative of an intracellular pathogen. Thus the organism owes its virulence due to its ability to survive with in macrophage rather than the production of toxic substance (Grange, 1998). The immune response to the bacillus is of the cell-mediated type which, depending on the type of T helper cells involved, may either lead to protective immunity and resolution of the disease or to tissue-destroying hypersensitivity reactions and progression of the disease process. The nature of the immune responses following infection changes with time so that human tuberculosis is divisible into primary and post-primary forms with quite different pathological features (Grange, 1998).

a. Primary pulmonary tuberculosis

The first infection of the lung with the tubercle bacillus is known as primary pulmonary TB and usually includes the draining lymph nodes in addition to the initial lesion. The great majority of Primary tuberculosis infection is usually asymptomatic, at least in young adults and adolescents (Seaton *et al*., 2000). The site of initial infection is usually the lung, following the inhalation of bacilli. These bacilli are engulfed by alveolar macrophages in which they replicate to form the initial lesion called as *'Gohn focus'*. Some bacilli are carried in phagocytic cells to the hilar lymph nodes where additional foci of infection develop. The Ghon focus together with the enlarged hilar lymph nodes form the *'Primary complex'*. In addition, bacilli are seeded by further lymphatic and haematogenous dissemination in many organs and tissues including other parts of the lung. When the bacilli enter the mouth, the primary complexes involve the tonsil and cervical nodes or the intestine, often the ileocaecal region, and the mesenteric lymph nodes.

Within about 10 days of infection, clones of antigen-specific T lymphocytes are produced. These release lymphokines which activate macrophages and cause them to form a compact cluster, or granuloma, around the foci of infection. These activated macrophages are termed epithelioid cells from their microscopical resemblance to epithelial cells. Some of them fuse to form multinucleate giant cells. Such hypersensitivity reaction leading to giant cell formation and epitheloid cell formation is called as *'Granulomatous reaction'*.

In a minority of cases one of the infective foci progresses and gives rise to the serious manifestations of primary disease, including progressive primary lesions. If a focus ruptures into a blood vessel, bacilli are disseminated throughout the body with the formation of numerous granulomata. This, from the miller seed-like appearance of the lesions, is known as *'miliary tuberculosis'* (Grange, 1998).

b. Post primary tuberculosis

Post primary tuberculosis is by far the most important type of tuberculosis, partly because it is most frequent and partly because smear positive sputum is the main source of infection

responsible for the persistence of disease in the community. This form of tuberculosis may arise in one of three ways:

- Direct progression of a primary lesion.
- Reactivation of a quiescent primary of post-primary lesion.
- Exogenous reinfection (Seaton *et al*., 2000).

It is generally a disease of the adults due to endogenous reactivation or exogenous re-infection in a patient who had infection in the past and has retained a degree of acquired immunity. Reactivation may occur spontaneously or after any immunocompromised state. In post primary TB, dissemination of bacilli to lymph node and other organ is unusual. Instead, the infection spread through the bronchial tree so that secondary lesion develops in lower lobes of the lung, trachea, larynx and mouth, and swallowed bacilli cause intestinal lesions; secondary lesions may also develop in the bladder and epididymis in cases of renal tuberculosis (Grange, 1998).

3.1.2.3 Human immune response in tuberculosis

IFN-γ is a crucial cytokine in the protective immunity to mycobacterial infections, which also plays a critical role in host responses to a wide variety of viral and microbial pathogens. Macrophages infected with mycobacteria secrete IL-10, a cytokine that down regulates production of proinflamatory cytokines such as TNF-α and inhibits IFN-γ production by T-cells (Fujiwara *et al*., 1999). Infection of macrophages with *M. tuberculosis* or exposure to *M. tuberculosis* 19-kDa lipoprotein for >16 h inhibits gamma interferon (IFN-γ)-induced major histocompatibility complex class II (MHC-II) expression by a mechanism involving Toll-like receptors (TLRs). *M. tuberculosis* was found to inhibit murine macrophage MHC-II antigen (Ag) processing activity induced by IFN-γ but not by interleukin-4 (IL-4), suggesting inhibition of IFN-γ induced gene regulation. Control of *M. tuberculosis* requires T cells and macrophages. T-cell function is modulated by the cytokine environment, which in mycobacterial infection is a balance of proinflammatory

(interleukin-1 [IL-1], IL-6, IL-8, IL-12, and tumor necrosis factor alpha) and inhibitory (IL-10 and transforming growth factor β [TGF-β]) cytokines. IL-10 and TGF-β are produced by *M. tuberculosis*-infected macrophages. The effect of IL-10 and TGF-β on *M. tuberculosis* reactive human $CD4^+$ and γδ T cells, the two major human T-cell subsets activated by *M. tuberculosis* was investigated. Both IL-10 and TGF-β inhibited proliferation and gamma interferon production by $CD4^+$ and γδ T cells. IL-10 was a more potent inhibitor than TGF-β for both T-cell subsets. Combinations of IL-10 and TGF-β did not result in additive or synergistic inhibition. IL-10 inhibited γδ and $CD4^+$ T cells directly and inhibited monocyte antigen-presenting cell (APC) function for $CD4^+$ T cells and, to a lesser extent, for γδ T cells. TGF-β inhibited both $CD4^+$ and γδ T cells directly and had little effect on APC function for γδ and $CD4^+$ T cells. IL-10 down-regulated major histocompatibility complex (MHC) class I, MHC class II, CD40, B7-1, and B7-2 expression on *M. tuberculosis*-infected monocytes to a greater extent than TGF-β. Neither cytokine affected the uptake of *M. tuberculosis* by monocytes. Thus, IL-10 and TGF-β both inhibited $CD4^+$ and γδ T cells but differed in the mechanism used to inhibit T-cell responses to *M. tuberculosis* (Rojas *et al.*, 2003).

M. tuberculosis remains a major cause of morbidity and mortality worldwide, infection approximately one-third of the world's population (Miller and Schieffelbein, 1998). Cellular immune responses control *M. tuberculosis* infection in most healthy individuals, resulting in fewer than 10% of infected persons developing active tuberculosis (Comstock, 1982). T cells and mononuclear phagocytes are required for successful control of *M. tuberculosis* infection. Mycobacterial antigens are recognized by a variety of T-cell populations, including $CD4^+$ αβT-cell receptor (TCR)-positive (TCR^+) T cells ($CD4^+$ T cells) and $V\delta2^+$ γδ T cells (γδ T cells) (Kaufmann, 1993). $CD4^+$ T cells have critical regulatory and effector functions in protective immunity to *M. tuberculosis* (Barnes *et al.*, 1993).

Infection of macrophages with *M. tuberculosis* results in the secretion of both

proinflammatory (interleukin 1 [IL-1], IL-12, and tumor necrosis factor alpha) and inhibitory (IL-10 and transforming growth factor β [TGF-β]) cytokines (Barnes *et al*., 1993). The balance of proinflammatory and inhibitory cytokines influences T-cell activation. Overproduction of IL-10 and TGF-β has been documented in tuberculosis patients and implicated as a cause of depressed T-cell function in these individuals (Dlugozitzky *et al*., 1999).

The systemic immune response in HIV-uninfected patients with pulmonary tuberculosis is characterized by depressed responses of PBMC to PPD and other crude MTB antigens in terms of blastogeneisis and particularly expression of the cytokines INF-γ and IL-2. Concurrently there is over expression of IFN-γ, IL-10 and TGF-β. The latter two separately and together suppress the expression of the Th1 cytokines. As patients are treated, expression of immunosuppressive cytokines decline within 3 months but decrease in IFN-γ production is sustained. Possible explanations include selective detection of IFN-γ producing cells from the circulation by apoptosis and compartmentalization to lung.

3.1.3 Epidemiology

3.1.3.1 Global aspect of TB burden

There were an estimated 8.3 million (7.3-9.2 million) new TB cases in 2000, or 137 (121-151) per 100 000 population; 3.7 million (3.1-4.0 million) were smear positive, i.e., 61 (51-66) per 100 000 population. Most new cases were in adults aged 15 to 49 years (5.4 million; 172/100 000). Among WHO regions, the African Region (essentially sub-Saharan Africa) had by far the highest annual incidence rates (290/100 000), while the South-East Asian Region had the largest number of cases (3.0 million). Half the new cases (4.4 million) were in the top 5 countries, all in Asia. Of 15 countries with the highest incidence rates per capita, 13 were in Africa (Corbett *et al.*, 2003).

The global burden of TB is growing. The total number of new TB cases increased at a rate of 1.8% per year between 1997 and 2000, and incidence rates per capita (all ages) at a rate

of 0.4% per year. Case numbers increased much more quickly in the former Soviet Union (6.0% per year) and in the WHO African Region (6.4% per year).

An estimated 9 million new cases of TB occurred in 2004 at the rate of 140/ 100000 population, of which 3.9 million (62/100000 pop) were smear positive and 741000 were in adults infected with the human immunodeficiency virus (HIV). 14.6 million were estimated to be prevalent TB cases at the rate of 229/100000 pop, of which 6.1 million were smear positive (95/100000 pop). More than 80% of all new TB patients in 2004 was in the Africa, South East Asia and Western Pacific Region. An estimated 1.7 million people (27/100000 pop) died from TB in 2004, including those co infected with HIV (248000) (Corbett *et al*., 2003; WHO 2002; WHO 2006).

A total of 183 countries and territories were implementing the DOTS strategy during 2004. By the end of 2004, 83% of the World's population lived in countries, or parts of countries, covered by DOTS. At the end of 2004, DOTS expansion was completed in nine High Burden Countries and nearing completion in five others. Pakistan reported full DOTS coverage by the end of 2005, and coverage has increased considerably in Afghanistan, Brazil, India and the Russian Federation. DOTS programs notified 4.4 million new and relapsed TB cases in 2004 of which 2.1 million were new smear positive. In total 21.5 million TB patients, and 10.7 million new smear positive patients, were treated in DOTS programs over the 10 years 1995-2004 (STC, 2006). There are estimated 17 million cases of active TB globally. Every year, about 9 million people develop active TB and 2 million die of the disease; 84% of all TB sufferers live in developing countries. Most are poor people aged between 15 to 54 years of age. Between 2000 to 2020, nearly 1 billion additional people will be infected with TB, 200 million will become sick and 35 million will die of the disease (Stephen *et al*., 2006).

3.1.3.2 TB burden within SAARC area

The SAARC region, which contributes 22% of global population, bears 29% of global TB burden. Approximately, 2.5 million of all forms of new TB cases occur per year (out of them 1.1 million are sputum positive) and 0.6 million deaths per year.

Despite the establishment of national TB programmes for over 3 decades along with the existence of cost-effective TB control strategies, TB remains a prevention and control challenge within SAARC region. In 1993, the urgency of the global TB epidemic prompted WHO to declare TB a global emergency and urged each National TB control programme (NTP) to work towards 2 objectives by the year 2000, these being 1) to treat successfully 85 percent of detected smear positive TB cases and 2) to detect 70 percent of all such cases by implementing DOTS strategy, an effective approach to TB control.

Almost 50% of the adult population of this region have already been infected with *M. tuberculosis* and are at high risk of developing tuberculosis. Almost 95% of TB cases and 98% of deaths occur within developing countries, where 75% of cases are within the economically most productive age group (15-49 years). On an average, 3-4 months of work time are lost if an adult is ill with TB.

3.1.3.3 TB burden in Nepal

Tuberculosis is a well-known disease, in the context of Nepal, recognized and feared for centuries. It has long been important disease in Nepal-reflected in language, culture and history of services. About 60% of the economically active adult population has been infected. It is estimated that 6 out of 10 adults in Nepal are infected with TB and 80,000 Nepalese populations have active TB disease. Every year, 40,000 people develop active TB, of whom 20,000 have infectious pulmonary disease. These 20,000 are able to spread the disease to others. Introduction of treatment by Directly Observed Treatment Short Course (DOTS) has already reduced the number of deaths; however, 5,000-7,000 people continue to die every year from this disease (DoHS, 2004/2005).

3.1.4 Extra pulmonary tuberculosis

TB is a disease that can affect any organ and tissue of the body but much less common than pulmonary TB. Extra pulmonary TB is most commonly found in the mediastinal lymph nodes, larynx, cervical lymph nodes, pleurae, meninges, central nervous system, spine, bones, joints, kidneys, pericardium, intestine, peritoneum and skin. Extra pulmonary TB occurs more frequently among persons who are infected with HIV, but pulmonary TB remains the most common type of TB in this group worldwide (WHO, 2003). Extra pulmonary tuberculosis can be classified as Severe Extra-PTB (e.g., Miliary TB, Meningitis TB, Genitourinary TB, Abdominal TB, Pericarditis TB, Peritionitis TB, Bilateral or Extensive plural effusion TB, Spinal TB) and less severe Extra-PTB (e.g., Lymph node TB, Pleural effusion (unilateral) TB, Bone TB (excluding spine), Peripheral joint TB, Skin TB).

3.1.5 Severity of disease

Bacillary load as reported by the microscopy examination, the radiological extent of pulmonary disease and the anatomical site of disease determine disease severity. A pulmonary TB case is classified as severe if parenchymal involvement is extensive.

Meningitis TB, Miliary TB, Pericarditis TB, Peritonitis TB, Bilateral or extensive pleurisy TB, Spinal TB with neurological complication, intestinal and genito-urinary TB are severe forms of extra-pulmonary TB.

The following forms of extra-pulmonary TB are classified as less severe: lymph node, unilateral and non-extensive pleurisy, bone (excluding spine), peripheral joint, and skin TB.

3.1.6 Mode of transmission

Tuberculosis is an airborne disease. When a patient with pulmonary TB coughs, sneezes, spits or talks, very small droplets containing TB bacteria are released into air. These droplets, which float in the air, if inhaled by another person, may cause infection in his/her lungs. Every person who inhales the droplets will not develop TB disease unless his

immunity status is poor. It is estimated that only 10% of infected people will develop the disease. Extra pulmonary TB is virtually never infectious. Transmission generally occurs indoors, where droplets foci can stay in the air for a long time. Ventilation removes droplets foci. Direct sunlight quickly kills TB bacteria, but they can survive in dark for several hours (STC, 2006).

Many reports emphasize the importance of droplet nuclei in the transmission of tuberculosis. Riley's study confirms that, general, prolonged contact with a highly infectious case was necessary before infection was acquired. At the other extreme, infection may be acquired by single exposure, e.g. in laboratories or postmortem room. Transmission to and from the HIV infected patient is more likely, particularly where cough inducing procedures such as sputum induction or pentamidine nevulization are being employed, recommended precaution should be taken (Seaton *et al*, 2000). Three types of contacts were identified in the transmission of tuberculosis. They are household contact, community contact and biomedical contact. Recent reports of studies using DNA finger printing suggest that person-to-person transmission may account for as many as one-third of new cases of tuberculosis in large urban population (Dhungana, 2002).

It has been reported that high prevalence of tuberculosis infection and tuberculin test conversion among close contact of pulmonary tuberculosis patients and PTB/HIV co-infected patients were less infectious than HIV negative PTB patients.

3.1.7 Laboratory diagnosis of TB

The definitive diagnosis of tuberculosis in laboratory is based on the detection of acid-fast bacilli in clinical specimens by microscopy, cultural techniques or by Polymerase Chain Reaction (PCR). Numerous attempts have been made to develop serological tests for the disease with little success (Grange, 1998). The diagnosis is established when tubercle bacilli are identified in the sputum, urine, body fluids, or tissues of the patients.

For the diagnosis of Tuberculosis (TB) in HIV positive people require specialized equipments and a well equipped laboratory because patients with smear negative TB constitute a significant proportion of HIV infected adults with respiratory disease. Similarly, tuberculin skin test may be less useful in people with HIV because immune response might be too weak in such persons. With minimal or no finding in chest X-ray, sputum negative for AFB and sputum culture being often unhelpful, additional tests are needed to arrive at the correct diagnosis. Such additional diagnosis methods for determination of mycobacteria infection in HIV infected people include the following:

- Mycobacterial culture of bronchoalveolar lavage (BAL)
- Bronchoscopy
- Polymerase Chain Reaction (PCR) using BAL fluid
- Blood culture on Bactec 460 (i.e. Radiometric method based on principle of monitoring 14 CO_2 produced during growth of mycobacteria).
- Blood culture on middle brook 7H4 agar.
- Rapid mycobacterial detection by mycobacterial growth indicator tube (MGIT).
- High performance liquid chromatography (HPLC)
- Serological Surveys

However, developing countries like Nepal where routine use of such sophisticated technique is troublesome, must relay on following conventional methods for diagnosis of TB in HIV/AIDS patients:

- Sputum microscopy
- Sputum Culture
- Tuberculin Test
- CSF investigation
- Examination of lymph node aspirates
- Biopsy.

Specimens: For the diagnosis of pulmonary TB, the most usual specimen is sputum but, if none is produced, bronchial washings, brushings, laryngeal swabs, and early morning gastric aspirates (to harvest any bacilli swallowed overnight) may be examined. Tissue biopsies are homogenized by grinding in Griffth's tubes for microscopy and culture. Cerebrospinal fluid, pleural fluid, urine and other fluids are centrifuged and the deposits are examined (Grange, 1998).

Contaminated samples are rejected in the laboratory. Saliva sample is rejected during sputum sample collection. Blood mixed CSF samples are also not accepted for microbiological examination.

a. Sputum microscopy

In high prevalence countries, TB case detection is largely based on microscopic examination of sputum for Acid-Fast Bacilli (AFB). The technical guidelines of WHO and International Union Against Tuberculosis and Lung Diseases (IUATLD) specify that this should be done by examination of three samples- the first spot, early morning and the second spot. It has been recommended that a minimum of 100 microscopic fields should be examined for maximum yield. A minimum of 10 AFB/100 fields is taken as the threshold for considering a result as positive, a definite case should have at least one such results confirmed by a second smear examination, a suggestive chest radiograph or alternatively there should be one positive mycobacterial culture result.

In microscopic examination, two types of staining methods are widely used in laboratory to detect AFB in clinical specimens. The staining characteristics of *M. tuberculosis* allow its rapid identification in clinical specimens. The specificity of stains for AFB typically is 99% or more and the sensitivity ranges form about 25% to about 75%. About 95% of infectious cases thus be detected by AFB microscopy. The main value of AFB-microscopy for diagnosis lies in its speed and extremely high specificity, while the main disadvantage is its low sensitivity. A high proportion (75%) of pulmonary cases positive in culture are also positive on smear. But, microscopy cannot distinguish between live and dead AFB, so that

some patients excreting non-viable bacilli at the end of treatment may be roughly considered as failure-cases (Duen, 2001).

AFB microscopy by Z-N staining method

Z-N staining method is widely used carbol fuchsin method to detect AFB in smears by microscopic examination of specimens. This method is a modification of Ehrlich's (1882) original method. It is also called 'hot stain' method because carbol fuchsin is heated for the better penetration in cell wall of mycobacterium. In Z-N stained smears, AFB typically appears as purple to red slightly curved rods (1-10um X 0.2-0.6 um) that occasionally are beaded or banded but also may appear coccoid or filamentous.

Fluorescence microscopy

This is the screening procedure recommended for those laboratories that posses a fluorescent (ultraviolet) microscope. With the flurochrome stain, such as auramine rodamine stain, Mycobacteria fluoresces with rodamine stain, with a bright orange color and can be easily seen on low power microscopy, increasing the sensitivity of the smear. This fluorescence method allows large numbers of specimens to be examined rapidly. When prepared smear is stained with fluorescent auramine-rhodamine, tubercle bacilli can be seen under usual high (100X) magnification.

b) Sputum culture

Sputum culture is the definitive diagnosis of tuberculosis by isolating the causative organisms in pure culture. So, culture remains the "gold standard" for diagnosis of tuberculosis. Diagnosis of tuberculosis by culture method is more sensitive than AFB staining method and can reliably find mycobacteria when they are present in a concentration of about 10^3 organisms/ml of specimen. Depending on the decontamination method and the type of culture medium used, as few as ten viable tubercle bacilli can be detected.

Media of choice

Solid media

(a) Egg based

Lowenstein Jensen (LJ) Media

Ogawa Media

Dorset egg Media

(b) Agar based media

Middle brook 7H10 and Middle brook 7H10Se

Middle brook 7H11 and Middle brook 7H11Se

Liquid media

BACTEC 12B broth

Middle brook 7H9 broth

Among these different types of media, the routinely used media are LJ and Ogawa. Liquid media are used for sensitivity test, biochemical test and preparation of antigens and vaccines. To prevent overgrowth by contaminants, a cocktail of antibiotics such as PANTA (polymixin, amphotericin, Nalidixic acid, Trimethoprim and Azlocillin) are added to the liquid media (Dhungana, 2004).

As the sputum specimens are submitted to the TB laboratory, are contaminated to varying degree by more rapidly growing normal flora, the specimens should be subjected to digestion and decontamination. Decontamination methods make use of the relatively high resistance of mycobacteria to acids, alkalis and certain disinfectants. In the widely used Petroff method, sputum is mixed well with 4% sodium hydroxide for 15-30 minutes, neutralized with potassium dihydrogen orthophosphate and centrifuged. The deposit is used to inoculate LJ or similar media (Grange, 1998).

Inoculated culture media are usually incubated at 35^0C to 37^0C in atmosphere of 5 to 10% CO_2 for at least 8 weeks and tubed media should be incubated in slanted position with caps lose for at least one week to ensure even distribution of inoculation over the surface. Most

strains of *M. tuberculosis* appear within 4 weeks, but may not be visible for 8 weeks or more if they originated from patients treated with anti-tuberculous agents.

c) Tuberculin test

The intracutaneous tuberculin skin test is indirect test method for diagnosis of tuberculosis and reliable means of recognizing prior mycobacterial infection. It is useful for identifying persons infected with *M. tuberculosis* complex (MTBC), but does not differentiate active disease from infection. Persons infected with MTBC develop a hypersensitivity reaction to proteins of the bacilli, which comprise the skin test reagent-PPD (Purified Protein Derivative). The preferred method of skin testing is Mantoux test, performed by intracutaneous injection of 0.1ml of intermediate strength (5 tuberculin units) PPD-S.

d) CSF investigation

The patients suspected of tubercle meningitis are generally processed for CSF investigation. Tubercle meningitis is common disseminated TB in AIDS. In AIDS patients suspected of tubercle meningitis; AFB staining and culture of CSF is highly appreciable. Centrifugation of the CSF followed by microscopic examination of the deposit and culture of that deposit increases the chance of detection of tubercle meningitis.

e) Examination of lymph node aspirates

Persistent generalized lymph adenopathy (PGL) is the first symptom (if present, otherwise asymptomatic) to appear in the progression of HIV infection. Thus early detection of suspected lymph node TB can be made by lymph node aspiration (Dhungana, 2004).

f) Biopsy

It is the invasive technique and is used in situation where tubercle bacilli are not frequently shed into body fluids. Isolated involvement of the pleura or other tissues that doesn't communicate externally may be diagnosed through examination of tissue (Rijal, 2005).

In miliary tuberculosis, multiple organs may be seeded with tubercle bacilli. Although organs like lungs and kidneys are frequently involved, in such setting sputum and urine specimens are found to have tubercle bacilli in 20-25% of cases. Tissue examination may be very helpful in such situation. Transbronchial biopsy has been reported to be diagnostic or highly suggestive of tuberculosis up to 85% of patients with miliary changes on the chest X-ray in negative sputum study (Rijal, 2005). Liver and bone marrow biopsy are important for diagnosis of miliary tuberculosis up to 40-90%.

g) Newer methods for tuberculosis diagnosis

With the recent advances in the laboratory diagnosis of tuberculosis; techniques are more directed towards the development of rapid culture identification and drug susceptibility system for use in TB specialist laboratories.

Serological test for the rapid diagnosis of tuberculosis that are based on the recognition of serum IgG antibody to selected mycobacterial antigens and that use ELISA techniques have been developed and appear with sensitivity similar to that of sputum microscopy. Serology has its greatest application in children and in patients with Extra-Pulmonary tuberculosis where sputum is not available. Gene amplification by PCR has been used with great sensitivity and specificity to identify mycobacterial DNA. This technique offers great promise for rapid diagnosis. In reference laboratories with sufficient instrumentation, high-performance chromatographic techniques are capable of rapidly identifying mycobacteria by their characteristic lipids (Issaelbacher *et al*., 1992).

Other newer techniques that has been applied for the diagnosis of tuberculosis are as follows:

Bactec 460 TB rapid radiometric Culture System

Bactec 9000 MB System

Septi-Check AFB System

Mycobacteria Growth Indicator Tube (MGIT)

ESP Culture System II

Chromatographic Analysis

Among these different techniques developed, Polymerease Chain Reaction (PCR) is used extensively for the diagnosis of TB (Forbes and Hicks, 1993). PCR enables the amplification of specific sequences of target nucleic acids. It is not only simple and fast, but also very sensitive and specific to amplify even a single molecule of DNA.

With the increased incidence of TB and the advent of MDR-TB strains, the demand of PCR is high in developing countries. The PCR microplate hybridization assay was also sensitive enough to detect as little as 1 pg of DNA which is equivalent to approximately three bacilli. Recently, a commercial PCR amplification kit for the detection and identification of *M. tuberculosis* complex bacteria has become available. The target for the PCR is the 16S rRNA sequence. The detection system is based on hybridization with *M. tuberculosis* complex specific capture probe in a microplate format.

h) Radiological diagnosis

For the diagnosis of TB, radiological methods have been the best options for the physicians. However, TB is difficult to diagnose with certainty on an X-ray alone. X-rays are expensive, unreliable as patients are often treated for TB when they do not have it. But X-ray are sometimes needed for difficult individual problems in particular for HIV infection. So, the chest radiograph is an important tool for both diagnosis and evaluation of TB. Multi-nodular infiltration in apical posterior segments of the upper lobes and superior segments of the lower lobes is the most typical lesion of PTB. Cavitation is frequently present and is usually accompanied by substantial amounts of infiltration in the same pulmonary segments. Laminagrams are very helpful in recognizing satellite nodular lesion, which are characteristics of TB and not usually seen in carcinoma (Issaelbacher *et al.*, 1992). Radiology suffers mainly of a lack of specificity. The place of radiology will thus be restricted to the second-line of diagnosis, in hospitals and used by medical officers for cases that stay negative on repeated smear microscopy (Duen, 2001).

i) Clinical diagnosis

Clinical signs and symptoms can be regarded as the basis for the diagnosis of tuberculosis. A careful history and physical examination often suggest the diagnosis of PTB before any laboratory test is ordered. Symptoms suggestive of Pulmonary TB are a) Persistent cough with expectoration for 2 weeks, b) Rise of body temperature in the evening, c) Chest pain, d) Weight loss, e) Loss of appetite, f) Haemoptysis (coughing up of blood in sputum), g) Lethargy, spontaneous pneumothorax. Chronic cough for 2 weeks or more (usually with haemoptysis) with or without fever and chest pain is the principal respiratory symptoms of pulmonary tuberculosis. With the progression of pulmonary tuberculosis, the normal pulmonary architecture is lost. Fibrosis, volume loss and upward contraction are typical. Rales that are accentuated or heard only pottussively are characteristics of apical disease. Amphoric breath sounds may be present with extensive cavitations (Issaelbacher *et al*., 1992).

The signs and symptoms of Extra-pulmonary tuberculosis depend on the organ involved. Swelling in the neck with or without discharge is symptom of lymph node tuberculosis. Symptoms like Headache, fever, drowsiness, confusing and neck rigidity may be suggestive of tuberculosis meningitis. Back pain, fever and in some cases swelling of the backbone are symptoms of spinal tuberculosis.

3.1.8 Identification tests

Identification tests are mainly done to confirm whether the isolate is a tubercle bacilli or mycobacteria other than tuberculosis (MOTT). *M. tuberculosis* can be differentiated from MOTT by the growth rate, pigmentation and some biochemical tests.

M. tuberculosis grow slowly, produce rough, tough and buff colored (not yellow) colonies, reduce nitrite to nitrate, gives niacin test negative and do not produce catalase at 68^0C. Thus a series of biochemical tests in combination with the observation of growth rate and pigmentation characters of mycobacterium aid to encounter mycobacterium species.

3.1.8.1 Growth rate

Observation of growth rate not only helps to separate *M. tuberculosis* from MOTT but also helps to distinguish different members of MOTT. For e.g. *M. avium* complex (slow grower) can be distinguished from *M. fortuitum-chelonae* complex (Rapid Growers). On the basis of growth rate mycobacterium are classified as:

- Slow grower (>7 days)
- Rapid grower (2-3 days)

3.1.8.2 Pigmentation

Presumptive identification of certain pigment producing mycobacteria can be done by the color of their colonies. For e.g. *M. tuberculosis* produce buff colored colonies, *M. kansasii* produce bright yellow or orange colors after 2 weeks of ambient light color whereas MAC (mycobacterium avium complex) do not produce color.

3.1.8.3 Niacin test

Niacin (Nicotinic acid) plays a vital role in the oxidation-reduction reaction that occurs during metabolic process in all mycobacteria. Although all mycobacteria produce niacin, comparative studies have shown that, because of a blocked metabolic pathway, *M. tuberculosis* accumulates the largest amount of nicotinic acid and its detection useful for its definitive diagnosis. Niacin negative *M. tuberculosis* strains are very rare, while very few other mycobacterial species yield positive niacin tests.

3.1.8.4 Nitrate reduction test

With combination of niacin test, nitrate reduction test can be used to differentiate *M. tuberculosis* from other mycobacteria as *M. tuberculosis* is one of the strongest reducers of nitrate among the mycobacteria. Culture isolates to be tested for nitrate reduction should be four weeks old and have abundant growth on Lowenstein-Jensen egg medium are recommended (Dhungana, 2004).

3.1.8.5 Catalase test

All mycobacteria posses catalase enzymes except for certain isoniazid resistant mutants of *M. tuberculosis* and *M. bovis*. As *M. tuberculosis* loses catalase activity at 68^{o}C, a performance of catalase test at this temperature is done for its identification.

3.2 HIV/AIDS

AIDS stands for Acquired Immuno Deficiency Syndrome, a pattern of devastating infections caused by a virus, which attacks and destroys certain white blood cells that are essential to the body's immune (defense) system. AIDS is also defined as an illness characterized by one or more reliably diagnosed diseases which are at least moderately indicative of cellular immunodeficiency; and where there is an absence of known underlying causes of cellular immunodeficiency or other causes of reduced resistance to infection. As the virus attacks and causes destruction and weakening of the body's immune system it is known as Human Immunodeficiency virus or HIV. The time period between HIV infection and the development of severe immunosuppression and AIDS is long and variable. AIDS represents the late clinical stage of infection. Patients with AIDS are considered to have CDC group IV disease and its diagnosis remains, in essence, a clinical definition.

In fact when HIV infects a cell, it may lie inactive for years and most of the people infected with HIV does not show any symptoms or may show only minor illness for 7-10 years. Those people infected with HIV can spread the infection to others but still they do not have AIDS (UNAIDS, 2004).

Gradually the virus becomes activated and breaks down the human body's natural defense mechanisms leaving it a prey to other opportunistic infections (among which TB is the most common) and other conditions including cancers that characterize AIDS (WHO, SEARO, 1999).

Till now there is neither any vaccine to prevent the AIDS nor any treatment to cure AIDS, presently available treatment can only extend life. So, for the moment prevention of transmission of infection remains the only method of control.

3.2.1 A history of the epidemic

A pattern of highly unusual infections in otherwise healthy young adults not responding to usual treatment emerged in the United States in 1981. This pattern, or syndrome, (symptom complex) was caused by an unknown entity that apparently attacked the body's immune system. It became known as AIDS. Between 1983 and 1984 researchers isolated a new virus responsible for AIDS and named it as HIV (UNAIDS, 2004).

Though AIDS was first recognized in the United States in 1981, it is clear that AIDS cases had occurred in several parts of the world before 1981. Evidence now suggests that the AIDS epidemic began at roughly the same time in several parts of the world, including the U.S.A. and Africa (UNAIDS and GTZ, 2003).

Although homosexual men from the United States and other developed countries were the first reported cases of AIDS worldwide, the scenario rapidly changed into that of global epidemic (pandemic). By early 1989, more than 140000 AIDS cases including men, women and children had been officially reported to WHO from around the world (UNAIDS and GTZ, 2003).

According to the latest estimate as of end 2003 an estimated 38 million people around the world were living with HIV/AIDS. During the year 2003, an estimated 5 million people acquired new infection. The epidemic claimed an estimated 3 million lived in 2003 (UNAIDS, 2004).

3.2.2 Etiological agent

AIDS is a fatal disease state caused by Human Immuno-deficiency Virus (HIV) 1 and 2, belonging to the retroviridae family and lentivirus sub-family. The causative agent of AIDS

was identified in 1984 almost simultaneously by research teams from the Pasture Institute (Paris) and the National Cancer Institute (United State). The French group, headed by Luc Montagnier, named the virus lymphadenopathy-associated virus (LAV), while the American group, led by Robert Gallo, called it the human T-cell lymphotropic virus type III (HTLV-III). In 1986, an international commission recommended the single name of Human Immunodeficiency Virus (HIV). In 1985, another retrovirus subsequently named HIV-2 was isolated in Africa. By 1993, French Luc Montagnier was acknowledged as the discoverer (Alcamo, 1997).

3.2.2.1 General properties of HIV

Human Immuno-deficiency Virus (HIV) is a unique type of virus that utilizes its RNA for replication, rather than DNA in mammalian genetic material. HIV has its genetic message in RNA. HIV-1 exhibits a characteristic dense, cylindrical core surrounded by a lipid envelop. HIV-1 is a single stranded plus-sense RNA virus. Reverse transcriptase is packaged within the virion core and is responsible for replication of the single-stranded RNA genome through a double stranded DNA intermediate.

The major structural core proteins are the p24 capsid protein and p18 matrix protein. The outer bilayered lipid envelope consists of envelope glycoproteins gp120 and gp41, which are encoded by viral specific gene and are responsible for cell attachment and entry. The HIV-1 genome is diploid, consisting of two identical viral RNA molecules assembled in a hydrogen bonded 70S complex. HIV-1 genome contains genes encodes structural and enzymatic proteins, including *gag*, *pol*, and *env*, but in addition it encodes a group of at least six additional regulatory proteins (*vif*, *vpr*, *vpu*, *tat*, *rec*, and *nef*) whose activities are critically important in regulating the life cycle and pathogenesis of virus. Though HIV-1 and HIV-2 are 40-50% similar in their overall nucleotide sequence homology, there are major differences in the genomic organization. HIV-1 contain *vpu* gene, which is not present in HIV-2 whereas HIV-2 contain an additional gene, *vpx* in its central region that is not present in HIV-1 (Dhungana, 2002).

3.2.2.2 Physical and chemical stability of HIV

HIV is completely inactivated ($\geq 10^5$ units of infectivity) by treatment for 10 minutes at room temperature with any of the following: 10% household bleach, 50% ethanol, 35% isopropanol, 1% Nonidet P40, 0.5% Lysol, 0.5% paraformaldehyde, or 0.3% hydrogen peroxide. The virus is also inactivated by extremes of pH (pH 1.0, pH 13.0). However, when HIV is present in clotted or unclotted blood in a needle or syringe, exposure to undiluted bleach for at least 30 seconds is necessary for inactivation (Brook *et al.*, 2004).

The virus is not inactivated by 2.5% Tween 20. Although paraformaldehyde inactivates virus free in solution, it is not known if it penetrates tissues sufficiently to inactivate all virus that might be present in cultured cells or tissue specimens.

Several studies have addressed the stability of HIV-1 with respect to variety of chemical and physical stresses. Survival of HIV-1 is reduced rapidly on drying (90% to 99% over several hours), although high concentration ($1x10^7$ TCID 50/ml), survival can extend for 1 to 3 days. In tissue culture fluid, cell free HIV-1 can be detected up to 15 days at room temperature, up to 11 days at 37^oC, and up to 1 day if virus is associated. At higher temperature ($56\text{-}60^oC$), HIV-1 is inactivated in approximately 30 minutes (Grange, 1998).

3.2.3 Pathogenesis of HIV

As the AIDS pandemic moves into its second decade, concepts regarding the mechanisms of HIV pathogenesis have made important contributions to the understanding of disease. Patients with AIDS are profoundly immunosuppressed. It was recognized early that the ratio of T helper to T suppressor (T4:T8 or CD4:CD8) lymphocytes in the blood was upset. In fact, the number of T helper cells declines during the asymptomatic period until it reaches a level where resistance to infection is seriously impaired. Monitoring the number of T4 cells is a useful guide to the onset of AIDS; in addition, the number of T8 cells increases as disease develops. In the circulation the virus is found in T4 cells and also in monocyte-macrophage cells, which may act as a reservoir for virus. Macrophages are also important in carrying the virus into the central nervous system across the blood-brain

barrier. The proportion of infected T4 cells rises as the infection progresses. Virus is also present in the plasma, and this increases with time too. Titres rise as the patient becomes symptomatic. Activation of latently infected lymphocytes can be achieved by contact with foreign antigen and lectins such as phytohaemagglutinin (PHA).

The mechanism explaining the actual loss of CD4 T cells is still unresolved. Specifically, it remains to be determined whether the virus can directly account for CD4 cell loss via infection followed by programmed cell death (apoptosis) or whether indirect mechanisms, such as gp120-CD4 antibody cross-linking, viral protein-induced cellular dysregulation or antiviral bystander effects are involved (Folks amd Khabbaz, 1998).

3.2.4 Cellular immune response to HIV

The Human Immune deficiency Virus is an RNA enveloped virus. Moreover, it is a retrovirus i.e. capable of propagating itself. The membrane envelope of the virus contains two linked glycoproteins, gp120 and gp41.

HIV invades host cells that are CD4+ like Th cells and cells of monocytes/macophages lineage, such as, dendrites cells of lymphoid tissues and skin. This process happens by binding of gp120 to CD4 molecules and then entering these cells carrying this marker. After that, HIV coat opens, RNA virus enters the cells and DNA is created from RNA by the enzyme reverse transcriptase. Viral DNA, then, is integrated into cellular DNA of the host cells. Consequently, cell products will include new virus structural components (Roitt *et al.,* 1998).

HIV contains a number of proteins that can be recognized by the Th cells as epitopes. Some of these epitopes are immunodominant i.e. frequently recognized by T cells of infected subjects. T1 and T2 are the two immunodominant epitopes on gp 120. (identified by Cease & colleagues). In vitro, IL2 is recognized and secreted by T cells of 85% of HIV infected subjects in response to one of the two epitopes. Moreover, asymptomatic sero-positive subjects show high proliferative response to HIV gp120, which decreases with the

onset of AIDS. The MHC class I-restricted CTL rise and proliferate in response to HIV protein. However, it decreases as HIV progresses to AIDS. Monocytes take a part in mediating ADCC against HIV-coated target cells in asymptomatic sero-positive subjects (De *et al.,* 1992).

However, the immune cells response to HIV decreases by time and several immunologic abnormalities can be detected.

The major immune cells affected by the virus are T4 (CD4+). As has been mentioned previously, the CD4+ molecule is the cellular receptor for the virus. This will not only lead to a quantitative defect in Th (Decrease in T4 number resulting in lymphopenia, the cytotoxic (CD8+) usually are normal or slightly elevated in amount. CD4: CD8 ratio decrease from 2 to less than 1% but the function of infected cells is also defected.

B cells are also affected by HIV. In addition to inadequate regulation by T cells, B cells are polyclonally activated resulting in increased level of circulating immunoglobulins. However, B cells will lose the ability to produce antibodies in response to a new antigen (Harawi and Ohara, 1989).

Monocytes/macrophages are the third type of cells affected by HIV. The ability of these cells for intracellular killing following phagocytosis is decreased. Beside that, monocytes/phagocytes become unable to respond to a variety of chemotactic stimuli, such as, EMLP and LDCF. Decreased expression of class II HLA antigens (HLA-DR) on circulating monocytes have been found. Two possible reasons for the reduced expression of HLA antigens, this can be due to either the lack of gamma interferon and other monocytes stimulating lymphokins or direct infection of monocytes/macrophages with HIV (Flaskeurd, 1989).

3.2.5 HIV as major public health problem

The HIV/AIDS epidemic continues to grow worldwide and posses a huge human and economic loss. HIV pandemic presents the global and public health communities with one of the most significant challenges. In one hand there is no widely accessible and effective chemotherapy and in other hand, the epidemic has mushroomed globally into an unforeseen and unpredicted nightmare.

HIV infection reveals varying patterns of transmission and evidence suggests that the impact globally has disproportionately affected the more vulnerable and marginalized persons with in the societies e.g. injecting drug users, commercial sex workers, migrants, poor, uneducated women and children. 95% of HIV infected people are living in less industrialized, developing countries. As most of the population of sub-Sahara African region has already been swept away to the HIV epidemic and HIV has been well established in Asia for many years. From recent studies, it has been shown that India has the single largest proportion of HIV positive cases within its border, second globally to South Africa. So, it can be well anticipated that sooner or latter Nepal may also be along with the list of these high burden nations if immediate action is not taken to fight this disease (Dhungana, 2004).

3.2.6 Epidemiology

3.2.6.1 The global problem

The HIV/AIDS epidemic is spreading throughout the world with ferocious speed. HIV has infected more than 60 million people worldwide, More than 20 million have died form AIDS, with 3 million dying in 2000 alone. There were around 40 million people living with HIV/AIDS at the end of 2002. Approximately, 14,000 new infections occur each day, more than half are among these below age 25. Over 95% of PLWHA are in low and middle-income countries (Stephen *et al*, 2006). Globally, only a small number of HIV infections are estimated to have occurred during the late 1970s and early 1980s. During the 1990s, HIV prevalence increased remarkably in sub-Saharan Africa. HIV has been well

established in Asia for many years. The HIV/AIDS epidemic is spreading rapidly in all South Asian countries. India has the single largest proportion of HIV positive cases within its border, second globally to South Africa. Over 4 million estimated HIV infections had been reported within the region and about 13,000 AIDS cases were reported by the year 2000 (STC, 2004).

At the end of 2003, some 37.8 million people (range 34.6-42.3 million) around the world were living with HIV/AIDS. An estimated 4.8 million people (range 4.2-6.3 million) acquired the HIV virus (infection) in 2003. The AIDS epidemic claimed 2.9 million lived (range 2.6-3.3 million) in 2003, and over 20 million since the first cases of AIDS were identified in 1981. More than 94% of the people living with HIV/AIDS are adults aged between 15-49 years and the rest (5.6%) are children aged below 15 years. Among the adults living with HIV/AIDS 47.6 % are women. The new infections included an estimated 6,30, 000 children comprising over 13 % of the total new infection (UNAIDS, 2004).

According to new estimates the total number of people living with HIV/AIDS after 9 years of its first detection in 1981 became nearly 10 million. The number became double (20 million) after another 4 years and became tripled in 1998. At the end of 2003 it became 38 million. In 1990 the adult (15-49 yr) rate of HIV infection was less than 0.5% and it has been increased to 1.1% at end of 2003 (STC, 2004).

HIV/AIDS has become pandemic in almost all countries of the world. There is no region throughout the world that has been spared by the scourge of HIV (Subedi, 1997). Sub Saharan Region of Africa is the worst hit (24 million cases) region in the world (UNAIDS, 2000). Since the HIV/AIDS epidemic has begun, almost 58 million people through out the world have been infected with HIV and almost 22 million people have died. HIV has continued to spread, causing more new infections everyday. Today, AIDS is the leading cause of death in Africa, and the fourth worldwide (Subedi, 1997).

According to the latest figures published on 21 November 2006 in the UNAIDS/WHO 2006 AIDS Epidemic Update, an estimated 39.5 million people are living with HIV. There

were 4.3 million new infections in 2006 with 2.8 million (65%) of these occurring in sub-Saharan Africa and important increases in Eastern Europe and Central Asia, where there are some indications that infection rates have risen by more than 50% since 2004. In 2006, 2.9 million people died of AIDS-related illnesses (UNAIDS/WHO, 2006).

3.2.6.2 HIV/AIDS in Asia

HIV/AIDS in Asia was first detected in the early to mid-1980s. Thailand was the first Asian nation to report HIV infection followed by an explosive epidemic. Moreover, initial AIDS cases were detected among MSM in several Asia Pacific countries such as Australia, Japan, Malaysia, New Zealand, Singapore, and Hong Kong during the early 1980s. Alternatively, extensive spread of HIV occurred in MSM sex workers in these Asia Pacific countries and such transmission probably peaked during the mid to late 1980s.

By the mid–to-late 1980s, it became evident that transmission of HIV was also increasing among other major HIV-risk behaviour groups within Asia. High HIV prevalence (up to 50% or more) was documented among female sex workers (FSW) in Thailand and in parts of India, notably Mumbai, during the mid–to-late 1980s. In addition, intense focal HIV epidemics were documented in Thailand, parts of north-east India**,** and the "golden triangle" area (where the borders of China, Myanmar and Thailand meet) in IDU populations beginning around the mid–to-late 1980s. 60% of all people with HIV in Asia are living in India (Stephen *et al*., 2006)

An explosive spread of HIV within IDU populations, which can lead to infection levels of over 50% within a year or two, continued to occur in several provinces of China, north-east India, Malaysia, Myanmar, Pakistan, Thailand and Viet Nam, and most recently, in the late 1990s, within Indonesia and Nepal (STC, 2004).

The estimates suggested that 7.4 (5.0 – 10.5) million people in Asia were living with HIV in 2003. During the year 2003, an estimated 1.1 million (range: 0.61-2.2 million) people have become newly infected and around half a million (range: 330 000 – 740 000) were

believed to have died of AIDS. Epidemic in this region remains largely concentrated among injecting drug users, sex workers, men who have sex with men, clients of sex workers and their sexual partners. But the region is also under threat of generalization of the epidemic (UNAIDS, 2004).

The region includes the world's most populous countries – china and India with 2.25 billion people between them. Though national HIV prevalence in these two countries is very low, both have extremely serious epidemics in a number of provinces, territories and states. India has the largest number of people living with HIV outside South Africa- estimated at 4.6 million in 2002. People's knowledge about HIV/AIDS in this region including India is poor and incomplete. Risk behaviour is on the rise and effective prevention programming coverage is inadequate (UNAIDS, 2004).

3.2.6.3 HIV/AIDS in Nepal

Nepal is a landlocked country sharing borders with India and China. In Nepal, the topography, environmental degradation, poverty and economic migration are linked with increase of the vulnerability to HIV (STC, 2006).

The first HIV infection in Nepal was identified in 1988. During the early 1990s, HIV seroprevalence survey detected HIV infection among STI patients and FSW throughout most regions in Nepal. Since 1988, the number has risen among the country's 27 million people. By the end of 2005, more than 950 cases of AIDS and over 5,800 cases of HIV infection were officially reported, with three times as many men reported to be infected as women. UNAIDS estimated that 75,000 people were living with HIV at the end of 2005.

IDUs in Nepal were initially believed to share injection equipment in relatively small and isolated networks. However, since the mid 1990s, an explosive increase in HIV infection (infecting about one-half of all IDU throughout the country and near about two-third in the Kathmandu valley) has occurred.

Nepal's HIV epidemic is largely concentrated in high-risk groups, especially female sex workers (FSW) and IDUs. Injecting drug use appears to be extensive in Nepal and to significantly overlap with commercial sex. Another important factor is the high number of sex workers who migrate or are trafficked to Mumbai, India to work, thereby increasing HIV prevalence in the sex workers' network in Nepal more rapidly. Poverty, ignorance and conflict are the root causes in undertaking high-risk behaviour (Singh *et al.,* 2005).

As reported to the National Center for AIDS and STD control, Teku, Kathmandu, Nepal, the cumulative number of HIV positive cases as of May 2007 is 9532. Among them, 69% were males and 31% were females with a male: female ratio of 2.3:1. Out of these total HIV positive cases, 1410 were full blown AIDS cases; 72% male and 28% female with male: female ratio 2.6:1. A total of 412 deaths due to AIDS were reported (NCASC, 2007).

3.2.7 Modes of transmission

The main modes of HIV transmission are through sexual intercourse, blood and from mother to child transmission (MTCT). Worldwide the most common route of HIV transmission is through unprotected sexual intercourse. Using anal route, presence of other sexually transmitted diseases (STD) (such as genital ulcers and discharges) and having multiple sex partners increase the risk of transmission. The risk increases four-six-fold particularly in presence of genital ulcer disease e.g. syphilis, chancroid or herpes. Blood borne HIV transmission occurs through contaminated blood or blood product transfusion, injections with contaminated needles and syringes, and the use of non-sterile instruments for piercing of ear, nose or skin. HIV is also transmitted from infected mother to their children during pregnancy, during childbirth or even through breast-feeding, chance of HIV transmission through breast-feeding is small (STC, 2006).

Sexual transmission: HIV-1 is transmitted by both homosexual and heterosexual contact and, as with other sexually transmitted infections, the likelihood of infections is related to numbers of sexual partners as well as to different sexual practices. The exchange of body

fluids such as semen, vaginal secretion during sexual intercourse acts as vehicle of infection.

Transmission via blood: Plasma, clotting factors, cellular blood component and whole blood have all transmitted HIV-1 infection, whereas other blood products (e.g., immunoglobulin, albumin, and hepatitis B vaccine) have not been implicated. A single unit of blood from an HIV infected persons nearly always transmits HIV-1 to the recipient. HIV is also transmitted among injecting drug users through use of contaminated needles and other equipment. Risk factors include frequency of needle sharing, frequency of injections, use of 'shooting galleries' and prevalence of HIV infection in the area. The risk of sustaining HIV infection from a needle stick with infected blood is approximately 1:300 (Dhungana, 2002).

Perinatal transmission: Transmission from an HIV-infected mother to the newborn has been shown to occur across the placenta but may also occur at the time of delivery through exposure to an infected genital tract or postnatally through breast-feeding. The rate of perinatal transmission has ranged from 13% to 40% with highest rates of being reported from Africa where rates of breast-feeding are highest and the severity of maternal HIV disease has been greatest.

3.2.8 Clinical features of HIV

The natural course of HIV infection is as follows:

Seroconversion illness - This is seen in 10% of individuals a few weeks after inoculation and coincides with seroconversion. These patients present with a mononucleosis like illness that comprises of fever, sore throat, enlarged lymph nodes, skin rash, joint aches and general malaise.

Incubation period -This is the period when the patient is completely asymptomatic and may vary from a few months to a more than 10 years. The median incubation period is 8-10 years. Children tend to have a shorter incubation period.

AIDS-related complex or persistent generalized lymphadenopathy - At the end of the incubation period, a number of signs and symptoms may appear which do not fulfill the definition of AIDS or other HIV-associated syndromes. These include slight immunological, dermatological, haematological and neurological signs. Constitutional symptoms, such as fever, weight loss, night sweats, and diarrhoea may develop. Generalized lymphadenopathy may be seen. Laboratory findings may show a decrease in the CD4 count, hyperimmunoglobulinaemia and cytopenias.

The lymphadenopathy syndrome is defined as the enlargement of lymph nodes to 1 cm or more at 2 or more body regions that persists for more than 3 months without any other recognizable cause other than HIV infection. AIDS-related complex is defined as fever, weight loss, night sweats or chronic diarrhoea of more than 1 month's duration in the presence of disturbances of CMI and in the absence of any other recognizable cause other than HIV infection. These definitions may be in part overlapping and are not mutually exclusive and they can be described generally as pre-AIDS.

AIDS - The first manifestation of HIV infection may be noted at any disease stage and the different stages may not occur consecutively. The transition to full-blown AIDS may occur rapidly or slowly. The disease progression is probably influenced by cofactors, such as other virus infections, stress, genetic makeup of the individual etc. Poor prognostic factors include the serial decrease in the number of CD4 lymphocytes, the reappearance of HIV antigen in the blood, the decline or disappearance of anti-core antibodies, and increased levels of B_2-microglobulin and neopterin. The diagnosis of AIDS is established with the appearance of opportunistic infections, or of certain neoplasms, such as Kaposi's sarcoma, primary lymphoma of the brain and other non- Hodgkin's lymphomas. In the US, the diagnosis of AIDS is also established by the finding of CD4 count of less than 200 cells/mm^3.

3.2.9 Diagnosis of HIV

A specific virological diagnosis of HIV infection can be achieved by:

1. Isolation of the virus in culture.

2. The detection of viral components, e.g. p24 antigen, by direct assay in the plasma or detection of proviral DNA or RNA (after reverse transcription) and amplification by the polymerase chain reaction (PCR)
3. The presence of antibody to HIV antigens in the serum (Grange, 1998).

Virus isolation and detection

Isolation of virus is a slow process, taking from 3-6 weeks. The usual sample is blood, from which the lymphocytes are separated and co-cultured with PHA-stimulated donor lymphocytes. Virus presence is detected by assays for reverse transcriptase and p24 antigens in the culture fields.

Detection of viral components

Detection of virus i.e. isolation of HIV is not routinely used for diagnosis. As an alternative to virus isolation, the detection of circulating viral antigen may be diagnostically useful because person infected with HIV, particularly early in the infection, may have circulating p24 antigen and Reverse Transcriptase (RT) antigen in their sera (Richard and Weiss, 1990).

Amplification by PCR can be a useful diagnostic tool in the investigation of patients with indeterminate serological results, and to detect infection in the early stages as in the partners of infected cases. The diagnosis of infection in babies born to infected mothers may require the use for anti-HIV for up to 18 months until maternal IgG disappears; if antibody persists beyond this time, this indicates infection. However, some babies do not produce anti-HIV. Detection of p24 antigen or a positive culture establishes that the baby is infected, but the PCR may be invaluable in confirming infection (Grange, 1998).

Serological detection of antibody to HIV antigens

This was the first practical approach and many different assays are available nowadays, most using enzyme tracing of the reaction (enzyme-linked immunosorbent assay or ELISA). The diagnosis of HIV infection is usually made on the basis of the detection of

antibodies to HIV in serum or plasma. Serological tests for detecting antibodies to HIV are generally classified as screening tests (sometimes referred to as initial tests) or confirmatory tests (sometimes referred as supplemental tests). Initial test provide the presumptive identification of antibody-positive specimens, and supplemental tests are used to confirm whether specimens found reactive with a particular screening test contain antibodies specific to HIV. A variety of simple, instrument free initial tests are now available, including agglutination, immunochromatographic and dipstick tests. Most of these tests can be performed in less than 10 minutes and are therefore called simple/rapid (S/R) assays (WHO, 2002).

ELISA test are the first screening test of choice for HIV antibodies. Different types of ELISA can be used depending upon their principal. Indirect ELISA, Competitive ELISA, Immunodot assays, Agglutination assay are different types of ELISA that can be used for screening test.

Confirmatory test

For the confirmation of HIV antibody in serum with a positive result, Western blot (WB) technique is the most widely used assay in a screening test to the date.

3.3 TB/HIV CO-INFECTION

TB and HIV/AIDS are two major public health problems. TB is an old public health problem and despite significant recent progress in the control of TB, different regions still has the disproportionate global burden of TB. Although HIV/AIDS is of relatively recent occurrence, it is rapidly spreading. The prevalence of HIV/AIDS in general population is still low but its prevalence has dramatically increased among high-risk groups in the world and it is simply a matter of time before it spreads to the general population. HIV infection has dramatically changed the epidemiology and clinical status of TB in the world. HIV/AIDS epidemic fueling each other in deadly spin (Bam and Rahman, 2002). One in three HIV infected people worldwide is co-infected with the TB bacterium. TB is responsible for death of one out of every three people with HIV/AIDS worldwide. TB

bacterium enhances HIV replication and might accelerate the natural progression of HIV infection. HIV is the most powerful known risk factor for reactivation of latent TB infection to active disease; HIV infected person who become newly infected by TB bacterium rapidly progress to active TB. HIV infection is common among PTB patients in Bangkok and is associated with injecting drug use and higher levels of *M. tuberculosis* antimicrobial resistance (Puntock and Pumprug, 1998).

The interaction between TB and HIV has implications for the public health approach to TB control among HIV-infected people. Untreated HIV infection leads to progression of immuno-deficiency and increased susceptibility to infections, including TB. TB in high HIV prevalence population is a leading cause of morbidity and mortality, and HIV is driving the TB epidemic in many countries (especially in sub-Saharan Africa). TB and HIV programmes therefore share mutual concerns: prevention of HIV should be a priority for TB control; TB care and prevention should be priority concerns of HIV/AIDS programmes (STC, 2004).

TB and HIV are closely interlinked and TB has been the leading cause of HIV-related morbidity and mortality. HIV is the most important factor fueling the TB epidemic in populations with a high HIV prevalence. Collaboration between TB and HIV/AIDS programmes is crucial in supporting general health services providers. To counteract the impact of HIV on TB, other interventions are required apart from effective TB case finding and cure. These interventions include: a) Measures to decrease HIV transmission (e.g. promotion of condoms, treatment of sexually transmitted infections, voluntary counseling and HIV testing, safe intravenous drug use, reduction in the number of sexual partners, prevention of mother to child HIV transmission, HIV screening of blood for transfusion and application of universal HIV precautions by health care workers). b) Antiretroviral therapy (ART) (to improve or maintain immune function in people living with HIV infection) c) Care for people living with HIV infection (e.g. treatment of HIV-related diseases, prevention of HIV-related infections, TB prevention, palliative care and nutritional support) (STC, 2006).

3.3.1 Facts on TB and HIV/AIDS

HIV increases a person's susceptibility to infection with *M. tuberculosis*. In a person infected with *M. tuberculosis*, HIV is a potential cause of progression of tuberculosis infection to active diseases. An individual infected with HIV, has a 30-50 times increased risk of developing TB, than a person who is not infected with HIV. One-third (>12 million) people are dually infected with TB and HIV globally. SAARC bears about 17 % of Global TB and HIV co-infection cases. In HIV infections the immune system is less able to prevent the growth and local spread of *M. tuberculosis*. Therefore disseminated and extra pulmonary TB disease is more common compared with the pulmonary TB. Weight loss and fever are more common in HIV positive patients than in those who are HIV negative. Conversely cough and haemoptysis are less common in HIV positive Pulmonary TB patients than in those who are HIV negative. Lung lesions or clinical picture of Pulmonary TB patients varies according to the stage of HIV infection. In early stage of HIV infection pulmonary TB often resembles post-primary TB with cavitations of lungs. Sputum smear result is always positive. In late stage HIV infections pulmonary TB often resembles primary TB with infiltrating lung lesions with no cavitations. Sputum smear result is always negative. Chest X-Ray changes in the TB/HIV patients reflect the degree of immunocompromise. In early stage of HIV infection (mild immunocompromise) the appearance is often classical with cavitations and upper lobe infiltration. In late stage of HIV infections (severe immunocompromise) the appearance is often a typical. Case fatality is less in TB/HIV patients treated with short course chemotherapy, yet it is higher than the HIV negative TB patients. More HIV infected people die due to TB than due to any other opportunistic infection. Increased incidence of adverse drug reactions may lead to interruption of treatment; facilitating emergence of drug resistant cases among TB/HIV patients (STC, 2006).

3.3.2 Epidemiology of TB/HIV co-infection

3.3.2.1 Global aspect of TB/HIV co-infection

Nearly 39.4 million people were living with HIV/AIDS worldwide, more than half of them in Sub-Saharan Africa and nearly about a fifth in South of South East Asia. By the end of

2000, about 11.5 million people were co-infected with HIV and *M. tuberculosis* globally, 70% of co-infected people were in Sub-Saharan Africa, 20% South East Asia and 4% in Latin America and the Caribbean.

In 2000, 11% of all new TB cases in adults (612 000) occurred in persons infected with HIV, and 9% of all new TB cases were directly attributable to HIV. The prevalence rate of HIV infection in new TB cases varied markedly between regions and countries: from 1% in the WHO Western Pacific Region, to 14% in industrialized countries, and 38% in the WHO African Region; from under 1% in Afghanistan, Bangladesh, China, and Indonesia, to 60% or more in South Africa and Zimbabwe. The proportions of cases directly attributable to HIV were necessarily smaller (e.g., 31% in the WHO African Region).

There were 1.84 million (1.59-2.22 million) deaths from TB in 2000, 226 000 attributable to HIV (12%; range, 8%-15%). The 246 000 (range, 167 000-298 000) TB deaths in adults infected with HIV represented 13% of all TB deaths and 11% of 2.3 million adult AIDS deaths, and most of these deaths (203 000) occurred in Africa. Across all countries, the aggregate CFR of HIV-infected TB cases were 40%. The number of people who died from TB was lower in the WHO African Region (482 000) than in South-East Asian Region (727 000), but the annual death rate was far higher (75 vs 47 per 100 000 population).

Tuberculosis death rates in high-burden countries varied dramatically, from 9 per 100 000 population in Brazil to 139 per 100 000 in South Africa. In these 2 countries overall CFRs for TB were 13% and 27%, respectively, and the difference was due largely to the difference in HIV infection rates.

Assuming lifelong MTB infection, and excluding effects on transmission of the recent increases in incidence in Africa and the former Soviet Union, 30% of the world population (1.8 billion people) carried MTB in 2000. Assuming no shared risk factors, the prevalence of MTB-HIV co-infection among adults aged 15 to 49 years was 0.36%, or 11.4 million people. Co-infection prevalence in adults aged 15 to 49 years equaled or exceeded 5% in 8 countries, all on the African continent. The largest numbers of co-infected adults were in

South Africa (2.0 million), India (1.7 million), and Nigeria (0.9 million) (Corbett *et al.*, 2003).

3.3.2.2 SAARC aspect of TB/HIV co-infection

Over 4 million estimated HIV infection are existing within the SAARC region. As HIV prevalence rate in SAARC region is still low, (<0.1% in Sri Lanka, Maldives, Bangladesh and Bhutan, where as >0.1% to <1% in Nepal, India and Pakistan), the available data show relatively low proportion of TB cases attributable to HIV- 0.8% in India, 0.6% in Nepal and 0.1% in Bangladesh (STC, 2004).

3.3.2.3 TB/HIV co-infection studies

In HIV positive persons, pulmonary tuberculosis is one of the most important opportunistic infection contributing for 67.2% of total AIDS cases (Subedi, 1997). The study conducted by Sherchand *et al.,* 2002 on 376 TB patients having a high risk factor for HIV infection to find TB/HIV co-infection of Nepal reported 6.1% TB/HIV Co-infection rate. Similarly, Napit 2001 reported that in UMHT, Nepal, out of 20 HIV positive hospital inpatients, TB was frequently encountered and made up to 40% (8/20) of the total cases.

According to a study conducted by Ministry of Health, the TB/HIV Co-infection is in a rising trend. It was observed that the total TB/HIV Co-infection infected cases as 14 (in 1988-1991), 99 (in 1992-1997) and 312 (in 1998-2003) (Subedi, 2003).

"A surveillance of HIV infection in patients with TB in Nepal" a study carried out in 2002 in five different testing sites in various parts of Nepal showed that HIV prevalence among TB patients continuous to rise and had increased four fold in the past eight years.

In another study conducted by central department of microbiology, T.U., during 2002 and Nepal Tuberculosis Centre (NTC) in Tansen mission Hospital by Dhungana *et al* showed that HIV prevalence in TB patients as 10.76% (Dhungana, 2002). Similarly, another study conducted by central department of microbiology, T.U., during late 2003, Kathmandu, in

order to find prevalence or respiratory pathogens in HIV infected people showed that 22.22% of PLWHA had tuberculosis (Gautam, 2003). Another recent study conducted by central department of microbiology, T.U., during 2004, in order to study TB/HIV co-infection in HIV/AIDS persons in Kathmandu valley showed 23% of studied population had TB (Dhungana, 2004).

A cross sectional study conducted at National Tuberculosis Centre (NTC, Thimi) lab, clinic and Dr. Iwamura Memorial Hospital and Research Centre, Bhaktapur during late 2005 showed HIV prevalence among diagnosed TB patients to be 1.5%. However, this study showed low HIV prevalence among TB patients in comparison to the previous study conducted by NTC, Nepal during 2001/2002 in five different testing sites, which revealed 2.44% HIV prevalence in TB patients (Jha *et al.*, 2005).

CHAPTER-IV

4. MATERIALS AND METHOD

A list of materials, reagents, media, equipments and chemicals for the study is presented in Appendix 5.

This thesis work was carried out in Health Research Laboratory, Institute of Medicine, Tribhuvan University Teaching Hospital, Maharajgunj during October 2006 to July 2007. 300 randomly selected patients who attended for treatment with suspected diagnosis of tuberculosis were included in the study. 900 sputum samples were collected from 300 patients clinically suspected of tuberculosis and TB/HIV Co-infection. Along with sputum sample, serum samples were also collected from these patients for diagnosis of HIV after counseling and taking consent from them.

All 300 patients were randomly selected, aged between 15 to 80 years. Patients were selected who visited DOTS center of TUTH, Maharajgunj and Infectious and Tropical disease research and prevention center, Tripureshwor. During the study, collected sputum samples of patients suspected of infected with TB were diagnosed of TB by microscopic examination of acid-fast bacilli by Ziehl-Neelsen staining method and were cultured using solidified Lowenstein-Jensen (L-J) media. Likewise, patients were further tested for HIV using Sandwich ELISA. To confirm the presence of one or more of the known risk factors of HIV and TB infections, all patients were asked some questions before collecting laboratory specimens.

4.1 Diagnosis of tuberculosis

All 300 patients who were randomly selected and were suspected to be infected by pulmonary tuberculosis were subjected for sputum examination. Those who were sputum smear negative but having clinical sign and symptoms, radiographic abnormalities, tuberculin test positive were subjected to culture.

4.1.1 Specimen collection (sputum sample)

Those patients who were randomly selected and were suspected of pulmonary tuberculosis infection were requested for the sputum sample in the laboratory. Patients were counseled for sputum collection according to the standard methods (WHO, 2000) after taking informed/written (if necessary) consent. During the collection of sputum sample, patients were instructed to inhale deeply 2-3 times and coughed up deeply from the chest and spitted closer to mouth. It was made sure that the collected sputum sample is of good quality i.e., thick and purulent and avoid of saliva. About 5 ml of sputum sample (but not saliva) was collected in plastic universal container.

Each patient was requested for three consecutive sputum samples. The first specimen was collected on the spot (i.e., during their first visit in clinic), second specimen in the early morning on next day and third specimen on spot during their visit in center next day. So, after collection of first sputum specimen, two containers were given to the patient to collect second and third specimen.

4.1.2 Acceptance or rejection of sputum sample

Physical examination of sputum sample was done. To eliminate the wrong evaluation, quality control of the sputum was done for possible cases. So, physical examination was done for the detection of presence or absence of mucopurulent portion of sputum. Specimen without mucopurulent portion was rejected if another sampling was possible.

4.1.3 Microscopic examination of sputum

4.1.3.1 Smear preparation and heat fixation

Collected sputum sample was opened carefully and if splitted outside the container, before processing it was decontaminated carefully following standard protocol. A small portion of the mucopurulent material was selected and separated from the remainder with the help of wooden stick and transferred to the slide. Mucopurulent part was separated evenly on a clean slide to a size approximately 1x2 cm. Smear was dried at room temperature completely inside the safety cabinet and was heat fixed by passing through the flame 3-4 times (but shouldn’t be over heated).

4.1.3.2 Staining of fixed smears by Ziehl-Neelsen (Z-N) method

1. Heat fixed smear slides were marked with the laboratory serial number and were placed on the staining rack with smeared slide facing upward.
2. The smear was flooded with Carbol Fuchsin stain and heated from below with spirit cotton until the vapor just begins to rise. It was noted that, carbol fuchsin was not allowed to boil or the slide to dry.
3. Heated Carbol Fuchsin was allowed to remain on the slide for 5-7 minutes and then, the slides were gently rinsed with tap water to remove the excess stain and slides were tilted to drain.
4. The smear was covered with 3% acid alcohol for 2-4 minutes or until the smear was sufficiently decolorized i.e. pale pink.
5. Smear was washed off with tap water and tilted to drain.
6. The smear was covered with malachite green (0.5%) or methylene blue for 1-2 minutes.
7. The smear was washed off by tap water and tipped to drain off the water.
8. Backside of the slide was wiped out by cotton and placed at the draining rack.

4.1.3.3 Observation of stained smear

The dried slides were examined microscopically using 40X lens to select suitable area of the slide and then examined using oil immersion objective i.e. 100X.

The interpretation of the AFB stain of microscopic examination was done according to WHO/IUATLD protocol.

4.1.4 Sputum culture

4.1.4.1 Processing of sample by modified Petroff's method

a) Collected sputum samples were aseptically transferred to the centrifuge tube.
b) Twice volume of 4% NaOH was added to the sputum and mixed properly.
c) The solution was left for 15 minutes at room temperature with occasionally shaking.
d) After 15 minutes, the solution was centrifuged at 3000 x g for 15 minutes.
e) The supernatant was discarded after centrifugation.

f) About 6 ml of distilled water was added to it and sediment was suspended and mixed properly.

g) The solution was centrifuged at 3000 x g for 15 minutes. The supernatant was discarded and sediment was used for inoculation (WHO, 1998).

4.1.4.2 Inoculating the primary culture and incubation

0.2 ml of decontaminated and concentrated sputum sample was pipetted out and inoculated into solidified L-J media. Tube was slightly rotated out to allow the dispersion of sample through out the media. The cap of tube was loosened slightly to create the microaerophilic environment and incubated at 37^{o}C for 24 hours in horizontal position at 20^{o} angle for complete absorption of sample into the media. After 24 hours cap was tightened and then kept in upright position. The inoculated tube was incubated at 37^{o}C for 6-8 weeks.

4.1.4.3 Observation

After incubation, growths of any contaminants were observed after 48 and 72 hours. Weekly observation was done to note growth rate, colony characteristics, pigmentation and contamination.

4.1.4.4 Sub culturing and preservation of organism

Those tubes showing sufficient growth in L-J media tubes were further sub-cultured on new L-J tube so that biochemical tests could be done. After that, tubes showing sufficient growth were stored in the cold at -70^{o}C.

4.2 Diagnosis of HIV

4.2.1 Specimen collection for HIV diagnosis

The specimen for the HIV diagnosis is blood sample. Oral / written (if necessary) consent from the randomly selected 300 patients were taken and counseled for HIV sero-testing. About 5 ml of blood was drawn from each patient using safe and disposable syringe and needles in laboratory aseptically. Collected blood samples were dispensed in the labeled dry and clean test tubes.

4.2.2 Processing of the blood sample

After dispensing the blood sample in the tubes, it was allowed to clot without disturbing the tubes for 5-10 minutes. Serum was separated as supernatant by centrifuging blood at 3000 rpm for 5 minutes and was transferred carefully to labeled clean screw-capped vials with the help of sterile plastic aspirator. Collected serum samples were stored at –70^{o}C till analysis. Then, samples were subjected for HIV testing as per Kit manufacturers instructions.

4.2.3 Principle of the procedure

The Advanced HIV Test is an ELISA based, double antigens "sandwich" immunoassay, which employs a variety of recombinant HIV antigens: some (gp120\ gp41\ gp36\ p24) immobilized at the bottom of the microtiter wells and others (gp120\ gp41\ gp36\ p24) coupled with horseradish peroxidase (HRP) as the conjugate solution. During the assay, the existing HIV antibodies in sample will react with those antigens to form an antigen-antibody-antigen-HRP immuno-complex. After the unbound material is washed off during the assay procedure, substrate is applied to indicate the test result. The appearance of blue color in microtiter wells indicates HIV reactive result. The absence of the color indicates non-reactive result in the specimen (ELISA TEST KIT, Instruction Manual). Detail of ELISA TEST KIT, Instruction Manual is given in Appendix 4.

4.2.4 Assay procedure

1. All reagents and specimens were brought to room temperature (18-25^{o}C) before the assay. All reagents and specimens were gently swirled before use. Incubator was adjusted to 37±1^{o}C.
2. The numbers of specimens and the wells were written on the data sheet; one well for blank, six additional wells from the controls and one well for each specimen.
3. 50ul of each specimen and control (3 positive control and 3 negative controls) was added into each appropriate well according to the data sheet (1 well was reserved for the blank).

4. 100ul of enzyme conjugate working solution was added into each reaction well except for the blank.

5. The plate was tapped gently to thoroughly mix the liquid in the wells, without splashing liquid onto the slip.

6. The plate was incubated in a 37^{o}C incubator for 60 minutes.

7. Each well was washed five times with wash buffer by wash procedure.

a) Washing must be performed strictly according to the instruction; incomplete wash may bring out false result.

b) The well contents were aspirated completely into a waste flask. Then, the wells were filled up with wash buffer (350ul or more), avoiding overflow and allowed to soak (approximately 30-60 seconds). Wash buffer was aspirated completely and the wash and soak procedure was repeated for four additional time for a total of five washes.

c) Make sure that no fluid remains on the strip holder and snips after the last aspiration (e.g. by blotting with absorbent tissue).

8. 50ul of color A and 50ul of color B were added to each well.

9. The plate was incubated in a 37^{o}C incubator for 20 minutes.

10. 50ul of 2M sulfuric acid was added into each well; the plate was tapped gently.

11. OD with micro-well reader at 450nm (single wavelength) or 450 and 630nm as reference (dual wavelength) was measured.

4.2.5 Calculations and result

1. Positive Control Mean absorbance (PCx):

PCx =(PC1+PC2+PC3)/3

2.Cut-off value:

Cut-off = PCx X 0.1+ 0.050

3. The Sample absorbance was divided by cut-off value

Positive: Sample absorbance is greater than or equal (≥) Cut-off value

Negative: Sample absorbance is less than (<) cut-off value.

4.2.6 Quality control

Results of an assay are valid if the following criteria are accomplished:

1. Substrate blank

   The absorbance value must be less than or equal (≤) 0.100.

2. Absorbance of the negative control (NC) after subtracting blank.

   Each NC must be less than or equal (≤) 0.100

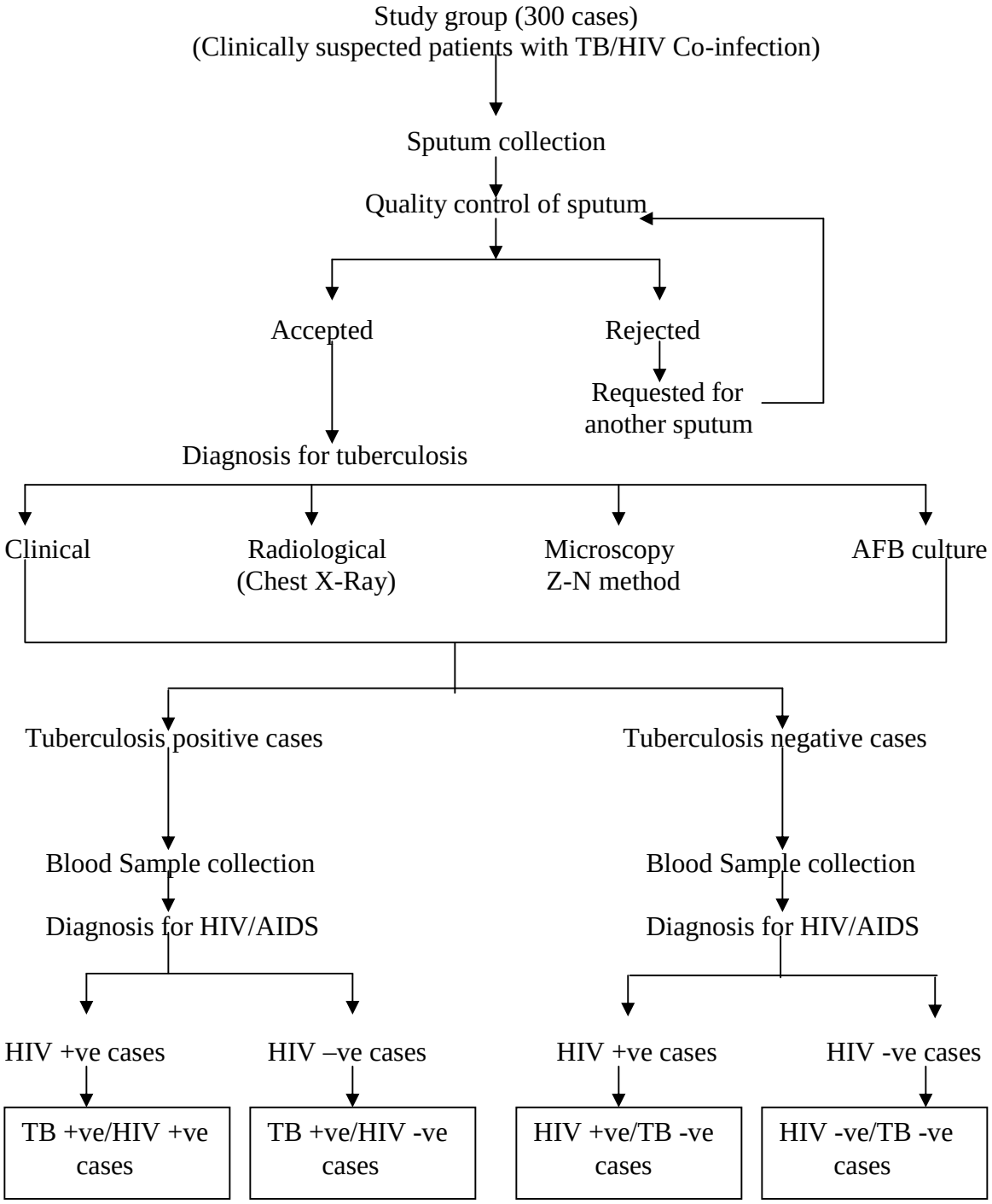
   If two values are out of this range, the run is invalid and the assay should be repeated.

3. Absorbance of the positive control (PC) after subtracting blank.

   Mean of PC must be greater than or equal (≥) 0.500

   If it is lower, the run is invalid and the assay should be repeated.

FLOWCHART OF METHODOLODY



CHAPTER-V

5. RESULT

The thesis work was carried out at 'Health Research Laboratory' Institute of Medicine (IOM) from October 2006 to July 2007. Samples were collected form DOTS center of Tribhuvan University Teaching Hospital (TUTH) Maharajgunj and Infectious and Tropical disease research and prevention center, Tripureshwor. The patients suspected of Tuberculosis infection and having risk behaviour towards HIV infection was included. Age between 15 to 80 years was included in the study. 300 patients with clinical symptoms of TB were included with clinical history of 2 or more weeks continuous cough, fever, marked weight loss.

Among 300 patients included, 79 were found to have pulmonary TB; diagnosed by sputum smear method and by culture method in solid L-J media as well as via their radiological X-Ray report. For all the included patients, HIV test of all collected serum samples were done using Sandwich ELISA. 34 out of 300 serum samples were found to be sero-positive for HIV. Among them 13 were found to be TB/HIV Co-infected cases. Data analysis was done using SPSS Program (Version-11), Excel and chi-square test as statistical tool.

5.1 Age and gender distribution of studied population

About 300 cases were enrolled in the study among which 205 (68.33%) were male and 95 (31.67%) were female. Among the studied population, highest percentage of population (26%) was in age group 21-30 followed by 51-60 age group (20.67%) and third predominant population from 31-40 age group with 19.67%. Statistical analysis showed the mean age as 40.7 years and median age as 39.4 years. Other percentage was in other age group as shown in fig2, Appendix 3.

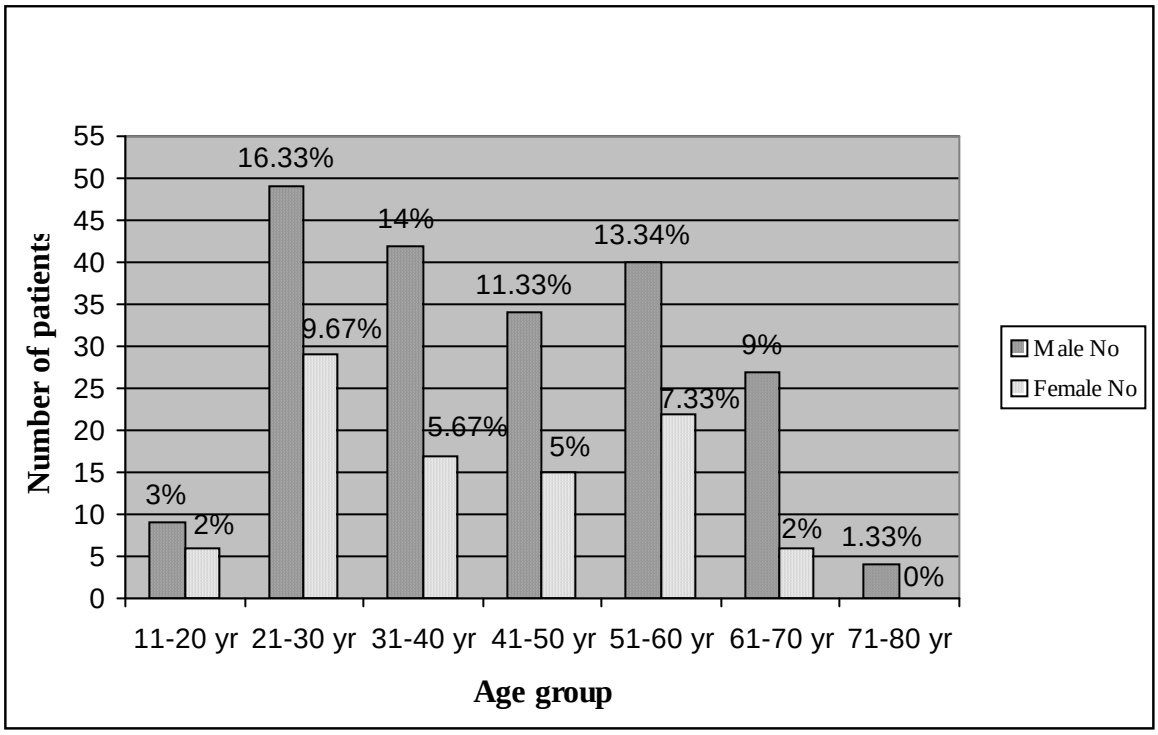


Figure 2: Age and gender distribution of studied population

5.2 Educational status of studied population

Of the 300 cases, educational status as recorded during questionnaire showed, 137 of them were illiterate which constitute 45.67% and 163 were literate which constitute 54.33%. Literate included the people who had formal education from school or colleges. Result is shown in fig 3.

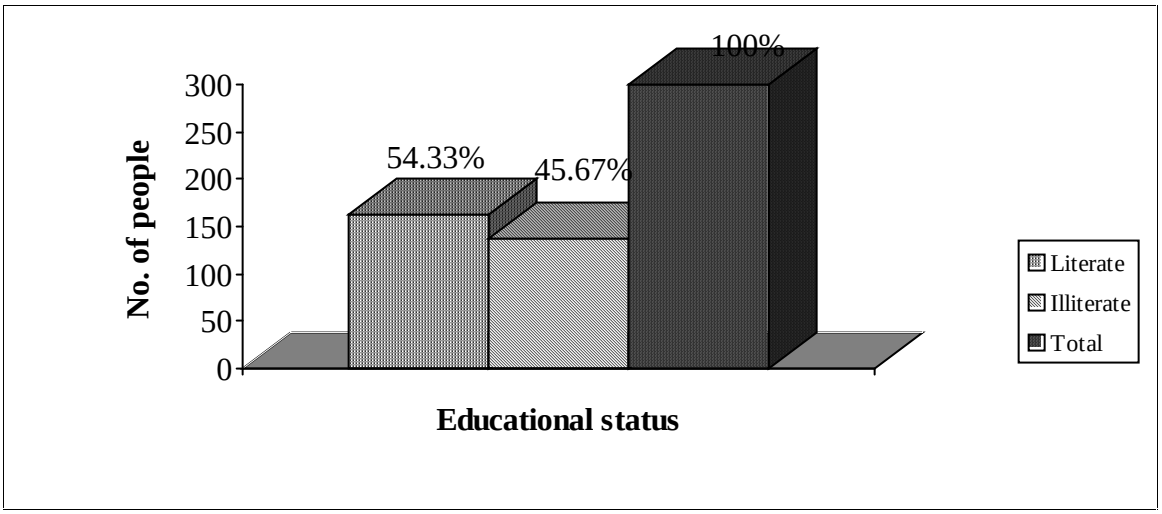


Figure 3 : Educational status of studied population

5.3 Age and gender distribution of total TB positive patients (Out of 300 suspected cases)

Out of 300 suspected tuberculosis cases, 79 of them were diagnosed to be TB positive by smear microscopy, AFB culture and radiology. Highest prevalence of tuberculosis patients were found in the age group 21-30 (18/79) which constitute 22.78%, followed by the age group 51-60 year (17/79). Two age groups 31-40 and 41-50 years were found to have same prevalence of TB patients i.e., (15/79) in each group. Out of 79 TB patients, 66 (83.54%) were male and 13 (16.45%) were female. More male patients were found to be tuberculosis infected and found to be statistically significant (χ^2= 11.47, P<0.01). Other data are shown in fig 4, Appendix 3.

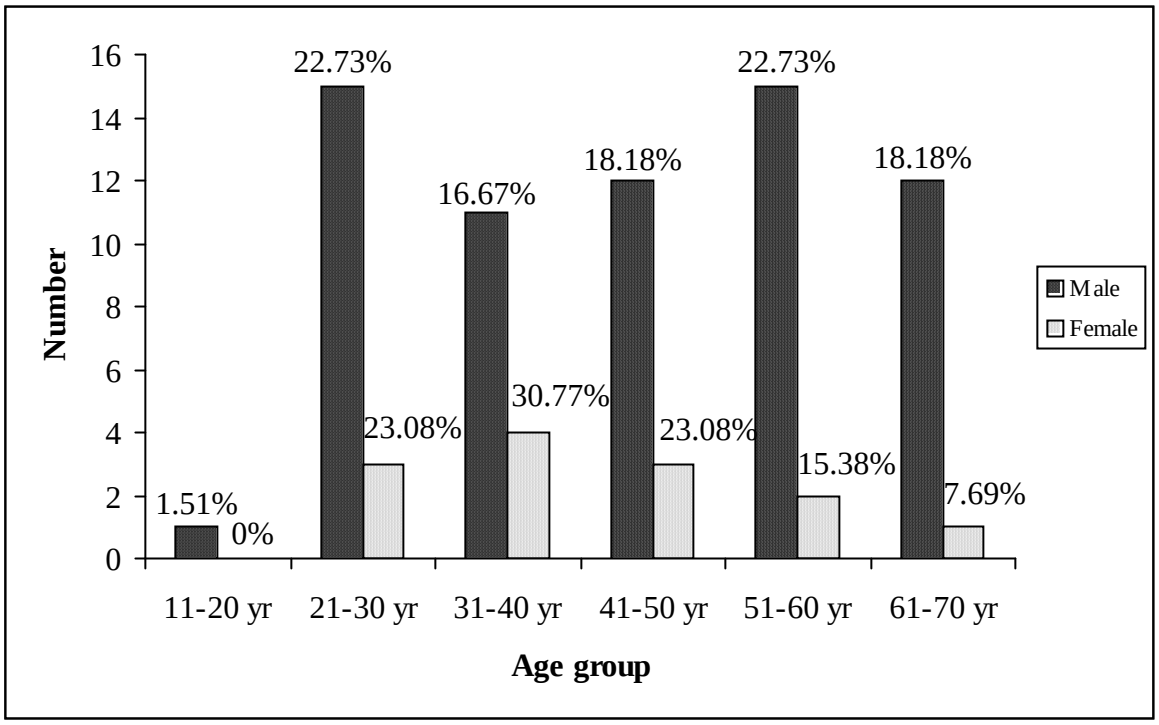


Figure 4 : Age and gender distribution of total TB patients

5.4 Age and gender distribution of 300 patients tested for both TB and HIV infection

About 300 patients included in the study were tested for both TB and HIV infection. Among them 13 (4.33%) were found to be TB/HIV co-infected cases. Out of 13 TB/HIV co-infected cases, highest co-infected cases were reported in 31-40, followed by 41-50 years. 21 HIV sero-positive cases were reported in whom TB infection was not found. As in TB/HIV co-infected cases, highest HIV sero-positive (10/21) was reported in age group 31-40 followed by 21-30 age group (6/21).

Out of 300 cases, 66 were TB positive in whom HIV infection was not detected by the test done. Highest TB positive cases without HIV infection was found in the age group 21-30 (16/66) followed by 51-60 age group (15/66). Detail data is given in table 1.

5.5 Distribution of patients tested for HIV

All 300 patients were tested for HIV infection, among which 34 (11.33%) were found to be HIV sero-positive. Out of 34 HIV sero-positive, higher number of male i.e. 24/34 (70.59%)

were found to be HIV sero-positive than female i.e. 10/34 (29.41%). However, the association was not significant i.e. there was no significant difference between male and female having HIV infection (χ^2 = 0.09, P=0.05). Detail data is shown in fig 5, Appendix 3.

Table 1: Age and gender distribution of patients tested for both TB and HIV infection

Sex / Age(yr)	HIV positive TB positive		HIV positive TB negative		HIV negative TB positive		HIV negative TB negative		Total
	Male	Female	Male	Female	Male	Female	Male	Female	
11-20	0	0	0	1	1	0	8	5	15
21-30	2	0	5	1	13	3	29	25	78
31-40	4	1	6	4	7	3	25	9	59
41-50	4	0	1	2	8	3	21	10	49
51-60	2	0	0	1	13	2	25	19	62
61-70	0	0	0	0	12	1	15	5	33
71-80	0	0	0	0	0	0	4	0	4
Total	12	1	12	9	54	12	127	73	300
Total(%)	13 (4.33%)		21 (7%)		66 (22%)		200 (66.67%)		300 (100%)

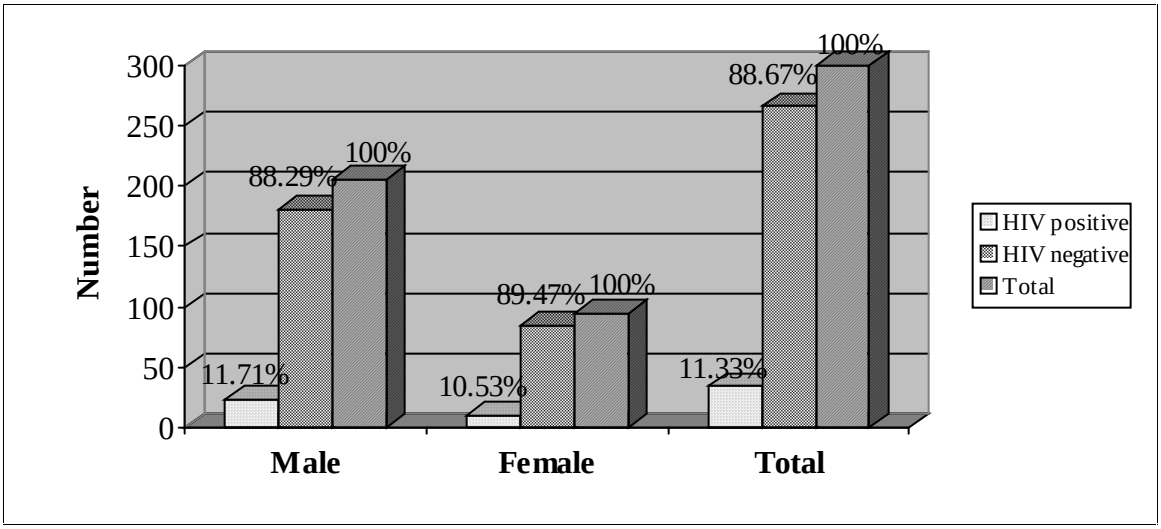


Figure 5 : Distribution of patients tested for HIV

5.6 Age and gender distribution of HIV patients

Out of 34 HIV sero-positive patients, 15 (44.12%) were found in age group 31-40 year, 8 (23.53%) in age group 21-30 year, which is followed by 41-50 age group with 7 (20.59%) HIV sero-positive patients. HIV patients were found in age group ranged form 15 years to 55 years. The detail distribution is shown in fig 6.

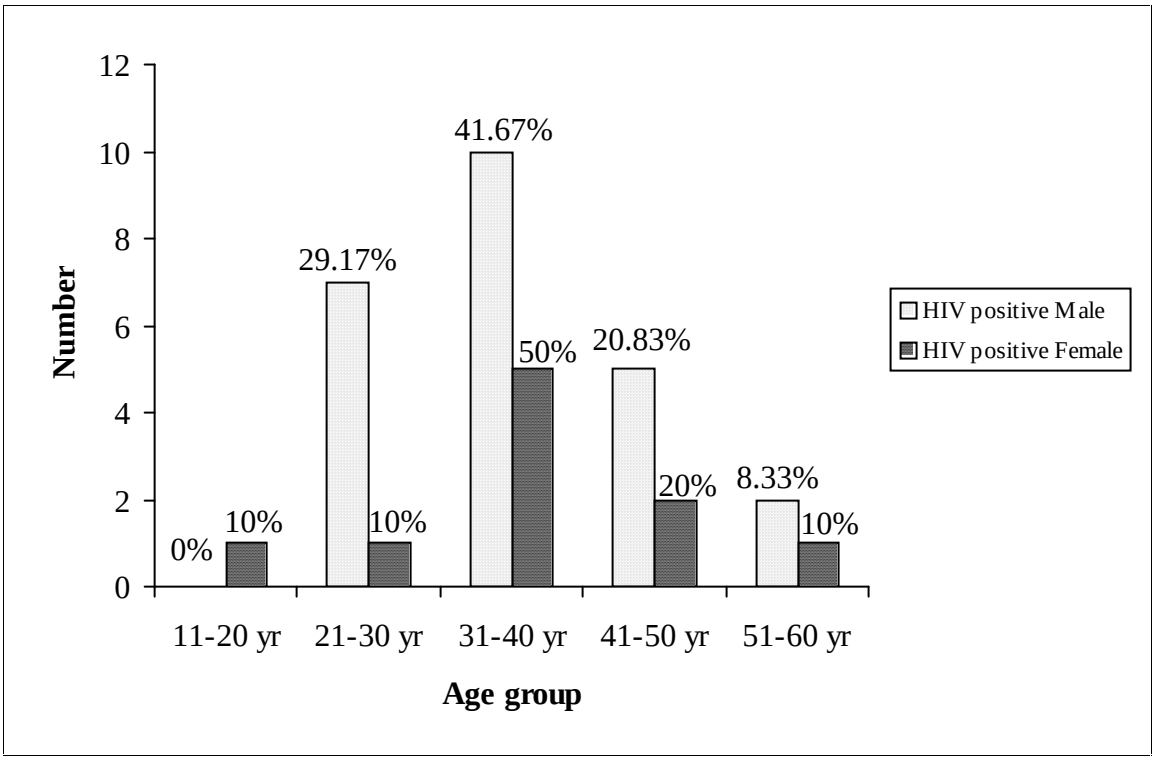


Figure 6 : Age and gender distribution of HIV patients

5.7 Age and gender distribution of TB/HIV co-infected cases

Among total patients enrolled, 13 TB/HIV co-infected patients were reported in this study. TB/HIV co-infected patients were ranged in age from 25 years to 55 years. Highest prevalence of co-infected patients 5 (38.46%) were reported in age group 31-40 years, followed by 41-50 years with 4 (30.77%) HIV sero-positive patients. Both 21-30 and 51-60 age groups have same number of TB/HIV co-infected patients i.e. 2 (15.39%). Among 13 co-infected patients, only one female (7.69%) belonging to age group 31-40 years was found to be co-infected, rest of 12 (92.31%) were males as shown in fig 7.

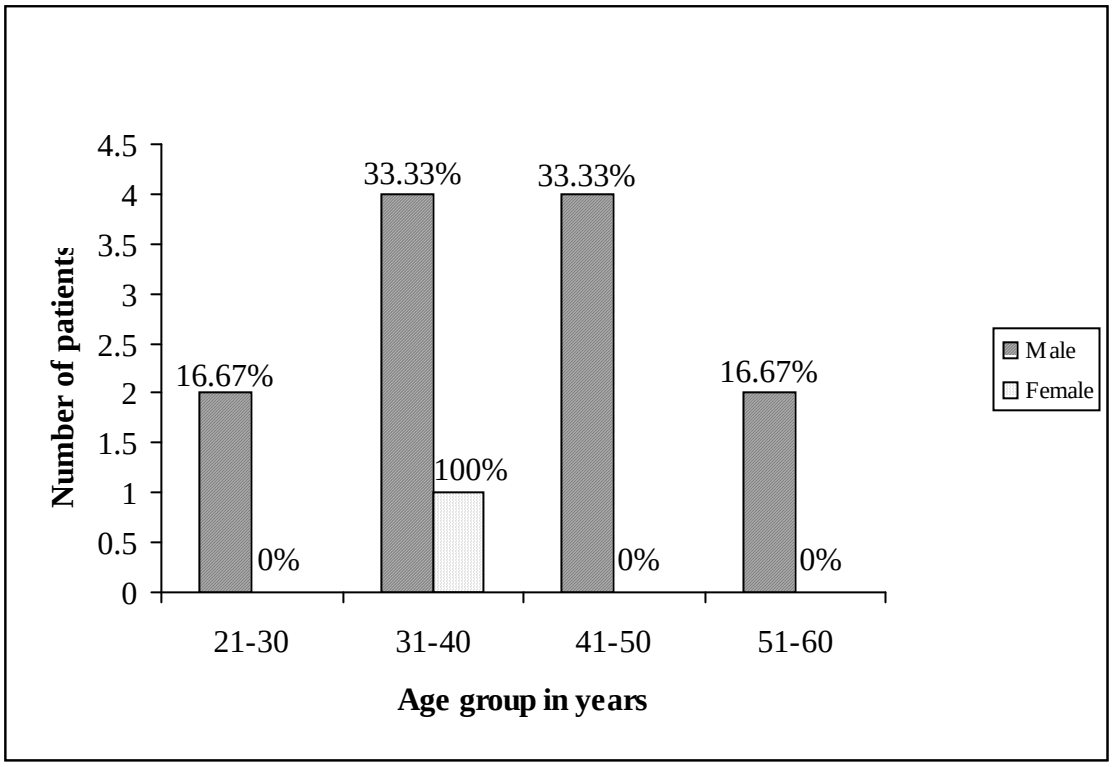


Figure 7: Age and gender distribution of TB/HIV co-infected patients

5.8 Distribution of HIV infection with gender in TB infected patients

Of 66 TB infected male patients, 12 males were found to be HIV sero-positive i.e. TB/HIV co-infected cases where as out of 13 female TB patients, only 1 female was found to be co-infected with HIV. From the result, males were found in higher number (18.18%) than female (7.69%). However, the values were found to be statistically insignificant ($\chi^2 = 0.67$, P=0.05). Detail data is given in fig 8.

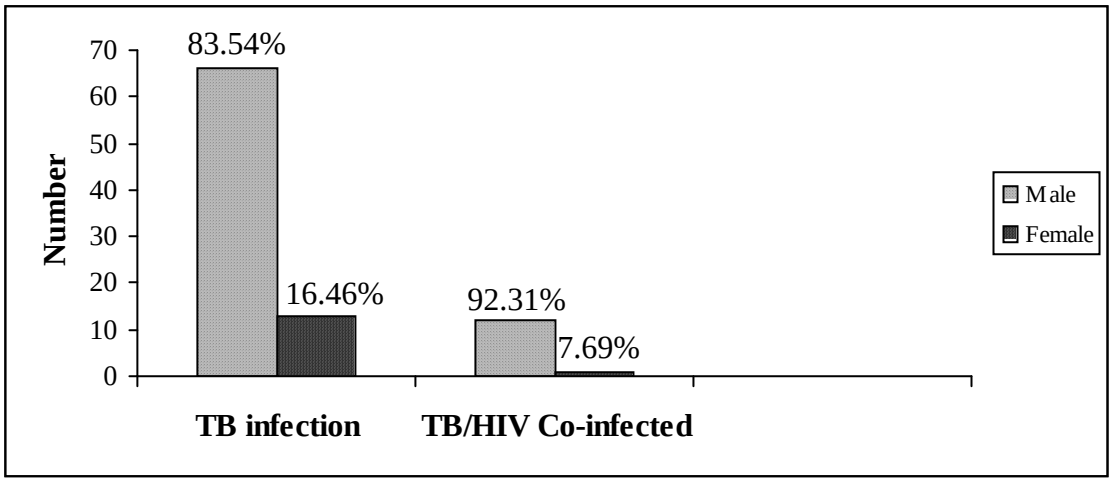


Figure 8 : Distribution of HIV infection with gender in TB infected patients

5.9 Distribution of Tuberculosis/HIV among tested patients

All 300 patients subjected for the study of TB and HIV infection, 13 (4.33%) of them were diagnosed with both TB and HIV infection. 66 out of 300 (22%) were diagnosed as TB infected patients without HIV infection. 21 (7%) were HIV positive patients without detected TB and 200 (66.67%) were found to be without both TB and HIV infection. Data is given in fig 9.

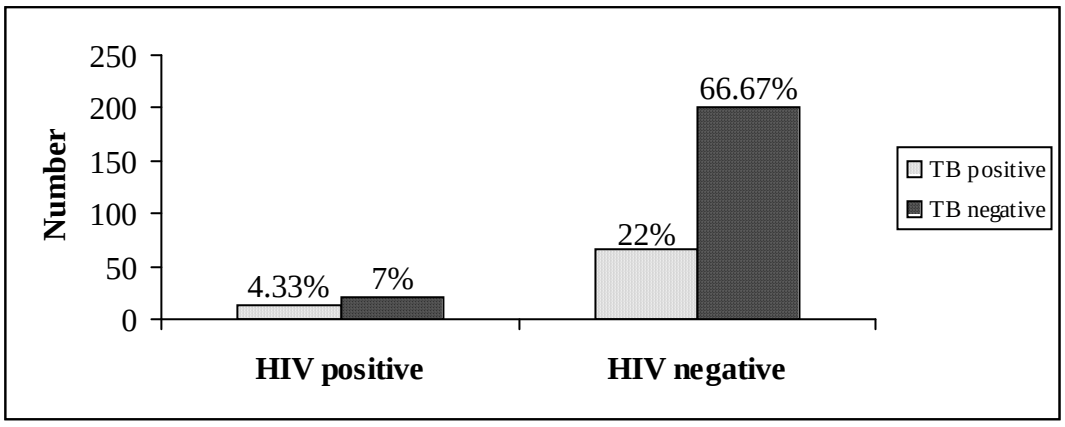


Figure 9 : Distribution of Tuberculosis/HIV among tested patients

5.10 Distribution of tuberculosis with smoking habit of patients

Of the 201 smokers out of 300 enrolled in the study, 64 (31.84%) were found to be infected with TB where as 15 (15.15%) out of 99 were diagnosed as TB patients who were non-smokers. χ^2 test applied for the data which showed the significant association between smoking habit and tuberculosis infection i.e. test was significant. (χ^2 = 9.52, P<0.01). (fig 10, Appendix 3)

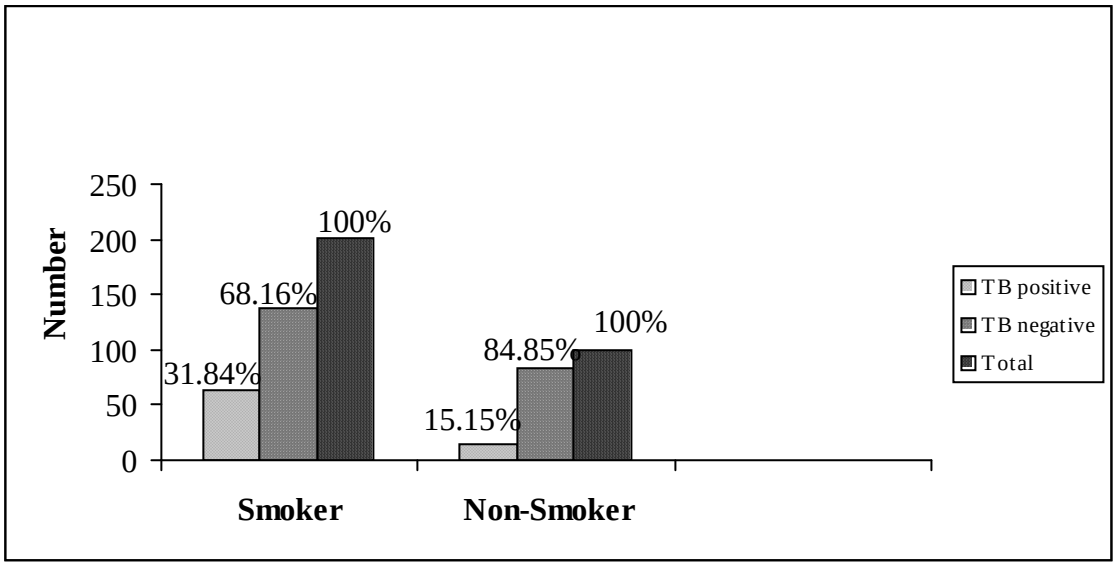


Figure 10 : Distribution of tuberculosis with smoking habit of patients

5.11 Distribution of tuberculosis with alcoholic habit of patients

Out of 180 alcoholics, 56 (31.11%) were diagnosed as TB patients where as 23 out of 120 (19.17%) were found to be TB patients who were alcohol non-users. When χ^2 test was applied, it was found that habit of taking alcohol and tuberculosis was statistically significant (χ^2= 5.297, P<0.05) as shown in fig 11, Appendix 3.

5.12 Occupational status of TB infected patients

Out of 79 TB infected patients in 300 cases, majority of them are involved in different services- 18 (22.78%), followed by factory employee- 16 (20.25%), Agriculture- 12

(15.2%), Housewives- 9 (11.39%), Unemployed- 9 (11.39%), Business-7 (8.87%), Drivers- 4 (5.06%) and Government employee- 4(5.06%) as shown in fig 12.

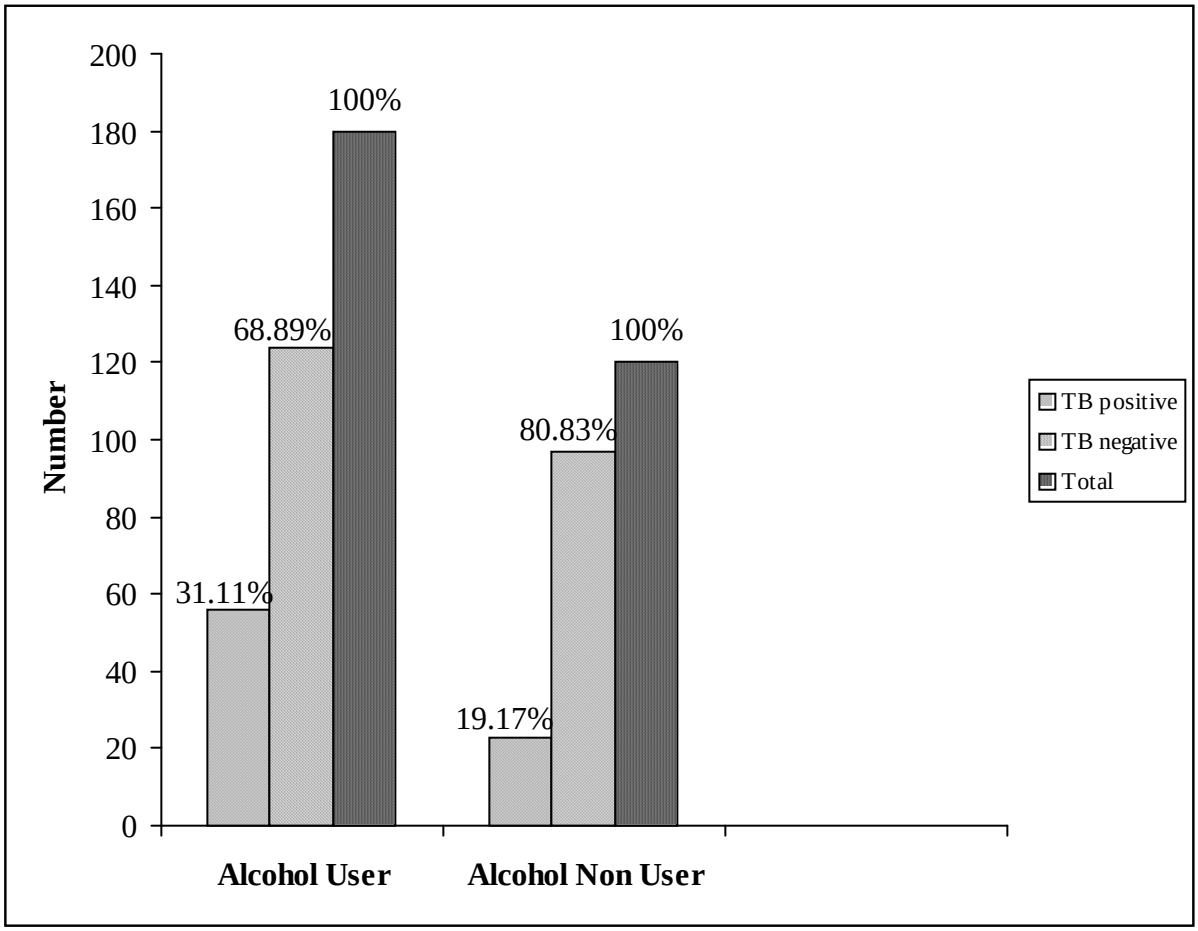


Figure 11: Distribution of tuberculosis with alcoholic habit of patients

5.13 Occupational status of HIV infected patients

Out of 34 HIV infected patients in 300 cases, majority of them are involved in different services- 14 (41.18%), followed by Business- 6 (17.65%), Factory employee-5 (14.71%), Housewives- 4 (11.76%), Drivers- 3 (8.82%) and Agriculture- 2(5.88%) as shown in fig 13.

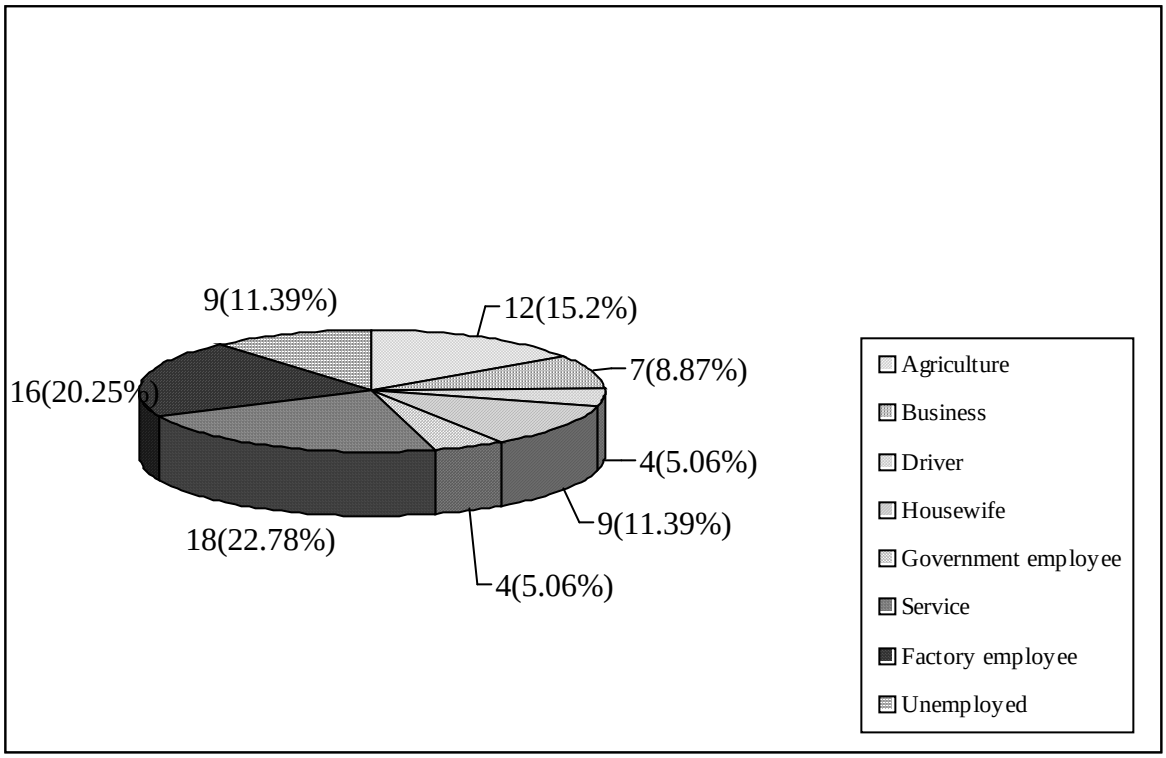


Figure 12 : Occupational status of TB infected patients

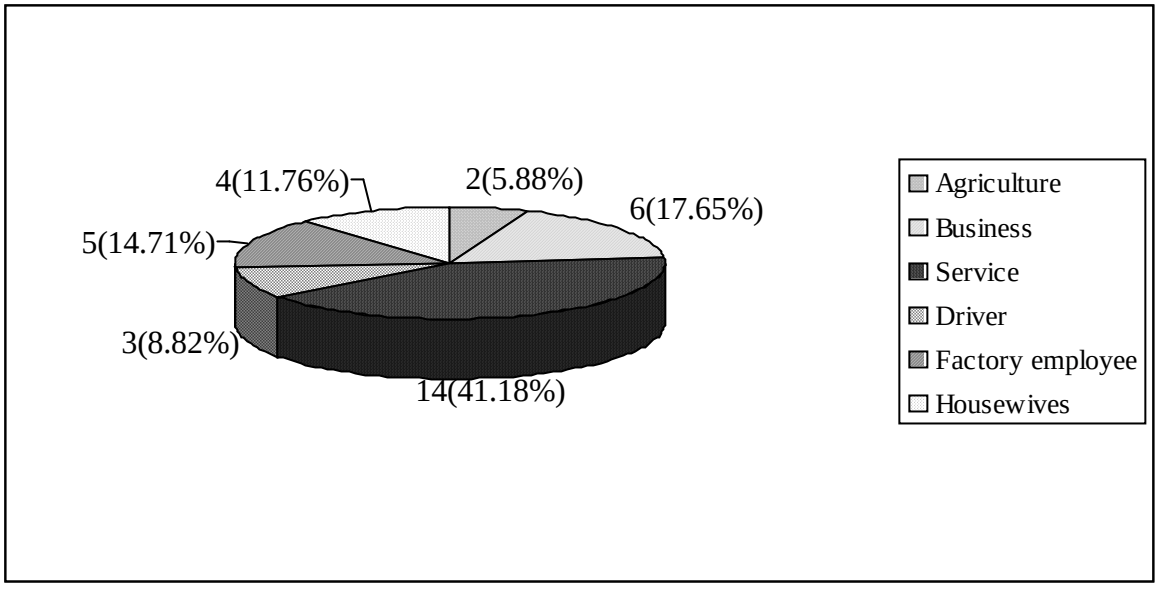


Figure 13 : Occupational status of HIV infected patients

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

About 300 suspected patients of having infected with tuberculosis and risk behaviour towards HIV/AIDS (IDUs, those who have visited to CSWs, migrators and who have been living far from family for long time) visiting DOTS center of Tribhuvan University Teaching Hospital (TUTH), Maharajgunj and Infectious and Tropical Disease Research and Prevention Center, Tripureshwor during October 2006 to July 2007 were enrolled in the study and analyzed in Health Research Laboratory, Institute of Medicine, Maharajgunj. Among 300 suspected patients, only 79 of them were confirmed to be infected with tuberculosis that were diagnosed by smear microscopy (Z-N stain), AFB culture and radiography (as directed by physician).

HIV infection increases the risk of TB. The HIV epidemic is also likely to have an indirect impact on the incidence of TB because of the increased number of infectious individual in the population. A retrospective cohort study (1991-1997) conducted in four gold mines in South Africa, involving 23,874 miners of whom 3371 were HIV positive on entry 2737 seroconverted during follow up, showed annual incidence of new pulmonary tuberculosis in mines rose from 0.53% to 1% in 1997. The incidence of TB was higher among HIV positive miner than HIV negative miner, and the proportion of TB attributable to HIV infection increased from 0% in 1991 to 14% in 1997 (Gautam, 2003)

Out of 300 patients included in the study, the prevalence of tuberculosis was found to be 26.33%. The highest prevalence of TB patients (22.78%) was found in age group 21-30 years followed by 51-60 years where prevalence of TB patients was found to be 21.52%. Age group of 31-40 and 41-50 years have been found to have same TB prevalence i.e. 18.99%. As study conducted by Dhungana, 2002 at United Mission Hospital Tansen (UMHT) and Dhungana, 2004 in TUTH had also found the highest prevalence of TB

among age group of 21-30 years. The highest prevalence of TB among this age group might be due to the exposure of young people to different environment during their work and activities that will make their health more prone to infection by TB organisms.

Followed by the age group 21-30 years, 51-60 years have second highest prevalence of TB patients. As people of this age group are exposed to the outer environment as well as due to the old age, the immunity is comparatively weaker; they are also prone to the infection with TB organisms. In this study, only one (1.27%) patient of age group 11-20 years was found to be infected.

Due to the higher prevalence of TB infection in 15-60 years, greater effected population is productive age group. So, it is greatly affecting the economic and social status of country. As it was difficult to obtain samples to diagnose extra-pulmonary tuberculosis, only pulmonary tuberculosis cases were enrolled by using sputum samples. As it is known, tuberculosis is a disease of lungs it mainly affects lungs than other organs. Acid-fast bacilli are usually detected in expectorated sputum specimens from the patient with active tuberculosis. From the studies done, the fact has been revealed that between pulmonary tuberculosis and extra-pulmonary tuberculosis, high prevalence of pulmonary tuberculosis has been reported in HIV patients. In the study of Dhungana at UMHT also, 88.11% of reported cases were pulmonary tuberculosis and others were extra-pulmonary tuberculosis. Higher number of pulmonary tuberculosis have been reported which may be due to poor quality of life, congested living in house and improper care of health in the developing countries like Nepal. In active tuberculosis, due to the congested living; tubercle bacilli from active TB patients can be transmitted to other healthy members in the family through the air or other means.

As extra-pulmonary tuberculosis in most cases, are self-limited and not frequently transmitted by tubercle bacilli; it is not frequently diagnosed in DOTS center and in most of the TB health centers.

Among 300 patients, 79 were diagnosed to be suffering from active TB in which 83.54% were male and only 16.45% were found to be female and these values were statistically significant ($P<0.01$). In TB/HIV co-infection study in UMHT also, 72.84% of male were found to have tuberculosis infection whereas only 27.15% of female have the infection. Similar type of result was reported by Ingole *et al.,* 2001 (India). The study conducted suggested that women are infected with TB 4-5 times less than men.

Female/male ratio of less than one was observed among the TB suspects undergoing sputum examination in all countries except in Pakistan where it was more than one. Significantly higher sputum positivity among male TB suspects has been observed in India, Nepal, Sri-Lanka and Bangladesh (STC, 2002). With the objectivity to assess the gender differences in TB suspects undergoing smear microscopy and smear positivity, the project conducted by STC in 2001, 61% were male and 39% were female with overall female/male ratio of 0.6.

The reason behind this gender differentiation in TB infection might be due to exposure of male to the external environment more than female. Exposure to the external environment may be during their daily activities as according to our social rules and norms, males are supposed to do their job outside the home while females are more restricted at home. From the occupational status of TB infected patients also, higher percentage of patients has been actively involving in jobs that need to get exposed to outer environment to greater extent and need to spend more time out of home. When male members of family gets ill, they have quick and easy access to the clinics/doctors and visit the health centers independently whereas female members of family has to depend on other male or senior members of family to have access to health centers as well as they can't freely express their health problems.

According to NTC, (2000), women visit to modern health care facilities less than men because of social pressure or stigma like to most developing countries of world. TB can be regarded as a symptom of poverty, instigated by unequal distribution of resources.

However, poverty itself within a society is not distributed equally among its social classes and between the two sexes. Estimates show that 70% of the world's poor are women. Poverty and genders are two key factors implicated in a women's vulnerability to TB. Hence, it could be assumed that women's poor health seeking behaviour perhaps be a possible reason for low case detection in TB.

Out of total 300 patients tested for HIV, 34 (11.33%) were found to be HIV sero-positive. Among 34 HIV sero-positive patients, males were found higher than females. Only 10 (29.41%) of female were found to be HIV sero-positive whereas 24 (70.59%) males were found to be HIV sero-positive as diagnosed using Sandwich ELISA. However these values were found to be statistically insignificant.

In both TB and HIV cases, gender differentiation in the infected number was found. The reason behind this may be due to the reasons as described above as in the case of TB. However, the recent report has shown the increase of HIV infected cases in housewives by greater percent that may be due to the development of new diagnosis tests and awareness in the people.

The major risk groups of HIV infection in this study were found to be IDUs, Housewives whose partners were out of hometown for long time in case of work and Migrators who were living far from their home and family. It has been reported that in most of the Asian countries, IDUs are the first community to be affected by HIV and Nepal was the first developing country to establish a harm reduction program with needle exchange for IDUs. HIV prevalence among Nepal's estimated 19,850 IDUs varies by location, 22% of IDUs are HIV positive in Pokhara, whereas 52%, 33% and 8% are HIV positive in Morang, Sunsari and Jhapa districts respectively (FHI, 2005). HIV prevalence among Kathmandu's 56,500 IDUs has decreased form 68% to 52%. Since highest percentage of IDUs is still being reported in Kathmandu, this study that is based on Kathmandu might have reported highest number of IDUs 16 (47.06%) with HIV sero-positive.

Followed by IDUs, second highest number 10 (26.42%) was found in case of migrators. In developing countries like Nepal, both internal and external migration for seasonal and long-term labor range form 1.5 to 2 million (The world Bank, 2006). Such migration has been necessity for the economic survival of many households in both rural and urban areas. Removal from traditional social structures, such as family, has been shown to promote unsafe sexual practices, such as having multiple sexual partners and engaging in commercial sex. 4 (11.76%) of HIV sero-positives were housewives who got the infection through their partners and remaining 4 (11.76%) of HIV sero-positives were those having multiple sex partners and women trafficked to Mumbai. None of HIV sero-postivies were found to have infection via blood transfusion.

Out of 34 HIV sero-positive patients, highest number of patients were in the age group 31-40 years where 15 out of 34 i.e., 44.12% of HIV sero-positive were diagnosed under this age group. Following 31-40 year, second highest HIV sero-positive were found in age group 21-30 years i.e. 8 (23.53%), then 41-50 year with 7 (20.59%) HIV sero-positive, 3 (8.82%) HIV sero-positive in 51-60 year and lowest HIV sero-positive in age group 11-20 year i.e. only one case (2.94%) was found to be sero-positive. Prevalence of HIV infection was found high in age group 21-50 years. Previous studies done have shown high prevalence of HIV infection in age group 20-40 years. As the development of nutrition intake and improvement of health status of people, the age bar of sexually active group may have increased which might have shown the result as given above. However, in the studies done before and in this study as well, highest HIV sero-positive patients were found in age group lying between 31-40 years, which has been supported by the study of Dhungana *et al* (2002) at UMHT.

From the report of cumulative HIV and AIDS situation in Nepal as of May 2007, highest cumulative HIV infection by age group was shown in 30-39 years (NCASC, 2007). Out of 300 patients subjected for both TB and HIV diagnosis, 66 (22%) were TB patients without HIV infection. 34 (11.33%) out of 300 patients were found to be HIV sero-positive that includes patients with TB as well as without TB infection. Among 34 HIV sero-positive

patients detected by Sandwich ELISA, 21 of them were HIV sero-positive patients without TB infection.

Since, HIV is the infection that is most commonly transmitted via sexual intercourse, the lowest prevalence was found in age group of 11-20 years, as the people in this age group are not so much sexually active. Only one HIV sero-positive (2.94%) out of 34 was found to be positive. None of the HIV sero-positive cases were reported in the age group of 61-70 years and above. The reason behind this might be due to the presence of low number of people survive at this age which resulted in lower number of patients of this age group enrolled in study. Another reason behind this fact is due to the sexually inactive age group.

Among 300 patients subjected for the study of both TB and HIV infection, it was found that there were 13 (4.33%) patients diagnosed as co-infected with TB and HIV. Among the TB/HIV co-infected patients, highest prevalence; 5 (38.46%) out of 13 was observed in age group 31-40 years, followed by second highest prevalence 4 (30.77%) in 41-50 years. Both 21-30 and 51-60 age group have same TB/HIV prevalence i.e.2 (15.39%) in each group. TB and HIV co-infected patients of this study were in the age group of 25-55 years. Among 13 TB/HIV co-infected patients, higher infected number was found in males i.e. 12 (92.31%) than female i.e. only one case (7.69%).

The study conducted to estimate incidence of all forms of TB and to evaluate clinical practice on TB by Diez *et al.,* 2001 in Spain found 17.7% of TB/HIV co-infection among 1755 TB patients. Most TB/HIV cases were (86.8%) male and in the age group 15-44 years (91.4%). Among the total male TB patients the percentage of HIV positive was much higher than among female patients. Regarding age, the most affected age groups were the 24-34 years with 40.7% followed by 35-44 years with 35.6%.

A cross sectional study conducted by SAARC TB and HIV/AIDS center (STC) Kathmandu, Nepal during 2005/2006 found HIV prevalence among diagnosed TB patients to be 1.5% (HIV prevalence among MDR-TB patients was 5% and Non-MDR TB patients

1.15%). Previous study conducted by National Tuberculosis Center (NTC), Nepal during 2001/2002 in five different testing sites revealed 2.44% HIV prevalence in TB patients (annual report 2001/2002, NTC). Similarly, the prevalence rate of TB/HIV co-infection at UMHT hospital was observed to be 10.76% (Dhungana, 2002). On the same year 2002, the prevalence rate of TB/HIV co-infection in the study of Sherchand *et al* in Kathmandu was found to be 6.1%. Study conducted by Gautam, 2003 in order to find prevalence of respiratory pathogens in HIV infected people showed 22.22% of PLWHA had tuberculosis. TB/HIV co-infection study conducted by Dhungana in 2004 in HIV patients from different expected sites of Kathmandu valley showed 23% of TB/HIV co-infected cases.

From the data collected, it has been shown different prevalence rate of TB/HIV co-infection in different study done at different places at various time period. Globally, different studies at different countries showed TB/HIV co-infection prevalence rate is in rapidly increasing order.

A cross-sectional study conducted by Berhane *et al*., 1999 (Ethiopia) also reported that a greater proportion of men (66%) were co-infected with HIV than women (34%) ($P>0.05$) and those infected tended to be younger. A prospective Epidemiological study in the Southern Region of Ethiopia on HIV and Tuberculosis co-infection in 2002 showed a strong association between area of residence and HIV with 15% (47/329) of the rural patients and 30% (45/150) of the urban patients being HIV positive ($P<0.001$). HIV prevalence was also associated with age. The highest number of TB cases occurred in the 15-24 year olds. TB/HIV co-infections however peaked in children and in the 25-34 year old adults, which was later than the peak for TB. The HIV prevalence was 19% and 26% among smear-positive and smear negative PTB cases, respectively.

Relatively low prevalence i.e. 13 out of 300 cases (4.33%) of TB/HIV co-infected cases were found in this study than that have reported by Dhungana, 2002 (10.76%), Sherchand *et al*., 2002 (6.1%). This may be due to nature of patients enrolled with in definite time period who have been suspected of having signs and symptoms of TB and some with risk

behaviour towards HIV infection. In case of study done in UMHT hospital, highest prevalence was observed as higher number of TB/HIV suspected patients form different regions of country and neighboring districts of India were referred to the hospital as its location is in an entrance to districts of migrants. In case of the study of Sherchand *et al*., the study was conducted from April 1999 to December 2001, which was relatively longer time period of study than this study, which brings higher possibility of enrolling more TB/HIV co-infected cases.

However, the TB/HIV co-infected cases in this study is comparatively higher than HIV prevalence among TB patients reported by a cross sectional study done by Jha *et al*., 2005/2006 in STC, Kathmandu, Nepal.

Prevalence of TB/HIV co-infection cases has been found to be changing with time and type of study population. The study done so far has included certain places and sites of country. So, it can be concluded through different studies that TB/HIV co-infection cases have been growing in alarming rate with increased risk of HIV in our country due to open border, increasing number of labor migrants, peak number of injecting drug user in youth mostly in urban areas and due to unsafe sex with CSW which are considered to be high risk groups.

6.2 CONCLUSION

In conclusion, the study has revealed that TB/HIV co-infection cases are increasing in the alarming rate in developing countries, like Nepal that is creating great problem in TB control programmes. Highest TB patients were found in age group 21-30 years where as highest HIV sero-positive patients were found in age group 31-40 years followed by 21-30 years. TB/HIV co-infection cases were found to be concentrated more in intravenous drug users, housewives whose partners are out of hometown for long time and in migrators. So these groups need special attention and priority in both TB and HIV control programmes.

Male were found to be more infected than female. Only one female was found to be TB/HIV co-infected among total 13 co-infected patients.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATION

7.1 SUMMARY

This dissertation work was conducted in Health Research Laboratory, Institute of Medicine, Tribhuvan University Teaching Hospital, Maharajgunj during October 2006 to July 2007 with general objective to study HIV infection in suspected Tuberculosis patients.

A total of 300 patients were enrolled in the study with clinical signs and symptoms suggesting of having TB infection and having risk behaviour towards HIV infection. From each patients, 3 sputum samples were collected in consecutive days and subjected for AFB staining by Ziehl-Neelsen staining and AFB culture in solidified L-J media. Serum samples from those patients were also collected after counseling them about HIV infection and taking oral consent (if necessary written consent was also taken). Serum samples were processed for HIV diagnosis by using Sandwich ELISA.

By using sputum sample, only pulmonary tuberculosis cases were included. In the studied population, people aged 15-80 years were included.

Out of 300 patients, 79 cases were diagnosed as tuberculosis patients i.e. 26.33% were the pulmonary tuberculosis cases. Regarding the sex wise prevalence of tuberculosis, male showed the higher value (83.54%) than female (16.45%) where these values were statistically significant ($\chi^2 = 11.47$, $P<0.01$). Highest TB prevalence (22.78%) was found in age group 21-30 years.

Total of 34 patients were diagnosed to be HIV sero-positive by using Sandwich ELISA of which there were 21 positive cases in which tuberculosis was not detected either by smear microscopy or AFB culture. Among 34 HIV sero-positive cases, 24 (70.59%) of them were male and 10 (29.41%) were female. However, there was found to be no association of HIV

infection in male and female patients i.e., these values were statistically insignificant. Highest prevalence of HIV sero-positive cases (44.12%) was found in age group of 31-40 years.

Among 300 cases detected for both TB and HIV infection, 13 (4.33%) of them were found to be Co-infected. Out of 13 TB/HIV Co-infected cases, 12 (92.31%) were male and only one (7.67%) was female but sex wise prevalence of TB/HIV Co-infected cases was found to be Statistically insignificant. As of HIV infection, highest prevalence of TB/HIV co-infected cases was seen in age group 31-40 years.

From the study, it was observed that age and sex were important factor in the distribution of TB/HIV Co-infection.

7.2 RECOMMENDATION

i. TB has been observed as one of the leading opportunistic infection in HIV infected patients. So, TB and HIV prevention, care and control programmes need to work in close collaboration.

ii. All diagnosed TB cases should be tested for HIV infection and HIV patients showing sign and symptoms of TB should immediately be subjected to diagnosis of TB.

iii. Co-infections to HIV/AIDS, especially tuberculosis, should be addressed through the provision of cost-effective and comprehensive services.

iv. TB care centers, giving special preferences to DOTS centers in country, need to follow the strategy to detect HIV infection as routine diagnosis in patients visiting the centers.

v. Males and females are almost equally distributed within the population of our country. With such population distribution between sexes, the low detection of female TB cases remains a troubling public health issue demanding urgent focused study.

vi. TB/HIV co-infection is emerging as a serious public health problem. Though, no studies till date have been done representing whole country. So, extensive study is strongly recommended to get more representative data of TB/HIV co-infection to develop national policy regarding this speedily emerging issue.

8. REFERENCES

Alcamo IE (1997) Fundamentals of Microbiology, 5th edition, The Benjaman/Cummings Publishing Company, pp 47-95

Bam DS and Rahman M (2002) HIV/AIDS and HIV/TB co-epidemic with SAARC region countries- Why are SAARC countries at risk? STC Newsletter 12(2): 22-27

Barnes PF, Abrams JS, Wang E, Yamamura M and Modlin RL (1993) Cytokine production at the site of disease in human tuberculosis 61: 3482-3489

Berhane K, Eyasu H and Atakilt G (1999) HIV testing among tuberculosis patients in Tigray region, Ethiopia; implications for tuberculosis treatment policy. International Journal of Tuberculosis and Lung Disease, pp 29-32

Bleed D, Dye C and Raviglione M (2000) Dynamics and control of the global TB epidemic.Current opinion in pulmonary medicine 6: 174-179

Brook GF, Butel JS and Morse SA (2004) Jawetz, Melnick and Adelberg's Medical Microbiology, 23rd edition, McGraw-Hill Companies, Inc, pp 275-284

Casal M, Vaquero M and Gurierrez J (1997) Evaluation of drug resistant tuberculosis in HIV infected patients. International Journal of Tuberculosis and Lung Disease 1(5): 36-41

Chakrabarti AK (1998) Female Genital Tuberculosis-A Retrospective study Indian Journal for Tuberculosis 45: 101

Chessbrough M (2003) Medical laboratory manual for tropical countries, ELBS edition, University Press, Cambridge, UK 2: 207-208

Chhetri GG (2002) Prevalence of tuberculosis among the suspected patients visiting Tribhuvan University Teaching Hospital and their antimicrobial resistance pattern (M.Sc Dissertation), Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Comstock GW (1982) Epidemiology of tuberculosis, Am Rev Respir Dis 125: 8-15

Corbett EL, Churchyard GJ and Charalambos S (2003) Morbidity and mortality in South African gold miners, impact of untreated HIV infection, Clinical Infectious Diseases 34: 1251-1258

Corper HJ and Cohn ML (1937) The thermolability of the tubercle bacilli, Am Rev Tuberc 35: 663

De V, Vincent T and Hellman S (1992) AIDS, Etiology, Diagnosis, Treatment, and Prevention. 3rd edition, Philadelphia, JB. Lippincott Company, pp 9, 80

Dhungana GP (2004) Tuberculosis and HIV co-infection in Kathmandu valley (M.Sc Dissertation) Central Department of Microbiology, Tribhuvan University, Kathmandu

Dhungana JR (2002) Tuberculosis and HIV co-infection in patients attending Tansen Hospital Palpa (M.Sc Dissertation) Central Department of Microbiology, Tribhuvan University, Kathmandu

Diez M (2001) Characteristics of HIV and TB patients in Spain: Results from the Multicenter Project for TB Research (MPTR). International Journal of Tuberculosis and Lung Disease, pp 28-51

Dlugovitzky D, Bay ML, Rateni L, Urizar L, Rondelli CF, Largacha C, Farroni MA, Molteni O and Bottasso OA (1999) In vitro synthesis of interferon-gamma, interleukin-4, transforming growth factor-beta and interleukin-1 beta by peripheral blood mononuclear

cells from tuberculosis patients: relationship with the severity of pulmonary involvement, Scand J Immunol 49: 210-217

DoHS (2004/2005) Annual Report, Ministry of Health and Population, Department of Health Services, Health Service Coverage Fact Sheet, pp 143-176

Duen AV (2001) Role of the microscopy network in the NTP. STC Newsletter, SAARC TB Publication 11(1): 18-23

Enarson DA, Rieder HL and Arnad OT (1994) Tuberculosis guide for low income countries, 3rd edition, International Union Against Tuberculosis and Lung Disease, pp 17-23

FHI (2005) National estimates of adult HIV infections, Kathmandu, pp 115-118

Flaskerud J (1989) AIDS/HIV infection a reference guide to nursing professionals, Phikadelphia, W.B Saunders Company, pp 37-44

Folks TM and Khabbaz R (1998) Retroviruses and associated disease in humans, Mahy BWJ, Collier L (editors) Topley and Wilson's Microbiology and microbial infection, Virology, 9th edition, Hodder Headline Group, Euston Road, London 1: 781-804

Forbes BA and Hicks KE (1993) Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory of polymerase chain reaction, Journal of Clinical Microbiology 31: 1688-1694

Forbes BA and Sahm DF (2002) Bailey and Scott's Diagnostic Microbiology, 11th edition, Mosby Inc. United States of America, pp 538-571

Fujiwara H, Nishio Y and Tsuyuguchi Y (1999) Production of Interferon-γ, Interleukin-10 and tumor necrosis factor-α by human blood mononuclear cells stimulated with mycobacteria. International Journal of Tuberculosis and Lung Disease, pp 121-130

Gautam D (2003) Prevalence of lower respiratory tract pathogens 'Bacterial' in Nepalese HIV/AIDS patients (M.Sc Dissertation) Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Godinho A and Joang L (2004) HIV/AIDS and tuberculosis in Central Asia; Country profiles, The World Bank, UN, Washington, D.C, pp 83-84

Good RC and Shinnick TM (1998) *Mycobacterium*, Balow SA, Deuerden BI (editors) Topley and Wilson's Microbiology and microbial infection, systemic bacteriology, 9th edition, Hodder Headline Group, Euston Road, London 2: 549-576

Grange JM (1998) *Mycobacterium*, Greenwood D, Slack RC, Peuthere JF (editors) Medical Microbiology, 15th edition, ELBS Churchill Livingstone, UK, pp 200-215

Hansen GA (1880) *Bacillus leprae*, Virchows Archiv 79: 32-42

Harawi S and Ohara C (1989) Pathology and pathophysiology of AIDS and HIV related diseases, St. Louis, The C.V MOSPY Company, pp 25, 28-32

Harries AD and Maher D (1997) WHO TB/HIV Clinical Manual, pp 9-23

Hasleton PS (1996) Spencer's Pathology of the Lung, 5th edition, McGraw Hill Companies Inc, pp 126, 223-231

Haslett C, Chilvers ER, Hunter JAA and Boon NA (1999) Davidson's principles and practice of Medicine, 8th edition, Churchill Livingstone. International Edition, pp 46-51

Ingole K, Gaikwad S and Jadhao G (2001) HIV seropositivity in Tuberculosis in rural district, India. Sixth International Congress on AIDS in ASIA and the PACIFIC. Melbourne, Australia, pp 116-119

Issaelbacher R, Braunwald D, Wilson T, Martin DU, Fauci S and Kasper GP (1992) Harrison's Principles of Internal Medicine, 13th edition, International Edition 1: 710-715

Jamison G, Dean T and Feachem RG (2006) Disease and mortality in sub-Saharan Africa, The World Bank, UN, Washington D.C, pp 12-15

Jha KK, Shrestha L, Karki BK, Piryani MR and Rahman MM (2006) HIV prevalence among diagnosed TB patients- A cross-sectional study in Nepal, Journal of Tuberculosis, Lung Diseases and HIV/AIDS 3(1): 60-64

Joshi K (1999) A prospective study on bacteriology of lower respiratory tract infection among the patients visiting Tribhuvan University Teaching Hospital (M.Sc Dissertation), Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Kaufmann SHE (1993) Immunity to intracellular bacteria Annu. Rev. Immunol. 11: 129-163

Kent PT and Kubica GP (1985) Public Health Mycobacteriology*:* A Guide for the Level III Laboratory, US Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, pp 1-207

Koch R (1882) Die Aetiologie der Tuberkulose, Berl Klin Wochenschr 19: 221-230

Lehmann KB and Neumann R (1896) Atlas und Grundis der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik, JF Lehmann, Munchen, Germany, pp 827-830

Mahajan BK (2004) Methods in Biostatistics, 6th edition, Jaypee Publication, pp 168-203

Marsik FJ and Denys GA (1995) Sterilization, decontamination and disinfection procedures for the microbiology laboratory Murray PR, Baron EJ (editors) Manual of Clinical Microbiology, 6th edition, American Society for Microbiology, Washington, DC, pp 86-98

Miller B and Schieffelbein C (1998) Tuberculosis, Bull, WHO 76: 141-143

Nakata K (1997) *Mycobacterium tuberculosis* enhances human immunodeficiency virus-1 replication in the lung. American Journal of Respiratory and Critical Care Medicine 155: 996-1003

Napit IB (2001) HIV Status In United Mission Hospital Tansen, Nepal, JNMA 40(137): 29-33

National Center for AIDS and STD Control (2002), pp 179-183

National Tuberculosis Center (2007) Newsletter of the National Tuberculosis programme World TB Day, March 24th, pp 1-7

NCASC (2004) National Center for AIDS and STD Control, pp 1-12

NCASC (May 2007) Cumulative HIV and AIDS situation of Nepal, Ministry of Health and Population, National Center for AIDS and STD Control, pp 1-4

Pizzo PA and Wilfert CM (1998) Pediatric AIDS, 3rd edition Lippincott Williams and Wilkins A Wolters Kluwer Company, pp 139-154

Puntock J and Pumprug U (1998) HIV infection and TB associated with injecting drug use and resistant *Mycobacterium tuberculosis* Bangkok, Thailand. International Journal of Tuberculosis and Lung Disease 2(11): 205

Richard ST and Weiss RA (1990) Retrovirus infection of human Collier LH, Timbury MC (editors), Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 8th edition, BC Decker Inc, Philadelphia Hamilton 4: 631-656

Rijal BP (2005) An overview of the conventional and recent advances in laboratory diagnosis of tuberculosis, A Journal of Nepal Medical laboratory student's society 6(1): 1-30

Riley RL (1957) Aerial dissemination of pulmonary tuberculosis, Am Rev Tuberc Pulm Dis 76: 931-941

Riley RL (1961) Airborne pulmonary tuberculosis, Bacteriol Rev 25: 243-248

Roitt IM, Brostoff E and Jonthan P (1998) IMMUNOLOGY, 4th edition, London, MOSPY, pp 6-7

Rojas RE, Gehring AJ, Canaday DH, Lakey DL, Harding CV and Boom WH (2003) *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through Toll-like receptor 2. Infect. Immun. 71: 4487-4497

SAARC Tuberculosis Center (2000) Report SAARC seminar for complication and updating of advocacy and IEC material relating to TB and HIV/AIDS control, pp 21-22

Sbarbaro JA (2001) Koch's tuberculosis strategy article. Bulletin of the World Health Organization 79(1): 69-78

Seaton A, Seaton D, Leitch A and Gordon H (2000) Crofton and Douglas's Respiratory Diseases, 5th edition, Blackwell Science 1: 476-543

Sherchand JB, Bam DS and Sherchand S (2002) Human Immunodeficiency Virus (HIV) infection in tuberculosis patients of Nepal. Journal of Nepal Association For Medical Laboratories Sciences 4(4): 1-5

Singh S, Mills E, Honeyman S, Suvedi BK and Pant NP (2005) HIV in Nepal: Is the Violent Conflict Fueling the Epidemic? PLoS Medicine 2: 705-709

Smith I (1996) Gender and tuberculosis in Nepal, Journal of the Nepal Medical Association TB special 24: 49-58

STC (2002) HIV/AIDS in SAARC Region (An Update), pp 5-9

STC (2004) HIV/AIDS in the SAARC Region (An Update), pp 1-25

STC (2004) SAARC regional strategy for TB/HIV co-infection, SAARC-CANADA regional tuberculosis and HIV/AIDS Project, pp 6-9, 12

STC (2005) Gender differences among tuberculosis patients in National TB programmes with in SAARC Countries; A publication by SAARC-CANADA regional tuberculosis and HIV/AIDS Project, pp 5-13

STC (2005) History of TB Control programme in the SAARC Region, SAARC TB and HIV/AIDS Centre, Bhaktapur, pp 17, 24-33

STC (2006) HIV/AIDS in the SAARC Region (An Update), pp 5-20

STC (2006) Tuberculosis in the SAARC Region (An Update), pp 7-15

Stephen M, Blanchard JF and Kang H (2006) AIDS in South Asia, Understanding and responding to a heterogeneous epidemic, The World Bank, UN, Washington D.C, pp 123-127

Subedi BK (1997) Ethics in relation to HIV/AIDS in Nepalese context. Journal of Nepal Medical Association 35: 10-13

Subedi BK (2003) The AIDS Situation in Nepal. Journal of Health Management 5(2): 205-212

Thakker RM, Mistry MA and Mistry AB (2002) Clinical, radiological and bacteriological spectrum of TB-HIV Co-infection: Amargadh Study, International Journal of Tuberculosis and Lung Disease, 33rd Conference Oct 8-16, Montreal, Canada, pp 67-80

The World Bank (2006) HIV/AIDS in Nepal, November, pp 21-32

UNAIDS (2004) Report on the global AIDS coidemic 2004 (PDF version), pp 5-20

UNAIDS and GTZ (2003) HIV/AIDS training tool kit developed by a joint initiative of the UN Theme Group on HIV/AIDS and GTZ in Nepal, February, pp 3

UNAIDS/WHO (2006) UNAIDS/WHO AIDS Epidemic Update, December, pp 17-20

Watt B, Rayner A and Harris G (1996) *Mycobacterium*, Collee JG, Fraser AG, Marmion BP, Simmons (editors), Mackie and McCartney Practical Medical Microbiology, 14th edition, Churchill Livingstone, pp 329-340

Webb GB (1936) Tuberculosis. Hoeber, New York

WHO (2002) Strategic framework to decrease the burden of TB/HIV, pp 296

WHO (1994) Guidelines for HIV surveillance among tuberculosis patients, 2nd edition, Geneva, pp 32-35

WHO (1997) Preventive therapy against tuberculosis in people living with HIV, Weekly Epidemiological Report 74: 385-398

WHO (2002) An expanded DOTS framework for effective tuberculosis control, Geneva, pp 21-30

WHO (2002) Global Tuberculosis Control WHO Report, Geneva Switzerland, pp 295

WHO (2003) Global Tuberculosis Control, Surveillance, Planning, Financing, Communicable Disease, Geneva, pp 28-35

WHO (2004) Tuberculosis and HIV, A framework to address TB/HIV co-infection in the Western Pacific Region, Geneva, Switzerland, pp 7-18, 20

WHO (2006) Global Tuberculosis Control, WHO Report, Geneva, Switzerland, pp 198

WHO, SEARO (1999) AIDS: The Challenge, pp 12

Zopf W (1883) Die Spaltpilze, Edward Trewendt, Breslau, pp 574-576

9. APPENDICES

Appendix-1

Questionnaire

Tribhuvan University

Central Department of Microbiology

Kirtipur, Kathmandu

Personal profile

Name of the patient……Code No……Age…….Sex……Registration No……..

Permanent address…… Ethnic group:……

Occupation……..Educational Status: Literate/Illiterate

Marital status: ( )married, ( ) unmarried Personal history: Smoking ( ), Alcohol ( )

Any other relevant points……

Clinical manifestations

Fever( ) If yes, Duration ( ) Weight loss ( ) If yes, how much ( ) diarrhea ( ) If yes, Duration.

Chest pain ( ) Night sweat ( ) Cough ( ) Blood in sputum ( )

Any other manifestations……..

Treatment history

Medical…….

Surgical (Duration)…….

How long have you been coming to DOTS / Clinic?…….

Effect shown after being treated in DOTS/ Clinic……..

Taken any other medicines: Yes/No

Any other surgery/treatment done before……

For TB diagnosis

Volume of Collected Sputum:…….

Time of collection……

Quality of sputum sample: Saliva mixed/Bloody/Muco-purulent

Sputum detected before( )If yes, result: Positive/Negative

Chest X-ray( )If yes, Diagnosis of TB from X-ray: Positive/Negative

For HIV sero-diagnosis

Any Risk behavior towards HIV ( )

If Yes, IDU/ Migrators/ Visitor of CSWs/ Blood transfusion/ Others…….

Volume of blood collected…….

Blood tested before for HIV ( ) If yes, HIV positive/negative

Laboratory findings (Laboratory use only)

1. AFB Staining of Sputum Sample (Grading based on American Lung Association)

Sample	Result
i)Spot	……
ii)Early morning	……
iii)Spot	……

2. Blood Test By Using ELISA Kit

Sample	Result	
i)Blood (C.N)	…….	Remarks:

Appendix-2

A. Composition and preparation of staining reagents

Ziehl-Neelsen Staining Reagents

a) Carbol fuchsin stain

Basic fuchsin	10 gm
Ethanol	100 ml
Phenol Crystal	50 gm
Distilled water	1 lit

Weighed basic fuchsin was dissolved in ethanol. To 50 gm of phenol crystal, some amount of distilled water was added to dissolve the crystal completely, and then basic fuchsin solution and the phenol were mixed well. Then, the remainder of the distilled water was added up to 1 liter.

b) Decolorizer: Acid alcohol (3%)

Ethanol (70%)	970 ml
Conc. HCl	30 ml

To 970 ml of ethanol 30 ml of conc. HCl was added to obtain 3% acid alcohol.

c) Counter Stain: Malachite green

Malachite green	0.5 gm
Distilled water	100 ml

To 0.5 gm of Malachite green 100 ml of distilled water was added and mixed well.

Appendix-3

Statistical tools

i. ¡ ^{2} test for tuberculosis infection in males and females among 300 patients

Gender Tuberculosis Status	Male	Female	Total
TB positive	66 (a)	13 (b)	79 (a+b)
TB negative	139 (c)	82 (d)	221 (c+d)
Total	205 (a+c)	95 (b+d)	300 (a+b+c+d)

Here,

Null Hypothesis (Ho): There is no association of tuberculosis infection in male and female patients.

Alternate Hypothesis (H_1): The infection of tuberculosis in males and females are associated with each other.

Using,

$$\chi^2 = \frac{(ad-bc)^2 \ (a+b+c+d)}{(a+b)\ (c+d)\ (a+c)\ (b+d)}$$

$$= \frac{12996025 \text{ X}300}{79 \text{ X } 221 \text{ X } 205 \text{ X } 9}$$

$$\chi^2 = 11.47.$$

Degree of freedom = (2-1) (2-1) = 1

From table, χ^2 tab at 1% level of significance= 6.635.

Since χ^2 cal> χ^2 tab, the Ho is rejected and H_1 is accepted.

This shows that there is significant relation between infection of tuberculosis in males and females.

ii. ¡^{2} test for HIV infection in males and females among 300 patients

Gender HIV Status	Male	Female	Total
HIV positive	24 (a)	10 (b)	34 (a+b)
HIV negative	181 (c)	85 (d)	266 (c+d)
Total	205 (a+c)	95 (b+d)	300 (a+b+c+d)

Here,

Null Hypothesis (Ho): There is no association of HIV infection in male and female patients.
Alternate Hypothesis (H_1): The infection of HIV in males and females are associated with each other.

Using,

$$\chi^2 = \frac{(ad-bc)^2 (a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

$$= \frac{52900 \times 300}{34 \times 266 \times 205 \times}$$

$\chi^2 = 0.09.$

Degree of freedom = (2-1) (2-1) = 1

From table, χ^2 tab at 5% level of significance= 3.84

Since χ^2 cal< χ^2 tab, the Ho is accepted and H_1 is rejected.

This shows that the HIV infection in male and female is found statistically insignificant or there is no significant difference of HIV infection in male and female.

iii. ¡^{2} test for development of tuberculosis infection and smoking habit of patients

Smoking Status Tuberculosis Status	Smokers	Non Smokers	Total
TB positive	64(a)	15 (b)	79 (a+b)
TB negative	137 (c)	84 (d)	221 (c+d)
Total	201(a+c)	99 (b+d)	300 (a+b+c+d)

Here,

Null Hypothesis (Ho): There is no association between smoking habit and development of tuberculosis infection.

Alternate Hypothesis (H_1): There is significant relation between smoking habit and development of tuberculosis.

Using,

$$\chi^2 = \frac{(ad-bc)^2 (a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

$$= \frac{11029041 \times 300}{79 \times 221 \times 201 \times 99}$$

$$\chi^2 = 9.52$$

Degree of freedom = (2-1) (2-1) = 1

From table, χ^2 tab at 1% level of significance= 6.635.

Since χ^2 cal> χ^2 tab, the Ho is rejected and H_1 is accepted.

This implies that there is significant relation between smoking habit and development of tuberculosis.

iv. ¡^{2} test for development of tuberculosis infection and alcoholic habit of patients

Alcoholic Status / Tuberculosis Status	Alcohol Users	Alcohol Non-Users	Total
TB positive	56 (a)	23 (b)	79 (a+b)
TB negative	124 (c)	97 (d)	221 (c+d)
Total	180 (a+c)	120 (b+d)	300 (a+b+c+d)

Here,

Null Hypothesis (Ho): There is no association between alcoholic habit and development of tuberculosis infection.

Alternate Hypothesis (H_1): There is significant relation between alcoholic habit and development of tuberculosis.

Using,

$$\chi^2 = \frac{(ad-bc)^2 (a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

$$= \frac{6656400 \times 300}{79 \times 221 \times 180 \times 120}$$

$$\chi^2 = 5.29.$$

Degree of freedom = (2-1) (2-1) = 1

From table, χ^2 tab at 5% level of significance= 3.84.

Since χ^2 cal> χ^2 tab, the Ho is rejected and H_1 is accepted.

This implies that there is significant relation between alcoholic habit and development of tuberculosis. There is high chance of developing tuberculosis in patients with habit of alcohol intake.

v. ¡ ^{2} test for tuberculosis and HIV Co-infection

HIV Status Tuberculosis Status	HIV positive	HIV negative	Total
TB positive	13 (a)	66 (b)	79 (a+b)
TB negative	21 (c)	200 (d)	221 (c+d)
Total	34 (a+c)	266 (b+d)	300 (a+b+c+d)

Here,

Null Hypothesis (Ho): There is no significant relation between HIV infection and development of tuberculosis.

Alternate Hypothesis (H_1): There is significant relation between HIV infection and development of tuberculosis.

Using,

$$\chi^2 = \frac{(ad-bc)^2 (a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

$$= \frac{1473796 \times 300}{79 \times 221 \times 34 \times 266}$$

$$\chi^2 = 2.8.$$

Degree of freedom = (2-1) (2-1) = 1

From table, χ^2 tab at 5% level of significance= 3.84.

Since χ^2 cal<χ^2 tab, the Ho is accepted and H_1 is rejected.

From above data it showed that there is no significant relation between HIV infection and development of tuberculosis.

vi. Mean age and Median age of the studied Population

Age gruop	Mid value (X)	Frequency (F)	Cumulative frequency (c)	F x X
10.5-20.5	15.5	16	16	248
20.5-30.5	25.5	78	94	1989
30.5-40.5	35.5	63	157	2236.5
40.5-50.5	45.5	52	209	2366
50.5-60.5	55.5	62	271	3441
60.5-70.5	65.5	25	296	1637.5
70.5-80.5	75.5	4	300	302
		∑f =300		∑fx =12220

$$\text{Mean age} = \frac{\sum fx}{\sum f} = \frac{12220}{300} = 40.7$$

∴Mean age = 40.7 years.

For Median,

N/2 =300/2 =150

∴Median lies in class interval 30.5-40.5.

Now,

I = 30.5, h = 10, c = 94, f = 63

Using formula,

$$\text{Median} = I + \frac{N/2 - c}{f} \times h$$

$$= 30.5 + \frac{150 - 94}{} \times 10$$

63

= 39.4

∴ Median = 39.4 years.

Appendix-4

HIV (1&2) ELISA TEST KIT instruction manual

Appendix-5
Materials used for the study:

1. Equipments

Autoclave	Bunsen burner
Centrifuge	Cryofreez
ELISA reader	Hot air oven
Incubator	Microscope with oil immersion
Refrigerator	Vortex mixer

2. Glass wares and materials

Bamboo stick	Bottles for reagent
Conical flasks	Droppers
Forceps	Graduated pipettes
Measuring cylinder	Pasteur pipettes
Plastic containers for sample collection	Screw cap centrifuge tubes
Screw cap test tubes	Serum tubes
Slides	Syringes
Test tube stand	

3. Chemicals

3% Acid alcohol	4% Sodium hydroxide
Basic fuchsin	Ethanol
Hydrochloric acid	Immersion oil
Lysol	Malachite Green
Methylene blue	Phenol
Tween 80	

4. Media

Solidified Lowenstein-Jensen Media

5. Kit

Sandwich ELISA Kit

6. Safety

Biological Safety cabinet, BSC II
Centrifuge safety cups
Discard pans
Disinfectant
Disposable masks
Protective clothing
Single use gloves

7. Miscellaneous

Alumunium foil
Blotting paper
Cotton wool
Diamond pencil
Marker
Slide drier
Staining racks