A REPORT OF THE RESEARCH WORK ENTITLED "PREVALENCE OF TUBERCULOSIS AMONG HIV INFECTED PERSONS OF KATHMANDU"

Submitted by

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ABSTRACT

Tuberculosis has been a major public health problem for centuries and HIV has become the most potent risk factor for progression of TB infection to active TB disease.

This study was conducted by Central Department of Microbiology, Tribhuvan University, in Collaboration with Tribhuvan University teaching hospital (TUTH) during January 2004 to August 2005 with a general objective to study tuberculosis in HIV infected people.

100 HIV infected cases, 66 males and 34 females, with mean age 30 years were included and 3 sputum samples of each person were collected for AFB staining, AFB culture and isolates of culture were identified by conventional methods.

From the studied population, 23 cases were diagnosed as tuberculosis, 22 cases by cultural technique and 1 case by AFB staining technique.

Statistical analysis showed that TB disease was significantly higher in HIV infected cases (χ^2 =11.65, P < 0.01) with highest incidence in the age group 21-30 and significant relationship was established between smoking and /or alcoholic habit and the subsequent development of tuberculosis (χ^2 =7.24, P < 0.05 for smoking habit and χ^2 = 4.39, P<0.05 for alcoholic habit).

Among 22 culture positive isolates, the predominant was *Mycobacterium avium* complex (41%) followed by *M. tuberculosis* (27%), *M. kansasii* (18%), *M. fortuitum* (10%) and *M. chelonae* (4%).

This study demonstrated that high incidence of TB in HIV infected people, younger adult were affected with co-infection and atypical types are predominant over typical type.

TABLE OF CONTENTS

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER – I

1. INTRODUCTION

Human immunodeficiency virus/Acquired immunodeficiency syndrome (HIV/ AIDS) has caused a resurgence of tuberculosis (TB), resulting in increased morbidity and mortality worldwide. HIV and *Mycobacterium tuberculosis* have a synergistic interaction, each accelerates the progression of the other. The rapid growth of the human immunodeficiency virus (HIV) epidemic in many countries has resulted in an equally dramatic rise in the estimated number of TB cases. HIV-related TB continues to increase even in countries with well organized national TB control programs (NTPs) that are successfully implementing the world health organization's DOTS strategy. This suggests that, where HIV is fuelling the TB epidemic, full implementation of the DOTs strategy is insufficient to control TB and control of HIV infection must become an important concern for NTPs. **The fact is that tuberculosis and HIV prevention and control programs / strategies have common concerns and interests e.g. the prevention of HIV in the first instance must be a priority for TB control and the provision of TB care, treatment and prevention should be a basic ingredient in HIV / AIDS strategies.** Thus it has been realized that HIV and TB programs need to collaborate to relieve the resultant suffering (STC, 2003)

Not all people infected with tubercle bacilli develop TB, but, certain groups termed as high risk groups, once infected have high chance of development of active TB. Among the high risk groups the majority of them are of HIV/AIDS patients followed by poverty, malnutrition, overcrowding, armed conflict and increased number of displaced persons (cheesbrough 2002). As HIV infection weakness the cellular immunity tubercle bacilli can grow more easily resulting the active TB disease in both persons recently and acquired latent TB infection. The rate of progression from TB infection to TB disease is 10-30 times higher among persons with HIV and TB infections than among persons with TB infection alone. If HIV status is negative, life time risk of developing TB is 5-10%, but if positive with HIV, then the lifetime TB risk may be up to 60%. TB stands as the most significant killer of the persons with HIV contributing to one third of AIDS death globally and 40% of AIDS mortality with in Asia.(STC2003).

The interaction between HIV and TB in personas co-infected with them is bidirectional and synergistic. The course of HIV infection is accelerated subsequent to the development of TB. Compared with CD4+ count matched HIV infected controls without TB, the relative risk of death and development of other opportunistic infections is higher in HIV-TB co-infected patients. Further, increased HIV replication has been demonstrated locally, at sites of disease affected by TB such as affected lung and pleural fluids in patients with HIV –TB (Sharma et. al. 2005).

Evidence also suggests that HIV can promote the emergence of multi drug resistance strains of *Mycobacterium tuberculosis*. Several factors such as (i) increased susceptibility to TB (ii) increased opportunity to acquire TB due to over crowding, exposure to patients with MDR –TB due to increased hospital visits and (iii) mal absorption of anti tuberculosis drugs resulting in suboptimal therapeutic blood levels in spite of strict adherence to treatment regimen (Sharma *et.al.*2005).

Particular features must be taken into account in the case detection, diagnosis and treatment of TB in HIV infected persons. The prevalence of extra pulmonary tuberculosis is increased in HIV-infected persons. This is because there is failure to develop characteristic granuloma of immunogenic origin and there is also a suppression of tissue necrotizing reaction and scar formation that would other wise limit the spread of infection (Grange 1990). The commonest form of extra pulmonary TB in HIV – infected persons are lymphadenopathy (especially cervical nodes), miliary disease, meningitis and tuberculous effusions in the affected areas , Pleurisy, pericarditis and peritonitis. Many HIV infected patients with extra-pulmonary TB also have coexistent pulmonary TB whenever possible the diagnostic specimen should be as many as possible and should be examined for acid-fast bacilli (AFB) and culture for mycobacteria (WHO 1998).

Tuberculosis can develop at any point during the progression of HIV infection. The presentation of pulmonary TB depends on the degree of immunosuppressant. At an early stage of HIV infection the clinical picture resembles the disease in non HIV – infected adult patients. with no difference in the frequency of smear positive findings. At late stage of HIV –infection the clinical picture resembles primary pulmonary TB. The sputum smear is negative and the chest radiology shows infiltrates without cavities. Cough and haemoptysis are less common in HIV patient then in non-HIV infected patients (WHO 1998).

CHAPTER – II

2. OBJECTIVES OF THE STUDY

General

- To find prevalence of tuberculosis in HIV infected people and to study tuberculosis in such people.

Specific

- To study the characteristics of HIV/TB infected people.
- To isolate *Mycobacterium* species from HIV infected people.
- To identify *Mycobacterium species* isolated from HIV infected people.
- To characterize the *Mycobacterium tuberculosis* and *Mycobacterium avium* complex isolated from HIV infected people.
- To assess the level of anti-retroviral therapy among the HIV infected people.
- To correlate AFB culture and AFB staining in HIV infected people.

tuberculosis has been referred "tabes pulmonali". The acid fast nature of the organism was discovered by Ehrlich in 1885 (Burke, 1995) 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

3.3 TUBERCULOSIS AS A MAJOR PUBLIC HEALTH PROBLEM AND ITS CONTROL

Accordingly to world health organization estimates, one-third of the world's human population has been infected by tuberculosis. An estimated 8-10 millions people developed Overt tuberculosis annually as a result of primary infection. This proportion of infected persons is similar in developing and developed countries but in the former most infected persons are in productive age (15-45) years whereas in latter most infected persons are in older age group. Thus in Nepal, for example, 60% of the infected persons are in the productive age while in USA and UK, for example, only about 12% of the persons in the age range principally exposed to HIV, 15-45 years are infected with TB.

Tuberculosis is the cause of 7% of all deaths and in developing countries 1 in 4 (i.e. 25%) preventable adult deaths, even though it is among the most effective of all adult disease to treat (Murray *et al.*,1990). However, MDR-TB has caused some difficulties to cure tuberculosis, resulting more suffering and deaths frm this disease.

Fortunately, the introduction of DOTS-plus system for the treatment of MDR-TB has created a ray of hope to reduce the case fatality rate. Thus a ray of hope is brighter to fight TB/MDR-TB through successful implementation of DOT/DOTS-plus.

3.4 HIV AS A ANOTHER MAJOR PUBLIC HEALTH PROBLEM AND ITS CONTROL

The HIV/AIDS epidemic continues to grow worldwide and poses 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 the other hand, the epidemic has mushroomed globally into an unforeseen and unpredicted nightmare so that the morbidity

societies e.g. injecting drug users, commercial sex workers, migrants, poor. Uneducated women and children. The facts are clear with 95% of HIV infected people living in less industrialized, developing countries. As most of the population of sub-Sahara Africa region has already been swept away by the HIV epidemic and the preview of this image has already been reflected to the India (our neighbour nation) 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.

The good news is that we are not powerless against HIV/AIDS for we do have evidence that prevention and intervention methods do work (e.g. the Thailand 100% condom programme). If someone acquires HIV infection, it can be treated via antiretroviral (ARVT) / High active antiretroviral Therapy (HART) and managed as a chromic disease. Besides these, now there is availability prevention of mother to child transmission of HIV (PMTCT) which affords the birth of healthy (HIV negative) child from HIV positive mother.

3.5 Double Pandemic Devastation of TB/HIV Co-infection

Tuberculosis is one of the most common opportunistic infection is HIV positive cases and recognized as the leading cause of death of people living with HIV / AIDS (PLWHA) as shown in table 1.

Table 1: Most Common HIV-related Condition –UNAIDS 1998

HIV Related Condition	Percent
Oral Candidiasis	53
Pneumocystis Carinni Pneumonia (PCP)	24
Tuberculosis	22
Esophageal Candidiasis	21
Cytomegalovirus	21
Kaposis Sarcoma	15
Toxoplasmosis	11
Cryptococcosis	9

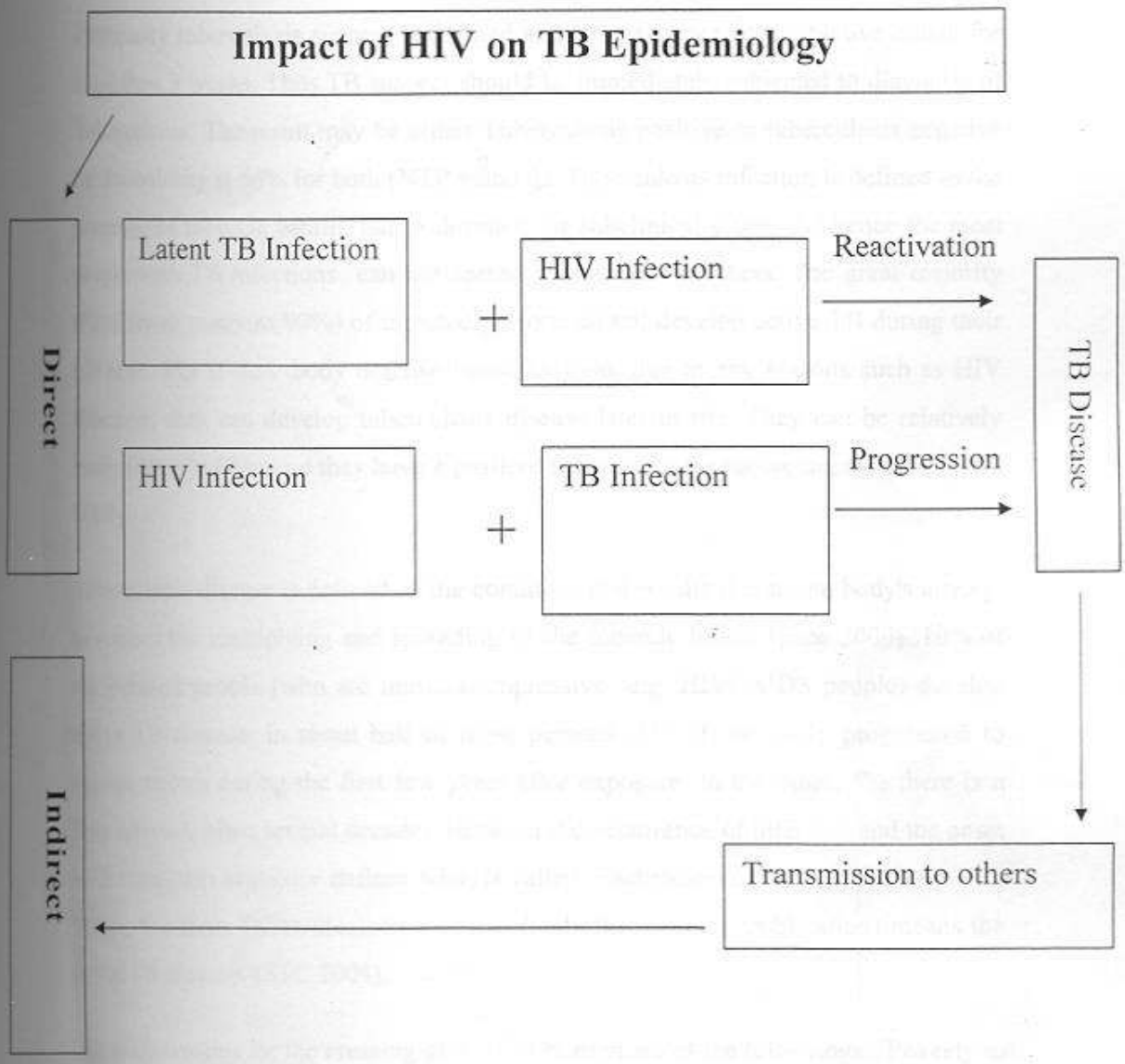
The co-existence of HIV infection and tuberculosis has been hailed as one of the most serious threats to human health. A person infected by only tubercle bacilli HIV negative cases have about a 10% chance of developing tuberculosis during the remainder of their lives; thus they have a less than 0.5% chance of developing overt disease annually. By contrast, an HIV-positive person already infected by tubercle bacilli has an 8% chance of developing overt disease annually, or up to 50% during the remainder of their relatively short life span (Doli, Raviglione and Kochi 1994) as shown in table 2. Thus HIV positive persons infected by tubercle bacilli have a 20-30 fold higher chance of developing tuberculosis than their HIV negative counterparts.

Table 2: Lifetime Risk of TB Development

PPD / HIV Status	Percentage Risk of Development of TB
PPD +ve / HIV –ve	10
PPD +ve / HIV +ve	50

From the global incidence of tuberculosis, it was estimated that 9% of all new TB cases in adults (aged 15-49 years) were attributable to HIV infection but the proportion was much greater on African region where the incidence of TB attributable of HIV was 31% (STC newsletter 2003).

The fueling mechanism of HIV on TB epidemic is shown below.



Flow Chart Showing Fueling Mechanism of HIV on TB Epidemic

3.6 Tuberculosis Suspect, Tuberculosis Infection and Tuberculosis Disease

Pulmonary tuberculosis suspect is defined as the persistence of productive cough for more than 3 weeks. Thus TB suspect should be immediately subjected to diagnosis of Tuberculosis. The result may be either Tuberculosis positive or tuberculosis negative i.e. Probability is 50% for both (NTP manual). Tuberculosis infection is defined as the presence of tubercle bacilli, but in dormant/ or subclinical stage, and hence the most people with TB infections can not spread the disease to others. The great majority (Possibly as many as 90%) of infected persons do not develop active TB during their life time. But if their body defense becomes weak due to any reasons such as HIV infection, they can develop tuberculosis disease later in life. They can be relatively easily identified because they have a positive response to the tuberculin skin test (STC 2004).

Tuberculosis disease is defined as the condition that results due to the body's attempt to control the multiplying and spreading of the tubercle bacilli (pace 2000) .10% of the infected people (who are immunosuppressive e.g. HIV/ AIDS people) develop active TB disease. In about half of these persons (5% of the total), progression to disease occurs during the first few years after exposure. In the other, 5% there is a long interval, often several decades, between the occurrence of infection and the onset of disease; this sequence defines what is called reactivation of latent infection . Hence, the term Tuberculosis in this text (and other articles, publication) means the actual TB disease. (STC 2004)

The main reasons for the creasing global TB burden are of the followings: **Poverty** in various population, not only in developing countries but also in inner city population in developed countries, **changing demographics**, with increasing world population, **insufficient and inadequate health coverage of the population**, especially in poor countries and of vulnerable groups of the population in all countries, **negligence and under finding of TB control programme with inadequate cases detection, inadequate case management and poor care rates in several countries, the impact of HIV epidemics** (WHO 2003).

Globally TB control is possible through the DOTS strategy, which represents an organizational framework for effective utilization of the existing tools for the diagnosis and treatment. The five component of DOTS strategy are:

- Sustained political commitment.
- Access to quality – assured sputum microscopy.
- Standardized short course chemotherapy for all cases of TB under proper case management conditions, including direct observation of treatment.
- Uninterrupted supply of quality assured drugs.
- Recording and reporting system enabling outcome assessment of all patients and assessment of overall programme performance (WHO 2003).

3.7 Anatomical Sites of the Disease:--

The main categories of TB by anatomical site of disease are pulmonary and extra-pulmonary TB. Generally, recommended treatment regimens are similar, irrespective of site. There are some exceptions such as tubercle meningitis, for which a prolonged continuation phase is recommended (WHO1998)

Pulmonary TB refers to disease affecting the lung parenchyma, Tuberculous intrathoracic lymph nodes (mediastinal and hilar)or tuberculous pleural effusion, without radiological abnormalities in the lungs , therefore make up the case definition of extra- pulmonary TB .Symptoms of pulmonary TB are : chest pain, cough, weight loss , night sweat , fever, appetite, tiredness.(WHO1998)

Extra- pulmonary TB is 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 , and joints, kidneys, pericardium, intestine, Peritoneum and skin. However ,TB may affect any organ or tissue of the human body. In miliary TB, acute haematogenous infection is observed. 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 world-wide.(WHO 1998)

3.8 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 server if parenchymal involvement is extensive.

The following forms of extra-pulmonary TB are classified as severe: meningitis, Miliary, pericarditis, peritonitis, bilateral or extensive pleurisy, spinal disease with neurological complication, intestinal, and genito –urinary 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.

3.9 Bacteriological Status

"Smear positive" and "smear negative" are the most useful bacteriological classification of pulmonary cases. Whenever a case is diagnosed as smear –positive or smear-negative it should be registered in the recording and reporting system.(Smear-positive cases are the only cases for which bacteriological monitoring of cure is available). In places where culture facilities are available, the culture diagnostic results are included in the bacteriological classification. In high-prevalence countries, among all bacteriological positive cases, about 70% of all bacteriologically positive cases are identified with the initial examination of a first set of 3 sputum smears, and up to 50% of the remainder with further repeated sputum- smear examinations. Only 10-15%will be positive by culture but negative by smear.

Under programme conditions- when microscopy laboratory services are available and diagnostic criteria properly applied –smear positive cases represent 65-80% of the total pulmonary cases in adults, and 50% or more of all TB cases.

3.10 Mode of Transmission

Transmission of tuberculosis is almost exclusively through respiratory routes. When droplet nuclei are created through coughing / sneezing / laughing /talking by untreated persons suffering from pulmonary tuberculosis (the most common form) in a confined environment, they may be inhaled by susceptible persons. who are in close contact

and become infected. This type of the transmission of infections diseases is known as air borne transmission. It is well established that, sputum smear positive cases of pulmonary TB are the main sources of transmission of infection. They are responsible for almost 95% of the transmission of infection in the community. They also suffer from extensive disease and thus are at higher risk of dying. If not treated properly they become the sources of drug resistance bacilli (STC 2004). Some documented facts about transmission are:

- The droplet nuclei produced by sneezing can carry viable tubercle bacilli up to 3 m.
- The droplet nuclei produced by coughing can carry viable tubercle bacilli up to 1.5m.
- One cough can produce 3,000 -5,000 droplet nuclei (Rijal *et.al.*)
- One cough is equivalent to about 5 minutes of loud talking in terms of resulting number of droplet nuclei (Rijal *et.al.*).
- One air borne particle (1-3μm) contains 1-10 bacilli.
- Persons who excrete 10,000 or more tubercle bacilli per ml of sputum are the main source of infection to others (Goothus *et.al.*)
- Lesser the size of droplet nuclei higher is the chance of transmission because, they are not trapped in the nose but may reach the alveoli of lungs.

3.11 Mycobacteria

3.11.1 Morphology and General Characteristics

Mycobacteria are cylindrical bacteria of size 1-4μm in length by 0.3-0.6 in length , which frequently forms small clumps (smith and easmon ,1990).They are weakly Gram positive non motile , non sporulating non capsulated and exhibit acid and alcohol fastness i.e., they are not readily decolorized by 3%acid and alcohol once stained with carbol Fuschsin. The acid and alcohol fastness is due to the presence of thick, complex , lipid rich , 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, mycobacteria possess peptidoglycan (inneimost) layer, Arabinogalactan (external to peptidglycan) layer and mycosides layer (forming species or strain specific surface lipid).

3.11.2 Cultural Characteristics

Mycobacteria are obligate aerobes and slow growers (average generation time 18 hours). Even the most rapid growers require 3-4 days to grow on simple media and most disease associated mycobacteria require up to 8 weeks on complex media enriched with eggs . Mycobacteria are usually cultivated in lowenstein –Jensen(LJ) media which consist of fresh egg, glycerol, asparagines several mineral salts and malachite green (to inhibit contaminants). In resent years several modification of L.J. medium have been developed, e.g. Ogawa Medium. Which is cheaper than LJ medium due to the exclusion of asparagines and some mineral salts.

Typical colonies of *M. tuberculosis* are rough, crumbly. Waxy, non- pigmented (cream colored) and slow growers I.e. only appearing after 3 weeks of incubation.

For preliminary identification of tubercle bacilli the fallowing characteristics are applied (WHO 1998).

- Tubercle bacilli do not grow in less than one week and usually take three to four weeks to give visible growth.
- The colonies are buff colored (not yellow) and rough having the appearance of bread crumbs or cauliflower.
- They do not emulsify in the saline for making smears but give a grander suspension.
- Microscopically they are frequently arranged in serpentine cords of varying length or show distinct linear clumping.

3.11.3 Mycobacteria Other than tuberculosis (MOTT)

MOTT, also known as Non- tuberculous mycobacteria (NTM) are the large No. of mycobacterial species frequently found in environment habitants that may colonize and occasionally cause infection in humans and animals.

MOTT are becoming more prevalent with the increasing prevalence of immunocompromised hosts, particularly in relation to the AIDS pandemic.

The most commonly encountered MOTT in the HIV patients are:

- *Mycobacterium avium* complex also known as *Mycobacterium avium intracellular* (MAI) complex, and
- M. *Kansasii.*

Most MOTT are acid fast and indistinguishable from *M. tuberculosis* except by a negative niacin test: other distinguishing characters are growth rate and pigmentation. Runyon classified the NTM into the following 4 groups by the growth rate and pigmentation (Brooks *et all*):

Group I Photochromogen -	Pigmentation only when exposed to light e.g. *M. Kansasii, M. Marinum & M. Simie*
Group II Scotochromogen -	Produce deep yellow to orange yellow pigment in dark.
Group III Non chromogen -	Do not produce pigment at all. e.g. MAC
Group IV Rapid growers -	Produce colonies with in 3-4 dap of incitation e.g. *M. Fortuitum, M. Chelonae.*

3.12 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 the macrophage rather than the production of toxic substance (Grange). The immune response to the bacilli is of the cell mediated type, which depending on the type of T-helper cells, involved, may either lead to the protective immunity and resolution of the disease or to the tissue destroying hypersensitivity reaction and progression of the disease process. Following infection, the nature of the immune response changes with tune so that human tuberculosis is derisible into primary and post primary form with quite different pathological features.

Primary tuberculosis is characterized by following features.

- Ghon focus formation.
- Primary complex formation.
- Granulomatous reaction.
- Miliary (Disseminated) TB.

Ghon focus is defined as the initial lesion formed by multiplication of tubercle bacilli inside the alveolar macrophage.

Primary complex is defined as the Ghon focus formation together with enlarged hilar lymph nodes.

Granulomatous reaction means the hypersensitivity reaction leading to giant cell formation and epitheloid cell formation.

Miliary (Disseminated) TB is defined as the formation of lesions in many organs via blood stream infection.

Table 3: Pathological Features of Primary Tuberculosis

Features	Symptoms
Hypersensitivity reactions	Erythema nodosum phlyctenular conjunctivitis dactylitis.
Pulmonary and pleural complications	tuberculosis pneumonia. Lobar collapse (bronchial compression)
Disseminated diseases.	lymphadenopathy (usually lenical) Meningitis. Pericarditis. Miliary diseases

Post Primary TB

In many individuals, the primary complex resolves and only evidence of infection is the tuberculosis reactivity. After an interval of months, years or decades, reactivation of dormant foci of tubercle bacilli may lead to post primary tuberculosis. Reactivation may occur spontaneously or after any

immunocompromised state. In post primary TB, dissemination of bacilli to lymph mode 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.

3.13 EPIDEMIOLOGY OF TB

3.13.1 Global aspect of TB burden

Nearly one third of the global population (2 billions) is infected with *Mycobacterium tuberculosis* and is at risk of development of active clinical TB disease. There were 8.8 million estimated new cases of TB in 2002, of which 3.9 million were smear positive (infections type). Everyday more than 5000 people are dying from the TB disease approximately 2 million per year. (STC October, 2004).

3.13.2 Tuberculosis Burden with in SAARC Countries

Almost 50% of the adult population of this region have already been infected with *Mycobacterium tuberculosis* and are at high risk of developing tuberculosis. In the year 2002, an estimated 2.4 million people newly developed TB disease of which about 1.1 millions are smear positive and capable to spread the disease to others. According to this estimate SAARC region is bearing 27.4% of the total global new TB cases. India, Bangladesh and Pakistan are occupying the 1^{st}, 5^{th} and 6^{th} position in the list of 22 high burden nations (according to the estimated incidence of TB: high burden countries, 2002), with India revealing the highest (20%) global absolute burden of TB (STC, October, 2004).

3.13.3 Tuberculosis Burden in Nepal

About 45% of Nepalese people are infected with TB. Every year 40000 people develop active TB, of whom 20000 have infections pulmonary disease. These 20000 are able to spread the disease to others. Fortunately introduction of treatment by DOTS has already reduced the numbers of deaths. (STC, October, 2004)

3.14 EPIDEMIOLOGY OF TB/HIV CO-INFECTION

3.14.1 Global Aspect of TB/ HIV Co-infection

According to the recent estimates by the WHO and joint united Nations Programme on HIV/AIDS (UNAIDS), nearly 39.4 million people were living with HIV/AIDS world wide, 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 *Mycobacterium tuberculosis* globally, 70% of co-infected people were in Sub-Saharan Africa 20% South East Asia and 4% in Latin America and the Caribbean. TB accounts for about 13% of HIV related deaths worldwide. Globally, 9% of all new TB cases (31% m Africa) were attributable to HIV/AIDS. (Sharma *et. al* 2005)

3.14.2 SAARC (Regional) Aspect of TB/HIV Co-infection

Over 4 million estimated HIV infection are existing within the SAARC region (STC 2003). 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 as shown below.(table 4)

Table 4: Percentage of Estimated New Cases of TB Attributable HIV in SAARC Region

Country	Percentage of New TB Cases Attributable to HIV
Bangladesh	0.1
Bhutan	NA
India	0.8
Maldives	NA
Nepal	0.6
Pakistan	*
Sri-Lanka	NA

Source: STC, 2003

Note NA = Data not available

* = According to surveillance report, no care detected

3.14.3 TB/HIV Co- infection in Nepal

Different NGOS / INGOS have estimated that there are 62,000 People Living with HIV / AIDS (PLWHA) in Nepal but the statistical date reported by National Centre for AIDS and STD Control (NCASC) during July 2005 shows that there are only 5069 (3696 male 1373 females) reported cases of PLWHA in Nepal. Further it has also been reported that among 5069, PLWHA 895 are AIDS.

With nearly half of the Nepal's population infected with Tuberculosis, the increasing incidence of the disease among HIV / AIDS people is a potent combination that could shatter the country's grossly ill-equipped health system.

According to a study conducted by Ministry of Health the TB/HIV Co-infection is a rising trend. It was observed that in 1998-1992, out of 14 AIDS cases 11 cases i.e. 78.5% had TB where as during 1998 -2002, out of 442 AIDS cases 357 cases i.e. 80.76% had TB.

According to a study conducted by the Nepal Anti-tuberculosis association (NATA) 75% of AIDS patients in Nepal had TB.

"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 such as NTC Kathmandu, INF Nepalgunj, Tansen Palpa, NATA Biratnagar and RTC Pokhara showed that HIV prevalence among TB patients continuous to rise and had increased four fold in the past eight years as shown in table 3.

Table 5: HIV in TB Patients Sentinel Surveys1993-2002.

Surveillance	% of HIV in TB
1993-1994	0
1995-1996	0.6
1998-1999	1.88
1999-2000	1.4
2001-2002	2.4

A study conducted by sherchand *et al* in Kathmandu during 2001 showed that HIV prevalence in TB prevent as 6.1%.

In another recent study (2003) conducted by central department of microbiology, T.U., and Nepal Tuberculisis centre (NTC) in Tansen mission Hospital by Dhungana *et al* showed that HIV prevalence in TB patients as 10.76%.

Another recent study conducted by Dipendra Gautam during late 2003,Kathmandu, in order to find prevalence or respiratory pathogens in HIV infected people showed that 22.22% of PLWHA had tuberculosis.

Above statistical data suggests that recently there is lacking of specific study concerning the surveillance of TB in HIV infected people and it is thought that this study to some extent measures this burning issue and unfolds the impact of HIV epidemic on TB epidemiology in Nepal.

3.14.4 Prevalence of *Mycobacterium avium* Complex (MAC) lung infection in HIV/AIDS patients

It is estimated that approximately 25% and perhaps as high as 50% of HIV infected patients develop MAC bacteremia and disseminated infection during the course of AIDS in western countries (Brooks *et. al*)

3.14.5 Prevalence of *Mycobacterium Kansasii* in HIV/AIDS patients

M. Kansasii is the second most common MOTT to affect paients with HIV/AIDS (Bamberger *et. at* 1994). Pulmonary and disseminated disease due to *M. Kansasii* has been reported in HIV patients (Hirasuna 1987 *et. al*, Parenti *et. al* 1995). Data from the CDC indicate that disseminated infection develops in 0.44% of HIV/AIDS patients.

3.15 LABORATORY DIAGNOSIS OF TUBERCULOSIS

The definitive diagnosis of TB 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 diagnosis with little success.(Greenwood)

3.15.1 Laboratory Methods for the Diagnosis of Tuberculosis in HIV Infected Persons

Laboratory methods of 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. It is found that 24% of people who were smear negative in multiple sputum examination had TB on bronchoscopy. Similarly, tuberculin skin test may be less useful in people with

HIV because immune response 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 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 CO2 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 (Where routine use of such sophisticated technique is troublesome) must rely on following. Conventional methods for diagnosis of TB in HIV/AIDS patients

i) Sputum microscopy.
ii) Fluorescence microscopy
iii) Sputum Culture.
iv) CSF investigation
v) Examination of lymph node aspirates.
vi) Biopsy

i) Sputum microscopy.

In high prevalence countries, TB case detection is largely based on microscopic examination of sputum from acid-fast bacilli (AFB). It represents one of the five pillars of DOTS strategy. 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, the a definite case should have at least one such result confirmed by a second smear examination, a suggestive chest radiograph, or alternatively there should be one positive mycobacterial culture result.

The simplest method for the detection of AFB is by Ziehl-Neelsen (ZN) staining technique. In Z-N staining, use is made of the acid fast property of mycobacteria i,e once started with carbol Fuschsin (a mixture of basic Fuschsin and phenol red) they are not easily decolorized by dilute mineral acids (3% HCl). This is because of the presence of mycolic acid in the Cell wall which tightly binds to the dye. The decolorizing agent remove the red dye, from the back ground cells, tissue fibres and any organisms except mycobacteria and hence, they are referred to as acid fast bacilli (AFB).

ii) Fluorescence microscopy

With the flurochrome stain, such as auramine rodamine stain, Mycobacteria fluoresce with rodamine stain, Mycobacterium fluoresces with a bright orange color and can be easily seen on low power microscopy, increasing the sensitivity of the smear.

Advantages

Low power objective is used.

One person can see 200 or more smears per day.

Disadvantages

High cost of the compete microscope unit and its maintenance.

iii) Sputum Culture.

Bacterial culture provides the definitive diagnosis of tuberculosis. Depending on the decontamination method and the type of culture medium used, as few as ten viable tubercle bacilli can be detected. Culture increases the no. of tuberculosis cases often by 30-50% detect cases earlier, often they become infectious. Since the culture technique can detect few bacilli, the efficiency of diagnosing failure at the end of the treatment can be improved considerably. Culture also provides the necessary materials for drug susceptibility testing. The turn around time of 6-8 weeks is one disadvantage of the culture.

Media of Choice

Solid Media

(a) Egg based

(i) Lowenstein Jensen (LJ) Media

(ii) Ogawa Media

(iii) Dorset egg media

(b) Agar Based Media

(i) Middle brook 7H10 & Middle brook 7H10Se

(ii) Middle brook 7H11 & Middle brook 7H11Se

(c) Liquid Media

(i) BACTEC 12B broth

(ii) Middle brook 7H9 broth

Among these media , the routinely used 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.

As the sputum specimen submitted to the TB laboratory are contaminated to varying degrees by more rapidly growing normal flora organisms, the specimen should be subjected to digestion and decontamination. This technique not only liquefies the organic debris, but also eliminates the unwanted normal flora. All currently available digesting/decontaminating agents are toxic to tubercle bacilli therefore , to ensure the survival of the maximum number of bacilli in the specimen, the digestion/ decontamination procedure must be precisely followed.

The most widely used decontamination technique is modified petroff;s method which utilizes 4% NaOH as a digesting decontaminating agent. I this technique sputum is treated with double volume of the NaOH, allowed stand for 15 min (with occasional shaking) and then centrifuged. Them the deposit thus obtained should be washed with normal saline, centrifuged to concentrate the bacilli and then inoculated into media.

iv) CSF investigation

If tubercular meningitis is suspected, which is common disseminated TB in AIDS. AFB staining & culture of CSF is highly appreciable. AFB, however, are difficult to detect in CSF. Centrifugation of the CSF followed by microscopic examination of the deposit and culture of the same increate the chance of tubercular meningitis.

v) **Examination of lymph node aspirates.**

Persistent generalized lymphadenopathy (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.

v) **Biopsy**

The invasive technique is not necessary when there is chance of obtaining specimen through less aggressive methods .Thus, pulmonary and genitourinary tuberculosis ,situation in which tubercle bacilli are frequently shed into body fluids generally don't require biopsy for diagnosis. Isolated invlovement of the pleura or other tissues that don't communicate externally may best be diagnosed through examination of tissue (Rijal) .

It should be noted, however, that the findings of histologically compatible tissue changes, most notably granuloma formation within the specimens, do not conclusively prove a diagnosis of tuberculosis. Other conditions, including those by non-tuberculous *Mycobacteria* and fungi as well as inflammatory diseases such as sarcoidosis may produces similar tissue changes. In some immunocompromised patients, such as those with advanced HIV infection, minimal tissue reaction may occur despite extensive disease.

In miliary tuberculosis, multiple organs may seeded with tubercle bacilli. Although organs such lungs and kidneys are frequently involved, in such setting sputum and urine specimens are found to be 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 patient with miliary changes on the chest x-ray in negative sputum study(Rijal).

Similarly, liver and bone marrow biopsy are important diagnostic opportunistic up to 40-90% of miliary tuberculosis. Percutaneous biopsy

of pleura, peritoneum, and synovium also persue and the yield of granuloma in this tissue has typically ranged 40-80%. And culture of this tissue and associated fluid can increase the yield (Glassroth, 1993).

3.15.2 Newer Techniques for Diagnosing Tuberculosis

Most of the recent advances in the laboratory diagnosis of tuberculosis have been directed at the development of rapid culture, identification and drug susceptibility systems for use in TB specialist laboratories.

A. Bactect 460 TB rapid radiometric Culture Systems:

Developed by Becton Dickinson diagnostic systems, the Bactect is an automated early detection system, in which specimens are cultured in a liquid media containing C14 - labeled palmitic acid. Growing mycobacteria utilize the acid, releasing radioactive CO_2 which is measured in the Bactec instrument. Growth of *M. tuberculosis* can be detected with in 12 days (WHO, 1998).

B. Bactec 9000 MB system:

This instrument based on the continuous growth monitoring system. Organisms are cultured in a modified middle brook 7H9 broth. The instrument detects growth by monitoring O_2 consumption by means of fluorescent sensor.

C. Septi-Check AFB System:

This system is also developed by Becton Dickison. This system consists of biphasic culture system made up of a modified middle brook 7H9 broth with a three sided paddy containing chocolate, egg-based and modified 7 H 11 solid agars. The bottle is inverted regularly to inoculate the solid media. Growth is detected by observing the three sided paddle.

D. Mycobacteria Growth Indicator Tube (MGIT):

Developed by Becton Dickson, consists of culture tube contains Middle brook 7H9 broth and a fluorescent compound embedded in a silicone sensor. Growth is detected visually using an ultra-violet light oxygen (O_2) diminishes the fluorescent output of the sensor, therefore, O_2 consumption by organisms present in the medium is detected as an increase in fluorescence (Forbes *et al*., 1998).

E. ESP Culture System II:

This works continuous growth monitoring systems. Organisms are cultured in a modified middle brook 7H9 broth with enrichment and a cellulose sponge to increase the culture's surface area. The instrument defects growth by monitoring pressure changes that occur as a result of O_2 consumption or gas production by the organism as they grow (Forbes *et al.*,2000).

F. Polymerase Chain Reaction (PCR):

PCR, a well developed technique, is used extensively for the diagnosis of TB (Forbes & Hicks, 1993; Notle *et al.*, 1993, Kent *et al.*, 1995). 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 defect as little as 1 pg of DNA; which is equivalent to approximately three bacilli. Nowadays, PCR could become a valuable alternative approach to the diagnosis of TB infections. 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 16 S rRNA sequence. The detection system is based on hybridization with an *M. tuberculosis* complex specific capture probe in a micro plate format.

G. Chromatographic Analysis:

The analysis of mycobacterial lipids by chromatographic methods, including thin layer chromatography (TLC), gas liquid chromatography (GLC), Capillary gas chromatography methods and reverse-phase high performance liquid chromatography (HPLC) has been used to identify mycobacteria. HPLC of extracted mycobacteria was a very specific and rapid method for identification of species.

3.16 IDENTIFICATION TESTS

The first step in identification is to determine whether an isolate is a tubercle bacilli or mycobacterium other than tuberculosis (MOTT).

Mycobacterium tuberculosis grow slowly, produce rough and buff colored (not yellow) colonies, reduce nitrate to nitrate, gives niacin test +ve and do not produce catalane at 68^0c.

Thus a series of biochemical tests in combination with the observation of growth rate and pigmentation characters he of mycobacterium aid to encountered mycobacterium species. Nevertheless there biochemical tests and colonial characterizes enable the precise identification of >95 of M. tuberculosis strain.

3.16.1Growth Rate

On the basis of growth rate mycobacterium are classified as

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

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

3.16.2 Pigmentation

Presumptive identification of certain pigment production mycobacteria can be done by the colour of their colonies for e.g. after 2 weeks of ambient light, *M. kansasii* produce bright yellow or orange.

3.16.3 Niacin Test

Nicotinic acid (Niacin) 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 detections useful for its definitive diagnosis. Niacin negative *M. tuberculosis* strains are very rare, while very few other mycobacterial species yield positive niacin tests.

3.16.4 Nitrate Reduction Tests

M. tuberculosis is one of the strongest reducers of nitrate among the mycobacteria, which allows for this test to be used in combination withy the niacin test in differentiating M. tuberculosis from the other mycobacteria.

Cultures to be tested for nitrate reduction should be four weeks old and have abundant growth Lowenstein Jensen egg medium are recommended.

3.16.5 Catalase Test

Catalase is intracellular, soluble enzyme capable of splitting hydrogen peroxide into water and oxygen, i.e. $2H_2O_2 \longrightarrow 2H_2O + O_2$. The oxygen bubbles into the reaction mixture to indicate catalase activity. Virtually all mycobacteria passes catalase enzymes, except for certain isoniazid-resistant mutants of *M. tuberculosis* and *M. bovis.*

As *M. tuberculosis* loses catalase activity at 68°C, performance of catalase test at this temperature is done for its identification.

Identification Chart of Same Commonly Isolated mycobacterial Species in HIV Patient is given in Table 6

Table 6: Identification chart of some commonly mycobacterium species.

Species	Growth rate	Pigmentation	Niacin test	Nitrate reductase	Catalase at 68^0c
Mycobacterium tuberculosis	Slow	-ve	+ve	+ve	-ve
Mycobacterium avium Complex	Slow	-ve	-ve	-ve	± ve
Mycobacterium Kansasii	Slow	+ve	- ve	+ ve	+ ve
Mycobacterium Fotuitum	Rapid	-ve	- ve	+ ve	+ ve
Mycobacterium Chelonae	Rapid	-ve	V	- ve	V

Sorce: Chakraborty

Note-V=Variable Reaction

CHAPTER - IV

4. MATERIALS AND METHOD

4.1. MATERIALS:

The materials, equipments, chemical and media used in this study are listed below.

List of materials:

Beakers	conical flasks	Glass slides	Glass rods
Measuring Cylinders	pipettes	Forceps	Labeling stickers
Spirit lamp	Bunsen burner	Inoculating loops	Markers
	Racks & Baskets	Soaps	Containers
Cotton	Tissue papers	Eggs	

Equipments:

Clean bench	Hitachi, Japan
Centrifuge	Remi, Japan
Incubator	N.S.W., India
Autoclave	Sakura, Japan
Coagulator	Te-Her, Japan
Water bath	Sakura, Japan
Hot air oven	Yamato, Japan
Microscope	Olympus, Japan
Distilling Apparatus	Yamato, Japan
Refrigerator	Hitachi, Japan

Chemicals and Reagents:

Sodium glutamate	Glycerol	Malachite green (2 %)
KH_2PO_4	Na_2HPO_4	Tween 80 (10 %)
$NaNO_3$	Sulfanilic Acid	N-napthyl ethylene diamine dihydrochloride
4 % NaOH	30 % Hydrogen peroxide	Carbol fuchsin
Basic Fuschsin	Ethanol	Phenol Crystals
0.1 % Methylene blue	Niacin strips	Tartaric acid

Media: (Composition in Appendix II)

3 % Ogawa media

Nitrate broth

4.2 METHODS

This study was conducted at TB laboratory of Teaching hospital. Altogether 300 sputum samples (and CSF too in possible cases) were collected from 100 HIV infected patients.

Sputum sample collection was done by periodic visit to the suspected sites such as OPD/indoor section of TUTH, Mahrajgunj; NaVa Kiran Plus, Budhanilakantha; Sparsha Nepal, Chobar gate; Karuna Bhavan, Basipati; Sheha Samaj, Lalitpur; Maiti Nepal, Gaushala; Nepal Plus, Dhumbarahi; Vision Plus,Putali sadak; SACTS-VCT, Thapa thali; Nepal Youth,Tokha; Aastha positive groupTokha and blue dimond society, Lazimpat.

For investigation of 100 HIV positive people 300 sputum samples are stained by Ziehl Neelsen technique and 100 samples (one sample of each person) were concentrated by modified Petroff's method and inoculated into ogawa medium. Then observation was done weekly to record the growth rate pigment formation, colonial morphology.

Those colonies which showed growth are submitted and biochemical tests namely. Niacin test, Nitrate reduction test and catalase test (at 68^0C) were performed.

4.2.1 SPECIMEN COLLECTION

As soon as HIV infected persons were identified, the investigator was reached to them with required number of wide mouthed, screw capped, leak proof, sterile sputum container. After taking informed consent the patients were instructed to collect the sputum of following type.

- Deep coughed
- Large amount of mucopurulent part (not saliva)
- Adequate amount (3ml-5ml)

Sputum collection of each patient was done with in 2 days in following order.

The first specimen was collected on the spot when a patient is identified as HIV infected - **"On the spot specimen"**

A container was given to the patient to collect the 2nd specimen early the following day - **"Early morning sample"**

A third specimen was collected under the direct observation of the investigator- **"A second on the spot specimen"**

4.2.2 ACCEPTANCE OR REJECTION OF SPUTUM SAMPLE

To eliminate the wrong evaluation, quality control of the sputum was done for possible cases .Thus observation was done for the presence or absence muco-purulent portion of sputum. If another sampling was possible specimen without mucopurulent part was rejected and asked for another sample..

4.2.3 MICROSCOPIC EXAMINATION OF SPUTUM

4.2.3.1 Smear Preparation and heat Fixation

A small portion of the mucopurulunt material is selected separated from the remainder, with a wooden stick and transferred to the slide. The material was spread evenly to a size of approximately 1×2 cm and dried it at room temperature completely inside the safety cabinet and heat fixed it by passing through the flame 3-4 times.

4.2.3.2Staining of Fixed Smears by Ziehl-Neelsen (ZN) staining to examine and fast bacilli (AFB)

Procedure

i. Heat fixed smears were placed on a staining rack.
ii. The smear was flooded with carbol Fuschsin stain reagent and heated from below (with spirit cotton) until the vapour just begins to rise (not to boil)
iii. Heated smear was allowed to remain on the slide for 5 minutes.
iv. Stain was washed off with tap water and the excess of water was drained out.

v. The smear was cowered with 3% acid alcohol for 5 minutes or until the smear was sufficiently decolorized i.e. pale pink.

vi. Smear was washed off with tap water and excess of water was drained out.

vii. The smear was covered with malachite green (0.5%) for 3 minutes.

viii. The smear was washed off by tap water.

ix. Backside of the slide was wiped out by cotton and placed at the draining rack.(Cheesbrough)

4.2.3.3 Observation of stained smear

The dried ZN ,stained slide was examined microscopically using oil immersion objective:

Te reporting of AFB stain was done according WHO/IUATLD standard (Appendix - III)

4.2.4 SPUTUM CULTURE

4.2.4.1 Digestion and Decontamination by Modified Pet ruff method.

a) Sputum sample (approximately 2mL) was aseptically transferred to the centrifuge tube.

b) Twice volume of 4% NaOH was added to the sputum.

c) The solution was left for 15 minutes at room temperature with occasionally shaking.

d) Them the solution was centrifuged at 3000 x g for 15 minutes.

e) The supernatant was discarded.

f) About 15 ml of sterile normal saline was added to it and sediment was suspended.

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

4.2.4.2 Inoculating the Primary Culture

0.1 ml of decontaminated and concentrated sputum sample was pipette out and inoculated into the 3% Ogawa media. Tube was slightly rotated out to allow the dispersion of sample through out the media. The tube was placed in horizontal position (20° angle) for 1 day (for complete absorption of sample into the media) and then kept in upright position.

4.2.4.3 Incubation

The inoculated tube was incubated at 37°C for 8 weeks.

4.2.4.4 Observation

After incubation weekly observation was done to note growth rate, colony characteristics, pigmentation and contamination (if any).

4.2.4.5 Sub culturing

Those tube which showed sufficient growth in Ogawa media were further sub-cultured on new Ogawa media so that biochemical tests could be done using primary culture tube. For this purpose 1 loopful of colonies are mixed with a drop of sterile water taken in a homogenizer and ground and then few drops of Water is added to make bacillary suspension which can be inoculated into the medium.

4.2.5 EXAMINATION OF CSF SPECIMEN

CSF sample from the suspected patient of tubercular meningitis were concentrated by centrifugation at 3000 × g for 15 minutes and deposit thus obtained were subjected to Z-N staining as well as culture.

4.2.6 IDENTIFICATION TESTS

4.2.6.1 Growth Rate Observation

After incubation the colonies were observed weekly and presumptive identification i,e. slow grower or rapid grower were noted.

4.2.6.2Observation of Colony

Characteristics and pigmentation - Colonies were observed in following aspects:

(i) Typical or a typical

Typical colonies of *M. tuberculosis* are rough, crumbly, Waxy, creamy and slow grower

(ii) Pigmented or non pigmented.

4.2.6.3Niacin test (Paper Strip Method)

Procedure

i. 1ml of sterile saline was added to the culture slant. If growth is confluent, the media are punctured with medium with a pasture pipette to allow contact of the saline with the medium.

ii. The tube was placed horizontally so that the fluid covers entire surface of the medium.

iii. 30 minutes was allowed for the extraction of niacin. The extraction time may be longer if the culture has few colonies.

iv. The slant was raised upright for 5 minutes to allow the fluid to drain to the bottom. 0.5ml of the fluid is remove to a clean screw cap tube.

v. The strip was inserted with the identification end up.

vi. It was left at room temperature for 15-20 minutes (occasionally agitated).

vii. The colour of the liquid in the bottom of the tube was observed against a white background. (Yellow = positive)

viii. Liquid was dipped into phenol solution and then sterilized by autoclaving.

NOTE: Extract from a culture of *M .tuberculosis* H37RV was used as positive control and an extract from an uninoculated tube of medium was used as negative control

4.2.6.4Nitrate red in test (Crystalline reagent method)

Reagents and chemicals

Given at the Appendix-II

Procedure

i. 0.2ml of sterile saline was added to a screw-cap tube.

ii. 2 loopfuls of colony were emulsified in the saline.

iii. 2 ml of $NaNO_3$ substrate was added.

iv. The tube was shaken well and incubated upright in a 37°C water bath for 3 hours and removed.

v. Small amount of crystalline reagents was added to the test solution. (Quantity of agent is not critical)

vi. The pink colour was observed immediately and compared to the standard chart.

4.2.6.5Catalase test at 68°C

Reagents and chemicals

Given at the Appendix-II

Procedure

i. 0.5ml of 0.067M phosphate buffer was added to a screw cap tube.

ii. Several loopfulls of test culture was suspended in the buffer.

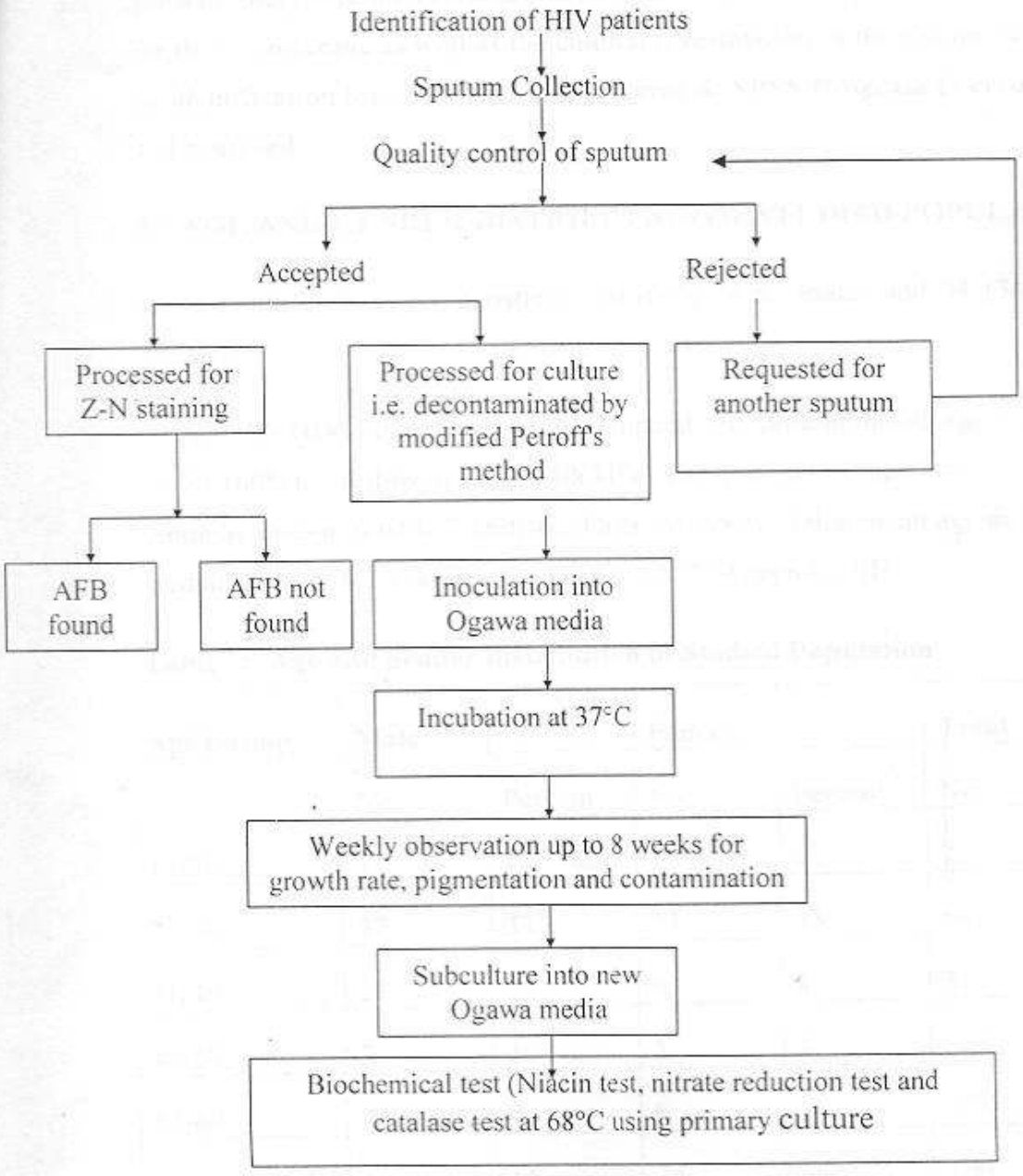
iii. The tube was placed in previously heated water bath at 68°C for 20 minutes. (Time and Temperature are critical).

iv. 0.5ml of freshly prepared tween-peroxide mixture was added to the tube and the cap was replaced loosely.

v. Formation of bubbles was observed on the surface of the liquid.

vi. Negative tubes were held for 20 minutes before discarding.

Flow chart of methods



Flow Chart of Methods

CHAPTER - V

5. RESULTS

Altogether 100 HIV infected people, 66 (66%) males and 34 (34%) females were included in the study to investigate tuberculosis. After taking informed consent, they were interviewed and then three sputum samples were collected for the microscopic as well as the cultural investigation of the sputum followed by identification tests .The data were entered on **SPSS Program (Verson-10)** and analyzed.

5.1 AGE AND GENDER DISTRIBUTION OF STUDIED POPULATION

Among 100 HIV infected patients 66 (66%) were males and 34 (34%) were females.

Of the 100 HIV infected cases investigated, the predominated age group was 21-30 (60%) followed by 31-40(31%).The rest percentage was in other age group as shown in table 7.Statistical analysis showed that mean age as 30 years , median age as 28.2 years and age range as 37 (Appendix III).

Table 7: Age and gender distribution of Studied Population

Age Group	Male		Female		Total	
	No	Percent	No	Percent	No	Percent
11-20	1	1	1	1	2	2
21-30	37	37	23	23	60	60
31-40	23	23	8	8	31	31
41-50	3	3	2	2	5	5
51-60	2	2	0	0	2	2
Total	66	66	34	34	100	100

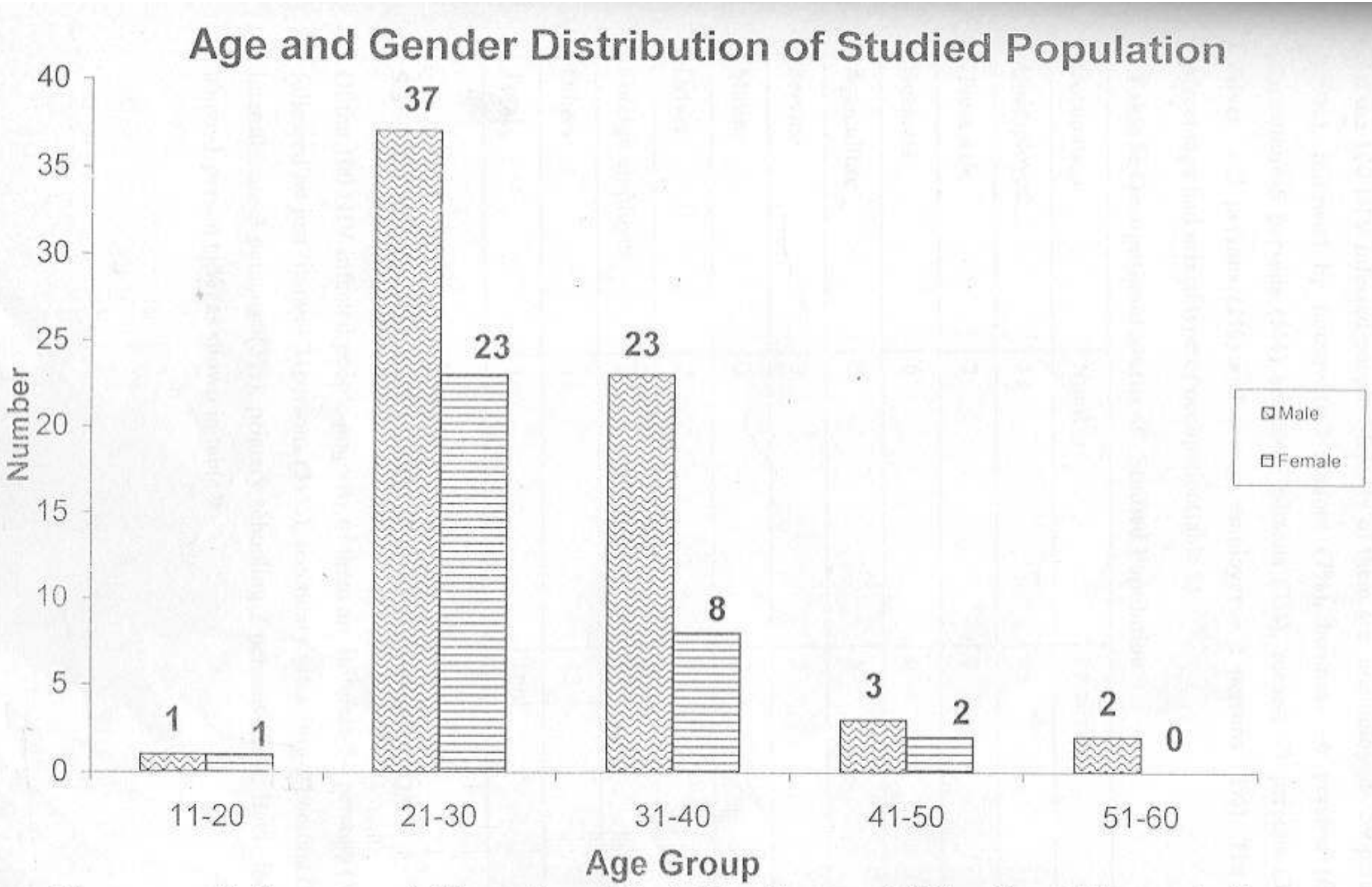


Figure - 1 Age and Gender Distribution of Studied Population

5.2 OCCUPATIONAL STATUS OF STUDIED POPULATION

Of the 100 HIV infected people Majority of them are unemployed –59 persons (59%), followed by housewife-7 persons (7%), business –6 persons (6%), agriculture-5 persons (5%) service-3 persons (3%), mason –3 persons (3%), driver - 2 persons (2%) and foreign employer – 2 persons (2%). The rest percentage had mixed type of occupation(table 8)

Table 8: Occupational Status of Studied Population

Occupation	Number	Percentage
Unemployed	59	59
Housewife	7	7
Business	6	6
Agriculture	5	5
Service	3	3
Mason	3	3
Driver	2	2
Foreign employer	2	2
Others	13	13
Total	100	100

5.3 EDUCATIONAL STATUS OF STUDIED POPULATION

Of the 100 HIV infected people majority of them are illiterate-54 persons (54%) followed by just literate- 31persons (31%), secondary schooling-9 persons (9%), intermediate-3 persons (3%), primary schooling-2 persons (2%), Bachelor and above-1 person (1%) as shown in table 9

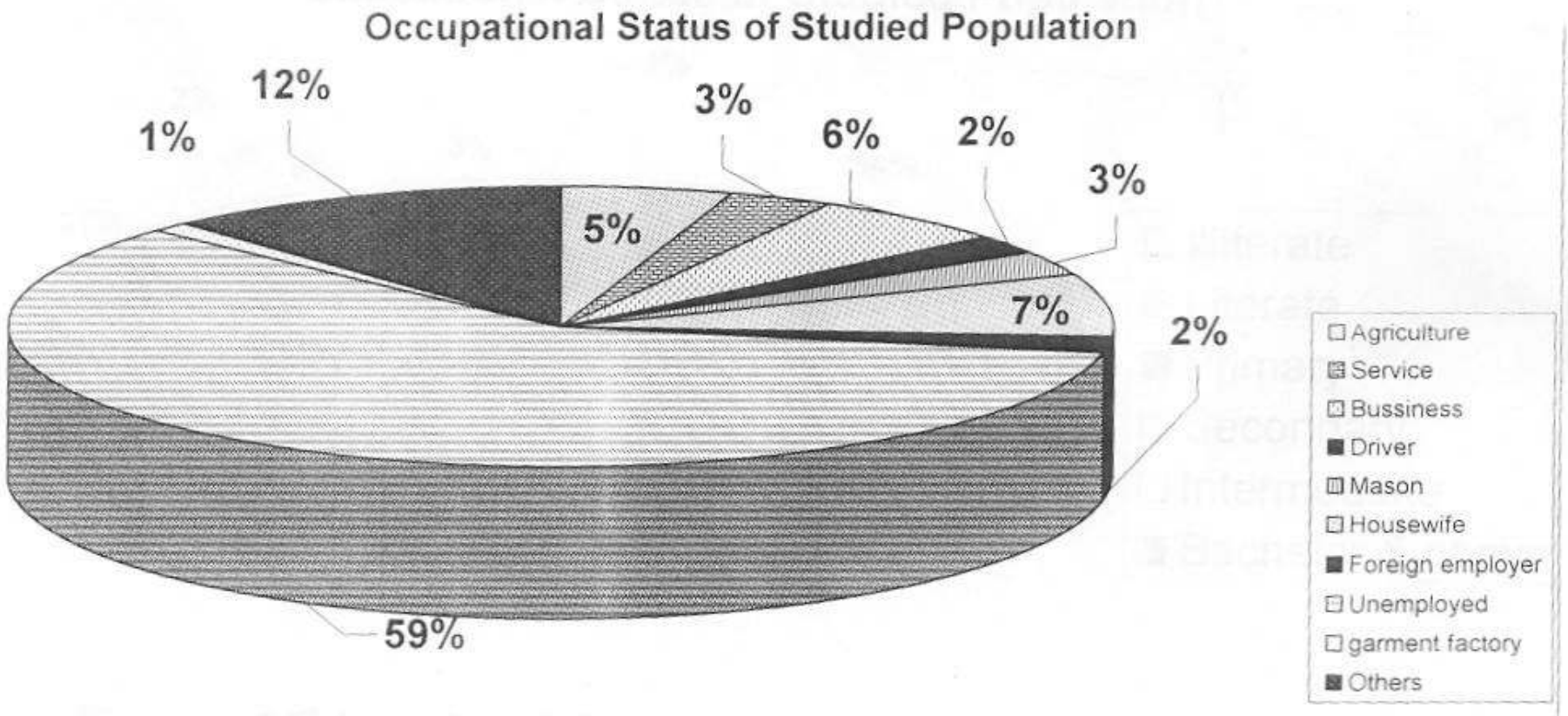


Figure- 2 Occupational Status of Studied Population

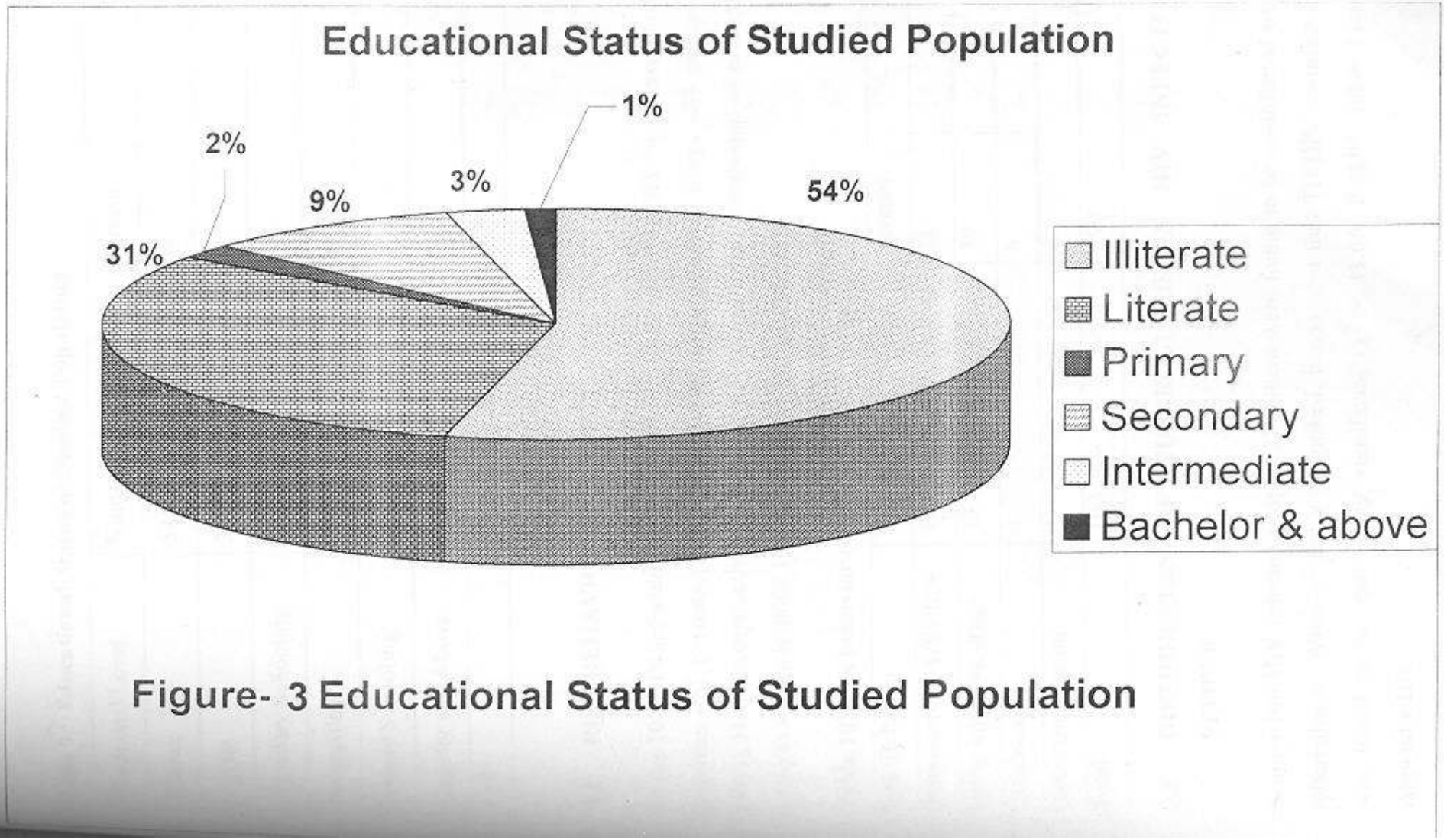


Figure- 3 Educational Status of Studied Population

Table 9 : Educational Status of Studied Population

Educational Level	Number	Percent
Illiterate	54	54
Literate	31	31
Secondary Schooling	9	9
Intermediate	3	3
Primary Schooling	2	2
Bachelor and Above	1	1
Total	100	100

5.4 RISK BEHAVIOR OF STUDY POPULATION

Of the 100 HIV infected people majority of them acquired HIV by heterosexual activities –51 persons (51%) followed by using injecting drugs –39 persons (39%), Homosexual activities –6 persons (6%) and blood transfusion –4 persons (4%) as shown in table 10.

Table 10: Risk behavior of HIV Studied Population

Risk Behavior	Number	Percent
Heterosexual Activities	51	51
Using injecting drugs	39	39
Homosexual	6	6
Blood transfusion	4	4
Total	100	100

5.5 DISTRIBUTION OF TUBERCULOSIS IN HIV INFECTED PEOPLE

Among 100 HIV infected people, 23of them were found to be co-infected with tuberculosis. When χ^2 test was applied, it was seen that TB/HIV co-infection was found to be statistically significant (χ^2 = 11.65, P<0.01, table 11and Appendix III).

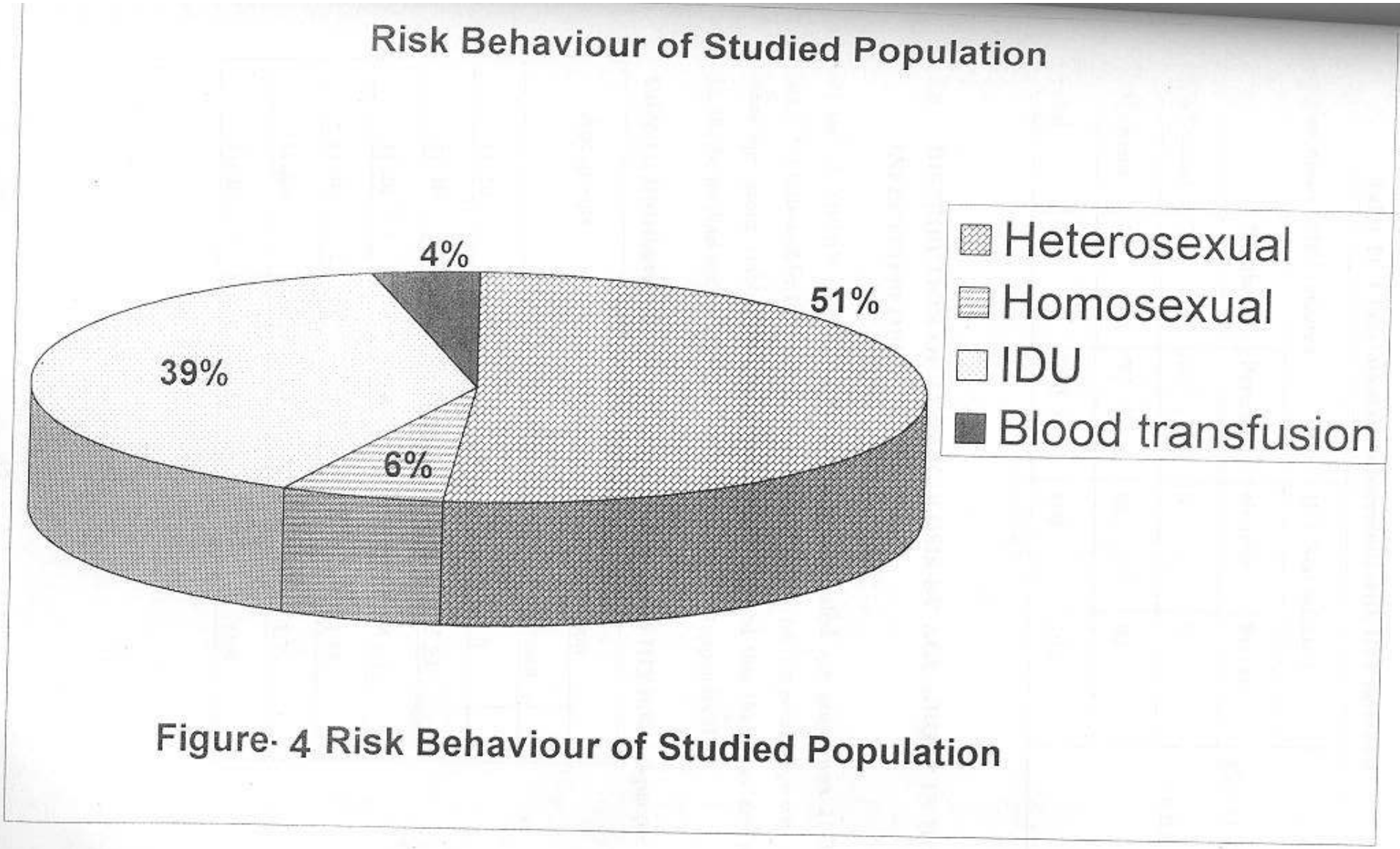


Figure- 4 Risk Behaviour of Studied Population

Table 11: Correlation of Tuberculosis with HIV infection

Tuberculosis	HIV infected		HIV Non infected		
	Number	Percent	Number	Percent	$\chi 2 = 11.65$
TB Present	23	23	6	6	$P < 0.01$
TB absent	77	77	94	94	
Total	100	100	100	100	

5.6 DISTRIBUTION OF TUBERCULOSIS BY AGE GROUP IN HIV INFECTED PEOPLE

Of the 22 TB/HIV co-infected cases the predominated age group was 21-30 (65.21%) followed by the age group 31-40 (26.09%) .The rest percentage was in other age group (table 12). Statistical analysis showed that the mean age as 30.28, the median age as 27.3 and the age range as 31 (appendix III)

Table 12: Distribution of tuberculosis by age group in HIV infected people

Age groups	TB/HIV co-infected person	
	Number	Percent
11-20	0	0
21-30	15	65.21
31-40	6	26.09
41-50	1	4.35
51-60	1	4.35
Total	23	100

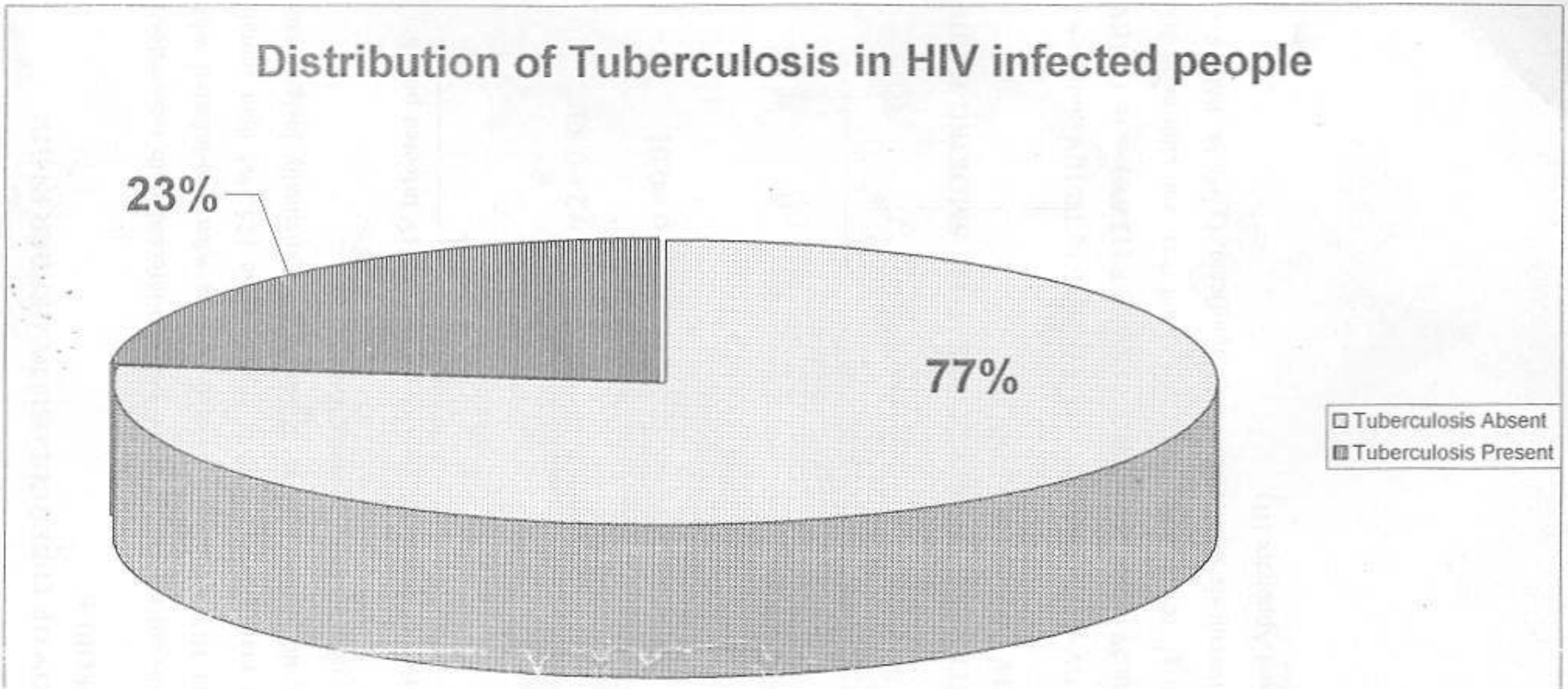


Figure 5 Distribution of Tuberculosis in HIV infected people

5.7 DISTRIBUTION OF TUBERCULOSIS BY GENDER IN HIV INFECTED PEOPLE

Of the 66 HIV infected males, 17 males were co-infected with tuberculosis where as of the 34 HIV infected females 6 females where co-infected with tuberculosis. Thus male showed slightly higher value (25.5%) than female (17.6%). However, the different in the 2 values are statistically insignificant (χ^2=0.56, p = 0.01 , table 13 and Appendix III)

Table 13: Correlation of Tuberculosis with gender in HIV infected people

Sex	HIV Infection	HIV/TB Co-infected		
	Number	Number	Percent	$\chi 2 = 0.83$
Male	66	17	25.75	p =0.01
Female	34	6	17.6	
Total	100	23	23	

5.8 DISTRIBUTION OF TUBERCULOSIS WITH SMOKING HABIT OF THE HIV INFECTED PEOPLE

Of the 41 smoker HIV people 15(36.6%) where found to be TB/HIV co-infected where as among the 59 non smoker HIV people only 8 (13.6%) were TB/HIV co-infected . When χ^2 test was applied it was found that smoking habit and development of tuberculosis was statistically significant. (χ^2=7.24, P<0.05 as shown in table 14 and Appendix III)

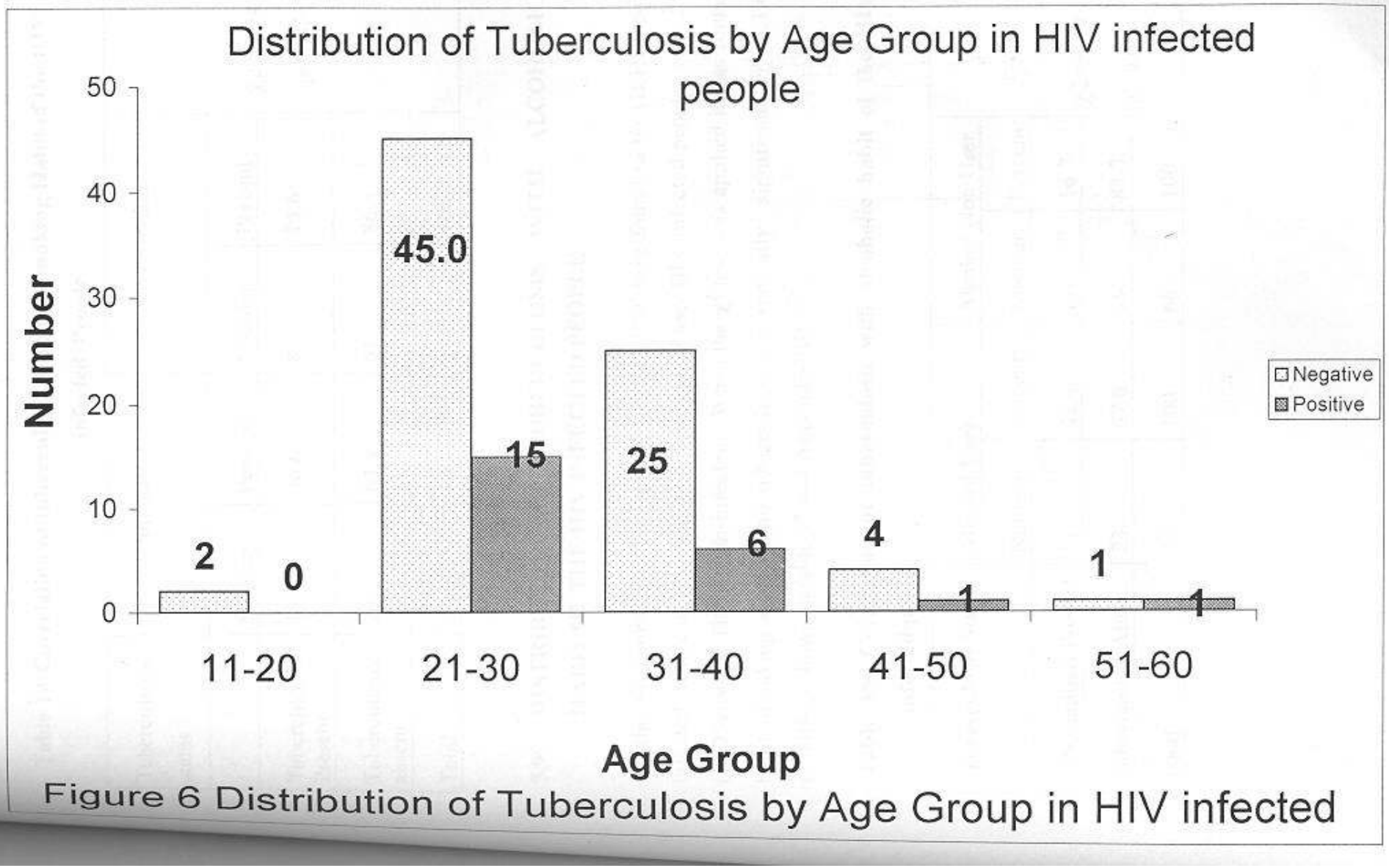


Figure 6 Distribution of Tuberculosis by Age Group in HIV infected

Table 14:Correlation of tuberculosis with the Smoking Habit of the HIV infected People

Tuberculosis status	Smoker		Non Smoker		
	Number	Percent	Number	Percent	$\chi 2 = 7.24$ $p<0.05$
Tuberculosis Present	15	36.6	8	13.6	
Tuberculosis absent	26	63.4	51	86.4	
Total	41	100	59	100	

5.9 DISTRIBUTION OF TUBERCULOSIS WITH ALCOHOLIC HABIT OF THE HIV INFECTED PEOPLE

Of the 34 alcohol user HIV people, 12 (35.3%) were found to be TB/HIV co-infected where as among the 66 non alcohol user HIV infected people , only 11 (16.7%) were TB/HIV co-infected . When the χ^2 test was applied it was found habit of taking alcohol and tuberculosis was statically significant. ($\chi^2=4.39$, $p<0.05$ as shown in table 15 and Appendix III)

Table 15: Correlation of tuberculosis with alcoholic habit of the HIV infected people

Tuberculosis Status	Alcohol User		Alcohol Non User		
	Number	Percent	Number	Percent	$\chi 2 = 4.39$ $p < 0.05$
Tuberculosis Present	11	32.4	11	16.7	
Tuberculosis Absent	23	67.6	55	83.3	
Total	34	100	66	100	

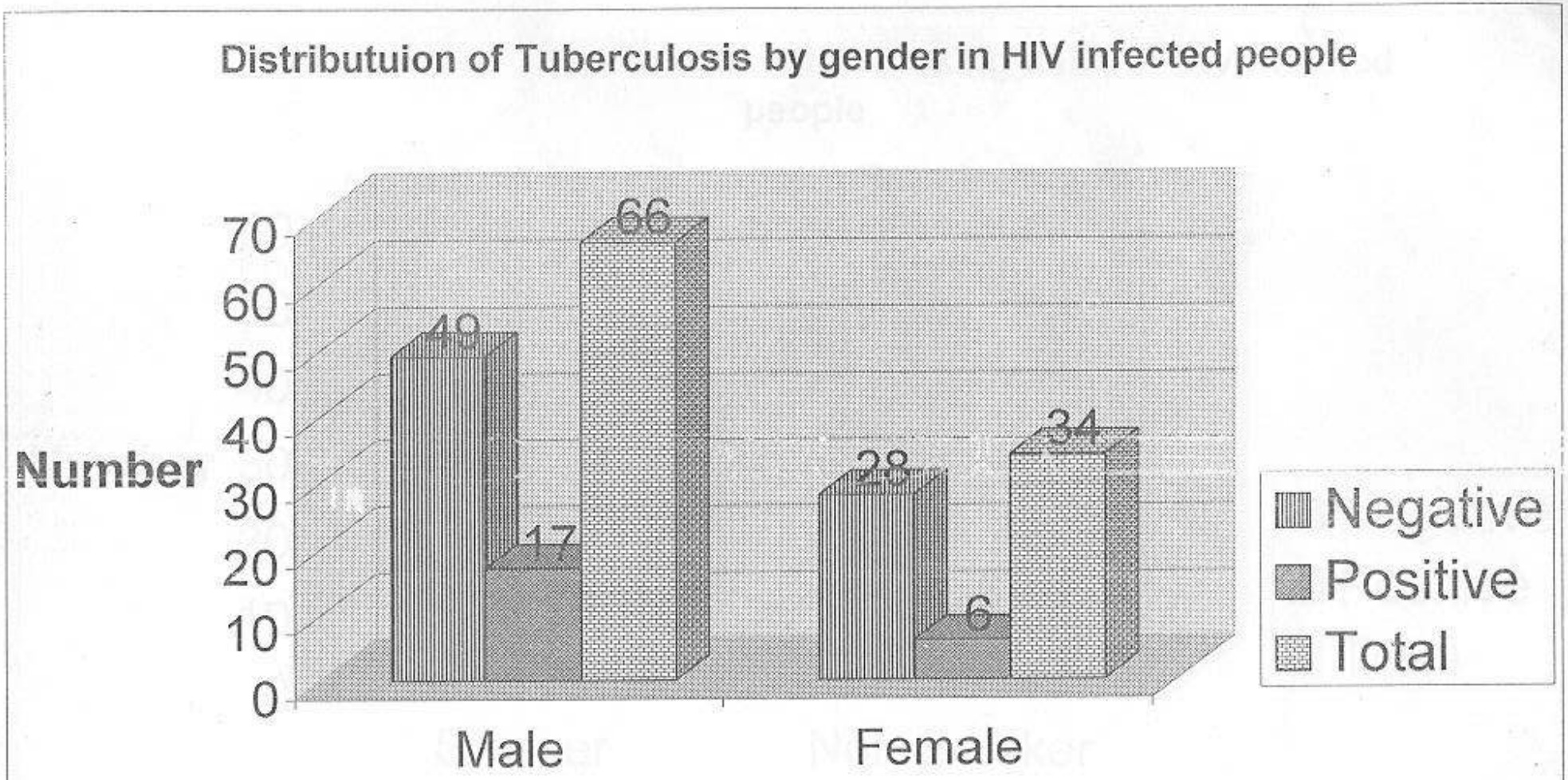


Figure 7 Distributuion of Tuberculosis by gender in HIV infected people

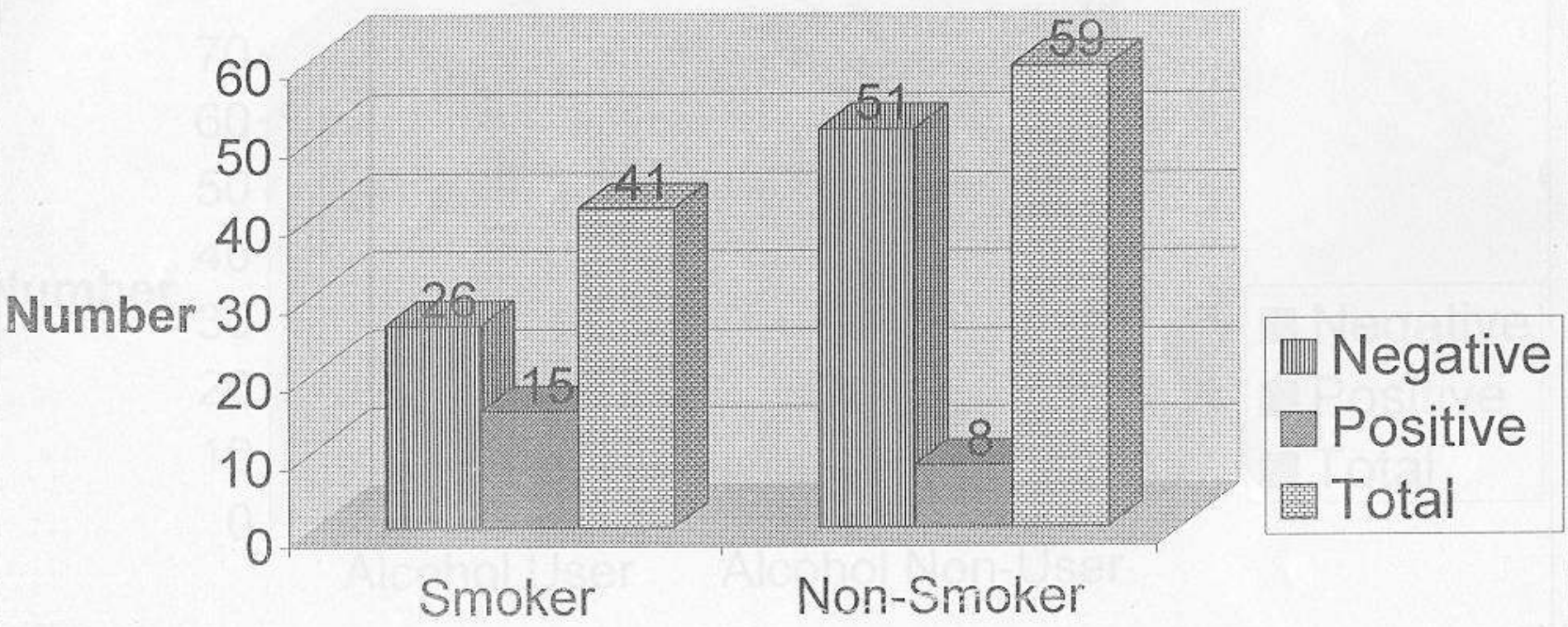


Figure 8 Distributuion of Tuberculosis with Smoking habit of HIV infected people

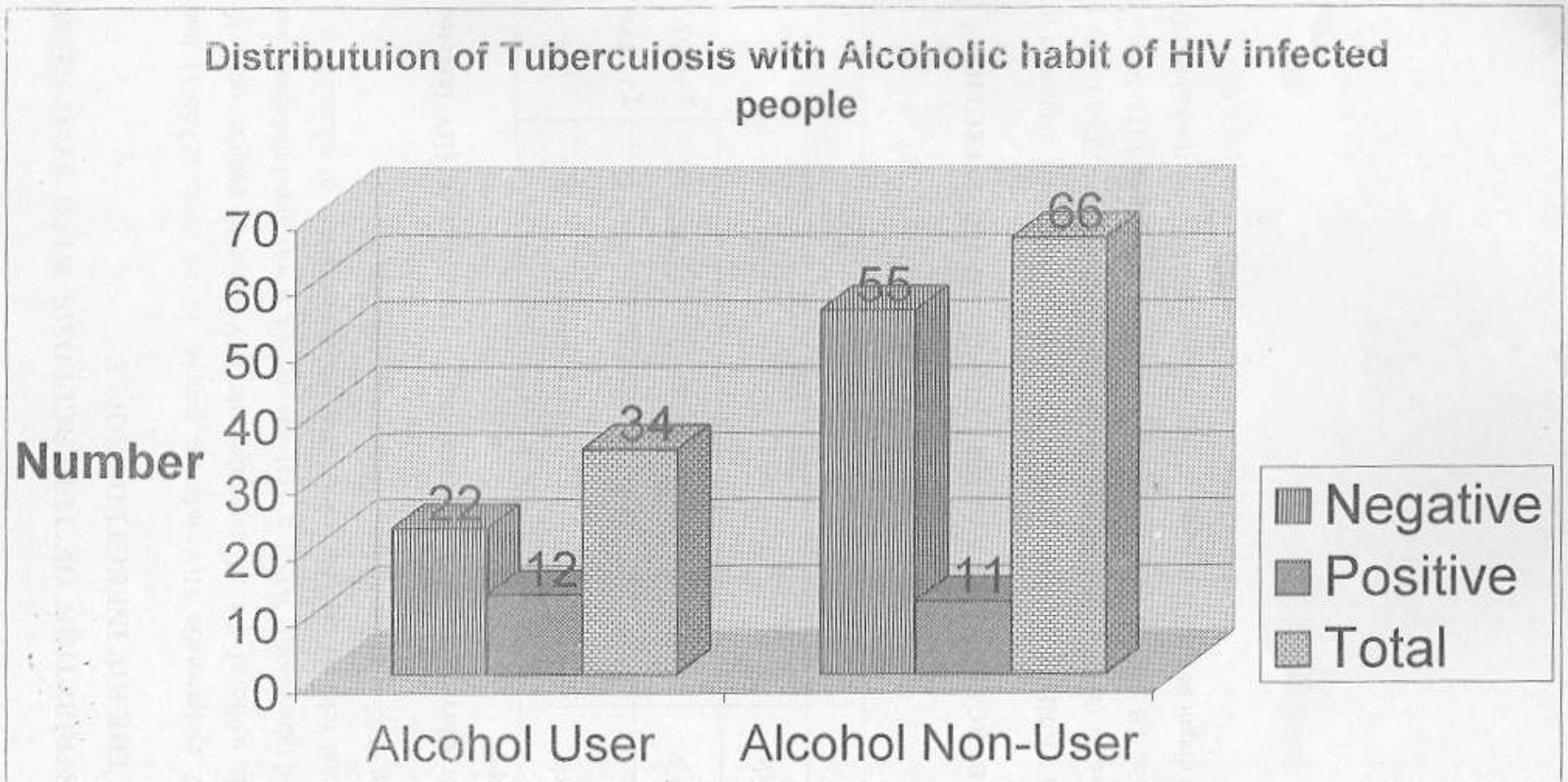


Figure 9 Distributuion of Tuberculosis with Alcoholic habit of HIV infected people

5.10 DISTRIBUTION OF TUBERCULOSIS WITH SYMPTOMS OF THE HIV INFECTED PEOPLE

Among 47 symptomatic HIV infected people, 13 of them (27.66%) had tuberculosis while of the 53 asymptomatic HIV infected peoples only 10 (18.9%) had tuberculosis. When χ^2 test was applied, it was found that there was no significant relation between symptoms and development of tuberculosis . (χ^2 =1.08, p=0.05 as shown in table 16 and Appendix III)

Table 16: Correlation of Tuberculosis with symptoms in HIV infected people

Tuberculosis Status	Symptomatic		Asymptomatic		
	Number	Percent	Number	Percent	$\chi2$ =1.08 p =0.05
Tuberculosis Present	13	27.66	10	18.9	
Tuberculosis Absent	34	72.34	43	81.1	
Total	47	100	53	100	

5.11: TUBERCULOSIS AT VARIOUS STAGES OF HIV INFECTION

OF the 23 TB/HIV co-infected cases, 9 (39.13%) of them were diagnosed as TB positive in the first year of HIV infection where as 5 (21.73%) of them diagnosed as TB positive in the fifth year of HIV infection and 3 (13.04%) of them were diagnosed as TB positive in the sixth year of HIV infection(table 17)

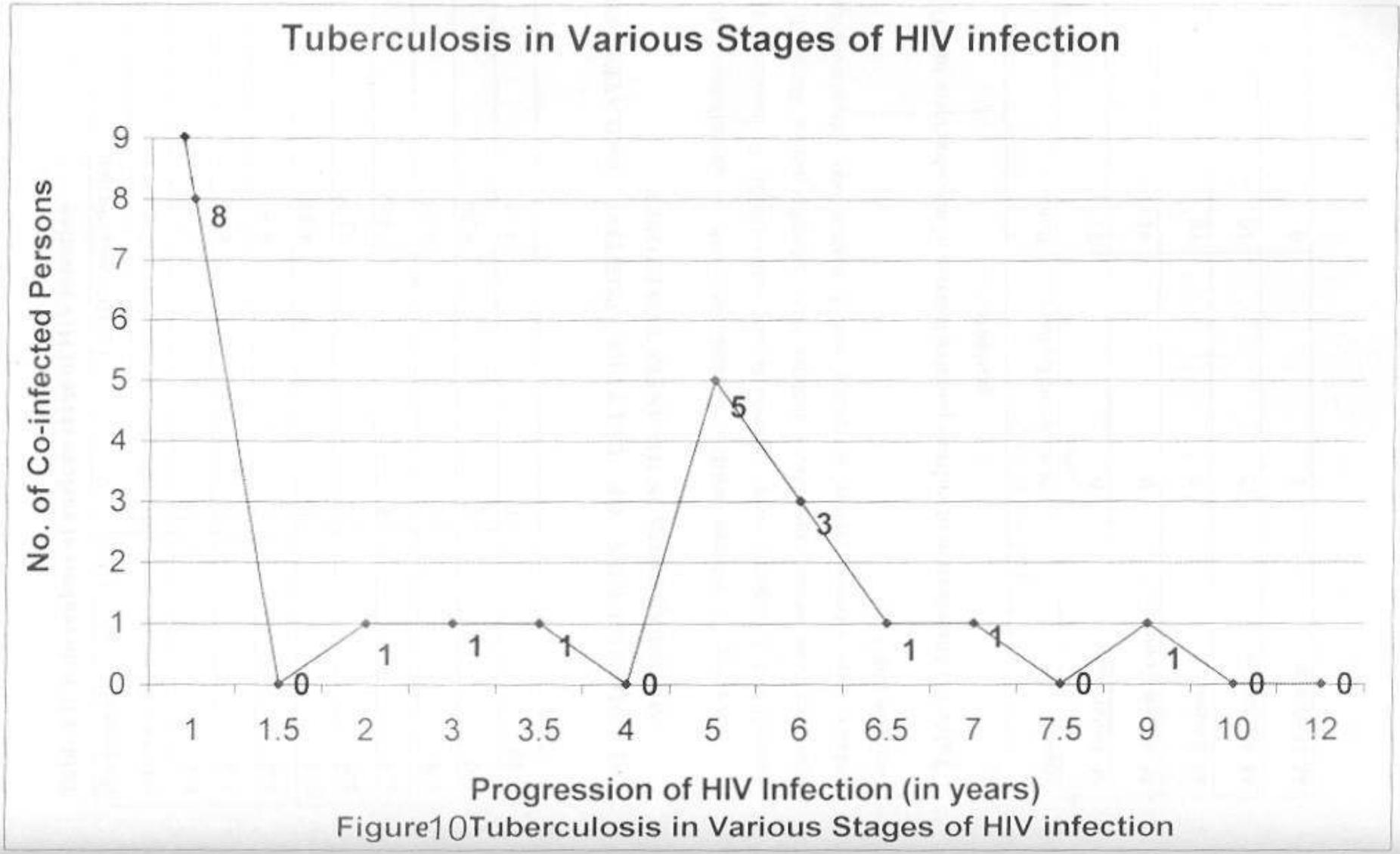


Figure10Tuberculosis in Various Stages of HIV infection

Table 17: Tuberculosis at various stage of HIV infection

Progression of HIV infection (in years)	TB/HIV co-infection	
	Number	Percent
1-2	9	39.13
2-3	1	4.34
3-4	1	4.34
4-5	1	4.34
5-6	5	21.73
6-7	3	13.04
7-8	1	4.34
8-9	1	4.34
9-10	1	4.34

5.12 DISTRIBUTION OF CULTURE POSITIVE ISOLATES OF MYCOBACTERIA IN HIV INFECTED PEOPLE

Among 22 cultures positive isolates the result of identification tests showed that 6 isolates were *Mycobacterium tuberculosis*, 9 isolates were *Mycobacterium avium* complex, 4 isolates were *Mycobacterium Kansasii*, 2 isolates were *Mycobacterium fortuitum* and 1 isolate was *Mycobacterium cholonae* (table 18)

Table 18: Distribution of culture positive isolates of mycobacteria in HIV patients

Species	Number of Isolates	Percent
M. Tuberculosis	6	27
M. Avium Complex	9	41
M. Kansasii	4	18
M. Fortuitum	2	10
M. chelonae	1	4

5.13 Distribution of *Mycobacterium tuberculosis* By Age and Gender

Mycobacterium tuberculosis was isolated from 6 HIV infected people. All of these 6 people were in the age group 21-30 (mean age 24) and they were all male (table 19 and Appendix III)

Table19: Age and gender distribution between of *Mycobacterium tuberculosis*

Age / sex	Number	Percent
22/M	1	16.66
23/M	2	33.33
26/M	3	50
Total	6	100

5.14 Distribution of *Mycobacterium avium* complex (MAC) by Age

Mycobacterium avium complex (MAC) was isolated from 9 HIV infected people . Majority of them were in the age group 31-40 (4 people), followed by 21-30 (3 people), 41-50 (1 people) and 51-60 (1 people), mean age being 37 years (table 20 and Appendix III)

Table 20: Distribution of *Mycobacterium avium* complex (MAC) by Age

Age group	Number	Percent
21-30	3	33.33
31-60	4	44.44
41-50	1	11.11
51-60	1	11.11
Total	9	100

5.15 Distribution of MAC by Gender

Of the 9 HIV infected people from where MAC was isolated 6 (66.66%)of them were male and 3 (33.33%)were female(table 21)

Table 21: Distribution of MAC by Gender

Sex	HIV infection	MAC-HIV co-infection	
	Number	Number	Percent
Male	66	6	66.66
Female	34	3	33.33
Total	100	9	100

5.16 DISTRIBUTION OF TUBERCULOSIS AND ANTI-RETROVIRAL (ARV) THERAPY

Among the 9 HIV infected people undergoing ARV therapy, 2 (22.22%) of them showed tuberculosis while of the 91 HIV infected people deprived of taking the ARV therapy, 21(23.08%) of them showed tuberculosis. When χ^2 test was applied the value thus obtained was statistically insignificant (χ^2=0.12, p=0.05 table 22 and Appendix III).

Table 22: Correlation of Tuberculosis and Anti-Retroviral (ARV) Therapy

Pulmonary infection	ARVT receiver		ARVT non-receiver		
	Number	Percent	Number	Percent	
Infected	2	22.2	21	23.08	$\chi2$ =0.12
Non infected	7	77.8	70	76.92	p =0.05
Total	9	100	91	91	

5.17 COMPARISON OF AFB CULTURE AND AFB STAINING

Of the 22 AFB culture positive cases, AFB was found on direct microscopy in 4 cases where as of the 78 AFB culture negative cases AFB was found on direct microscopy in 1 case. When χ^2test was applied the value thus obtained was statistically significant. (χ^2 = 10.3, P<0.01, table 23 and Appendix III)

Table 23: Correlation between of AFB Culture and AFB Stain

AFB culture	AFB staining		Total	
	AFB found	AFB not found		
Positive	4	18	22	$\chi2$ =10.3
Negative	1	77	78	p<0.01
Total	5	95	100	

CHAPTER - VI

6. DISCUSSION

The findings of this study were found to be valuable for the documentation of new and updated information related to TB/HIV co-infection with the fulfillment of objectives set above. The general objective of this study was to find prevalence of tuberculosis in HIV infected people and to study tuberculosis in such people and the specific objectives were: to study the characteristics of TB/HIV infected people, to isolate identify and characterize *Mycobacterium* species, to assess the level of ARV therapy and to correlate AFB culture and AFB staining..

In this study the majority of HIV infected people were found to be in the age group 21-30 years (60%) which is similar to HMG's data i.e. data of National centre for AIDS and STD control (NCASC) shows that >50% HIV positive people are in the age group 21-30. Similar study done by Dhungana *et al* (2002) in Tansen also showed that 40% of HIV infected people were in age group 21-30. Similarly, A study done on HIV positive people of Chennai (South India) by Thomas *et al* showed that 47% of HIV positive people were in the age group 15-29. High burden of HIV in this age group may be due to fact that early adolescent age group is supposed to be the age of freedom, sexually active and more inclination to vulnerable works.

The sex wise distribution of HIV in our study was higher in male (60%) than female (34%) which is similar to HMG (NCASC) data (72.7% male and 27.3% female). Similarly Dhungana et al (Tansen based study) and Thomas *et al* (Chennai based study) had shown that HIV prevalence was higher in male group i.e. 77.77 % (Tansen based) and 64% (Chennai based).High prevalence of HIV in male than female may be due to the high exposure of male to the vulnerable environment i.e. female are more restricted to household work while male has to visit different places.

In this study, the majority of HIV infected people were unemployed (unskilled) and illiterate -59% and 54% respectively. Considering the similar study done by Thomas *et al*, Chennai, they had found that the percentage of unemployed

(unskilled) and illiterate are 425 and17% respectively. As the literacy rate of Nepal is lower than Chennai, the illiterate percentage is relatively higher in our study. Further, illiterate persons have no (or very little) knowledge about HIV/AIDS and in combination with unemployment the chance of vulnerability will be increased.

The major risk factor of HIV infection in our study is heterosexual activities (51%).Similar findings was observed in HMG's data (According to NCASC report of July 31st, 2005, 65% of HIV/AIDS cases are due to heterosexual activities).Similarly, a study done by Rajasekaran *et al* during 1999 showed that that heterosexual activities were responsible for 74.4% of HIV infection

In this study, the prevalence of tuberculosis among HIV infected people (23%) was found to be nearly same as the statistical data published by UNAIDS 1998 (table 1). Our findings also support the fact given by WHO which states that one third of the death of HIV/AIDS people is due to TB .Similar result was obtained in a study done in Kathmandu during late 2003 by Dipendra Gautam who found 22.22 % of TB in HIV infected people.

In this study, high incidence of tuberculosis in HIV infected people was found in the age group 21-30 . Similar result was obtained by Dhugana *et ai* during 2002 in Tansen. As the majority of HIV infected people were higher in age group 21-30, there might be higher chance of development of TB in this age group of HIV people because it had already been verified that TB/HIV co-infections were statistically significant. Additionally, People of this age group have relatively higher exposure to the outside environment and hence there may be chance of development of tuberculosis.

Slightly higher incidence of tuberculosis in male than females, in our study, may be by chance as the males have higher exposure to the outside environment during life activities. Dhungana *et at* also documented that TB-HIV co-infection was higher in males than females.

It has been well established that smoking and unlimited use of alcohol reduces the immune response of on body. In this study also it was statistically verified that there was significant relation between smoking habit and /or alcoholic habit

and the subsequent development of tuberculosis. Thus, one of the important findings of this study is that if HIV infected people smokes and /or takes alcohol the chance of development of tuberculosis is significantly higher..

Although, *Mycobacterium avium* complex considered as the most common opportunistic infection in HIV/AIDS patients of Western countries, where as high as 50% prevalence is found (Brooks *et al*), our study suggests that it is not uncommon to other countries. However, its prevalence rate is comparatively lower i.e. 9% in our study. Similarly 4% prevalence rate of *Mycobacterium Kansasii* in HIV/AIDS patient is in accordance with Hira Suna *et al* 1987, parent et al 1995 who reported the pulmonary and disseminated desease due to *M. Kansasii.* Another interesting finding of this study is that isolation of *M.tuberculosis* is from younger HIV positive people (mean age 24) where as isolation of *M.avium* complx from older HIV positive people(mean age 37).

As the $\chi 2$ value between HIV symptoms and development of tuberculosis is insignificant it suggests that tuberculosis may occur before the development of the signs and symptoms of HIV.

In this study the tuberculosis burden in HIV positive people was significantly higher in two points during the progression of HIV /AIDS – The first year of HIV infection and then the fifth and the sixth year of HIV infection .The first year (with in a year) suggest the late diagnosis of HIV. Thus TB and HIV are diagnosed at the same time. The second peak (during fifth and sixth year)suggests that HIV has caused the resurgence of tuberculosis.

As the $\chi 2$ value between ARV therapy and tuberculosis is statistically insignificant this study suggests that simple ARV therapy does not prevent the development of tuberculosis. Thus it is suggested that HIV/TB co-infected patients should br subjected to highly active antiretroviral therapy (HAART) tuberculosis patient

As the $\chi 2$ value between AFB culture and AFB culture is significant it further supports the validity of the laboratory findings of this research.

CHAPTER - VII

7. SUMMARY AND RECOMMENDATION

7.1 SUMMARY

This study was conducted by central department of microbiology, Tribhuvan University in collaboration with Tribhuvan University, Teaching Hospital (TUTH) during January 2004 to August 2005, with a general objective to study tuberculosis in HIV infected people. Altogether 100 HIV infected people (66 males and 34 females) were included in this study. 3 Sputum samples of each people were collected and subjected to AFB staining by Ziehl-Neelsen method ,AFB culture in Ogawa media and identification tests.

In the studied population ,the age group 21-30 was predominant(60%) followed by 31-40 (31%).The mean age ,the median age and age range was found to be 30 years ,28.5 years and 37 years respectively .Majority of the HIV people were illiterate and unemployed .

Out of 100 HIV/AIDS cases 22 showed culture positive (in Ogawa media) for tuberculosis of which 4 cases were smear positive (by Ziehl-Neelsen technique) where as 1 smear positive case was found to be culture negative. Thus overall prevalence of tuberculosis in HIV infected people was found to be 23%. Statistical analysis showed that TB/HIV co-infection was statistically significant ($\chi^2 = 11.65$, $P<0.01$),the age group 21-30 were highly susceptible to TB/HIV co-infection and significant relation was established between smoking and /or alcoholic habit and the subsequent development of tuberculosis. Regarding the Sex wise prevalence of tuberculosis , male showed the slightly higher value (25.52%) than female (17.6%), but these values were statically insignificant.

Among 23 culture positive isolates, the predominant was *Mycobacterium avium* complex (9 isolates i,e 41%), followed by *Mycobacterium tuberculosis* (6 isolates i,e 27%) *Mycobacterium Kansasii* (4 isolates i,e 18%),

Mycobacterium fortuitum (2 isolates i,e 10%) and *Mycobacterium chelonae* (1 isolate I.e 4 %).

Both symptomatic and asymptomatic and asymptomatic HIV positive people, regardless of ARV therapy, are equally susceptible to tuberculosis.

This study was funded by Nepal health research Council (NHRC)

7.2 RECOMMENDATION

i. Surveillance of HIV-TB should be done not only with epidemiological and microbiological aspect but also with social aspect. Thus development of prevention, care, treatment and advocacy policies in combination with effective implementation of case detection, case management of these diseases should be in the priority action area of the nation.

ii. TB & HIV prevention and control programs / strategies should be done in close collaboration.

iii. In every VCT centre / Hospital / Clinics where HIV is detected, the patients should be immediately subjected to investigate TB and vice versa.

iv. More extensive study is recommended to get the more representative data of the TB / HIV co-infection to develop national policy regarding this burning issue.

8. REFERENCES

Bam DS and Kumar P (2003) Involving medical colleges and private sector in Tuberculosis and HIV control, STC , Thimi, Bhaktapur

Bam DS and Smith I (1996) Principles of effective TB control in Nepal . Journal of the Nepal Medical Association, TB special 34:3-7

Bam DS Piryani RM and Rijal BP (2004) SAARC Journal of Tuberculosis, lung Disease an HIV/AIDS Vol-1, no.-1 year.

Bam DS, Rahman MM ., and Samaratunga M (2002) Involving Medical Colleges in tuberculosis and HIV Control , STC , Thimi ,Bhaktapur

Brooks GF, Butel JS and Morse SA (2002) Medical Microbiology 22nd Edition, MC Graw Hill Publication, pp.275-284

Chakvaborty P (2001) *Mycobacterium tuberculosis.* A text book of Microbiology, First Edition New Central Book Agency (P) LTD, pp 396-414

Cheesbrough Monica (2002) District Laboratory Practice in Tropical countries, Part 2. Cambridge University Press 329-331, 71-76, pp-207-211.

Chhetri GG , Rijal BP and, Sharma AP (2001) Prevalence of Tuberculosis among the Suspected Patients Visiting Tribhuvan University Teaching Hospital and their Antimicrobial Resistance Pattern (Dissertation) Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Collec TG , Marmion BP Fraser AG and Simmons A (1996) Mackie and MC Cartney Practical Medical Microbiology, 14th edition, churchil livingstone publication,

Deun AV (2001) Role of Microscopy Network in NTP. Vol. 1, Jan- March: 18-23.

DGHS (2002) Technical guidelines for tuberculosis control. Central Of TB division, Nirman Bhavan, New Delhi, India.

Dhungana JR, Ghimire P, Bam DS and Rijal BP (2002) Tuberculosis and Human immunodeficiency virum Co-infection in United Mission Hospital-Tansen (Dissertation) , Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal..

Dye C, Garnett GP, Selman K, William BG, (1886) Prospects for worldwide Tuberculosis control under the WHO Dots strategy. *The lancet.*, vol. 352

Espinal MA (2003) The global situation of MDR-TB Tuberculosis *Journal of Biochemical Research in tuberculosis* ; 83, 44-52.

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

Forbes BA Sahm DF and weissfeld AS (2002) Bailey and Scott's Diagnostic Microbiology. 11th edition, Mosby Inc. USA.

Forbes,BA. Sahm, DF.and weissfeld AS (1998) Bailey & Scott's Diagnostic Microbiology. 10th edition, Mosby Inc. USA.

Grange JM (1996) *Mycobacterium* and human disease . Arnold, Londen.

Grange JM (1990). Tuberculosis, Topley and Wilson's Principles of Bacteriology, Virology and Immunity Volume 3. 8th Edition, BC Decker. Philadelphia Hamilton.

Greenwood D, slack RC and Peuthere JF (1997) Medical Microbiology, 15th edition. Churchill Livingstone, U.K pp 200-215.

Gupte S (2001) Viva and recent Advances in Medical Microbiology, 5th Edition. Jaypee Publication , India

Gurubacharya VL and Gurubacharya DL (2004) HIV Prevalence among Nepalese Migrant Workers Working in Nepal and Indian cities, Journal of Nepal Medical Association Vol.43, No.154, July-August

Harries AD and Maher D (1997) WHO TB/HIV Clinical Manual..

Hornick D.B(1993) Tuberculosis; Public Health and Preventive Medicine, 1993; 208-219.

Kunimoto DY and . Peppler MS (2003) Comparison of PCR and cultural technique for detection of tuberculosis in people with high prevalence of HIV, Journal of Clinical Microbiology, ASM Publication January, Vol.1.

Madigan MT., Martinko J.M. and Parker J. (2000) *Brock* Biology of Microorganisms, 9th edition, Prentic hall international

Mahajan BK(1999) Methods in Biostatistics sixth edition Tayoee Publication India .

Malla P(1996), Factors Associated with Treatment failure in TB patients at National TB Centre. *Journal of Nepal Medical Association*, TB special, 34:41-46.

NCASC (2062). AIDS Newsletter, A quarterly Booklet Published by, Year 16, No.54

NTC (1997) National Tuberculosis Programme of Nepal, General Manual

NTC (1998) National Tuberculosis Programme: A clinical manual for Nepal

NTC (2002) National Tuberculosis Control Programme, Manual, Tuberculosis in Nepal

NTC (2002) Surveillance of HIV Infection in Patients with Tuberculosis Thimi, Bhaktapur 2004.

NTC (2003) Annual report of National Tuberculosis control program Nepal, HMG Ministry of Health , Thimi, Bhaktapur.

NTC (2003) National Tuberculosis control programme "Tuberculosis in Nepal." Thimi, Bhakatpur

NTC/JICA (1998) Laboratory Manual for national tuberculosis programme of Nepal. National tuberculosis center JICA/ HMG National TB control project (II). Second, edition march.

NTI (1998) Manual on Isolation, Identification sensitivity testing of *Mycobacterium tuberculosis*. Government of India,

Pace B. (2000)"Tuberculosis," Patient Page: *Journal of American Medical Association*, vol. 108; 1408.

Park K.(2000) Park's Text book of Preventive and Social Medicine, M/S Baorsidas Bhanol Publishers, India 137-138.

Pio Antonio and Chaulet Pierve. (1998) Tuberculosis hand book. WHO Publication

Pokherel, B.M. H hand book of clinical Microbiology 1st Edition, Gorakhnath Desktop & Printing Supports, 2004.

Prez D, RM. and Heim CR. (1991) *Mycobacterium tuberculosis*, in principles and practices of infectious diseases 3rd edition, churchhill livingstone, New York.

Rajasekarans, Lima A, Kamakshi. S, Jeyaganesh, Sntha Mighchelvan A, Savithr's and Gopi Nathan (200) Indian Journal of Tuberculosis, Vol.47, Page 223-226.

Rieder HL, Cauthen GM., Comstock GW .and Snider DE(1989) Epidemiology of tuberculosis in the United States, Epidemiology. *Journal* Rev ; 11:79-98.

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,Vol.6, No.1, July .

Rijal BP, Banjade N., Joshi HH., Pokharel BM.and Tuladhar NR(1996) The incidence of TB infection in suspected TB patients. *JNMA*. : 48.

Rijal BP, Rahman Md. M. and Bam DS(2002) "Multi-Drug Resistant Tuberculosis: an overview of the SAARC Region. STC Newsletter, Vol. XII No.1, January-June : 13-14.

Rijal KR, Ghimire, P, Bam DS and Rijal BP (2004). An Epidemiological Study Of Anti-Tuberculosis Drug Resistance Pattern In The Pulmonary Tuberculosis Patients Visiting National Tuberculosis Centre (dissertation) , Central Department of Microbiology Tribhuvan University, Kathmandu

Sharma SK, Mohan A, Kadhiravum T(2005) HIV/TB Co-infection: Epidemiology, diagnosis & management, Indian Journal of Meidical Research .

Shen Y, Zhou D, Sehgal P and Simon M(1996) , Mycobacterium bovis enhances the pathogenicity of Siman Immunodeficiency Virus infection and accelerates the progression to AIDS in Macaques. International Journal of Tuberculosis and Lung ; Vol.2, No.11, supplement 2. .

Sherchand JB, Bam D Ş and Sherchand S (2001). Human Immunodefieicncy Virus (HIV) infection in Tuberculosis patients of Nepal. Journal of Nepal Association for Medical Laboratories Sciences, Vol.4, No.4. 1-5.

Smith GR. and Easmon CS.. (1990) Topley &Wilson's Principles of Bacteriology, Virology and Immunity, Volume 3. 8th edition .BC Decker. Philadelphia.

Smith, I. Gender and Tuberculosis in Nepal. *Journal of the Nepal Medical Association TB special 1996*; 24; 117; 49-58.

Snider DE, Roper W1 (1992)., The new tuberculosis (Editoriol*). N. England J. Med*; 326; 703-705.

Sriyabbaya N, Silarug N, Chunnchaiviboonwat D. Increasing surveillance notification of TB cases extending to all regions of Thailand in the situation of the HIV / AIDS epidemic. International Journal of Tuberculosis and Lung Disease Vol. 2, No.11, supplement 2, S208.

STC (2002) SAARC Guidelines for Partnership with Media in Prevention & Control of TuberculosisNational Tuberculosis Programme Nepal, Souvenir Page 27-29.

STC (2003) Situation analysis of TB, HIV / AIDS and TB / HIV Co-infection in the SAARC region Thimi, Bhaktapur.

STC (2003) Articles on Tuberculosis and HIV / AIDS in the SAARC region -I Thimi, Bhaktapur.

STC (2003) Commercial Sex Workers in SAARC Region, "The Major Transmitter of HIV / AIDS to General Population". Thimi, Bhaktapur.

STC (2003) HIV / AIDS in the SAARC region, Thimi, Bhaktapur.

STC (2003) Report of Second Round External Proficiency Testing of Smear Microscopy in National TB Reference Laboratories in SAARC Region Thimi, Bhaktapur.

STC (2003) Report of the first round external proficiency testing of the smear microscopy in National TB reference laboratories in SAARC region Thimi, Bhaktapur.

STC (2003) TB / HIV Co-Epidemic in the SAARC Region Thimi, Bhaktapur.

STC (2004) STC Newsletter, Vol. SIV, No.1 Jan-June

STC (2004) Abstracts book of 1st SAARC conference on TB, HIV/AIDS and respiratory disease, December, Thimi Bhaktapur

STC (2004) Gender Differences among Tuberculosis Patients in National TB Control Programmes within SAARC Countries, Thimi, Bhaktapur.

STC (2004) Gender Issue in Tuberculosi and HIV / AIDS in the SAARC Region, Thimi, Bhaktapur.

STC (2004) HIV / AIDS in the SAARC Region, Thimi, Bhaktapur.

STC (2004) HIV / AIDS, What everyone should know About it? A special publication of STC on the Occasion of SAARC awareness year for TB and HIV / AIDS, Thimi, Bhaktapur.

STC (2004) Situation Analysis of Quality assurance of Sputum Microscopy in Bhutan Thimi, Bhaktapur.

STC (2004) Tuberculosis in the SAARC region, An update 2004

Thakker RM, Mistry MA and Mistry AB (2002). Clinical, Radiologicl & Bacteriological spectrum of TB-HIV Co-infection: Amargadh Study. International Journal of Tuberculosis and Lung Disease; 33rd Conference Oct 8-16. Montreal, Canada.

Thomas BE, Arckiaselvi J, Suryanarayan and R Fathis Soumya (2005) SAARC Tournal of Tuberculosis Lung Disease and HIV / AIDS Volume II, No. 1.

Toungoussova OS, Caugant DA, Sandven PB and Junc G (2002) Drug Resistance of *Mycobacterium tuberculosis* Strains isolated from patients with pulmonary tuberculosis in Archangels, Russia. *The international Journal of TB and Lung disease*, 6 (5) 406-414.

Training for Better TB control, Human Resource Development for TB control. A strategic Approach with in Country Support WHO, 2002.

Tuberculosis in the SAARC Region, STC Publication, 2003, Thimi, Bhaktapur.

UNAIDS / WHO (1995) HIV / AIDS / STI Surveillance

Wayne LG (1982) Microbiology of tubercle bacillus .*Am Rev Respir Dis*, 125, 3141.

WHO (1994) Guidelines for HIV Surveillance among Tuberculosis Patients, 2nd Edition. Geneva

WHO (1999) Guidelines for the prevention of Tuberculosis in Health Care Facilities Resource Limited Settings, Geneva.

WHO (2002) An Expanded DOTS Framework for effective Tuberculosis Control, Geneva,

WHO (2003) Global Tuberculosis Control, Surveillance, Planning, Financing, Communicable Disease. WHO, Geneva.

WHO (1998) Laboratory Services in Tuberculosis Control. Part I, Organization and management, WHO, Geneva

WHO (2001) Training for Better TB Control, WHO,Geneva

WHO (1993) Treatment of Tuberculosis, Guidelines for National Program,WHO, Geneva

9. Appendices

Appendix- I

Questionnaire

Tribhuvan University

Central Department of microbiology

Kirtipur, Kathmandu.

Personal Profile

Name of the patient .....code No.....Age..... sex.....Registration No....

Permanent address.....Occupation.....Education.....

HIV tested Positive on.....

Antiretroviral therapy: yes ( ), No ( ), If Yes Since.....years.....months back

Marital status: ( ) married, ( ) unmarried: If married, Spouse HIV+ve ( ) HIV-ve ( )

Child affected yes ( ), No ( )

Risk factors:( )Homosexual( )IDU( )blood transfusion/organ transplant ( ) CSW ( )

Others 1.....2.....3.....

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 ( )

Oral Candidiosis ( ) Capo sis Sarcoma ( )

Treatment history:

medical.....

Surgical (Duration).....

Laboratory Findings:

1. AFB Staining of Sputum Sample

Sample	Result
i) Spot	.....
ii) Early morning	.....

2. Culture on Ogawa media

Weeks	Result
First	.....
Second	.....
Third	.....
fourth	.....
Fifth	.....
Sixth	.....
Seventh	.....
Eighth	.....

3. Biochemical test

Test	Result
Catalase	.....
Nitrate Reduction	.....
Niacin	.....

Appendix- II

A. COMPOSITION AND PREPARATION OF 2% MODIFIED OGAWA MEDIA

1.	Preparation of salt solution	500 ML Flask
	i) Potassium Dihydrogen Phosphate	2.0 gm
	ii) Magnesium citrate	0.1 gm
	iii) Sodium Glutamate	0.5 gm
	iv) Distilled Water	100 ml

- Mix well and heat at 100^0C for 30 minutes in a water bath (or autoclave at 121^0C for 15 minutes).
- Add glycerol 4 ml into the salt solution while it is hot.
- add 4 ml of 2% malachite green solution.

2. Preparation of whole egg homogenate.
   - Wipe of the egg shell with spirit cotton.
   - Break down the egg into a plate to cheek the decomposition.
   - Transfer the egg into the beaker (500 ml).
   - Homogenize the egg with a pair of chopsticks until the egg become watery.
   - Place the two layers of sterile gauze piece on the funnel.
   - Filter the egg homogenate until you get 200 ml.
3. Mix 1&2 (Raw modified ogawa media)
4. Distribution of raw medium
   - Dispense the medium 6 ml into each tube (avoid bubble formation)
5. Inspissation
   - Arrange the tube in the slant position and coagulate them at 90^0C for 1 hour with caps closed loosely.
6. Store at 4^0C - 6^0C with caps closed tightly. Keep them into the plastic bags and store into the refrigerator for 1-3 months.

B. Composition and Preparation of Different types of biochemical test media

1. **Nitrate reduction** test (A liquid regents)

Sodium nitrate substrate in buffer

KH_2PO_4	3.02g

Distilled water 1000 ml

Weighed KH_2PO_4 was dissolved in 1000 ml of water to produce a 0.022 M solution

Na_2HPO_4 ⟶ 1.316g

Distilled water 1000 ml

Weighed sodium phosphate was dissolved in 1000 ml of distilled

Water to provide an 0.022 M solution ⟶ Solution 2

To 389 ml of Solution 1,611 ml of Solution 2 was added and mixed well to produce a buffer solution of PH 7.0 ⟶ Solution 3

NaNo3 0.85g

Solution 3 1000ml

Weighed sodium nitrate was dissolved in the buffer solution (Solution 3) and sterilized by autoclaving at 121 C for 15 minutes.

C. Composition and Preparation of Test Reagents

1. Catalase reagents

Tween 80 (10%):

Tween 80 10 ml

Distilled water 90ml

To 90 ml distilled water, 10ml of Tween 80 added and mixed well to produce 10% tween 80. The mixture was autoclaved at 121C for 10 minutes. The Tween settle down during autoclaving, it was resuspended by swirling.

Tween peroxide mixture (Complete catalase reagent)

Equal parts of 10% Tween 80 and 30% hydrogen peroxide was mixed well to produce Tween peroxide mixture a complete catalyse reagent.

2. Crystalline Reagent

Tartataric Acid – 10 gm

Sulfanilic Acid – 1 gm

N-napthylethylenediamine dihydorchloride – 1 gm

These 3 reagents are thoroughly mixed (if needed they are ground by using mortar and pestle)

3. Decontamination Reagents

Sodium hydroxide (NaOH) solution (4%).

Sodium hydroxide pellets	4 g
Distilled water	100 ml

Weighed sodium hydroxide pellets were dissolved in 100 ml of distilled water and sterilized by autoclaving at 121 C for 15 minutes.

D. Composition and Preparation of Staining Reagents

Ziehl-Neelsen Staining Reagents

i. 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.

ii. 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.

iii. Counter stain (Malachite green)

Malachite green	0.5gm
Distilled water	100 ml

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

Appendix- III

Statistical Tools

I. Mean age, median age and range of the studied population.

Age group	Mid value(x)	Frequency (f)	Cumulative frequency (c)	F x X
10.5-20.5	15.5	2	2	31
20.5-30.5	25.5	60	62	1530
30.5-40.5	35.5	31	93	1100.5
40.5-50.5	45.5	5	98	227.5
50.5-60.5	55.5	2	100	111
		$\sum f = N = 100$		$\sum f x = 3000$

$$\text{Mean age} = \frac{\sum f x}{\sum f} = \frac{3000}{100} = 30$$

For median,

$N/2 = 100/2$

$= 50$

∴ Median lies in class interval 20.5-30.5

Here,

$l = 20.5, \quad h = 10, \quad c = 2, \quad f = 60$

Using,

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

$$= 20.5 + \frac{50 - 2}{60} \times 10$$

∴ Median = 28.5 years

Age Range = 54 years -17 years
= 37 years

II. Mean Age, Median Age and Age Range of TB/HIV Co-infected People.

Age group	Mid value(x)	Frequency (f)	Cumulative frequency (c)	F x X
20.5-30.5	25.5	15	15	382.5
30.5-40.5	35.5	6	21	213
44.5-50.5	45.5	1	22	45.5
50.5-60.5	55.5	1	23	55.5
Total		$\sum f = 23$		$\sum f x = 696.5$

$$\text{Mean age} = \frac{\sum f x}{\sum f} = \frac{696.5}{23} = 30.28$$

For median,

N/2 = 23/2
= 11.5

∴ Median lies in class interval 20.5-30.5.

Here,

$l = 20.5, \quad h = 10, \quad c = 0, \quad f = 14$

using,

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

$$= 20.5 + \frac{11.5 - 2}{14} \times 10$$

$\therefore$ Median = 27.3 years

Age Range = 54 years -23 years

= 31 years

III. χ^2 test for TB/HIV coinfection.

No. of HIV/AIDS people with Tuberculosis =23

No. of non-HIV/AIDS people with Tuberculosis = 6.

No. of HIV/AIDS people without Tuberculosis = 77.

No. of non HIV/AIDS people without Tuberculosis = 94.

Here,

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

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

2 x 2 Categorized table :

Tuberculosis status / HIV status	Tuberculosis Present	Tuberculosis absent	Total
HIV infected	23 (a)	77 (b)	100 (a+b)
Non HIV infected	6 (c)	94 (d)	100 (c+d)
Total	29 (a+c)	171 (b+d)	200 (a+b+c+d)

Using,

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

$$= \frac{(23 \times 94 - 77 \times 6)^2}{100 \times 100 \times 29 \times 171}$$

$$= 11.65$$

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

From table, χ^2tab at 1% level of significance = 6.64.

Since χ^2 cal > χ^2 tab, the Ho is rejected and alterative hypothesis is accepted. This implies that there is significant relation between HIV infection and development of tuberculosis .

IV. χ^2 test for smoking habit and development of tuberculosis in HIV infected people

No. of smokers with .tuberculosis= 15

No. of smokers without tuberculosis = 26

No. of non smokers with tuberculosis = 8

No. of non smokers without tuberculosis = 51.

Here,

Null hypothesis (Ho): There is no significant relation between smoking habit and development of tuberculosis.

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

2 x 2 categorized table.

Tuberculosis status / Smoking Status	Tuberculosis present	tuberculosis absent	Total
Smoker	15(a)	26 (b)	41 (a+b)
Non smoker	8(c)	51 (d)	59 (c+d)
Total	23 (a+c)	77(b+d)	100 (a+b+c+d)

Using,

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

$$= \frac{(15 \times 51 - 8 \times 26)^2 \times 100}{23 \times 77 \times 41 \times 59}$$

$$= 7.24$$

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

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

Since χ^2 cal $> \chi^2$ tab, the Ho is rejected. This implies that there is significant relation between smoking and development of tuberculosis.

IV. χ^2 test for alcoholic habit and development of tuberculosis in HIV infected people

No. of alcohol user with tuberculosis = 12

No. of alcohol user without tuberculosis = 22

No. of non alcohol user with tuberculosis = 11.

No. of non alcohol user without tuberculosis = 55.

Here,

Null hypothesis (Ho): There is no significant relation between alcoholic habit and Development of tuberculosis.

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

2 x 2 Categorized table

Alcoholic Status / Tuberculosis Status	Alcohol user	Non alcohol user	Total
tuberculosis Present	12(a)	11 (b)	23 (a+b)
tuberculosis absent	22(c)	55 (d)	77 (c+d)
Total	34 (a+c)	66(b+d)	100 (a+b+c+d)

Using,

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

$$= \frac{(12 \times 55 - 11 \times 22)^2 \times 100}{23 \times 77 \times 34 \times 66}$$

$$= 4.39$$

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

V. χ^2 test for Tuberculosis and Gender

Total No. of males with tuberculosis = 17

Total No. of males without tuberculosis = 49

Total No. of females with tuberculosis = 6

Total No. of females without tuberculosis = 28

Here,

Null hypothesis (Ho): There is no significant relation between gender and development of tuberculosis.

Alternative hypothesis (H_1): There is significant relation between gender and development of tuberculosis.

2 x 2 categorized table

Sex / Infection	Male	Female	Total
tuberculosis present	17(a) .	6 (b)	23 (a+b)
Tuberculosis absent	49(c)	28 (d)	77 (c+d)
Total	66(a+c)	34(b+d)	100 (a+b+c+d)

Using,

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

$$= \frac{(17 \times 28 - 6 \times 49)^2 \times 100}{23 \times 77 \times 66 \times 34}$$

$$= 0.83$$

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

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

Since χ^2 cal < χ^2 tab, the hypothesis is accepted. This implies that there is no significant difference between the development of tuberculosis and and gender .

VI. χ^2 test for HIV symptoms and development of Tuberculosis.

No. of Symptomatic HIV infected people with tuberculosis = 13

No. of Symptomatic HIV infected people without tuberculosis = 34

No. of Asymptomatic HIV infected people with tuberculosis = 10

No. of Asymptomatic HIV infected without tuberculosis = 43

Here

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

Alternative hypothesis (H_1): There is significant relation between HIV symptoms and development of tuberculosis.

2 x 2 Categorized table :

Mycobacterial status / Symptomatic Status	tuberculosis	tuberculosis	Total
Symptomatic	13(a)	34 (b)	47 (a+b)
Asymptomatic	10(c)	43 (d)	53 (c+d)
Total	23(a+c)	77(b+d)	100 (a+b+c+d)

Using,

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

$$= \frac{(13 \times 43 - 34 \times 10)^2 \times 100}{47 \times 53 \times 23 \times 77}$$

$$= 1.08$$

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. This implies that there is no significant relation between HIV symptoms and development of tuberculosis

VII. Mean age, median age and age range of the *Mycobacterium tuberculosis* / HIV co-infected people

Age (x_)	Frequency (f)	Cumulative frequency (c)	F x X
22	1	1	22
23	2	3	46
26	3	6	78
	$\sum f = 6$		$\sum f x = 146$

$$\text{Mean age} = \frac{\sum f x}{\sum f} = \frac{146}{6} = 24$$

For median = $\frac{N+1}{2} = \frac{6+1}{2} = 3.5$

∴ Median age = 26 Years

Age range = 26-22

= 24

VIII. Mean age, median age and age range of MAC / HIV Co-infected people.

Age (x_)	Frequency (f)	Cumulative frequency (c)	F x X
24	1	1	24
26	1	2	26
30	1	3	30
38	1	4	38
40	3	7	120
43	1	8	43
54	1	9	54
Total	$\sum f = 9$		$\sum f x = 335$

Mean age = $\frac{\sum f x}{\sum f} = \frac{335}{9} = 37$

For median ,

$$\frac{N+1}{2} = \frac{9+1}{2} = 5$$

∴ Median age = 40

Age range = 54 - 24

= 30

IX. χ^2 test between ARV therapy and Development of Tuberculosis in HIV infected people

No. of ARVT receiver HIV infected people with tuberculosis = 2.

No. of ARVT receiver HIV infected people without tuberculosis =7 .

No. of ARVT non receiver HIV infected people with tuberculosis = 20.1

No. of ARVT non receiver HIV infected people without tuberculosis = 70

Here,

Null hypothesis (Ho): There is no significant relation between ARV therapy and development of tuberculosis in HIV infected people.

Alternative hypothesis (H_1): There is significant relation between ARV therapy and development of tuberculosis in HIV infected people.

2 x 2 categorized table.

ARV status / Tuberculosis status	ARV Receiver	ARV non receiver	Total
Tuberculosis Present	2(a)	21 (b)	23 (a+b)
Tuberculosis absent	7(c)	70 (d)	77 (c+d)
Total	9 (a+c)	91(b+d)	100 (a+b+c+d)

Using,

$$\chi^2 \text{ (with Yate's correction)} = \frac{(|ad-bc| - N/2)^2 (a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

$$= \frac{(|2 \times 70 - 21 \times 7| - 100/2)^2 \times 100}{23 \times 77 \times 9 \times 91}$$

$$= 0.12$$

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

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

Since χ^2 cal $< \chi^2$ tab, the Ho is accepted. This implies that there is no significant relation between ARV therapy and development of tuberculosis in HIV infected people.

X. χ^2 test between AFB culture and AFB staining.

Total No. of samples with smear as well as culture positive = 4.

Total No. of samples with culture positive but smear negative = 78.

Total No. of samples with smear positive but culture negative = 1.

Total No. of samples with negative for both smear as well as culture = 77.

Here,

Null hypothesis (Ho): There is no significant difference between the AFB staining and AFB culture.

Alternative hypothesis (H_1): There is significant difference between the AFB staining and AFB culture.

2 x 2 categorized table

AFB culture / AFB staining	Culture +ve	Culture negative	**Total**
AFB Found	4(a)	1 (b)	5 (a+b)
AFB non found	18(c)	77 (d)	95 (c+d)
Total	22(a+c)	78(b+d)	100 (a+b+c+d)

Using,

$$\chi^2 \text{ (with Yate's correction)} = \frac{(|ad-bc| - N/2)^2 (a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

$$= \frac{(|4 \times 77 - 1 \times 18| - 100/2)^2 \times 100}{5 \times 95 \times 22 \times 78}$$

$$= 7.06$$

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

From table, χ^2 tab at 1% level of significance = 3.84.

Since the χ^2 cal $> \chi^2$ tab, the new hypothesis is rejected and alternative hypothesis is accepted. This implies that there is significant relation between AFB culture and AFB staining.

Appendix-IV

Table No. 4.1 Reporting of Sputum Microscopy:

No. of AFB observed/Field	Report
No AFB found /300F	Negative
1 to 9 AFB found per 100 VF	Record exact no. of AFB per 100 VF
10 to 99 AFB found per 100 VF	1+
1 to 10 AFB found per VF in at least 50 VF	2+
More than 10 AFB found per VF in at least 20 VF	3+

No. : - Number

VF : - Visual Field

AFB : - Acid Fast Bacilli (Source: WHO/IUATLD, 2000)

Table No.4.2 Method for reporting culture

The grading of primary culture based on WHO/IUATLD:

Reading	Report
No growth	Negative
1-19 colonies	Positive (Number of Colonies)
20-1000 Colonies	1+
100-200 Colonies	2+
200-500 Colonies (almost confluent growth)	3+
>500 Colonies (Confluent growth)	4+
Contaminated	Contaminated