TITLE: PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION AND IMMUNE STATUS IN NEWLY DIAGNOSED ADULT TUBERCULOSIS PATIENTS IN ADAMA HOSPITAL, ETHIOPIA

BY
TADESSE LIGIDI

A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfillment of the requirements for the degree of masters in medical microbiology

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<td>AFB</td>
<td>Acid Fast Bacilli</td>
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<td>Ag</td>
<td>Antigen</td>
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<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
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<td>ART</td>
<td>Antiretroviral therapy</td>
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<tr>
<td>BCG</td>
<td>Bacille Calmette-Guerin</td>
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<tr>
<td>CFP-10</td>
<td>Culture Filtrate Protein-10</td>
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<tr>
<td>DOTS</td>
<td>Directly Observed Therapy -short course</td>
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<td>DC</td>
<td>Dendritic cells</td>
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<td>EDTA</td>
<td>Ethylene Diamine tetra-acetic acid</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>EPTB</td>
<td>Extra pulmonary tuberculosis</td>
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<td>ESAT-6</td>
<td>Early Secretary Antigenic target 6</td>
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<tr>
<td>FACS</td>
<td>Fluorescent Activated Cell Sorter</td>
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<td>FDC</td>
<td>Follicular dendritic cell</td>
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<td>FMOH</td>
<td>Federal ministry of health</td>
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<td>FRPC</td>
<td>Faculty Research and Publication Committee</td>
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<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
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<td>HAPCO</td>
<td>HIV/AIDS prevention and control office</td>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>IPT</td>
<td>Isonized preventive therapy</td>
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<tr>
<td>IUATLD</td>
<td>International Union Against Tuberculosis and Lung Disease</td>
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<td>MC</td>
<td>Mast cell</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>MGIT</td>
<td>Mycobacterial growth indicator tube</td>
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<td>MTB</td>
<td>Mycobacterium tuberculosis</td>
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<tr>
<td>OI</td>
<td>Opportunistic Infections</td>
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<td>Abbreviation</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PIHCT</td>
<td>Provider initiated HIV counseling and testing</td>
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<td>PLWHA</td>
<td>People living with HIV/AIDS</td>
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<td>PPD</td>
<td>Pure protein derivative</td>
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<tr>
<td>PTB+</td>
<td>Pulmonary Tuberculosis smear positive</td>
</tr>
<tr>
<td>PTB-</td>
<td>Pulmonary Tuberculosis smear negative</td>
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<tr>
<td>QC</td>
<td>Quality control</td>
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<td>TB</td>
<td>Tuberculosis</td>
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<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>TNFR2</td>
<td>Tumor necrosis factor receptor 2</td>
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<tr>
<td>TST</td>
<td>Tuberculin Skin Test</td>
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<td>UNAIDS</td>
<td>United Nations program for AIDS</td>
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<td>VCT</td>
<td>Voluntary counseling and testing</td>
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<td>WHO</td>
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Abstract

Tuberculosis (TB) is a major public health problem disproportionally affecting the low income countries. It is estimated that one third of the world’s population is latently infected with mycobacterium tuberculosis (MTB). Each year, 8 million cases of active TB with over 2 million deaths are estimated to occur globally. The vast majority of individuals with TB live in Africa, South-East Asia and Western Pacific regions. TB and HIV form a lethal combination, each speeding the other’s progress. Globally the number of people living with HIV continues to grow, as does the number of deaths due to acquired immunodeficiency syndrome (AIDS). A total of 39.5 million (34.1 million – 47.1 million) people were living with HIV in 2006 of which about two-thirds live in sub-Saharan Africa. Sub-Saharan Africa thus bears the overwhelming burden of the HIV/AIDS epidemic and TB.

The aim of this study was to determine the prevalence of HIV infection and assess the immune status of newly diagnosed untreated tuberculosis patients. Sample of blood from 258 patients aged 18 and 70 years was collected and screened for HIV by rapid HIV test kits according to the country national testing algorithm. The CD4+ T cell count of each patient was also performed after collecting 4 ml whole blood to k2EDTA tube using fluorescent activated cell sorter (FACS count- Becton Dickinson-USA) and expressed as cells/mm³.

Out of the total 258 specimens, 68 (26.4%) were found to be positive for HIV antibodies. The prevalence was 28.8% (36/125) in females and 24.1% (32/133) in males. The prevalence was high in urban patients (37.9%) than rural (15.7%) (p=0.00), in divorced and widowed patients (65% and 55% respectively) than married TB patients (21.3%) (p=0.00). Occupationally, government employees were the most affected group of new TB patients by HIV. The findings suggest that higher risk of HIV co-infection is present among urban residents, divorced TB patients and government employees. Type of clinical TB, educational status of patients and sex has no association with HIV infection in TB patients.

Of all 68 study participants found HIV positive, only 14.7% knew their HIV sero status before the study (pre ART patients) and the remained around 85% do not know their status, so learned it from the study. The median CD4+ T-cell count was 233 cells/mm³ for those newly diagnosed TB who tested positive for HIV during this study and 295 for the pre ART patients. The median count was significantly higher in HIV negative TB patients (702 cells/mm³, p=0.00) than HIV positives. Large proportion of newly diagnosed HIV positive TB patients who did not know their HIV status prior to this study had CD4+ T-cell count < 200 cells/mm³, account which could have made them eligible for ART. In this regard, the provider initiative counseling and testing (PICT) recently started in health institutions, especially in TB clinics should have to be appreciated and strengthened.

In conclusion, HIV sero prevalence in TB patients is a sensitive indicator of the spread of HIV into the general population and this information is essential to respond to the increasing commitment to provide comprehensive HIV/AIDS care and support among the high risk groups identified, including antiretroviral therapy (ART) to HIV-positive TB patients. Therefore, the study will give an understanding of the epidemiological relationship between HIV and TB diseases at the community level in Adama town and the surrounding villages.

Key words- Tuberculosis, HIV/AIDS, Immune status, prevalence, pathogenesis.
1. INTRODUCTION

Bacteria belonging to the *Mycobacterium tuberculosis* complex cause tuberculosis, one of the oldest diseases known to affect humans. It was classified as a family of Mycobacteriaceae and the order Actinomycetales. Of the pathogenic species belonging to this complex, the most frequent and important agent of human disease is *M. tuberculosis* itself. The complex includes *M. bovis*, *M. africanum* and *M. microti*. *M. tuberculosis* is a rod-shaped, non-spore-forming, thin aerobic bacterium measuring about 0.5 um by 3 um and it do not stain readily and are often neutral on Gram's staining. However, once stained, the bacilli cannot be decolorized by acid alcohol, a characteristic justifying their classification as acid-fast bacilli (AFB). Acid fastness is due mainly to the organisms' high content of mycolic acids, long-chain cross-linked fatty acids, and other cell-wall lipids. Transmission usually takes place through the airborne spread of droplet nuclei produced by patients with infectious pulmonary tuberculosis (Raviglione et al., 2001).

HIV (human immunodeficiency virus), a causative agent of AIDS (acquired immunodeficiency syndrome) belongs to the family of human retroviruses (Retroviridae) and the subfamily of lentiviruses. The most common cause of HIV infection throughout the world is HIV-1, which comprises several subtypes with different geographic distributions. HIV-2, another type of HIV, was first identified in 1986 in West African patients and was originally confined to West Africa (Fauci et al., 2001).

Someone can be infected with HIV in several ways. It can be transmitted through unprotected sexual intercourse with an infected partner, injection or transfusion of contaminated blood or blood products, sharing unsterilized injection equipment that has been previously used by someone who is infected; maternofetal transmission (during pregnancy, at birth, and through breastfeeding). Occupational infections of healthcare or laboratory workers may occur; however, it was not frequent. The risk of occupational HIV transmission from contaminated needles to healthcare workers was found to be around 0.3 % in case series performed prior to the availability of potent Anti Retroviral Therapy (Fauci et al., 2001).
1.1. Epidemiology of Tuberculosis and HIV infection

Tuberculosis (TB) is a major public health problem particularly in low-income countries. Each year, 8 million cases of active TB with over 2 million deaths are estimated to occur globally. For example in 2004, there were 8.9 million new TB cases in the world of which more than 80% of all cases were in the African, South-East Asia and Western Pacific regions (WHO, 2006a). The highest number of deaths was in the World Health Organization (WHO) Africa region and the estimated TB incidence was also exceptionally rising in this region. It was thought that high prevalence of human immunodeficiency virus (HIV) infection particularly in sub-Saharan Africa has fuelled the incidence of TB in the African region. The six WHO regions are Africa, America, Eastern Mediterranean, Europe, South East Asia and Western Pacific (WHO, 2006b).

In Ethiopia, available data suggest that the incidence of TB has risen in recent years, partly because of the impact of the HIV/AIDS epidemic. The country ranks 8th out of the 22 high burden countries in the world based on estimated number of incident cases of all forms of TB in 2004. The country has an estimated TB incidence rate of 353, prevalence of 533 and mortality rate of 79 per 100,000 populations per year (WHO, 2006c).

More than two decades after the discovery of the first clinical symptoms of the acquired immunodeficiency syndrome (AIDS), the HIV/AIDS epidemic is continuing to expand and globally the number of people living with HIV (PLWHA) continues to grow. An estimated 33.2 million (30.6–36.1 million) people were living with HIV in 2007 (UNAIDS, 2007). The estimated number of new infections in 2007 was 2.5 million of which over two third (68%) occurred in sub-Saharan Africa. Nearly 90% of children infected with HIV live in this region. Overall, Sub-Saharan Africa is home to an estimated 22.5 million adults and children infected with HIV in 2007 (UNAIDS, 2007). 76% of 2.1 million total world death due to AIDS in 2007 occurred in sub-Saharan Africa (UNAIDS, 2007) and it is lower than 2.1 million (63%) deaths out of global total death of 2.9 million in 2006 (UNAIDS, 2006). The observed decline of death due to this disease in the world was partly attributable to the scaling up of antiretroviral treatment
services. However, AIDS remains a leading cause of mortality worldwide and the primary cause of death in sub Saharan Africa (UNAIDS, 2007).

Ethiopia is among those sub-Saharan African countries most severely affected by the HIV epidemic. The first evidence of positive sera for HIV antibody was obtained in 1984 (Tsega et al., 1988) and the diagnosis of the first AIDS cases in Addis Ababa hospitals, the capital city, was two years later (Lester et al., 1988). Since then HIV has continued to spread rapidly in different population groups, mainly through heterosexual contact. The estimate in 2005 indicated a national HIV prevalence of 3.5% (3% for male and 4% among females). The estimated prevalence in urban areas was 10.5% (9.1% among males and 11.9% among females) and 1.9% in rural areas (1.7% among males and 2.2% among females). The overall HIV incidence estimate for Ethiopia in 2005 was 0.26% (0.99% in urban and 0.12% in rural). It was also estimated that 1,320,000 people were living with HIV/AIDS. According to the sentinel surveillance result done on antenatal care clinic attendants of the Adama town, the prevalence of HIV seems gradually decreasing. The respective prevalence rates for the years 2001, 2002, 2003 and 2005 were 18.7%, 16%, 10.8% and 9% (MOH, 2005a).

1.2. Pathogenesis of Mycobacterium tuberculosis

Tuberculosis can involve a delay between infection and clinical disease ranging from several weeks to several decades. Active disease may arise almost immediately after infection in about 5% of exposed individuals. Most of the others infected individuals develop latent infections in which the tubercle bacilli persist in vivo without causing any clinical symptoms (Kaufmann, 2001). The consequences of inhaling or ingesting tubercle bacilli depend on both the virulence of the organism and the resistance of the host. At one extreme, organisms with little virulence for the particular host disappear completely, leaving no anatomic trace behind. At the opposite extreme, the bacilli flourish with in macrophages and disseminate widely, and cause death within a few months (Kaufmann, 2002). Generally, four potential outcomes of Mycobacterium tuberculosis (MTB) infection can occur according to the fate of the microorganism inside the macrophages: the bacterium can be immediately eliminated, becomes dormant indefinitely inside the
host, causes primary tuberculosis or reactivates many years after the primary infection (Giacomini et al., 2001).

MTB infection occurs mainly at the lung through the respiratory route (Neil, 2001). Following its penetration of the mucosal barrier, the bacteria is associated with intraepithelial leukocytes and subsequently conveyed to the draining lymph nodes. Then it spread from the site of initial infection in the lung through the lymphatics or blood to other parts of the body (Teitelbaum et al., 1999; Muñoz et al., 2003). It is obvious that mycobacterium is an intracellular pathogen in host macrophages and therefore the success of it as a pathogen relies on its ability to survive within this cell. In this type of cells, it resides within early endosome-like phagosomes, which make it persistent to the effect from the cell and even multiply (Pieters, 2001). The mycobacterium containing phagosomes are hampered in maturation and fail to fuse with lysosomes, which enable the cells to kill the bacillus (Fratti et al, 2000). The intralysosomal acidic hydrolases are released from lysosomes to degrade the phagocytized microorganism only upon phagolysosome fusion. This is the reason why prevention of phagolysosomal fusion was one hypothesized mechanism by which the MTB survives inside macrophages (Raja, 2004).

1.3. Host responses against *Mycobacterium tuberculosis*

Epidemiologic evidence on the frequency of tuberculosis in various populations has long suggested that malnutrition, overcrowding, and stress decrease resistance to the disease (Van Lettow et al, 2003; Schwenk and Macallan, 2000). However, this kind of evidence does not firmly establish a casual relation because these conditions are generally also associated with a high rate of other infection (Hawker et al, 1999). Overall, one-third of the world's population is currently infected with the TB bacillus. Among persons infected with the tubercle bacillus, as detected by a positive Tuberculin test, only a small proportion develop overt disease (5-10%) at some time during their life (WHO, 2006b). The transition from infection to mild or severe disease depends strongly on various factors notably socio-economic factors, co-infection with HIV and genetic predisposition of the host (Ulrichs and Kaufmann, 2002).
Humans exhibit a range of responses to MTB. The major host immune response components against it include innate immunity (mainly macrophages and dendritic cells (DCs), T-cell mediated adaptive immunity (including CD4+ and CD8+ T cells) and production of cytokines (inflammatory and anti-inflammatory) and chemokines. These responses are directed towards containing or eliminating the tubercle bacillus within the tissue of the host. The series of cytokines are derived from various host cells including mast cells, macrophages and T cells (Kaufmann, 2002; Flynn, 2004). Mast cells (MC) are inflammatory cells typically found in relatively large numbers in the mucosa of the respiratory, gastrointestinal, and urinary tracts and near blood or lymphatic vessels. Since these sites are also common portals of infection, mast cells are likely to be one of the first inflammatory cells to make contact with invading pathogens. Following activation, they release a myriad of pro-inflammatory cytokines (histamine, tumor necrosis factor-alpha, and Interleukin-6), proteases, and inflammatory mediators. Findings suggest that mast cells derived histamine in addition to its well-known roles of inducing bronchoconstriction, mucus secretion, increment of vascular permeability and edema production, have the potential to play an important regulatory role in the immune response to MTB (Munoz et al., 2003). It was demonstrated that MC is also an important source of TNF-α following exposure to MTB. MC produces IL-6, another cytokine with both pro- and anti-inflammatory properties, after activation by MTB during early infection (Ladel et al., 1997; Schindler et al, 1990). This cytokine contributes to host defense by activating neutrophils and stimulating the growth and function of T cells (Munoz et al., 2003). These mast cell mediators are crucial for mobilizing and recruiting various other inflammatory cells to the site of infection (Mekori and Metcalfe, 2000). Another protective immune response is the production of the cytokine Interferon (IFN)-γ by CD4+ T helper 1 (Th1) type cells. CD8+ T cells can also produce IFN-γ and TNF which are critical cytokines in the activation of the microbicidal mechanisms of macrophages (Flynn and Chan, 2001a; Flynn and Chan, 2001b). In humans, some experimental evidence suggests that CD4+ T cells perform an additional cytolytic function, particularly in the local immune response in the lung (Collins and Kaufmann, 2001).
While innate immune responses initially predominate, the subsequent recruitment of T lymphocytes to the lung is necessary to the containment of MTB within granulomas, which consist of activated macrophages surrounded by T-lymphocytes, fibroblasts, and epitheloid cells (Giacomini et al., 2001; Saunders and Cooper, 2000). Macrophages infected with mycobacterium promote Th1 lymphocyte activity by the release of IL-12 and IL-18. These Th1 lymphocytes then drive macrophage activation by the release of IFN-γ and TNFα. Macrophage apoptosis occurs within the granuloma, which is essential for successful immunity to tuberculosis. Granuloma, which is a major histological feature of mycobacterial infection, is a dynamic entity, with macrophages dying and being replaced by newly activated monocytes. Similarly, lymphocytes are continuously replaced as cell mediated immunity improves the specificity of cells. Both apoptotic macrophages and lymphocytes can be seen within the granuloma. Macrophage apoptosis is beneficiary to the host since macrophages undergoing apoptosis kill intracellular mycobacteria. A major communication between infected macrophages and the adaptive immunity is via MHC-2 antigen presentation to CD4+ T lymphocytes (Fairbairn, 2004).

The ability of mycobacteria to avoid the induction of macrophage apoptosis has been demonstrated as a virulence factor. A number of mechanisms by which virulent MTB prevents macrophage apoptosis have been identified, including increased secretion of soluble TNFR2 which mops up TNFα, increased levels of anti-apoptotic proteins and inactivation of pro-apoptotic proteins (Fairbairn, 2004).

1.4. HIV pathogenesis.

The pathogenesis of HIV infection is a function of the virus life cycle, the host cellular environment and quantity of viruses in the infected individual. Therefore, disease progression is a direct reflection of virus replication (Wei et al., 1995). In the absence of active viral replication, or when viral replication is low (blood viremia < 100 genome copies per milliliter), HIV infection does not progress to clinical immunodeficiency. Conversely, when viral replication is rapid (viremia > 100,000 genome copies per milliliter), disease progression is correspondingly rapid (Pantaleo et al, 1995). Among HIV-infected patients studied in the pre-HAART era, most progress from infection to
AIDS in 5 to 15 years. A fraction of patients, about 5% to 10%, progress more rapidly (rapid progressors), and another fraction, perhaps 5%, show no evidence of progression over many years (non-progressors) (Reynold et al, 2006). Individuals who have been infected with HIV for a long period (10 years), whose CD4+ T cell counts are in the normal range and have remained stable over years, and who have not received antiretroviral therapy are considered to be long-term non-progressors (Fauci et al., 2001).

The hallmark of HIV pathogenesis is immune dysfunction due to depletion of CD4+ T cell count. Many types of cells express CD4+ receptors under different conditions and are susceptible to HIV infection, including cells of the mononuclear phagocyte lineage (principally blood monocytes and tissue macrophage), T lymphocytes, natural killers cells, dendritic cells (langerhans cells and follicular dendritic cells in lymph nodes), hematopoietic stem cells, endothelial cells, microglial cells in the brain and gastrointestinal epithelial cells. CD4+ T cells, the primary targets of HIV may become infected as they encounter HIV trapped on follicular dendritic cells (FDC) which is considered as a significant reservoir of infectious HIV (Fauci et al., 2001).

Three major mechanisms of CD4+ T-lymphocyte killing by HIV have been suggested: direct virus-mediated cytolysis, virus-induced apoptosis, and indirect killing through immune effector mechanisms. Direct virus-mediated cytolysis has been demonstrated in vitro and syncytium formation may accelerate the cytolytic process. Here infected cells are killed because of viral replication in these cells, disrupting the cell membrane (Fauci et al., 2001). The turnover rate of CD4+ T lymphocytes is correspondingly turbulent. Each day in an infected patient, up to 10^9 new virions are made and about 10^9 CD4+ T lymphocytes are killed and replaced (Hu et al., 1996). Recent data shows that the destruction of billions of CD4+ T cells overwhelms the immune systems regenerative capacity. Loss of CD4+ T cells results in a loss of recognition of antigens that are presented on class II MHC molecules. With gradual decrease of CD4+ T cells, Th1 function is damaged with loss of cell-mediated immune functions and Th2 functions are impaired with gradual loss of humoral responsiveness to newly presented foreign antigens (Reynold et al, 2006).
Syncytia formation gives chance for the spread of HIV from cell to cell which result in
the death of uninfected cells. CD4⁺ T lymphocytes from infected patients undergo
apoptotic death when HIV proteins, probably leading to their suicide, distort cellular
regulation. Apoptosis perhaps reflects the high rate of ongoing immune activation in
HIV-infected persons. Immune destruction of infected cells may not likely to be a central
mechanism of CD4⁺ T-lymphocyte depletion: because some times persons with weak
immune responses show more rapid depletion and more rapid clinical disease progression
(Reynold et al, 2006). The model, which is currently supported, stated that a concerted
effect of the different mechanisms is responsible for the depletion of CD4⁺ T cells in HIV
infection (McCune, 2001).

1.5. TB-HIV co-pathogenesis
Infection with HIV constitutes the strongest risk factor for development of TB in subjects
with latent MTB infection. Due to the high incidence of both HIV and MTB infection in
the developing countries, TB has emerged as the most common opportunistic infection
(OI) in HIV-infected patients worldwide (WHO, 2006a). Thus, the interaction of these
two pathogens currently and in the future will potentiate morbidity and mortality
associated with either. Globally, more than one-third of HIV positive individuals are co
infected with MTB and 12% of AIDS deaths are attributed to TB (Raviglione, 2003;
Corbett et al., 2003). In Africa, HIV is the single most important factor determining the
increased incidence of TB in the past 10 years (WHO, 2006b). HIV/AIDS accounted for
32% of the estimated 141,000 cases of tuberculosis in Ethiopia in 2005 (MOH, 2005a).

The co pathogenicity between MTB and HIV is best illustrated by the high susceptibility
of the HIV-infected persons for reactivation of a remote TB infection or early progression
of a newly acquired disease. This dual interaction is also demonstrated by the negative
impact of TB on the natural history of HIV, which is characterized by increased
incidence of clinical progression and increased mortality rates (WHO, 2006b; Gerard,
2000). An HIV-positive person infected with MTB has a 50 - 60% lifetime risk of
developing TB disease as compared to HIV-negative person who has only 5-10% risk.
HIV-infected persons who become newly infected with MTB rapidly progress to active TB disease. Clinical presentation of TB is also atypical and severe when immune suppression is advanced in TB-HIV co-infected patients (Gerard, 2000).

The progressive loss of CD4+ T cells in HIV-infected patients is the basis of increased TB incidence since CD4+ T cells are considered to be the primary cellular component involved in immune protection against TB via their ability to produce IFN-γ, activate macrophages, and kill infected cells (Flynn and Chan, 2001a; Ottenhoff et al., 2003). Furthermore, CD4+ T cells are believed to be required either for primary activation of CD8+ T cells or for the maintenance of immune protective CD8+ T cells (Janssen et al., 2003; Shedlock and Shen, 2003; Sun and Bevan, 2003; Badri et al., 2001).

Since, MTB can spread through the air, the increase in active tuberculosis cases among dually infected people means more transmission of the TB germ, meaning more TB infections and disease in the whole population. Consequently, the HIV/AIDS epidemic is reviving an old problem in developed countries and exacerbating an existing one in the developing countries (Fauci et al., 2001). For these reasons, it is believed that HIV increases the spread of TB. Active TB is most common in patients aged 25 to 44 years in developing countries. In these demographic groups, 20 to 70% of the new cases of active TB are in patients with HIV infection. This active TB often develops relatively early in the course of HIV infection and may be an early clinical sign of HIV disease (Fauci et al., 2001).

Clinical TB accelerates the progression of underlying HIV disease by stimulating HIV infected macrophages and CD4+ lymphocytes to produce more viruses. A cohort study undertaken to assess the effect of TB on the natural history of HIV infection in patients from a high TB prevalence setting (Badri et al., 2001), demonstrated a significantly reduced survival and an increased frequency of AIDS-defining illness in HIV-infected patients with TB. The median time of progression to AIDS according to this study, in patients free of AIDS at baseline, was 6 months for tuberculosis cases compared to 14.5
months for patients with HIV but no TB (comparison group). Mortality rate was significantly higher in TB cases compared to the comparison group (Badri et al., 2001).

Furthermore, TB has been shown as one of the leading opportunistic diseases diagnosed in patients with AIDS as well as the most common cause of death in autopsied African patients with AIDS (Lynn et al., 2003). In one observational cohort study of HIV-infected adults in South Africa (Day et al., 2004), viral load was compared between patients experiencing episode of TB and those non-TB control group. The result revealed that the mean HIV viral load was higher in the TB group than in the non-TB control group showing that an episode of TB could have some effect on HIV disease progression or HIV transmission at the population level (Day et al., 2004). On the other hand, HIV-positive patients with active TB, who receive anti-TB therapy and HAART, are just as likely as HAART-treated HIV-positive patients without TB to benefit from antiretroviral therapy (Hung, 2003).

Taken together, the onset of TB in HIV-infected patients is associated with an increased risk of AIDS and death. Although a causal link cannot be established in an observational study, the above findings support the view that prolonged immune activation induced by tuberculosis leads to increased HIV replication and consequent accelerated disease progression (Badri et al, 2001).

1.6. TB and HIV diagnosis

Presumptive diagnosis of TB is commonly based on the finding of acid fast bacillus (AFB) on microscopic examination of a diagnostic specimen such as a smear of expectorated sputum or of tissue (for example, a lymph node biopsy or fine needle aspiration) (Hudson et al., 2000; Johnson and Ellner, 2006).

Definitive diagnosis depends on the isolation and identification of MTB from a diagnostic specimen (in most cases a sputum) using Mycobacterial Culture on Lowenstein-Jensen or Middlebrook 7H10 media by incubating at 37°C under 5% CO₂.
In today's laboratories, the use of liquid media with radiometric growth detection (e.g., BACTEC-460) and mycobacterial growth indicator tubes (MGIT) have replaced the traditional methods of isolation on solid media. These new methods have decreased the time required for isolation to 2 to 3 weeks compared to the 8 weeks required for the traditional culture methods. Advances in knowledge about genetic structure of tubercle bacillus helped develop gene probes and gene amplification methods for identification and detection of tubercle bacillus, from culture or directly from clinical specimens and molecular detection of drug resistance. While the gene probes can help in rapid identification of isolates, gene amplification methods (e.g. PCR) developed for diagnosis of TB is demonstrably highly sensitive and detection can be done within hours (Katoch, 2004). Alternative specimens for diagnosis of TB can be aspirated effusions, blood for cultures, early morning urine for TB culture and bone marrow biopsy (Hudson et al., 2000; Johnson and Ellner, 2006).

Skin testing with PPD is most widely used in screening for MTB infection. The test is of limited value in the diagnosis of active TB because of its low sensitivity and specificity (Johnson and Ellner, 2006). False-negative reactions are common in immunosuppressed patients. Positive reactions are obtained when patients have been infected with MTB but do not have active disease and when persons have been sensitized by non-tuberculous mycobacteria or Bacilli Calmette-Guerin (BCG) vaccination. In the past years, new diagnostic methods like Enzyme-linked immunospot (ELISPOT) and Enzyme-linked immunosorbent assay (ELISA) for the diagnosis of infection with MTB have been developed. The ELISPOT and ELISA detect the secretion of \( \gamma \)-interferon by mononuclear cells in venous blood, specific for MTB peptides, ESAT-6 and CFP-10. These tests are more sensitive and specific for the diagnosis of MTB infection and are superior to the tuberculin skin test (TST) in patients with immunosuppression (Ferrara et al., 2006). ESAT-6 and CFP-10 are peptides that mediate MTB virulence (Brodin et al., 2005).

Radiographic Procedures and clinical sign and symptoms can also be used in the process of diagnosing TB. The initial suspicion of pulmonary TB is often based on abnormal
chest radiographic findings in a patient with respiratory symptoms (Johnson and Ellner, 2006). Of the clinical features, cough is reported less frequently in HIV patients, probably because of weak cough reflex due to debilitated condition of the patients in advanced disease, absence of cavitations, and less endobronchial irritation (MOH, 2005b).

The following are the criteria to diagnose the various clinical forms of TB:

1. Pulmonary smear positive (PTB⁺): at least 2 sputum smear examinations positive for AFB, or one sputum positive for AFB and radiographic abnormalities consistent with active pulmonary TB, or one sputum specimen positive for AFB and culture positive for *M. tuberculosis*.

2. Pulmonary smear negative (PTB⁻): at least three sputum examinations negative for AFB, radiographic abnormalities consistent with active pulmonary TB and not responding to a course of general antibiotics, or diagnosis based on positive culture but negative AFB sputum examinations. Others consider the patient is PTB⁻ when three sputum smear examination is negative and bronchoscopy samples (BAL) show ‘scanty’ to 1⁺ positivity or if two of any samples were positive after concentration (Harries et al., 2001).

3. Extra-pulmonary tuberculosis (EPTB): one culture-positive specimen from an extra pulmonary site, or histological evidence, or strong clinical evidence consistent with active extra-pulmonary TB (MOH, 2005b).

Tuberculosis may affect any organ system. Extra pulmonary TB results from hematogenous dissemination of tubercle bacilli with incomplete immunologic control of the disease, either during primary infection or because of reactivation from a site of latent infection. In order of frequency, extra pulmonary tuberculosis involves the lymph nodes (40% of all EPTB), the pleura, the genitourinary tract, bone and joints, the meninges and the peritoneum (Johnson and Ellner, 2006).

For HIV diagnosis currently different testing methods can be used. These methods detect the presence of infection by detecting one of the following: HIV antibody, HIV antigen, combined HIV Ab/Ag, HIV viral nucleic acid and HIV virus by viral culture method.
HIV antibody detection can be done using ELISA methods, rapid tests and western blot assay methods. For surveillance as well as diagnostic purpose in developing countries, WHO recommends alternative testing strategies using combination of ELISA or rapid tests (WHO, 2001).

As stated above an HIV infection may also be diagnosed through the direct detection of virus. Because of its cost, virus detection is only necessary in certain situations. Alternatively, assays for the detection of HIV p24 antigen are available. Although the p24 antigen ELISA has generally been replaced by the more sensitive nucleic acid detection assays, fourth generation antibody screening tests incorporate p24 antigen detection in addition to HIV antibody detection, to shorten the "diagnostic window" period. The detection of viral nucleic acid (i.e. of virus genome) may be achieved by different laboratory techniques. These methods may be used to detect either proviral cDNA in leucocytes or viral RNA in the cell-free compartment (Preiser and Korsman, 2006).

The close relationship between clinical manifestations of HIV infection and CD4+ T cell count has made measurement of the latter a routine part of the evaluation of HIV-infected individuals. The CD4+ T cell count provides information on the current immunologic status of the patient (Fauci et al., 2001). Patients with HIV infection should have CD4+ T cell measurements performed at the time of diagnosis and every 3 to 6 months thereafter (DHSS Panel, 2005). CD4+ T cell count is stated in treatment guidelines for determining when to start or change ART and for deciding when to initiate prophylaxis for opportunistic infections (MOH, 2005c). According to most guidelines, a CD4+ T cell count <500/mm³ is an indication for consideration of initiating antiretroviral therapy, and a decline in CD4+ T cell count of >25% is an indication for considering a change in therapy (Fauci et al., 2001). In the Ethiopian setting currently clinical symptoms and CD4+ T-cell count of <200 cells/mm³ are in use for initiating antiretroviral treatment (MOH, 2005c). In untreated HIV patients or in patients in whom therapy has not adequately controlled virus replication, the CD4+ T cell count falls below a critical level after a variable period and the patient becomes highly susceptible to opportunistic
diseases (Fauci et al., 2001). Different opportunistic infections occur at different CD4+ T cell levels in HIV/AIDS patients. Unlike most other opportunistic infections (OIs) associated with AIDS, TB can occur at relatively high CD4+ counts (Lynn et al., 2003).

1.7. Prevention of TB in HIV infected patients

Antiretroviral therapy (ART) is the cornerstone of the overall strategy to reduce morbidity attributed to HIV related infections. Potent combination ART has reduced the incidence of OIs for certain patients with access to care. However, it does not replace the need for antimicrobial prophylaxis among patients with severe immune suppression (Benson et al, 2004). A number of trials have evaluated the efficacy of primary preventive therapy against TB among HIV-infected individuals (Hawken et al., 1997; Whalen et al., 1997). In all the trials, preventive therapy reduced the incidence of TB among HIV-infected people with a positive tuberculin skin test. Most of the trials that evaluated the effect of preventive therapy irrespective of tuberculin test result found that preventive therapy protected against TB. In support of these findings, meta-analyses showed that both produced statistically significant results among all individuals regardless of tuberculin status, with pooled estimates of reduction in TB incidence of 42% (Grant et al., 2001). In some studies, it is associated with a 60% reduction of risk of development of tuberculosis (Gerard, 2000).

Isoniazid preventive therapy (IPT) for HIV–TB co infected individuals reduces the reactivation of latent MTB infections and is being evaluated as a potential community-wide strategy for improving TB control. Projected effects of IPT intervention on TB and TB drug resistance indicated that, in the first few years after program introduction, community-wide IPT was associated with reductions in the prevalence of both latent infection and active infectious TB (Cohen et al., 2006). Despite these reductions in the overall burden of TB, increasing IPT coverage led to an increasing proportion of drug-resistant TB. The increase in drug-resistant TB observed was not the result of acquired resistance generated by IPT. Rather, IPT promoted the emergence of drug resistance in two ways: (i) IPT prevented disease among individuals infected with
drug-sensitive MTB strains, thereby decreasing further transmission of these strains. Because infection with one strain provided partial protection against infection with another, there was competition between sensitive and resistant strains, so this effect promoted the spread of resistant strains. (ii) IPT cured drug-sensitive latent infections in patients dually infected with sensitive and resistant strains and thus increased the likelihood that reactivation with a resistant strain would occur (Cohen et al., 2006).

An alternative approach to prevent evolution of drug-resistant TB would be to use preventive therapy regimens with more than one drug, (e.g rifampicin plus pyrazinamide) which would resolve the problem of persons with active disease unintentionally receiving isoniazid mono therapy. However, multidrug preventive therapy regimens are expensive, and if unsupervised preventive therapy regimens including rifampicin were introduced, resistance to this key anti tuberculosis agent might be promoted (Grant et al., 2001).

1.8. TB-HIV co infection in Ethiopia

Varying HIV seropositivity rates among tuberculosis patients have been reported in different parts of the world and even within a country. Several studies done elsewhere globally have reported TB-HIV co-infection rates ranging from <1% up to as high as 65% (Vander Werf et al., 2006; Vander Werf et al., 2005; Jain, 2000; Prasanthi and Kumari, 2005; Attili et al., 2005; Daniel et al., 2004; Schoch and Rieder, 1996). Similarly, studies from Central, North and Southern part of Ethiopia revealed varying rates of HIV seropositivity in active TB patients ranging from 6.6% to 52.1% (Kefene et al., 1990; Mitike et al., 1997; Gellete et al., 1997; Demisse et al., 2000; Yassin et al., 2004; Kassu et al., 2007). On the other hand, very limited studies in Ethiopia tried to assess the immune status of patients when they develop active TB (Wolday et al., 2003). While no published reports are available from Adama area.

The purpose of this study was therefore, to determine the magnitude of the problem in patients attending TB clinic of Adama hospital. In this study, the HIV seroprevalence was determined in new adult TB patients to understand the degree of co infection of HIV and
TB in the area. The prevalence was determined in different groups of TB patients and it was hypothesized that the prevalence of HIV in new TB patients will be high especially in those who reside in town. The study also tried to estimate the median CD4$^{+}$T cell count around which HIV positive patients develop clinical TB and come to health institutions for treatment. The median range hypothesized was 300-400 cells/mm$^3$, based on published reports elsewhere (Bartlett, 2004), showing that tuberculosis is one of the early occurring opportunistic infection.

2. SIGNIFICANCE OF THE STUDY

Rising TB case rates over the past decade in many countries in sub Saharan Africa and in parts of South East Asia are largely attributable to the HIV epidemics. This opportunistic disease often appears before other opportunistic infections occur in persons infected with HIV. Different opportunistic infections (OI) in HIV infections occur at different CD4$^{+}$ T-cell counts. Therefore this study will help the people working in the filed to know the epidemiological distribution of the HIV infection in a study population, which also help to understand the contribution of HIV infection for the development of clinical tuberculosis. The study also helps to identify the high-risk groups of the study population for HIV infection and the median CD4$^{+}$ T cell count around which clinical TB develops in HIV infected patients, which will be used in the process of TB prevention.
3. OBJECTIVES OF THE STUDY

3.1. General Objective: The purpose of this study was to determine HIV sero prevalence as well as assess the immune status of newly diagnosed tuberculosis patients by counting their CD4$^+$ T cell during their first visit to the TB clinic of Adama hospital.

3.2. Specific objectives
- To determine sero prevalence of HIV infection in different groups of new adult tuberculosis patients.
- To determine the median CD4$^+$ T cell level in new adult patients with different sexes, HIV status and clinical TB type at their first visit for tuberculosis treatment.
- To make necessary recommendations based on the research findings.
4. MATERIALS AND METHODS

4.1. Study design, time and place

Cross sectional hospital based 3/12 study done at Adama town (May18 - August 6, 2007). Adama town is located about 100 km east of Addis Ababa and it has more than 200,000 populations.

4.2. Study population

Adult newly diagnosed TB patients (n=258). The criteria for inclusion was adult (age ≥ 18 years) newly diagnosed pulmonary TB (smear positive and negative) and extra pulmonary TB patients. New TB patients are those patients who did not take anti-TB drugs before or have taken it for less than 30 days (MOH, 2005b).

Different Medical examinations were performed by the hospital physicians including, observation of complex symptoms suggestive of TB such as fever more than two weeks, cough more than two weeks, night sweats, weight loss. In addition, information on household contact with proved or highly suggestive cases of TB and suggestive chest X-ray findings were collected. TB is classified as pulmonary TB when it is bacteriologically confirmed (smear positive), pulmonary TB bacteriologically not confirmed but clinically or radiologically suggestive of PTB (smear negative), and extra pulmonary TB (MOH, 2005b).

4.3. Sample size determination

The minimum sample size was calculated using the formula required for determination of sample size for estimating single proportions (Daniel, 1987). An estimated 21% prevalence of HIV-TB co infection for Ethiopia (WHO, 2006c), 95% confidence interval and a precision of 5% was used in sample size determination. Accordingly, a total of 258 patients were included in the study.
4.4. Sampling procedure

All eligible consecutive patients who came to the clinic were included in the study until enough samples were collected. The health personnel assigned in TB clinic was a nurse trained as a counselor and gave appropriate provider initiative HIV counseling. Blood collection was done for those who gave their consent. HIV antibody testing and CD4+ T cell counts were performed for all eligible patients. After posttest counseling, the HIV positive patients were referred to hospital antiretroviral therapy (ART) clinic with their CD4+ T cell count result for further management.

Basic social and demographic information’s about all included TB patients were collected from their medical records (cards, referral forms) and by interviewing each of them using structured questionnaire.

a) Blood sample collection, handling and transportation.

Four-milliliter (4 ml) blood sample for HIV testing and CD4+ T cell count was collected from arm vein to K3 EDTA vacutainer tube after appropriate disinfection of vein puncture site by 70% alcohol. Blood collection was done between 2:00 pm and 5:00 pm each day by a laboratory personnel in a separate room in the TB clinic assigned for sample collection and HIV testing. After HIV testing the sample for CD4+ T cell count was transported to regional laboratory the same day. The sample transporter wear glove and has taken all necessary safety precautions during transportation.

b) Sputum sample collection handling and transportation

All pulmonary tuberculosis (PTB+ and PTB-) diagnosed patients has given their sputum to the laboratory for smear microscopy examination before they come to TB clinic for treatment. For each patient three consecutive samples (spot, early morning, spot) were collected to sterile sputum cup. A spot specimen was collected at the first attendance day,
an early morning specimen was collected the next day at home and a third spot specimen collected when the patient brought his/her morning sputum to the laboratory. The sputum samples were collected to the sputum cup and transported to the laboratory by the patients or their relatives after appropriate orientation was given on how to collect the right type of sample and transport it safely.

4.5. Laboratory procedures

a) HIV testing

Blood samples were tested for HIV antibodies according to the Ethiopian national testing algorithm for voluntary counseling and testing by using Determine HIV1/2 (Abbott laboratories, Japan co LTD), Capillus HIV1/2 (The Trinity Biotech, Ireland) and Unigold HIV1/2 (Trinity Biotech, Ireland) rapid test kits (Fig 1). Tests were done according to the manufacturer’s instruction.
Quality control: The quality control (QC) for HIV rapid test was performed according to the recommendations of the kit manufacturers. For HIV rapid tests, in addition to strictly looking for the appearance of internal procedural control band (for Determine and Unigold), and performing internal control tests for Capillus as per the manufacturers recommendations, in-house known positive and negative controls were utilized during the study.

b) CD4$^+$ T cell count

From the sample collected into the K$_3$EDTA test tubes, CD4$^+$ T-cell count was done using a fluorescent activated cell sorter (FACS)Count and fluorescent labeled monoclonal antibodies (Becton Dickinson Immunocytometry System, San Jose, California, USA) and expressed as cells/mm$^3$. The CD4$^+$ count was done in Adama regional laboratory the same day of sample collection.

Quality control: The quality control (QC) for CD4$^+$ count was performed according to the recommendations of the kit manufacturers. For CD4$^+$ count, quality control was done by running BD FACS count control beads every other day when the instrument is turned on and whenever new lot of reagent is opened. These control runs set up the instrument and check the linearity as well as reagent activity. As an additional QC, the sum of CD4$^+$ T cell and CD8$^+$ T cell was compared with total CD3$^+$ T cell number. The CD4$^+$ result was valid when the sum of CD4$^+$ and CD8$^+$ T cells was within the range of $\pm$ 10% of total CD3$^+$ T cell counts.

All left over specimens and disposable materials used to perform these different tests were disposed according to the waste disposal system of the institution in which the tests were performed (incineration).

c) Direct smear microscopy
In Adama hospital, TB diagnosis was made by identification of acid-fast mycobacteria from sputum and/or aspirate after staining with Ziehl Neelsen stain. Pathologic examination for extra pulmonary TB diagnosis is usually performed in private diagnostic laboratory and the result was submitted to hospital to start treatment.

**Quality control:** All laboratories performing AFB-smear microscopy should ideally do a quality control for AFB staining in a routine series daily by using known positive (1+) and known negative samples. Adama hospital laboratory reported that they use this stated method for controlling the quality of their AFB microscopy.

### 4.6. Data entry and analysis

HIV prevalence in over all patients, different age groups and different sex groups of new adult TB patents was analyzed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Chi square test at \( \alpha = 0.05 \) level of significance was done to explore the association of different categorical variables included in the study. Predictive factors for HIV infection in TB patients were examined by logistic regression. The median CD4\(^+\) T cell level at which TB disease appear (for both HIV negative and HIV positives), for pre ART patients and for HIV positive with different clinical TB type patients was calculated using STATA statistical software (STATA, Version-8, Texas, USA). Median CD4\(^+\) T cell counts were compared using the nonparametric Mann-Whitney U test using the STATA statistical software. P values < 0.05 were considered statistically significant.

### 4.7. Ethical considerations

The study was performed after obtaining ethical clearance from Addis Ababa University medical faculty research and publication committee (FRPC), and the Oromiya Regional State Health Bureau research committee. Informed consent was obtained from each participant before blood sample collection. Strict confidentiality was maintained throughout the study. All patients were given code numbers to keep their identity confidential.
5. RESULTS

5.1. Socio demographic characteristics and proportion of clinical tuberculosis type in a study group (n=258)

The overall response rate of patients included in the study was 95.6% (258/270). The main reasons for refusal were poor perception of personal risk factors or thinking that TB should be treated first for better outcome before going to be tested for HIV. As summarized in Table-1 below, of the total 258 newly diagnosed TB patents included in the study 133 (51.6%) were males and 125 (48.4%) were females with an age range of 18-70 years (median 25 years). In terms of residence, 124 (48%) of the patients were living in urban and 134 (52%) in rural areas. Most of the TB diagnosed patients at Adama hospital were in the age group of 18-29 years (56.6%) and educationally illiterate (38%).
Table 5.1. Demographic characteristics and HIV prevalence of newly diagnosed TB patients in Adama hospital (May-August 2007)

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of patients</th>
<th>Percent of the group</th>
<th>Number of HIV positives</th>
<th>Prevalence of HIV</th>
<th>P-value</th>
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*Other occupations include urban housewife, daily laborers, private employees, beggars and household servants. TB = tuberculosis; OR = odds ratio; CI = confidence interval; PTB+ = pulmonary TB bacteriologically confirmed by smear microscopy; PTB– = pulmonary TB smear negative; EPTB = extra pulmonary TB.
The most frequently diagnosed types of TB were smear negative pulmonary TB (40.7%) followed by extra pulmonary TB (38.4%) and smear positive pulmonary TB (20.9%) (Figure 5.1).

![Pie chart showing the proportions of various types of TB](image)

Figure 5.1. Proportions (%) of diagnosed clinical tuberculosis types among newly diagnosed patients in Adama Hospital, Adama

### 5.2. HIV testing result

The calculated overall HIV prevalence in TB patients included in the study was 26.4% and was higher in urban (37.9%) than rural (15.7%) residents (P < 0.05). The median age was 32.5 years for HIV positive and 25 years for HIV negative patients. Higher HIV positivity rate was observed in the age group 30-39 (46.5%) followed by the age group 40-49 (37%) (Table 5.1). In terms of occupation, high HIV prevalence rate was observed among governmental employees (50%) followed by unemployed patients (44%) and the lowest prevalence (12.5%) was observed in students (P<0.05). On the other hand, type of clinical TB, educational status and sex were found to have no association with HIV infection (P>0.05). However, the significant association of HIV infection with residence and occupation (Table 5.1), except for farmers, disappeared after multivariate adjustment (Table 5.2).
Table 5.2. Association of socio demographic factors and clinical TB types with HIV seropositivity in newly diagnosed TB patients in Adama Hospital, Adama

<table>
<thead>
<tr>
<th>Variable</th>
<th>No (% HIV infected)</th>
<th>Univariate, OR (95% CI)</th>
<th>Multivariate, OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>125 (28.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>133 (24.1)</td>
<td>0.78 (0.45-1.36)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-29</td>
<td>146 (17.8)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>30-39</td>
<td>58 (46.5)</td>
<td>4.0 (2.06-7.83)</td>
<td>3.69 (1.58-8.58)</td>
</tr>
<tr>
<td>40-49</td>
<td>27 (37)</td>
<td>2.7 (1.1-6.6)</td>
<td>2.20 (0.724-6.70)</td>
</tr>
<tr>
<td>≥50</td>
<td>27 (18.5)</td>
<td>1.0 (0.36-4.30)</td>
<td>0.50 (0.12-2.1)</td>
</tr>
<tr>
<td><strong>TB classification</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPTB</td>
<td>99 (28.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PTB +</td>
<td>54 (18.5)</td>
<td>0.57 (0.25-1.30)</td>
<td></td>
</tr>
<tr>
<td>PTB -</td>
<td>105 (28.6)</td>
<td>1.01 (0.55-1.86)</td>
<td></td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>134 (15.7)</td>
<td>1.00</td>
<td>1</td>
</tr>
<tr>
<td>Urban</td>
<td>124 (37.9)</td>
<td>3.28 (1.82-5.9)</td>
<td>1.86 (0.74-4.6)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>117 (21.3)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unmarried</td>
<td>101 (18.8)</td>
<td>0.85 (0.43-1.66)</td>
<td>0.788 (0.31-2.0)</td>
</tr>
<tr>
<td>Divorced</td>
<td>20 (65)</td>
<td>6.83 (2.46-18.9)</td>
<td>5.15 (1.48-17.9)</td>
</tr>
<tr>
<td>Widowed</td>
<td>20 (55)</td>
<td>4.5 (1.68-12.05)</td>
<td>4.47 (1.26-15.8)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government employee</td>
<td>20 (50)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Merchant</td>
<td>19 (36.8)</td>
<td>0.58 (0.16-2.1)</td>
<td>0.4 (0.094-1.68)</td>
</tr>
<tr>
<td>Student</td>
<td>48 (12.5)</td>
<td>0.14 (0.04-0.48)</td>
<td>0.26 (0.06-1.11)</td>
</tr>
<tr>
<td>Farmer</td>
<td>95 (14.7)</td>
<td>0.17 (0.06-0.49)</td>
<td>0.17 (0.04-0.69)</td>
</tr>
<tr>
<td>No job</td>
<td>25 (44)</td>
<td>0.78 (0.24-2.55)</td>
<td>0.4 (0.09-1.66)</td>
</tr>
<tr>
<td>Other *</td>
<td>51 (39.2)</td>
<td>0.64 (0.22-1.82)</td>
<td>0.4 (0.11-1.380)</td>
</tr>
</tbody>
</table>

*Other occupations include urban housewife, daily laborers, private employees, beggars and household servants. TB = tuberculosis; OR = odds ratio; CI = confidence interval; PTB+ = pulmonary TB bacteriologically confirmed by smear microscopy; PTB– = pulmonary TB smear negative; EPTB = extra pulmonary TB.
Analysis of HIV status by clinical TB type showed that HIV seroprevalence was lower in smear positive pulmonary TB patients (18.5%) compared to those diagnosed as smear negative pulmonary TB (28.6%), and extra pulmonary TB (28.3%) patients. The difference is not statistically significant (P>0.05) (Table 1).

Both male and female divorced TB patients had high HIV prevalence, which is 55.6% and 72.7% respectively. Widowed females also had high prevalence of HIV infection (57.9%). These findings showed that marital status of the patients had significant effect on the observed HIV infection (P= 0.00) (Table 5.1) and the HIV infection among male and female patients vary significantly by marital status (P< 0.05) but not by age group (P>0.05)(Table-5.3).
Table 5.3. HIV sero-prevalence of different sex by age group and marital status in tuberculosis patients of Adama hospital, Adama.

<table>
<thead>
<tr>
<th>Age (years) groups and socio demographic characteristics</th>
<th>Male</th>
<th>Female</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total tested</td>
<td>HIV positive No (%)</td>
<td>Total tested</td>
</tr>
<tr>
<td>18-29</td>
<td>75</td>
<td>10 (13.3)</td>
<td>71</td>
</tr>
<tr>
<td>30-39</td>
<td>26</td>
<td>14 (53.8)</td>
<td>32</td>
</tr>
<tr>
<td>40-49</td>
<td>14</td>
<td>5 (35.7)</td>
<td>13</td>
</tr>
<tr>
<td>≥50</td>
<td>18</td>
<td>3 (16.7)</td>
<td>9</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>62</td>
<td>15 (24.2)</td>
<td>55</td>
</tr>
<tr>
<td>Unmarried</td>
<td>61</td>
<td>12 (19.9)</td>
<td>40</td>
</tr>
<tr>
<td>Divorced</td>
<td>9</td>
<td>5 (55.6)</td>
<td>11</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td>0 (0)</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>32 (24.1)</td>
<td>125</td>
</tr>
</tbody>
</table>

5.3. CD4+ T cell count result

The median CD4+ T cell count of HIV negative and HIV positive TB patients included in the study was 702 cells/mm³ (range 95-1760) and 233 cells/mm³ (range 36-773) respectively. The difference was statistically significant (p<0.05)(Fig 5.2).The median CD4+ T cell count for PTB smear positive, PTB smear negative and EPTB was 294 (range 133-409), 129 (range 58-751) and 295 (range 36-773) cells/mm³, respectively for HIV positives. Smear negative PTB patients had significantly lower median count than PTB+ and EPTB patients did (P<0.05) (Table 5.5). HIV infected TB patients had
significantly lower median CD4$^+$ T cell counts, high CD8$^+$ T cell counts and markedly inverted CD4+/CD8+ ratios compared to their HIV negative counterparts irrespective of TB types and gender (Table 5.4).

Female patients had comparatively higher median CD4$^+$ T cell counts than their male counterparts. The difference was not statistically significant in HIV infected patients (P>0.05) but marginally significant (P=0.06) in HIV non-infected patients (Table not shown).

Among the study subjects, there were ten patients who already knew their HIV status when they visit the TB clinic. These patients develop TB while waiting to start ART (pre-ART) and come to TB clinic after TB diagnosis. The median CD4$^+$ T cell count of these patients was 295 cells/mm$^3$ with comparatively narrow range (range 134 - 472 cells/mm$^3$). The median age of the Pre ART TB patients was 25 years, which was much younger than the total HIV positive subjects (32.5 years).

Table 5.4. Median CD4$^+$, CD8$^+$ T cell counts /mm$^3$ blood and CD4+/CD8+ ratio of newly diagnosed TB patients in Adama hospital, Adama, by clinical TB type, gender and HIV status

<table>
<thead>
<tr>
<th>TB Type</th>
<th>Sex</th>
<th>HIV positive</th>
<th>HIV Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of patients</td>
<td>CD4$^+$ cell</td>
<td>CD8$^+$ cell</td>
</tr>
<tr>
<td>PTB+</td>
<td>Male</td>
<td>4</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>6</td>
<td>249</td>
</tr>
<tr>
<td>PTB-</td>
<td>Male</td>
<td>14</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>16</td>
<td>133</td>
</tr>
<tr>
<td>EPTB</td>
<td>Male</td>
<td>14</td>
<td>259</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>14</td>
<td>318</td>
</tr>
<tr>
<td>All TB Types</td>
<td>Both sexes</td>
<td>68</td>
<td>233</td>
</tr>
</tbody>
</table>
As depicted in the figure 5.2, HIV-TB co infected patients had a significantly lower CD4+ T cell counts (P<0.0001) compared to HIV non-infected TB patients. Moreover, of major concern, as can be seen in the figure (Figure 5.2), large proportions of the study subjects of whom the majority did not know their HIV status before this study (85%) had CD4+ T cell counts below 200 (close to 50%), a count, which is considered as a criterion for eligibility to start Anti Retroviral Therapy (ART). In addition, one patient had CD4+ T cell count <50 cells/mm³ and only 3 patients (5%) had CD4+ counts >500 cells/mm³. The data indicated that these patients are already in an advanced stage of HIV disease when they visit the TB clinic. Among the HIV non-infected TB patients, only 1.6% (n=3) had CD4+ T cell count of <200, none had < 50 cells/mm³ while the majority (75%) had CD4>500 cells/mm³. On the other hand, the CD4+ T cell count of the Pre-ART patients was relatively higher at the time of new TB diagnosis compared to those who did not knew their HIV status at the same visit, underscoring the significance of knowing HIV status for early seeking of medical care, early diagnosis of OIs and better survival in HIV infected patients.

Figure 5.2. CD4+ T cell count of HIV positive (filled diamonds; n=68) and HIV negative (open diamonds; n=190) newly diagnosed TB patients in Adama Hospital, Adama.

*Each point represents CD4+ counts of individual patients and horizontal bar lines indicate median values.
Tables 5.5 show median CD4+ T cells/ mm³ and Inter quartile ranges [25th and 75th IQR] of active TB patients in Adama Hospital by clinical TB type and HIV status. Regardless of the TB type, HIV negative patients develop TB at higher CD4+ T cell counts compared to HIV co-infected patients. For example, about 50% of the patients had CD4+ T cell count above 500 cells/ mm³ and below 900 cells/mm³ when they were first diagnosed for TB [IQR 500-900 cells/ mm³ for all HIV negatives]. The IQR was lower for PTB+ HIV negative patients [464-828 cells/ mm³] compared to the other TB types; the difference was statistically significant (P<0.01) compared to HIV negative EPTB patients [IQR 555-940 cells/ mm³]. In HIV co-infected subjects, smear negative pulmonary TB patients (PTB-) had significantly lower median CD4+ counts compared to both smear positive TB patients (PTB+) and EPTB patients (P values<0.05 for both) (Figure 5.3). Besides, 63.3% of smear negative TB patients, 35.7% of EPTB patients and 20% of PTB+ patients had CD4+ T cell counts of less than 200.

Table 5.5. Median CD4+ cells/ mm³ and Inter quartile ranges [25th and 75th IQR] of active TB patients in Adama Hospital, Adama, by clinical TB type and HIV status

<table>
<thead>
<tr>
<th>HIV status</th>
<th>TB Types</th>
<th>PTB+</th>
<th>PTB-</th>
<th>EPTB</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(HIV-)</td>
<td></td>
<td>596</td>
<td>664</td>
<td>785</td>
<td>702</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[464-828]</td>
<td>[500-900]</td>
<td>[555-940]</td>
<td>[500-900]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=44</td>
<td>n=75</td>
<td>n=71</td>
<td>n=190</td>
</tr>
<tr>
<td>HIV positive</td>
<td></td>
<td>294</td>
<td>129</td>
<td>295</td>
<td>233</td>
</tr>
<tr>
<td>(HIV+)</td>
<td></td>
<td>[215-330]</td>
<td>[95-276]</td>
<td>[129-444]</td>
<td>[112-328]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=10</td>
<td>n=30</td>
<td>n=28</td>
<td>n=68</td>
</tr>
</tbody>
</table>

*significantly higher compared to PTB+ HIV negative patients,  **P<0.01, Wilcoxon rank test

*significantly lower compared to both PTB+ and EPTB HIV positive patients,  **P<0.05, Wilcoxon rank test

*significant difference compared with HIV negative TB patients (all TB types),  ***P<0.0001, Wilcoxon rank test
Figure 5.3. CD4$^+$ T cell counts by clinical TB types in HIV co-infected active tuberculosis patients in Adama Hospital, Adama.

Note: PTB+ (filled diamonds); PTB- (filled circles), EPTB (open diamonds). Each point represents CD4$^+$ T cell counts of individual patients and horizontal bar lines indicate median values.
6. DISCUSSION

The published reports about sero prevalence of HIV among tuberculosis patients are highly variable worldwide. The recent WHO estimate of Ethiopian adult TB cases who are HIV positive was 21% (WHO, 2006c). The 26.4% prevalence of HIV observed among newly diagnosed adult tuberculosis patients in the present study was slightly higher than the national estimate by WHO, but within the range of previous reports from Ethiopia, which ranged from 6.6% to 45% (Kefene et al., 1990; Mitike et al., 1997; Gellete et al., 1997; Demisse et al., 2000). However, the prevalence rate reported herein is much lower than a recent report of 52.1% HIV prevalence among TB patients from the northern part of Ethiopia (Kassu et al., 2007) and higher than a previous report of 19% from the southern part of Ethiopia (Yassin et al., 2004).

Taken together, the HIV-TB co infection rate reports from different parts of Ethiopia showed variations as in the other continents and countries. In Africa, a cross-sectional study of adult tuberculosis patients admitted into the DOTS program of one of the Nigeria’s university teaching hospital showed HIV seroprevalence of 14.9% (Daniel et al., 2004) while an earlier report from Zimbabwe showed a co infection rate of 65% (Schoch and Rieder, 1996). Likewise, in India wide variations in HIV seroprevalence among tuberculosis patients have been observed. For example, a study done among newly diagnosed untreated TB patients in Delhi showed 0.68% positivity for HIV antibodies (Jain et al., 2000) while another study done in 2005 in newly screened Indian TB patients, demonstrated an overall HIV prevalence of 15.5 % (Prasanthi and Kumari, 2005). Moreover, in a study done in Ukraine, Kiev City in 2002, the prevalence of HIV infection among TB patients was 6.3% (Van der Werf et al., 2005) and 2 years later an increased prevalence of 10.1% was reported from the same city (Van der Werf et al., 2006).

In the present study, 53% (36/68) of HIV positive TB patients were females and 47% (32/68) were males. This gross difference between genders coincides with the Ethiopian national HIV report, which shows high percentage of female PLWHA (55 %) compared to males (45%) (MOH, 2005a). In contrast to these observations and reports, a study by
Demisse et al (2000), showed a high rate of HIV seropositivity in males (61.7%) compared to females (38.3%) out of the HIV positive PTB⁺ patients.

According to one study done in southern region of Ethiopia by Yassin et al (2004), the HIV prevalence was 18% for female and 21% for male TB patients, which was 28.8% and 24.1%, respectively in our study. Although the prevalence of HIV in males was relatively coinciding in both studies, the prevalence was higher in females in this study.

In the present study, the degree of HIV infection vary significantly with age and the highest number of HIV cases occurred in the age group 30-39 years (OR 3.69, 95% CI 1.58-8.58, P<0.05) followed by 40-49 years. The significant association between age and HIV infection was also observed in a study done in Addis Ababa by Demissie et al (2000). Similarly these findings agree with a study from Ukraine (Van der Werf et al., 2006) which showed a high frequency of HIV infection in patients with age <50 years and with the Netherlands ( Haar et al., 2006) and Indian studies ( Jain et al., 2000) which found high peak of HIV infection in TB patients in their 4th decade. However, others have reported a high HIV prevalence in relatively lower age groups. For example, one African study showed peak HIV prevalence in TB patients in the 3rd decade accounting for 42.5% of cases, followed by 32.5% in the 2nd decade (Daniel et al., 2004).

The finding of high rate of HIV positivity in females compared to males in the younger age groups (18-29 years) and remarkably more males than females in the age group 30-39 years reflect the general notion that females are sexually active at younger ages than males(Table 5.3). In addition, as expected HIV prevalence significantly varies by residential area with higher rates in urban (39.9%) compared to rural (15.7%) residents (p<0.05). Nevertheless, the strength of the association was not found in the regression analysis (Table 5.2). Yassin et al (2004) also observed differences by residential location with a prevalence rate of 15% and 30% for rural and urban TB patients, respectively in consistent with our study.
Compared to married TB patients, divorced and widowed TB patients showed high HIV prevalence in our study and the variation was significant (P<0.05). The prevalence of HIV was 65% in divorced and 55% in widowed new TB patients. The odds of HIV infection for divorced and widowed patient was about 5 and 4 times higher than the odds of married patients (OR = 5.1, 95% CI 1.48-17.9) and (4.4, 95% CI 1.26-15.8), respectively, showing that being divorced and widowed are the strongest independent predictors for HIV infection in TB patients (Table 5.2). The married males and females showed greater HIV prevalence than their unmarried counterparts (Table 5.3). Contrary to our finding, in Jain et al (2000) study, seroprevalence of HIV was higher among unmarried males and those living singly than male tuberculosis patients living with their spouses.

HIV prevalence of 18.5%, 28.6% and 28.3% was observed in our study for PTB+, PTB- and EPTB patients respectively. Demisse et al (2000) in their cross sectional study done in 1998, in Addis Ababa, on smear positive TB patients found 45.3% HIV positivity, which is much higher than the present study. They further stated that the percentage of HIV positive samples of patients from different health centers included in their study vary greatly showing the heterogeneity of HIV prevalence in the same city (Demisse et al., 2000). Likewise, the 18.5% HIV positivity of PTB+ patients reported in our study was lower than the seropositivity rate of 26.9% recently reported in PTB+ patients by Kassu et al (2007) from the northern part of Ethiopia but consistent with the findings of Yassin et al, who demonstrated 19% in PTB+ patients from the south (Yassin et al., 2004). Moreover, the prevalence of HIV in smear negative TB patients also agreed between the present study (28.6%) and the study from the southern Ethiopia (26%) (Yassin et al., 2004) and India (Prasanthi and Kumari, 2005), but not with the study from the north (38.8%)(Kassu et al., 2007). However, the 28% HIV prevalence in EPTB patients in our study was much higher than the 11% HIV positivity reported by Yassin et al (2004) but lower than the report by Kassu et al (2007) who showed 34.4% HIV seropositivity in EPTB patients.
The HIV prevalence observed in our study for all PTB patients (PTB+ plus PTB+) which was 25% (40/159) was much lower than the reported 57.1% seropositivity for pulmonary TB patients from Addis Ababa (Burchfeld et al., 2002).

In contrast to our result, Jain et al. (2000) from India, found low Prevalence of HIV infection in PTB- patients (0.71%) than PTB+ (1.86%) and EPTB (1.52%) patients. In our case, this condition can be explained by immunocompromised patients usually develop EPTB and smear negative pulmonary tuberculosis. Several published studies have shown that smear negative TB is about twice as common in HIV infected people, with rates of around 50% in some reports. Likewise, extra pulmonary disease is also much more common. As CD4+ T cell counts decline, both extra pulmonary TB and smear negative TB become more frequent (Saranchuk, 2005).

Taken together, the reported prevalence of HIV in the different clinical TB type category varies between studies from the different regions of Ethiopia (Demisse et al., 2000; Kassu et al., 2007; Yassin et al., 2004; Burchfield et al., 2002) and studies done by Jain et al. (2000). A possible explanation could be the proportion of co-infected patients often correlates with the prevalence of HIV in a population during the study. Hence, the prevalence of HIV in the population of Addis Ababa during Demisse et al and Bruchfeld et al study (1998 and 2002, respectively) was much higher than the prevalence of HIV in Adama town by now (MOH, 2005a).

It was also observed that sputum positivity was less in HIV positive patients than in patients negative for HIV (Prasanthi and Kumari, 2005). Attiti et al. (2005) reported that sputum positivity in HIV patients ranged from 15.4% to 85% depending on the immune status of the patients. Chances of AFB isolation were high in patients with mild immunosuppression (CD4>500 cells/mm³) compared with the advanced disease (CD4<200cells/mm³). The overall rate of PTB+ in the present study was 20.9% of all forms of TB, which was lower than the 27% reported by Kassu et al. (2007), 34.1% by W/michael et al. (2004), the national figure of 54% (WHO, 2003) and more than 3 times lower than the global target of 70% (WHO, 2000). On the contrary, PTB- cases were
slightly higher (40.7%) than that reported from Gondar (37%) (Kassu et al., 2007) and much higher than a 24.2% prevalence report from southwest Ethiopia (W/michael et al., 2004) but close to the 42.6% report from the northern part of Ethiopia (Mesfin et al., 2005a). Whereas EPTB cases accounted 38.4% of all TB forms in the present study, a figure, which is close to the 36% report from Gondar (Kassu et al., 2007) and another study from north (39.7%), but lower than the report from south west (41.7%) (W/michael et al., 2004; Mesfin et al., 2005a). It has been suggested that the high number of PTB- and EPTB cases could be partly due to the referral system adopted in the country, where health centers are allowed to treat only PTB+ patients and must refer other suspected TB patients to hospitals (Kassu et al., 2007; Mesfin, 2005). The situation in Adama is not an exception to this general practice in the country. On the other hand, non-adherence to diagnostic algorithm, poor quality of sputum processing and microscopy, lack of culture facility as well as high HIV co infection might contribute to such high sputum smear-negative rates (Kassu et al., 2007; Burchfeld et al., 2002; Lambert et al., 2003; Mesfin et al., 2005b).

A study done by Bruchfeld et al (2002) have shown that diagnosis of PTB based on clinical symptoms, sputum microscopy for acid-fast bacilli and chest radiography was sensitive (86.7%) but unspecific (64.1%). On the other hand, the HIV pandemic poses a serious diagnostic and therapeutic challenge as a co infection in high-burden countries. As a result, in HIV-positive patients, both sensitivity and specificity were lower and HIV-related pulmonary infections are often misinterpreted as smear-negative PTB (Burchfeld et al., 2002).

A very crucial observation, especially which has an implication on the ongoing HIV prevention and control efforts in the country, is that only 14.7% of the study subjects found HIV positive knew their HIV status prior to this study and the vast majority (85.3%) learned their HIV status through this study. In this regard, one of the striking findings in this study was almost half of the newly diagnosed TB patients co infected with HIV, but who were also unaware of their status before this study, had CD4+ T cells counts of less than 200 cells/mm³, a count which could have made these patients eligible to start ART. These patients were immediately referred to Adama hospital ART clinic for
further management after a post test counseling. The finding of such a high proportion of
individuals with an advanced stage of HIV disease as evidenced by their low CD4+ count
despite all the efforts of advocating for voluntary HIV counseling and testing and above
all the availability of free ART, calls for a more strengthened effort to initiate people
know their HIV status voluntarily. Interestingly, those patients who knew their HIV
status before the study were younger than all positive TB patients (median age of 25
versus 32.5 years, respectively), showing that the younger ones tend to know their HIV
status than the older people.

Concerning the immune status of TB patients, the overall median CD4+ T cell counts for
all HIV positive new TB patients in this study was 233 cells/mm³ and HIV negative
patients had a median count of 702 cells/mm³. The opportunistic TB infection occurs
when the immune status of an individual drops below normal. One literature stated that
TB opportunistic infection (OI) usually occur when the CD4+ cell count is between 200-500 cells/mm³. According to this literature, the common presentation of extra pulmonary
TB and atypical pulmonary TB is at CD4+ T cell count less than 100 cells/mm³ (Bartlett,
2004). In our study, however, the median count for EPTB patients was higher (295
cells/mm³), a finding which needs further study, and the lowest count was observed in
PTB smear negative (129 cells/mm³) patients. Besides, large proportion of HIV positive
smear negative TB patients (63.3%) had CD4+ T cell count of less than 200 compared to
35.7% of EPTB patients and 20% of PTB+ patients. The finding of large proportion of
smear negative TB patients with advanced stage of HIV disease agrees with our
expectation, since HIV infected patients with advanced immunosuppression are unable to
form cavities in their lungs and expectorate fewer bacilli in sputum (MOH, 2005b)

According to Badri et al (2002) most of the HIV-TB co-infected patients (67%) had CD4+
T cell level of more than 200 cells/mm³ and it was about 54% in our case. Although there
is a possibility that clinical TB can occur at any CD4+ level, the present study showed that
HIV positive individuals develop the disease at a significantly lower median CD4+ count
than their HIV negative counter parts for all types of clinical TB.
In areas where TB is endemic, certain patients have higher CD4\(^+\) T lymphocyte counts at the time HIV related TB disease develops and in countries with low rates of TB disease (e.g., United States and countries in Western Europe), more patients have advanced HIV disease at the time TB develops (Benson et al., 2004). For resource-limited settings other than Africa, the average CD4\(^+\) T cell counts at which TB is detected was 125 cell/mm\(^3\). This is statistically significant when compared to the average CD4\(^+\) T cell counts in Africa (200 cells/ mm\(^3\)) showing that even among the TB prevalent countries, TB occurs at a relatively higher CD4\(^+\) T cell level in HIV patients of Africa (Scano et al 2005).

A cohort study done in Ethiopia by Wolday et al (2003) have demonstrated that among the 10 HIV positive study participants who subsequently develop TB, the CD4\(^+\) T cell count was low with a median of 201 cells/ mm\(^3\) and range of 45-419 cells/ mm\(^3\). For the ten pre-ART patients who develop TB before starting ART in our study, the median CD4\(^+\) T cell count was 295 cells/mm\(^3\) with comparatively narrow range (range 134-472 cells/mm\(^3\)) than the overall median CD4\(^+\) T cell count for all HIV positive new TB patients in the study (median 233 cells/ mm\(^3\)). Here different explanations can be given based on knowledge of HIV status by study subjects prior to the respective studies. That is, those who already learned their HIV status prior to the studies and seek health services for any illness like our Pre-ART patients and the long term cohort study participants of Wolday et al (2003 ) versus those active TB patients who learned their HIV status during this study. Our median CD4\(^+\) T cell count for the pre-ART patients (295 cells/ mm\(^3\)) was somewhat higher than the median CD4\(^+\) T-cell count of 201 cells/mm\(^3\) of the above cohort study, which may be attributable to the time of sample collection and the methodologies used to count the CD4\(^+\) T cells. The FACSCount (a single platform technology) was used in the present study whereas Wolday et al (2003) used the dual platform FACScan. In case of FACS count absolute CD4\(^+\) T cell count was directly measured by the machine but in case of FACScan the absolute CD4\(^+\) T cell count was calculated from lymphocyte count produced by other machine or the same machine. Concerning the sample collection time, in our case sample was collected between 2 pm and 5 pm, after the patients completed their visit at the out patient department (OPD) of the hospital and referred to the TB clinic. It was known since early times that the CD4\(^+\) T cell count is high in the afternoon than morning even in
the same individual (Malone et al., 1990). Malone et al (1990) reported that the diurnal variation ranges between an average CD4+ T cell count of 506 cells/mm³ for HIV negative people and an average variation of only about 60 cells/mm³ each day for HIV positive people. They further stated that generally people with lower baseline CD4+ T-cell counts had much less diurnal variation compared to people with high baseline counts.

The low CD4+ T-cell count for the total HIV TB co-infected patients than the Pre-ART patients in our study could be explained by prolonged diagnosis delay of the patients and therefore they came for treatment after worsening of their condition. Yimer et al (2005) in their study done to determine the length of delay between onset of symptoms of PTB and commencement of treatment in Amhara region, Ethiopia, found a median total delay of more than 30 days while another study from Tigray region, north Ethiopia have reported a median delay between 60 to 90 days (Mesfin, 2005). The studies noted that both health facility and patients contributed to the delay (Mesfin, 2005; Yimer et al., 2005). Such findings can also be true for other regions in the country including Adama hospital. The prolonged delay can explain that patients might have been referred to TB clinics for treatment after they become weak and immunologically deteriorated. The immunological deterioration observed in our study subjects when they were newly diagnosed for TB might have been reversed if the subjects were aware of their HIV status earlier and were managed properly. Nevertheless, knowing their HIV status and having pre- and posttest counseling, the pre-ART patients most likely seek for treatments as soon as possible.

In HIV patients, Primary prophylaxis is indicated for patients with a positive tuberculin skin test (induration of more than 5 mm) who have never been treated for TB, and for patients with recent exposure to someone with active TB (Gerard, 2000). Nine-month regimen was a consensus recommendation, since a 6-month regimen was less effective than longer regimens and regimens lasting more than 12 months did not appear to provide additional benefit (Kovacs and Masur, 2000).

Preventive therapy is only of proven benefit to HIV-infected people with a positive result on a tuberculin skin test. However, performing tuberculin tests in low-income countries is not easy; staff needs special training, tuberculin is not always available, individuals must
return to have their tests read at 48 hr (Grant et al., 2001). Tuberculin skin test also has limited value for the diagnosis of TB infection especially in developing countries because of the difficulty in excluding active TB disease in those with HIV TB co-infection in these countries and false negativity of the test due to cutaneous anergy as the number of CD4$^+$ T cell count decreases (Johanson and Ellner, 2006). TST is positive in the majority of patients with pulmonary disease or infection and CD4$^+$ T lymphocyte counts $>$200 cells/mm$^3$ (American thoracic society, 2000). In HIV infected patients with CD4$^+$ T cell counts of less than 200/mm$^3$, the tuberculin skin test is usually non-reactive. On the other hand, false positive results may be found in patients who were BCG-vaccinated or who had contact to non-tuberculous mycobacteria (Fisk et al., 2003).

WHO and the Joint United Nations Programme on HIV/AIDS (UNAIDS) recommend that if tuberculin skin testing is not feasible, HIV-infected individuals may be considered for preventive therapy if the prevalence of TB infection in the population is more than 30% (Grant et al., 2001). Another interesting option according to one literature is to administer TB chemoprophylaxis to HIV/AIDS patients with CD4$^+$ T cell counts below 100 cells/mm$^3$ (Palmero, 2007). However, according to the findings of the present study this seems very low count to consider preventive therapy since almost 80% of new TB cases in HIV positives occur above this count.

7. CONCLUSION AND RECOMMENDATIONS

Measuring HIV prevalence among tuberculosis patients is increasingly being recognized as important, as the HIV epidemic continues to fuel the global TB epidemic. The HIV prevalence in TB patients is a sensitive indicator of the spread of HIV into the general population. This study has demonstrated a frequency of 26.4% sero-prevalence among new adult TB patients. It suggests that prevalence of HIV among tuberculosis patients in Adama town and the surrounding village is lower than that reported from Addis Ababa, the capital of the country, and Gondar but almost similar with the report from the south region of the country.
High prevalence of HIV infection was observed in divorced and widowed patients, age group 30-49 years and urban residents. The HIV prevalence is also high in PTB smear negative patients than the other types of clinical TB diagnosed at this hospital. The Acid fast bacilli (smear) positivity rate observed in the TB patients included in present study (20.9%) was lower than the national data of 54% and more than 3 times lower than the global target of 70%.

The median CD4⁺ T cell count at which clinical TB disease occur in HIV positives and pre ART HIV patients were 233 cell/mm³ and 295 cell/mm³, respectively. About 65% (44/68) HIV positive TB patients had CD4⁺ T cell count of less than median count for pre-ART TB patients. If supplemented by further studies, this later median CD4⁺ T-cell count limit (295 cell/mm³) below which most HIV patients develop TB, might be considered in the initiation of TB Prophylaxis in PLWHA, In addition to the currently used criteria, that is exclusion of active TB only according to the manual of the Federal Ministry of Health (WHO, 2006c). In spite of giving prophylaxis for all HIV infected people, it is better to exclude HIV infected peoples with low risk of developing TB from prophylaxis considering the complications they may cause (e.g. peripheral neuropathy, drug interactions, drug-induced hepatitis). In case of INH prophylaxis, severe drug-induced hepatitis occurs in about 1 in 1,000 patients and can be fatal (Means Markwell and O’Neil, 2000).

Generally, this study will give the concerned bodies the understanding of the epidemiological relationship between HIV and TB diseases at the community level and the CD4⁺ T cell count levels around which different clinical tuberculosis occur in HIV patients in Adama town and the surrounding villages. Another very important point is that as discussed above only few new tuberculosis patients knew their HIV status before coming to TB clinic and many of the HIV positives had CD4⁺ T cell count below 200 cells/mm³ (eligible to start ART). As knowledge of the HIV sero status of TB patients may also influence the treatment of their tuberculosis and their further management, all patients with newly diagnosed TB should be strongly encouraged to undergo HIV testing. Therefore, the
provider initiative HIV counseling and Testing (PIHCT) recently started in health institutions, especially in TB clinics should have to be appreciated and strengthened.

8. LIMITATIONS OF THE STUDY

The major limitation of this study was the time of blood specimen collection for CD4$^+$ T-cell counting. Sample collection was performed in the afternoon while it is known that CD4$^+$ T-cell counting is affected by diurnal variation. However, it was not ethically sound to return patients home after they were diagnosed for active TB at the OPD and referred to the TB clinic to start treatment in the afternoon. Requesting patients to come the next morning for the sake of this study might further delay the time these patients start TB treatment or some patients may even be missed totally. Moreover, if any diurnal variation affects the current result, the impact would be underestimation of the proportion of patients with more advanced condition (i.e., those with CD4<200 cells/mm$^3$).
### A. Data collection format by which information are collected in TB clinic

Date_________________________

#### I. Identification

1. 1 Serial Number____________________________________________________
2. 2 Code ____________________________________________________________

#### II. Background information (circle one for those which have options)

2.1. Age (write in years) ________________________________________________
2.2. Sex  a) Male           B) Female
2.3. Marital Status. A. Married. B. Unmarried    C. Divorced     D. widowed
2.4. Residence       A) Rural          B) Urban
                      D) Farmer E) No job. F) Others_______________________
2.6. Duration in that occupation stated on 2.5 ____________________________
     (Years)

2.7. Educational status
     A. Illiterate.    B. Primary school.  C. Middle school   D. High school
     E. Tertiary level

#### III. General medical history (circle the appropriate case and write where necessary)

                          C) Extra-pulmonary (if possible write type of TB) ________.
2. ART status (for those who know their sero status)
                   A) ART started. B) ART not started (pre ART) e) not applicable

#### IV. Willingness to participate in the study.

     A. willing to participate. B. Not willing to participate

* If “B” Reason for not participating _________________________________.
B. Laboratory result reporting format

1. HIV test result (Rapid tests) Test 1____ Test 2_______ Test 3______
   
   Finally reported result________________

2. CD4$^+$ T cell count /mm3__________ CD8$^+$ T cell/mm3____________
   
   CD4$^+$/CD8$^+$Ratio_________________________.
C. consent form

The objective of this study is to know the prevalence of HIV in newly diagnosed TB patients and to see the level of CD4\(^+\) T-cell count at which opportunistic active tuberculosis develops. HIV prevalence in TB patients is a sensitive indicator of the spread of HIV into the general population. It gives the concerned body the detailed understanding of the epidemiological relationship between HIV and TB diseases at the community level. The information on HIV levels in TB patients is essential to respond to the increasing commitment to provide comprehensive HIV/AIDS care and support including antiretroviral therapy to HIV-positive TB patients. This study specifically helps the participants to know their sero status and immune status at this comparatively early stage (first TB clinic visit) and take care of themselves according to the counseling given to them to make conditions less severe.

Therefore, here we request your kind participation in this study, which requires your willingness to give blood samples for laboratory examination and to respond to an interview. You have full right and free choice to either participate or not in this study and it will never affect your right of getting appropriate treatment in Adama hospital or elsewhere. Results will be confidential and reported only to the counselor and if necessary to ART clinic depending up on your interest for appropriate treatment and management.

I ____________________________, after being fully informed about the purpose of this study, hereby give my consent to participate in this study as the counselors find best for me.

Signature___________________ Date____________________
D. HIV testing procedures

1. Determine HIV 1/2(T1) Procedure
   1. Remove the protective foil cover from each test and label it with client code
   2. Apply 50 \( \mu l \) of sample (whole blood) to the sample pad.
   3. Wait until blood is absorbed in the sample pad, then apply one drop of chase buffer to the sample pad.
   4. After waiting a minimum of 15min (up to 60 min) read the result.
      Interpretation:
      - Reactive: red bars appear in both control and patient window
      - Non-reactive: one red bar appears in the control window of the strip.
      - Invalid: Absence of red bar in the control window

2. Capillus (T2) Procedure
   1. Reagents and client sample are allowed to reach room temperature (if stored in refrigerator) before use.
   2. Label patient sample identification number on slide.
   3. Place slide on black interpretation station (including both Positive and Negative internal controls).
   4. Mix the latex reagent well by gently agitating the bottle to ensure that the Latex suspension is homogeneous.
   5. Draw the latex to the black calibration mark of the dropper and then drop the Latex on the slide at the edge of mixing well.
   6. With pre calibrated pipette add 10\( \mu l \) of sample or control to the latex dropped on slide, then mix and move the well mixed sample and latex solution to the opening of channel until the capillary flow begins using a sample pipette tips.
   7. Allow the latex mixture to flow through the entire capillary channel into the viewing box before interpreting. The result takes approximately 3-7min.
      Interpretation:
      - Reactive –any sample showing aggregation
      - Non reactive-sample showing no aggregation

3. Uni-gold (T3) Test procedure
1. If reagents/samples have been stored in refrigerator, remove and allow it to stand for 20 minutes to reach room temperature.
2. Remove the device from their protective wrappers.
3. Label each test device appropriately.
4. Add two drops of sample (approximately 60 μl) at the sample port carefully.
5. Add two drops (approx. 60 μl) of the wash reagent to sample port.
6. The result should be read immediately after the end of 10 minutes incubation time N.B. Do not read result after 20 minutes following sample addition.

Interpretation: Reactive: pink line of any intensity forming in the test region, plus a line forming in the control region.
Non-reactive: A pink line in control region only.
Inconclusive: no line appears in the control region.
E. CD4⁺ T cell count determination

(The sample will not be stored longer than 48 hrs at room temperature.)

Test Principle

The CD4⁺ T-cell count using the FACSCount uses the principle of flow cytometry. The basic mechanics of this method consists of injecting cells in suspension through nozzles into a flowing sheath fluid, which focuses the cells into the center of the stream. The cells are then passed single file through a focused light beam usually generated from a laser or a mercury arc lamp. Each cell traversing the beam scatters light and will generate an emitted signal when a fluorescent reagent is tagged with the cell. The various optical signals scattered light and fluorescence are then collected through appropriately arranged filters and photo detectors. The flow cytometry utilizes a combination of two light scatter parameters (forward and side scatter) together with two fluorescence signals (fluorescein and phycoerythrin (PE)). Light scatter signals provide information about size as well as cytoplasmic and nuclear characteristics. The fluorescence signals are usually generated from reagents directed at specific cell surface markers and can be used to characterize cell subsets. A variety of peripheral blood mononuclear cell are identified with monoclonal antibodies that react with cell surface antigens or markers.

Preparing patient samples

1. Label reagent pair tube with the patient’s accession number.

2. Mix the pair upside down for 5 seconds and then upright for 5 seconds.

3. Open the reagent tube with the coring station.

4. Mix the patients whole blood by inverting the tube five times.

5. Reverse pipette 50 µl of patient’s whole blood to each tube.

6. Cap the tubes and mix upright for 5 seconds.
7. Incubate for 60 to 120 minutes at room temperature in the dark.

8. After uncapping the tubes, reverse pipette 50 μl of fixative solution into each tube.

9. Recapping the tubes, mixing (vortex) upright for 5 seconds and then incubation for at least 30 minutes at room temperature in the dark will follow.

10. Finally run the test on FACSCount instrument with in 24 hrs of preparation.

Entering patient and reagent information

1. Press (sample) from the FACS Count screen or the CONTROL results screen.

2. Enter or verify the reagent lot code and bead count.

3. Press (confirm).

4. Enter the patient accession number.

Running patient’s sample

1. After the end of recommended incubation period, mix the reagent pair (vortex) upright for five seconds. The CD4+ tube will be uncapped and reagent pair is placed in the sample holder so the CD4+ tube is in the run position.

2. Press (RUN).

3. The reagent pair removed and CD4 tube recapped.

4. Uncapping the CD8 tube and placing the reagent pair in the sample holder will follow so the CD8 tube is in the run position.

5. Press (RUN).

6. Remove the reagent pair and discarded in an appropriate biohazard container.
Preparing and running control. It follows the same procedure as for the sample except that we use normal blood sample and add the control beads at end of procedure and read. First two reagent pairs will be labeled CD4-zero, CD8-low, CD4-midium, CD8-high and processed as for the sample. Then at end of the process the following was done.

1. The zero/low control beads pair was mixed and 50μl of zero control beads added in to the CD4 reagent tube labeled zero.

2. Reverse pipette 50μl of low control beads in to the CD8 reagent tube labeled low.

3. Vortex the medium/high control beads pair and reverse pipette 50μl of medium control beads in to the CD4 reagent tube labeled medium.

4. Reverse pipette 50μl of high control beads in to the CD8 reagent tube labeled high and then run on FACSCCount instrument with in 2 hrs of adding the control beads.

Entering control and reagent information

The following procedures should be done sequentially. 1. Pressing “control” from the FACSCount screen. 2. Entering the eight–digit control bead lot code. 3. Entering the bead counts for low, medium, and high controls. 4. Pressing “confirm”. 5. Entering the eight-digit reagent lot code. 6. Entering the CD4 and CD8 reference bead counts for the reagent lot. 7. Pressing “confirm”. 8. Enter the normal control.

Running controls

1. Vortex the first reagent pair (CD4-zero and CD8-low) upright for five seconds

2. Uncap the CD4-zero tube and place the reagent pair in the sample holder so the CD4-zero tube is in the run position.

3. Press (RUN).

4. Remove the reagent pair and recap the CD4–zero tube.
5. Uncap the CD8-low tube and place the reagent pair in the sample holder so the CD8 low tube is in the run position.

6. Press (RUN).

7. Remove the reagent pair and recap the CD8-low tube.

8. Step1 through 7 are followed for the second pair of controls (CD4-medium and CD8-high) and then discard the reagent pair.

F. Sputum smears preparation and Acid Fast Staining procedures

1. Label the slide with patient code/number

2. Make the appropriate thickness and width sputum smear on the slide

3. Air dry by placing this slide with smear horizontally

4. Fix the dried smear by passing 3 times over the top of Bunsen- burner or sprit flame

5. Stain the fixed smear with Ziehl-neelsen hot method

A) Place the slide on the slide rack with the smear upper most, their edges separated.

Make sure that the slides do not touch each other.

B) Cover the whole surface of the slide with filtered Ziehl carbol fuchsin

C) Heat very gently until steam appears. Use the flame of a cotton wool in methylated Sprit fixed on end of metal rod or stick for heating.

D) Leave the warm stain for 5 minutes

E) Tilt the slide to drain off excess stains. Rinse each slide individually in a gentle stream of running water until the all free stain is washed away.
F. Decolorize by 3% acid alcohol. Leave until the solution runs clear and then wash the slide with a gentle stream of running water.

G. Counter stain the smear by flooding it with 0.3% methylene blue and leaving it for 1-2 minutes.

H. Pour off methylene blue stain and wash the slide with gentle stream of running water.

I. Tilt and place the slide on the rack to dry in the air

Microscopic slide examination for AFB

1. Select the well-distributed smear area on the slide using 10 times objective

2. Add a drop of oil immersion and switch the objective to 100x (oil immersion objective)

3. Read the slide systematically. Look at least for 100-oil immersion filed.

Reporting of microscopic reading by grading (Quantitation scale recommended by the World Health Organization and the International Union Against Tuberculosis and Lung Disease-IUATLD)

<table>
<thead>
<tr>
<th>Report</th>
<th>grading report</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB/100 oil immersion filed</td>
<td>No AFB seen</td>
</tr>
<tr>
<td>1-9/100 oil immersion fields</td>
<td>exact count</td>
</tr>
<tr>
<td>10-99/100 immersion fields</td>
<td>1+</td>
</tr>
<tr>
<td>1-10/ immersion field</td>
<td>2+</td>
</tr>
<tr>
<td>&gt; 10/ immersion field</td>
<td>3+</td>
</tr>
</tbody>
</table>
10.REFERENCES


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WHO. (2006b) Tuberculosis facts sheet number104. Geneva, Switzerland


Declaration

I, the undersigned, declare that this MSc thesis is my original work and has not been presented for a degree in any other university, and that all sources of material used for this have been duly acknowledged.

Name Tadesse Ligidi

Signature_______________________ Date_________________

Advisors

1. Solomon G/silassie(MD MSc). Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University.

Signature_________________ Date__________________

2. Aster Tsegaye (MSc, PhD). School of Medical Laboratory Technology, faculty of Medicine, Addis Ababa University.

Signature_________________ Date__________________