EPIDEMIOLOGY OF TUBERCULOSIS AND HUMAN IMMUNODEFICIENCY VIRUS CO-INFECTION, CLINICAL PRESENTATIONS AND IMPACT ON IMMUNOHAEMATOLOGICAL PARAMETERS IN MOMBASA COUNTY, KENYA.

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A thesis submitted in fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Public Health and Epidemiology in the School of Public Health of Kenyatta University.

JANUARY 2015
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or for any other award.

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This work is dedicated to my mother Mrs. Agneta Amollo Yonge whose commitment to see me through the formative years of my education gave me aspiration for continued learning.
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ACRONYMS AND ABBREVIATIONS

AIDS – Acquired Immune Deficiency Syndrome

AFB- Acid fast bacilli

ARV’s- Anti retroviral drugs

BD FAC’s- Becton Dickson fluorescent Activated cell sorter

BCG – Bacillus of Calmette and Guerine

BMI- Body mass index

BSC II- Biological safety cabinet II

CBC-Complete blood count

CD4+ --Cell Differentiation no. 4 (Helper T- Cells)

CDA – Coast Development Authority

CDC – Center for Disease Control and Prevention

COMBDOT- Community based DOTs

CPGH- Coast Provincial General Hospital

CPT- Cotrioxazole Preventive Therapy

CTRL- Central Tuberculosis reference laboratory

CXR- Chest X-ray

DLTLD- Kenya’s National Division of Leprosy, TB and lung disease

DOT—Directly Observed Treatment
EDTA - Ethylene diamine-tetraacetic acid

EMB - Ethambutol

ELISA - Enzyme-linked immune Absorbent Assay

EPTA - Extra pulmonary Tuberculosis

FBDOT - Facility based DOT provider

HAART - Highly Active Anti-Retroviral Therapy

HCL - Hydrochloric acid

HIV - Human Immuno-deficiency Virus

HPLC - High performance liquid chromatography

INH - Isoniazid Antibiotic

IPT - Isoniazid preventive therapy

KANCO - Kenya Aids NGOs Consortium

LJ Media - Lowenstein Jensen media

MABA - Microplate Almar Blue drug susceptibility assay

MDR-TB - Multi-Drug Resistant TB

MIC - Minimum inhibitory concentration

MIRU’s - Mycobacterial interspersed repetitive units

MOEST - Ministry of Education, Science and Technology

MTB - *Mycobacterium tubercle bacilli*

NASCOP - National Aids and STDs Control Program

NaOH - Sodium hydroxide

NLTP - National Leprosy and Tuberculosis Program

OI’s - Opportunistic infections
OR- Odds Ratios

PCR- Polymerase chain reaction

PMTCT- Prevention of mother to child transmission

PPM- Public-private mix DOTS

PLWHAs- People Living With HIV/AIDS

ROK – Republic of Kenya

SPSS – Statistical Package for Social Sciences

SSA- Sub Saharan Africa

STR- Streptomycin

TB – Tuberculosis

UNAIDS – Joint United Nations Program on AIDS

VCT – Voluntary Counseling and Testing

VNTR- Variable number of tandem repeat

WHO – World Health Organization

XDR-TB- Extra drug resistant Tuberculosis

ZN- Ziel Neelsen staining
OPERATIONAL DEFINITION OF TERMS

**Case of TB:** A patient whom tuberculosis has been bacteriological confirmed or has been diagnosed by physician.

**Smear-positive pulmonary:** A patient with at least two initial smear examinations smear positive for AFB by direct microscopy or a patient with one initial smear examination positive for AFB by direct microscopy and culture positive with a patient with one initial smear examination positive for AFB by direct microscopic and radiographic abnormalities consistent with active TB as determined by a clinician.

**Smear negative:** Pulmonary tuberculosis not meeting the above criteria for smear positive diseases, diagnostics criteria should include at least 3 sputum smear examinations negative for AFB, and radiographic abnormalities consistent with active pulmonary TB, and no response to a course of broad spectrum antibiotic, and decision by a clinician to treated the patient with full course of antibiotics therapy or positive culture but negative AFB sputum examination

**Extra-pulmonary cases:** patient with tuberculosis of organs other than the lungs

**New cases:** patients who has never had a treatment for tuberculosis, or who has taken anti tuberculosis drugs for less than one month

**DOTS program:** Directly observed short courses treatment and it is WHO recommended policy package for tuberculosis control program.
Recurrences: Refers to rediagnosis of smear-positive TB in patients who were declared cured or treatment completed in the past with or without smear positive result. It also includes relapse cases.
ABSTRACT

Tuberculosis still represents an important global public health threat and it is one of the world’s leading causes of death and HIV/AIDS has substantially altered the epidemiology of the infection especially in Sub-Saharan Africa. Most of death due TB and TB-HIV co-infection could be averted if clinicians recognized the signs and symptoms of the two diseases and instituted appropriate measures without delay. The magnitude of TB and TB-HIV co-infection is increasing despite progress made in the ART/DOTs control programs. There is no adequate knowledge on TB-HIV co-infection and effect on immune system since immunohaematological cell counts are not routinely carried out on TB patients. This hospital and laboratory based descriptive cross-sectional study was carried in Mombasa County. The main objective was to determine the magnitude of TB and TB-HIV co-infection and their relationship with clinical markers. Sputum from five hundred tuberculosis suspects were examined for AFB and cultured on solid and liquid media. Drug susceptibility test was done using BACTEC MGIT 960 incubator. Blood samples from tuberculosis suspects were screened for human immunodeficiency virus. Complete blood cell count was done using Sysmex Kx-2 and CD4+T cells analysed using FACS count flow cytometer. A questionnaire was used to collect demographic and medical history of the tuberculosis suspects. The data was entered in MS Excel 8.0 and analysed using Epi-Info 6.04b and statistical package for social sciences (SPSS) version 16.0 software. Pearson’s chi-square test of independence was used to determine level of associations between TB-HIV co-infection and clinical outcomes. Student t test was used to test differences of means between two or more groups and Odds ratio to assess risk factors related to outcomes. Results showed tuberculosis prevalence was 42.0% and it was significantly higher in females (45.9%) than males 38.7% (P<0.05). Two hundred and two patients (96.2%) had pulmonary tuberculosis and eight (3.8%) extra-pulmonary tuberculosis. Smear positivity rate was 81.1% and culture positives 100%. Tuberculosis recurrence rate was 14.3% and was significantly associated with HIV infection (p<0.05). The majority of the TB cases (38.6%) were aged between 25-34 years (OR=58; CI: 0.34-0.94; p<0.05). Tuberculosis-HIV co-infection rate was 37.1% and it was not significantly associated with gender (P>0.05). Clinical features of chronic dry cough, fever, night sweats and weight loss were common in both TB and TB-HIV co-infected patients. Tuberculosis patients had higher CD4+T cell counts (474.5±198.8 cells/mm³) than co-infected patients (276.4±142.71) (t=5.6,df=461,p<0.05) but lower than reference group (1054.9 ± 156.1 cells/mm³, t=34.6, df=485, p<0.05). HIV/AIDS patients had significantly higher body mass index (19.9±2.2 kg/m²) than tuberculosis HIV/AIDS co-infected patients (BMI 18.8±2.7, t=0.70, df=58, p<0.05). The mean packed cell volume in TB-HIV co-infected patients was (32.31±4.8%) lower than TB patients without HIV/AIDS (34.21±4.4%) and the control group (36.41±4.2%). A high rate of drug resistance was observed in isoniazid (17.6%) and rifampicin (2.1%). Eight patients had multi-drug resistant-TB (4.8%). Any type of drug resistance in TB-HIV co-infection patients was 19.1% suggesting a positive correlation (p<0.05). The high prevalence of tuberculosis and high co-infection in this study underscores the need for more efforts and resources to increase knowledge and access health care. There is also need to improve drug susceptibility testing to all newly diagnosed tuberculosis patients in all health facilities to monitor drug resistance. Immunohaematological indices (CD4 count, FBC and ESR) be performed routinely to monitor both TB and TB-HIV co-infection patients.
CHAPTEr ONE
INTRODUCTION

1.1 Background of the study

Tuberculosis is a communicable disease resulting from infection with Mycobacterium tuberculosis whose principal reservoir is man and also, but infrequently with other mycobacterium belonging to the Mycobacterium tuberculosis complex (Ahmed et al., 2004). In 1993, the World organization (WHO) declared TB a global emergency (Aisu et al., 1995). It is estimated that between years 2009-2025 nearly one billion people will be newly infected, 200 million people will get TB and 40 million are likely to die from it if control programs do not improve (Benard, 2006). Tuberculosis is a disease of the poor and under privileged. With improved socio-economic conditions and availability of effective drugs, spread of TB infection has been effectively controlled in many parts of the world. In industrialized countries this disease is generally associated with identified high risk groups such as the elderly, immigrants from TB high prevalence areas, the homeless, drug and alcohol addicts (Espinal et al., 2000; Al Quuiz et al., 2002, Cook and Zumla 2003 ; Bacha et al., 2004).

The spread of the human immunodeficiency virus (HIV) has also contributed dramatically to the re-emergence of TB infection. HIV infection is recognized as a powerful risk factor for the development of active and often lethal TB and for the reactivation of latent TB infection to active disease (Davis, 2003; De Cock et al., 2006). The HIV infection affects cell mediated immunity and individuals infected
with *Mycobacterium tuberculosis* are at risk of developing active disease after being infected with HIV (Halvird *et al*., 2000, Corbette *et al*., 2009). According to WHO about a third of the 40 million people with HIV/AIDS are co-infected with *Mycobacterium tuberculosis* (Kirk *et al*., 2000, Fitzgerald *et al*., 2000; Badri *et al*., 2001; Cahn *et al*., 2003). In a population, the lifetime risk of developing active TB once infected in absence of HIV infection is about 10% (Yassin *et al*., 2006). However, it increases tenfold in HIV infected individuals and this has resulted in a large increase in the number of TB cases (Datiko *et al*., 2008). The proportion of smear-negative pulmonary TB (PTB) and extrapulmonary TB (EPTB) is higher among HIV co-infected TB patients (Reid *et al*., 2006).

HIV infection is characterized by CD4+T lymphocyte depletion manifested through the loss of the immune response capacity. The resulting immunodeficit is expressed by the blocking of immune surveillance mechanisms and thus, by the establishment of favourable condition to the development of opportunistic infections and or malignant process (Tegbaru *et al*., 2011). In tuberculosis, the immunodeficiency associated with HIV infection makes possible the evolution of a latent infection to a clinically manifest disease. Latent tuberculosis is characterized by the intracellular persistence of some metabolically inactive TB bacillus forms which are incapable of multiplication. The blocking of such mechanisms in case of CD4+T cell depletion will allow the multiplication of metabolically active TB bacillus forms and the development of clinically manifest tuberculosis (Yassin *et al*., 2006). CD4+T lymphocyte depletion is the result of facilitating antibodies and certain cytokines and some autoimmune processes which also affect the non-infected CD4+T cells (Cook and Long, 2007).
Control of TB infection has been further complicated by the worldwide increase in the incidence of drug resistance *tuberculosis* strains. The high morbidity and mortality due to multi-drug resistant TB (MDR) has caused major concerns regarding the clinical management and prevention of dissemination of the disease (Campos *et al*., 2003). World health organization (WHO) recommends inclusion of HIV testing in the algorithm for diagnosis of tuberculosis in countries with adult HIV prevalence rate of $\geq 1\%$ or settings where HIV prevalence rate in tuberculosis patients is $\geq 5\%$ (Cauthen *et al*., 1999; Bell *et al*., 2001; Corbet *et al*., 2003; Antonucci *et al*., 2004). Amongst the 22 high-burden countries that have 80% of all the global TB cases, Kenya is ranked 13th globally and 4th in Africa (WHO, 2011). The total number of TB cases reported in 2011 was 200,000 with notification rate of 268/100,000 which was more than five-fold higher than in 1990 (DLTLD, 2010). The prevalence of TB-HIV/AIDS co-infection in Kenya is around 60% per year.

### 1.2 Statement of the problem

Tuberculosis still represents an important global public health threat and it is one of the world’s leading causes of death. The cumulative human suffering and economic losses caused by TB and HIV in Kenya is immense. The country is ranked 13th on the list of 22 high burden tuberculosis countries in the world (WHO, 2009). The HIV/AIDS pandemic has substantially altered the epidemiology of tuberculosis. Many persons with *Mycobacterium tuberculosis* and HIV have a 5-10% annual risk of developing active TB. The double impact of TB and HIV co-infection is keeping large number of people trapped in poverty with these diseases that reinforce each other. An estimated 170 million working days are lost each year as a result of TB and the health
sector is burdened by the cost of drugs and treatment (MOH, 2010). Surveillance of TB-HIV co-infection, immuno-haematological markers and MDR-TB requires well developed laboratory networks, expensive equipment and trained personnel that are not widely available in many settings.

In Mombasa County, tuberculosis and HIV co-infection ranks first among the ten most common disease morbidity and mortality. It accounts for 40% of outpatient visits and 45% of inpatient admissions (HIS, 2009). The increased burden of the disease has been attributed to informal settlement, high population and poverty. The magnitude of TB and TB-co-infection is increasing despite progress made in the ART/DOTs implementation and control programs. This verifies that there could be several reasons for this situation including deficiencies in the health system that leads to lack of access to TB and HIV control interventions and low effectiveness of these interventions than expected. Thus it is essential that a research is conducted to identify the gaps. There is no sufficient knowledge on TB and HIV co-infection and effect on immune system since Immuno-haematological cell counts are not routinely carried out on TB patients. There is need to examine clinical symptoms of both TB and TB-HIV co-infection, enumerate the distribution of CD4+T cell count, full blood count and Erythrocyte Sedimentation Rate which are not known in these patients. This is critical in developing interventions and formulating policies for TB and TB-HIV control.

1.3 Justification of the present study

Rising TB cases 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. The
difficulties in diagnosing tuberculosis in HIV infected patients are among the challenges which are facing the national tuberculosis control programmes in Kenya. Lack of rapid and effective methods for TB diagnosis is also a major problem in developing countries. This complicates means of addressing the prevalence of HIV/AIDS and tuberculosis co-infection in resource constrained areas. Given this worrying trends, there was a clear need to assess the prevalence of TB and TB/HIV co-infection and their effect on immunological markers among newly diagnosed tuberculosis patients. The study was done when Kenya is scaling up ARV to HIV/AIDS patients with medical eligibility and also planning to offer Isoniazid prophylaxis to PLWHA. There is a knowledge gap in understanding TB and HIV co-infection and interplay between clinical outcomes, drug resistant and immunological markers which is not well understood. Earlier studies in the area concentrated on TB patients who were on treatment while this is the first study on newly diagnosed patients.

There are no studies done on tuberculosis and HIV co-infection, clinical outcomes and their impact on immunohaematological markers. There is little information on the pattern of drug sensitivity tests to all first line anti-tuberculosis drugs. In line with this, the findings can play paramount role in identifying anti-TB drugs resistant to Mycobacterium tuberculosis. It will also help to identify the high-risk groups of the study population for HIV infection and the median CD4+T and CD8+T cell counts around which clinical TB develops in HIV infected patients. The study provides information on the immunological and hematological indicators that can help predict the extent and severity of both TB and TB-HIV/AIDS co-infection and therefore informed intervention. These results will also help people working in the field to
understand the epidemiological distribution of HIV/AIDS infection in newly diagnosed TB patients and its contribution to clinical disease. The data from normal subjects (controls) can serve as reference ranges for adults in Kenya. The results will be used by policy-makers and health managers in designing algorithms for appropriate interventions at community level.

1.4 Research questions

i. What is the prevalence of tuberculosis among newly diagnosed TB suspects in Mombasa, Kenya?

ii. What is the prevalence of HIV/AIDS in newly diagnosed tuberculosis patients?

iii. What are the clinical features of tuberculosis in HIV sero-positive and sero-negative patients?

iv. What is the CD4+T and CD8+T count cells in tuberculosis, HIV/AIDS and tuberculosis-HIV/AIDS co-infection?

v. What is the susceptibility pattern of Mycobacterium tuberculosis isolates to first-line anti-tuberculosis drugs among tuberculosis patients in Mombasa?

1.5 Null Hypotheses

H₀ 1: Tuberculosis and human immunodeficiency virus co-infection have no effect on immunohaematological cells (ESR, PCV, platelets, CD4+T and CD+T cells).

H₀ 2: There is no correlation between TB and HIV co-infection and resistance of Mycobacterium tuberculosis to first line anti-TB drugs.

H₀ 3: There is no relationship between socio-demographic characteristics and tuberculosis human immunodeficiency virus co-infection.
1.6 Objectives of the study

1.6.1 General Objective

To evaluate the occurrence of tuberculosis and TB-HIV co-infection, clinical outcomes and assess the immune status of patients attending chest-TB clinics in Mombasa County.

1.6.2 Specific Objectives

i) To determine prevalence of pulmonary tuberculosis in Mombasa County.

ii) To determine the prevalence of HIV/AIDS among newly diagnosed tuberculosis patients in Mombasa County.

iii) To compare the clinical features of tuberculosis in HIV sero-positive and sero-negative patients.

iv) To determine the haematological cells count in tuberculosis, HIV/AIDS, tuberculosis-HIV/AIDS co-infected patients and in healthy subjects.

v) To analyze the susceptibility pattern of *Mycobacterium tuberculosis* isolates to first line anti-tuberculosis drugs among tuberculosis patients.
Conceptual Frame Work

The conceptual framework below represents the influence of clinical outcomes and Immuno-haematological markers on TB and HIV co-infection.

**Figure 1.1:** Researcher own conceptualization
CHAPTER TWO
LITERATURE REVIEW

2.1 Historical background

Tuberculosis has been present in human since antiquity. The origins of the disease are in the first domestication of cattle. Skeletal remains of pre-historic humans were found to have TB (Behr et al., 1999). Gaspard Laurent and Rene Laennec established the forms and stages of tuberculosis as a disease entity. The American Doctor Edward Livingstone Trudeau who was afflicted with tuberculosis established the Trudeau laboratory in 1874 (Narain et al., 2004), which became a modern sanatorium, the kind that for many years was the mainstay of tuberculosis treatment. The bacillus-causing tuberculosis, *Mycobacterium tuberculosis* was identified and described by Robert Koch in 1882 and developed the tuberculin test of diagnosis of the disease in 1890. The first genuine success in immunization against tuberculosis was developed from attenuated bovine strain tuberculosis by a French bacteriologist Albert Calmette and Camille-Guerine in 1996 and it was called ‘BCG’ (Bacillus of Calmette and Guerine).

The BCG was first used in humans in 1921 (Nimery et al., 2003). The first specific drug for tuberculosis became available in 1944 when Selman Abraham discovered streptomycin. This discovery was followed by the development of PAS (Para-amino salicylic acid) and later Isoniazid and other antibiotics that revolutionized the treatment of tuberculosis in 1955 (Pape et al., 1999). Sir John Crofton and colleagues developed multidrug chemotherapy regimens in the 1950s. Beginning in 1986, an expected resurgence of tuberculosis occurred in most parts of the world due to HIV/AIDS (Barnejee et al., 2008). Infection with HIV appears to be the high risk factor for reactivation of tuberculosis in populations who are infected with
2.2 Epidemiology of tuberculosis

Tuberculosis is a highly contagious disease that is mainly transmitted from person via inhalation of small cough droplets. In 2009, there were 9.4 million new cases of TB and 1.7 million deaths, including 380,000 deaths from TB among people with HIV/AIDS worldwide. Each person with active TB if left untreated will infect an average between 10 and 15 people each year (Blumberg et al., 2005). According to Styblo, the progression of TB infection into active disease is influenced by factors like; The risk of an individual in the community being infected with TB bacilli at a given period of time, The risk of disease following shortly after such infection and the risk of disease occurring long after the original infection owing to reactivation. The first risk factor is dependent upon the rate of active disease in a given population (Girardi et al., 2004).

The higher the rate of active diseases, the greater is the chance of acquiring infection (Davies, 2003). It is generally believed that most TB infections in high endemic areas occur early in childhood. In infected individuals the risk of disease following infection is known to vary between 5 and 12% (Selwyn et al., 2004). Majority of tuberculosis individuals mount an effective immune response and do not develop active disease. As immunity decreases with age, these individuals are at an increased risk of developing active TB due to reactivation of primary infection or exogenous infection (Behr et al., 1999; Dye et al., 2000). The relative contribution of reactivation of endogenous infection versus exogenous re-infection depends upon the rates of active disease transmission (Rob et al., 2005). In populations with low rate of active
transmission, reactivation of dormant infection will be a major contributor towards the total disease burden. In high prevalence areas re-infection with new TB is likely to be responsible for most of the active disease cases contributing significantly to all (Alland et al., 1994; Harris et al., 1998 WHO, 2007). About 15-25% of all new tuberculosis cases in adults between 15-49 years are also HIV/AIDS co-infected (Sharma et al., 2004). Worldwide, around 50% of all HIV/AIDS patients are co-infected with TB (WHO, 2009). In 2009, Kenya had approximately more than 162,000 new TB cases and an incidence rate of 150 new sputum smear-positive (ss+) cases per 100, 000 population (USAID, 2009). The world health organization also estimates that there were around 2500 cases of multi-drug resistant (MDR-TB) in Kenya in 2007 and 4000 cases in 2008. Proper diagnosis and correct treatment of tuberculosis will result in reduction of prevalence provided the infectious cases are detected and brought to treatment (Stephene et al., 2006). However, there are difficulties in achieving the goal of reducing tuberculosis in Kenya due to a number of challenges which include prevailing problems in the control program, diagnosing tuberculosis in HIV/AIDS patients and increase in extra pulmonary tuberculosis (EPTB). Stigma associated with tuberculosis and its link to HIV/AIDS, poor drug adherence associated with high pill burden in cases of co-infection, high mortality rates in HIV/AIDS and tuberculosis also complicates management (Murray 1998; Volmink et al., 2000; Wright et al., 2004; Wandwalo et al., 2005).

Macrophages, dendritic cells, CD4+ T and CD8+ T cells play important role in controlling TB infection (Tsegaye et al., 2002). It has been found that there is considerable reduction in cell mediated immunity during TB infection (Corbet, 2003). Another study documented significant reduction in CD4+ T cells but it was without
notable reduction in CD8+ T cells. However, reduction in CD4+ T cells with increase in CD8+ T cells has also been reported. Less than 300 mm3 CD4+ T cells were found associated with poor prognosis (Jones et al., 1993). Further, CD4+ and CD8+ T cells did not vary at different times of treatment with ATT and there was no significant change in CD4+ and CD8+ T cells between chronic TB patients and healthy controls (Mientjes et al., 1992; Sonnenberg et al., 2005; Cailhol et al., 2005).

One of the most important cell populations in the control of tuberculosis infection is the CD4+ T cell population. It is clear that an intact CD4+T cell population correlates with a better outcome as studies in patients with compromised helper cell populations have shown including those with HIV infection (North and Jung 2004, Kaufmann 2005). Absent levels of Th1 cytokines (interferon-γ and IL12) are associated with an inability to control tuberculosis disease (Miaini et al., 1996; Whalen et al., 1997; Prins et al., 1999). This suggests that the regulatory T cell population may possibly have a pivotal role in determining the outcome of the disease process by inhibiting a TB specific CD4+T cell response. Currently the role of Tregs is uncertain in the pathogenesis of tuberculosis. A small pilot study suggested that the numbers of Tregs are increased in active tuberculosis disease (Guyot-Revol et al., 2006). These preliminary findings were confirmed by further studies (Hougardy et al., 2007; Bacha et al., 2007). These cells may aid the bacterium (in addition to the mechanism described above) in immune evasion. Further investigations (Chenz et al., 2007) suggest that regulatory T cells, as defined by their expression of CD4, CD25 and FoxP3 are increased in absolute number and frequency in the blood of patients with active tuberculosis which compared with uninfected controls and patients with latent tuberculosis. The effects of these cells appear to be diverse including secretion of IL-
10 and suppression of tuberculosis specific interferon-γ secretion (Chenz et al., 2007). It seems unclear as to the role of these cells in suppression of infection. Studies in mice that have had preferential depletion of the FoxP3-expressing CD4+ T cell population showed a log reduction in the colony forming units of *Mycobacterium tuberculosis* (Hougardy et al., 2007). In addition, adoptive transfer of FoxP3 expressing CD4+ T cells into mice resulted in suppression of a tuberculosis-specific effector CD4+ T cell response (Kursar et al., 2007).

### 2.3 Influence of risk factors on tuberculosis and transmission

Several studies have cited various risk factors implicated in TB and spread of the disease in order to assist in proper utilization of public healthcare resources and prioritize targets for TB control. In this connection, the most significant independent risk factor in association with active pulmonary or extra-pulmonary TB is HIV infection contributing to ongoing transmission among individuals (Corbett et al., 1999; Godfrey-Faussett and Ayles, 2003; Cailhol et al., 2005; Reid et al., 2006). Age has also been shown as a risk factor in increasing TB incidence; while women have been found to be more susceptible to TB than men probably due to the effect of female hormones or underreporting of TB cases (Sonnenberg et al., 2004; Cailhol et al., 2005). Other risk factors include; past history of TB in the family, smoking, place of residence, place of origin, malnutrition and alcoholism (Cantwell et al., 1998; Lienhardt et al., 2005; van et al., 2005; Patel et al., 2007). In countries with low TB incidence, immigrants from countries with high TB prevalence constitute potential increased risk for recent transmission of infection to local populations (Borgdorff et al., 1998; Dale et al., 2005). In some industrialized countries, TB revival has been linked to certain risk factors, such as overcrowding, reduced funding, poverty,
homelessness, improper TB management and negligence in implementing TB control programme (Bhatti et al., 1995; Gutierrez et al., 1998; Elender et al., 1998; Dye et al., 2009). In different parts of the world, consumption of unpasteurized milk was observed in association with TB caused by \textit{M. bovis} (Coker et al., 2006). Silicosis, an occupational disease occurring among mine workers exposed to silica dust, predisposes TB and non-tuberculous mycobacteria (NTM) infections (Sonnenberg et al., 2005). Other researchers reported the occurrence of TB transmission in hospitals in association with healthcare workers born in countries with high TB burden but without further continued spread (Anderson et al., 2007). An association between tuberculosis (TB) and body wasting has been long recognized. Malnutrition impairs host immunity and predisposes to TB while TB itself can cause malnutrition (Grange, 1999; Zacharia et al., 2002; Hill et al., 2006).

Malnutrition or poor diet and food low in calories, puts a person at greater risk of TB and it may impair survival in TB patients. It is thought that chronic lack of appetite can be one of the causes of malnutrition associated with TB and therefore may be a potential independent risk factor for latent TB (Brouwer et al., 2006). Latent TB infected people with poor appetite develop active TB rapidly, and then these patients have poor treatment outcome. Studies have shown that supplementation with vitamins and Zinc (mineral) during treatment of pulmonary TB may reduce mortality in those co-infected with HIV (Lienhardt et al., 2005; Patel et al., 2007; Kumar et al., 2008). Furthermore, malnutrition and intestinal parasites cause immunosuppression, which in turn may cause false-negative tuberculin skin tests (TST) and failure to identify TB infection (Zacharia et al., 2006; Lawson et al., 2008).
The association between smoking and TB has been investigated for several decades. Both passive and active exposure to tobacco smoke has shown to be associated with TB infection and with the transition from being infected to developing active TB disease (Borgdorff et al., 1998; Auer et al., 2000; Dale et al., 2005). There may be several reasons for the association between smoking and TB. Smoking may decrease immune response or damage the protective effect of tiny hair–like structures called cilia in the airways, resulting in increased TB risk. It has been shown that heavy smokers are more likely to have cough, dyspnea, chest radiograph appearances of upper zone involvement, cavity and miliary appearance, and positive sputum culture, but are less likely to have isolated extra-pulmonary involvement than non-smokers. Smoking has been found to be associated with both relapse of TB and TB mortality (Singh et al., 2005; Coker et al., 2006). There appears to be enough evidence to conclude that smoking is causal associated with TB disease. Patients with TB need and should receive counseling and assistance in stopping smoking.

A meta-analysis study reported smokers were 73 percent more likely to become infected with TB and more than twice as likely to develop active TB. Overall, smokers are 40 to 60 percent more likely to develop active TB after being infected with TB bacteria (Lienhardt et al., 2005; Davies et al., 2006; Patel et al., 2007).
2.4 Epidemiology of HIV and AIDS

About 3.5 million people in Kenya are living with HIV/AIDS (KANCO, 2009). The epidemic is spreading fast in rural areas with less health facilities compared with the urban areas. The spread in rural area is accelerated by poverty, ignorance and lack of information about proper methods of prevention. Although the epidemic is reported to decrease in some areas of Kenya like central province with prevalence of 4.8%, in other regions like coast province it was 15.3% compared to the country prevalence of 7.9% (USAID, 2009). About 59% of the HIV cases in Kenya are women (KANCO, 2010). On average, 81,000 Kenyans die annually from HIV/AIDS and related conditions and on average almost every minute two people are infected HIV virus (NACC, 2010).

The World Health Organization (WHO) estimates show that where the HIV prevalence in the general population is high, the prevalence of HIV in tuberculosis patients is also relatively high and vice versa. For example, the 1991 World Health Organization estimates show that in Botswana, with HIV prevalence of 36% in the general population, the prevalence of HIV in tuberculosis patients was 77%. In Sub Saharan Africa with HIV prevalence of 8.7% in the general population, the prevalence in tuberculosis patients was 37% (Barnejee, 2008; CIK, 2009). Efforts to control HIV/AIDS are in progress countrywide through the National AIDS Control Council (NACC). The control program includes information, education and communication (IEC) about the prevention of HIV/AIDS and behaviour change and communication (BCC). Emphasis is on abstinence, faithfulness and promotion of safer sex through condom use in high risk groups. Prevention of mother to child transmission of HIV/AIDS (PMTCT) is also promoted by administering anti-retro viral drugs (ARVs)
during the third trimester or at onset of labour, and by education about breastfeeding options (Odhambo, 1999). The Government of Kenya initiated the roll out of ARVs program in 2001 which aimed at scaling up ARVs to reach those in need in resources constrained areas. In the roll out ARVs program all patients with medical eligibility for ARVs are treated free of charge according to Kenyan national policy for HIV/AIDS managements. In order for the HIV/AIDS patients to start ARV treatment among other screening they should also be screened for tuberculosis before initiation and on the course of treatment with ARVs. The World Health Organization recommends screening of HIV-infected person for TB diseases after HIV diagnosis, before initiation of ARVs and during routine follow up care. In this strategy TB, if diagnosed, is treated promptly before starting ARVs or for a few days before introduction of ARVs to minimize overlapping of the drugs side effects (Williams et al., 2003). To achieve the target of treating many HIV/AIDS patients in need of ARVs, there is need to screen those with features or diseases suggesting HIV infection such as tuberculosis and this will serve as the entry point to HIV/AIDS care and treatment since tuberculosis patients co infected with HIV/AIDS are eligible for ARVs (Zachariah et al., 2006).

The available drugs for treating HIV/AIDS patients can suppress the viral replication, which results in increase in cellular immunity (CD4+ T lymphocytes) and improved response/fight against opportunistic infections including tuberculosis. However, due to difficulties in diagnosing active tuberculosis in HIV/AIDS patients, starting isoniazid preventive therapy may be challenging. Isoniazid, if given to HIV/AIDS patients reduces the increased risk of these patients to develop tuberculosis (Wilkinson et al., 1998). If isoniazid prophylaxis is given to patients with active TB
there is a risk of developing resistance due to anti TB mono-therapy in patients with active tuberculosis.(Pape et al.,1993; WHO, 2008a).

2.5 HIV/AIDS and tuberculosis co-infection

In sub Saharan Africa including Kenya, the HIV and AIDS infection has contributed significantly to the rising levels of tuberculosis incidence. Kenya’s Ministry of Health figures show that the TB incidence has increased tremendously since 1981 and has intensified because of the HIV/AIDS infection. Whereas the country had 30,000 reported cases of TB in 1983 when the first case of HIV/AIDS in the country was reported, this has increased to 3.6 million (USAID, 2009). People with HIV infection are increasingly infected with TB because HIV weakens their immune system. HIV/AIDS is the most important risk factor for the development of tuberculosis (Antonucci et al., 2004) patients with TB infection co-infected with HIV, have a 20-30 times higher risk of developing tuberculosis disease during their lives, than TB infected person without HIV infection. In immunocompetent individuals with TB infection the lifetime risk of developing active TB disease is 10% in contrast with TB infected patient co-infected with HIV where the annual risk of developing TB disease is (5-8%) (Korenromp et al., 2003).

Tuberculosis is the most common opportunistic infection (OI) in HIV/AIDS patients in developing countries. Autopsy studies have found disseminated TB in 40-54% of HIV infected people in HIV prevalent countries many of whom were undiagnosed prior to death (Pape, 2004). Tuberculosis is the common pre AIDS opportunistic infection and accounts for about 40% of all presentations seen in HIV patients in Haiti. Other common presentation is the wasting syndrome, which includes weight loss of more than 10% of normal weight and prolonged fever or diarrohea. The
wasting syndrome is also associated with TB and more often the symptoms of TB are misattributed to HIV/AIDS (Corbet, 2003).

Tuberculosis can occur at any stage of CD4⁺T cells depletion but it is common during the early stage when the CD4⁺T cells is relatively normal (Tsegaye et al., 2002). In Haiti for example, 56% of the TB patient infected with HIV were diagnosed when CD4⁺T cells were >350/microlitre, 23% and 12% of the patients infected with HIV has TB at the CD4⁺T cell levels of 200-350/microlitre and <200/ microlitre, respectively (Nwachukwu and Peter, 2010). The pattern of chest radiography in TB patients co-infected with HIV/AIDS varies diversely, the typical upper-lobe cavitatory picture usually seen in reactivated adult pulmonary tuberculosis (PTB) occurs when the CD4⁺T cells are still relatively normal. As the CD4⁺T cells continue to fall with the progression of HIV/AIDS atypical presentation such as pleural effusion, mediastinal and lower lobe consolidation, milliary pattern and hilar lymph node enlargement become more common. Some of these changes are similar to presentations of other opportunistic infections affecting the lungs in HIV/AIDS patients making interpretation of radiography for assisting diagnosis difficult (Mientjes et al., 1992; Elliot et al., 1993). It is recognized that HIV infection worsens the risk and clinical course of TB, similarly co-infection with M. tuberculosis accelerates progression of disease by HIV-1 infection (Narain et al., 2004).

Tuberculosis infection enhances local HIV-1 replication in-vitro. Cytokines produced during TB infection may result in activation of lately HIV infected cells with virus. Expression and induction of virus replication, increased IL-2, IL-6 and TNF- & (TH-2 type cytokine) generated by infection with TB may be responsible for the increase in
the viral load (Miaini et al., 1996; Whalen et al., 1997; Prins et al., 1999). The HIV prevalence is higher among TB cases compared to general population in Kenya and is currently the greatest risk factor for the progression of latent TB infection to active TB including progression of new TB infection. It is also a potential risk for recurrence of tuberculosis (Daley et al., 1992; Long and Shwartzman 2007).

An episode of recurrent TB has been defined as a case re-notified at least 12 months from the date of the initial notification (Crofts et al., 2010). This is based on the reporting criteria of Enhanced Tuberculosis Surveillance that a year has to elapse before a case in the same patient can be notified again, and that any case reported from the same patient twice within a year is considered a single episode. The assumption is that most cases would be expected to have completed their treatment within 12 months, which is also why 12 months is the standard cut-off time at which treatment outcomes are recorded (Dobler et al., 2008; Crofts et al., 2010). Previous studies have shown that recurrent TB develops in about 2-5% of the patients after curative treatment with short-course anti-TB chemotherapy (Weis et al., 1994). Among TB patients cured by short-course treatment in trial conditions, up to 7% develop recurrent TB needing retreatment within 1 to 2 years (Verver et al., 2005). Also re-infections with new strains of *M. tuberculosis* complex may play a significant role in the recurrence of TB especially in settings with a high prevalence of the disease (WHO, 2011).
2.6 Clinical presentation of tuberculosis with and without HIV-infection

Tuberculosis is a disease with protean manifestations. The clinical presentation of tuberculosis can mimic several diseases and can be a diagnostic problem even in endemic areas. Virulence and dose of the infecting mycobacterium, the immune status of the host, the organ systems(s) involved, all influence the clinical manifestations of tuberculosis. Patients with tuberculosis often develop the symptoms insidiously and present with constitutional symptoms of tuberculosis toxaemia and with symptoms and signs related to the organ/system(s) involved (Haileyeus et al., 2007).

Human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) have a profound impact on the clinical presentation of tuberculosis. Clinical presentation of tuberculosis in patients with HIV infection depends on the severity of immunosuppression (Majid and Abba, 2008). In patients with early stages of HIV disease, the clinical presentation of tuberculosis tends to be similar to that observed in persons without HIV infection. Pulmonary disease is most common often with focal infiltrates and cavities. When the immunosuppression is more marked (CD4 count <200ml⁻³), the features of tuberculosis are a typical with a much greater frequency of extra-pulmonary involvement, especially of the lymph nodes (Pape, 2004).

Diffuse pulmonary disease without cavitation often involving the lower lobes and prominent paratracheal and mediastinal adenopathy is common. In patients with advanced disease, disseminated and military disease is more often seen (Kumarasamy et al., 1995). Usually patients with tuberculosis present with fever, which often develops in the late afternoon or evening. The fever is usually low grades at the onset
and may become high grade with progression of disease. Some patients may remain afebrile. Patients also manifest weight loss, which may sometimes precede the other symptoms (Affussim et al., 2011). The weight loss can be profound reducing the patient to a “skin and bones” appearance. Tiredness, lassitude, fatigue, night sweats, and anorexia are other important constitutional symptoms observed in patients with tuberculosis (Bacha et al., 2004). Extra pulmonary TB occurs more frequently among HIV seropositive individuals than among seronegative (Badri et al., 2000; De Cock et al., 2000). The symptoms of active TB are often non-specific and mainly pulmonary; the patient may present with fever, chronic persistent cough with or without blood, lack of appetite, night sweats, weight loss and severe thoracic pain. Failure of the immune system to combat tubercle bacilli causes haematogenous spread of organisms to various sites to produce extra-pulmonary disease (Carrol et al., 2001). The extra-pulmonary form has many manifestations and accounts for only 20% of all cases (Martin and Lazarus, 2000). Haematogenous dissemination to the brain or meninges results in tuberculosis meningitis (TBM) (Aderaye, 2007). The TBM is a debilitating disease characterized by symptoms of fever, headache, and lowered level of consciousness and stiffening of the neck (American Thoracic Society, 2000; Carrol et al., 2001). In childhood, it is mainly a manifestation of severe extra-pulmonary TB (Zugar and Lowy, 2006). There is evidence to suggest that there is a close relationship between TBM strains isolated in the UK (Dye et al., 2009).

The defined course of infection with *M. tuberculosis* is dependent upon the inter-play of a number of factors such as the host, environment and the organism. In addition, the clinical presentation of the disease has been shown to vary between ethnic groups, in relation to the infecting strain (Malik and Godfrey-Faussett, 2005). For instance, in
England and Wales, one study showed that the majority of people originating from the Indian subcontinent were prone to extra-pulmonary TB, dominated by mycobacterial lymphadenopathy (Kumar et al., 1997; Millen et al., 2008). Genito-urinary TB was found to be more frequent in white populations, while pericardial TB was a more common phenomenon in Sub-Saharan Africans (Braun et al., 1999). This could be attributed to genetic susceptibility and host-pathogen interaction. In the presence of co-infection with HIV the clinical picture is altered with extra-pulmonary signs predominating (Harries et al., 2001). Clinical presentations of TB and virulence may further vary with respect to strain genetic changes. Infection with the Beijing strain causes a febrile response during early treatment with anti-tuberculosis drugs (Ngoc Lan et al., 2003). Increased virulence of these strains and their ability to fight against hostile microenvironment of the host immune system is thought to be related to genetic alterations in mutator (mutT) genes and other factors (Rad et al., 2003; Vynnycky and Fine, 2007). Treatment failures and relapses and ability to spread more quickly to persons in contact leading to outbreaks have also been reported in association with the Beijing strain (Ngoc Lan et al., 2003; Rad et al., 2003).

The features of pulmonary tuberculosis (PTB) and the clinical presentation are influenced by the degree of immune-suppression. Patients with a well preserved CD+T counts are likely to present with symptoms and signs similar to HIV- negative patients (cough for at least three weeks, night sweats and weight loss). Patients with more advanced immuno-suppression often present with more atypical symptoms and signs. Symptoms may be nonspecific or absent and it may be difficult to distinguish from HIV disease or other opportunistic infections (Elston and Thaker, 2008). Haemoptysis which is usually considered to be hall mark of PTB in developing
countries is not as frequently reported by patients with advanced HIV infection (Majid and Abba 2008). Because of this reason, the clinicians need to have a high index of suspicion for PTB in symptomatic HIV-positive patients (Elston and Thaker, 2008). Non-specific generalized skin rash on examination is normally associated with low CD4+T cell count (Majid and Abba 2008); Prins et al., (1999). Moore et al., (2008) studied the patterns of skin manifestation and their relationships with CD4+T cell counts among 384 HIV/AIDS patients in Cameroon and the findings showed that up to 68.8% patients presented with at least one type skin problem. Generalized prurigo, oral candidiasis, herpes zoster and vaginal candidiasis were the most common skin problems associated with CD4+T cells count $\leq 200$ (P$<0.05$). Generalized skin rash was associated with lower mean CD4+T cell counts less than 100 cells. A study by Tsegaye et al. (2002) among TB-HIV co-infected showed that skin infections and enlarged lymph nodes had the strongest prognostic effect in all the models considered.

2.7 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 opportunistic infections (OIs) for certain patients with access to care. However, it does not replace the need for antimicrobial prophylaxis among patients with severe immune suppression (Bernard, 2006). A number of trials have evaluated the efficacy of primary preventive therapy against TB among HIV-infected individuals (Blower et al., 1996; Whalen et al., 1997; Sarita et al., 2007). 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 (Bock et al., 2007). 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% (Sarita et al., 2007). In some studies, it is associated with a 60% reduction of risk of development of tuberculosis (Gerardi et al., 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, community-wide IPT was associated with reductions in the prevalence of both latent infection and active infectious TB (Aisu et al., 1995; 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 however not the result of acquired resistance generated by IPT (Sarita et al., 2007). 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 (Braitstein, 2006). 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 (Padmapriyadarsini and swaminnathan, 2005). 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 (Korenromp et al., 2004; Gothi and Joshi, 2004; Jeon et al., 2005 Sharma et al., 2006).

2.8 Treatment of Tuberculosis

Active TB disease can almost be cured with combinations of antibiotics. The variety of treatment and drug options depend on the country (WHO, 2009). Effective treatments quickly make a person with TB non-contagious and therefore prevent further spread of TB. Achieving a cure takes about eight months of daily treatment (Volmink et al., 2000). To ensure thorough treatment, it is often recommended that the patient takes his/ her pills in the presence of someone who can supervise the therapy. This approach is called DOTs (Direct Observed Treatment, short course) and it cures 95 percent of TB cases (Bell et al., 1999; Pape 2004; Wandwalo et al., 2005).

2.9 Treatment of Tuberculosis in HIV/AIDS

It is virtually important for people with HIV to have treatment if they have active TB. This will cure them and prevent transmission to others even in a setting where antiretroviral drugs are unavailable (Wilkinson et al., 1998). It is crucial that health system is able to offer HIV- positive people the simple antibiotics needed for DOTs (WHO, 2006). The risk of active tuberculosis among individuals with dual tuberculosis and HIV infections can be reduced by treatment for 6 to 12 months with isoniazid for two months with rifampicin and pyrazinamide (Williams et al., 2003).
This treatment is also administered to prevent recurrence in HIV-infected tuberculosis patients who have completed tuberculosis therapy (Girardi et al., 2000). For some people it can be difficult to take drugs for both TB and HIV at the same time. Some HIV drugs can interact with some TB drugs making the treatment too difficult. Antiretroviral therapy slows the development of immuno-deficiency in HIV-infected persons, may restore immuno-competence, and delays the onset of TB (Libamba et al., 2005; Chiang et al., 2008).

2.10 Drug-resistant tuberculosis

Chemotherapy is the most potent weapon available in the fight against tuberculosis. When used properly, available anti-TB drugs are able to reach cure rates above the 85% target recommended by the World Health Organization (WHO, 2006). Early in the chemotherapy era, resistance associated with treatment failures emerged and has become a common occurrence worldwide. Of particular concern are the increasing prevalence of organisms resistant to isoniazid and rifampicin, the two drugs that form the backbone of modern short-course therapy. Rifampicin (RIF) resistance occurs mostly in conjunction with INH resistance (90% of cases) and can be used as a surrogate marker for multidrug resistance (Quy et al., 2006).

Drug resistance in *M. tuberculosis* occurs as a result of random spontaneous chromosomal mutations during natural cell replication. These mutations are neither drug induced nor drug linked. The probability of a drug-resistant mutant occurring is directly proportional to the size of the bacterial population (Mermin J. 2007). The frequency of primary resistant organisms varies for each drug; however, it is usually between $10^{-6}$ to $10^{-8}$. Spontaneous resistance to isoniazid is estimated to occur once in
every 106 organisms, and to rifampicin once in every 108 organisms. The probability of spontaneous mutants being simultaneously resistant to two or more drugs is the product of the individual mutants. The development of drug resistance is a man-made amplification of a naturally occurring phenomenon. Previous treatment for tuberculosis predisposes to the selection of multi-drug resistant organisms. Non-compliance is a major factor in allowing the resistant organisms to survive (Sarita et al., 2007). Multi-drug therapy is used to prevent the emergence of drug resistant mutants during the long duration of treatment. Resistance can be defined as single-drug, multi-drug, or poly-drug resistance depending on the number of drugs and/or which drugs are involved (Van et al., 2005; Raviglione et al., 2007).

Although an unequal global distribution of drug resistance exists between poor and rich countries, the problem is global (WHO, 2007). The regions where drug-resistant TB is more prevalent lack the resources to implement adequate measures to control even the susceptible types of the disease. Recent reviews have reported a high prevalence of primary multidrug resistant tuberculosis in Latvia (1998: 9.0%), Estonia (1998, 14.1%) and Dominican Republic (1994-1995: 6.6%). The case fatality rate is high among patients with HIV/AIDS who are infected with strains of drug resistant M. tuberculosis (WHO, 2009). MDR-TB in AIDS patients is on the rise and the future it may be alarming (Fitzgerald et al., 2000; Quy et al., 2006; Cohen et al., 2006).

WHO estimates there were around 2,000 cases of multidrug-resistant (MDR)-TB in Kenya in 2007, although only 4.1 percent of these cases were diagnosed and notified. This was based on only 4,000 (14% of all retreatment cases) sample analyzed at the central reference laboratory (NLTB, 2009). As of 2010 one case of Extra drug
resistant tuberculosis (XDR-TB) had been reported in the western part of Kenya in Busia (NLTB, 2010). The high rates of drug resistance TB currently being reported in Kenya are alarming. In 2006, WHO estimated a total of 400,000 multi-drug resistant TB (MDR-TB) to have occurred in the world of which 10,000 of them were reported from African countries (WHO, 2008a). There is a policy supporting MDR-TB diagnosis and treatment and a laboratory testing facility and in 2008, USAID continued to support routine MDR-TB surveillance (Zwarenstein et al., 1998; WHO, 2009). In most areas of the world where TB is common, reliable pretreatment drug susceptibility results are not available. However epidemiological studies have shown that most previously treated patients with drug resistance initially had primary drug resistance. *Mycobacterium tuberculosis* strains resistant to anti-TB drugs can be transmitted the same way MTB strains susceptible to anti-TB drugs are transmitted. Human immunodeficiency virus (HIV) infection curtails the effects of TB control programme by lowering the life expectancy of those receiving TB treatment (Githui et al., 1998; Taylor et al., 1999; Zhiyuan et al., 2008).

### 2.11 Global picture of TB and HIV/AIDS

The importance of TB to the global HIV is enormous (WHO, 2011). Tuberculosis is a serious health problem in its own right but it is also the cause of the death for HIV positive people. In endemic arrears it primarily affects the adolescents and youth adults (Rosenstock et al., 1994; Netto et al., 1999). In some parts of the world TB is increasing after almost 40 years of decline. Escalating TB rates over the past decades in many countries in sub-Saharan Africa and parts of South Asia are mainly due to HIV epidemic. Between 1990 and 2005, TB incidence rates triplets in African
countries with high HIV prevalence. Rates TB are now rising in the worse affected African countries by around 5 percent annually (Odhiambo 1999, Moore et al., 2007). In 2008, Africa accounted for 81% of the estimated 941,000 cases of TB among HIV positive people worldwide (USAID, 2009). The largest number of TB cases occurs in the South East Asia region which in 2008 accounted for an estimated 4,000,000 new cases (1/3 of the Global total) (WHO, 2009).

The estimated incidences per capita in sub-Saharan Africa are nearly twice that of South East Asia at 756 cases per 100,000 populations in 2007. Tuberculosis is not only a problem in low and middle income countries. There are 18,517 new cases reported in the U.S in 2008 and 8,167 new cases reported in England, Wales and Northern Ireland in the same year. About 10 percent of people with TB in London are likely to infect with co-infected with HIV. It is estimated that the least 1/3 of the world’s population (2.6 billion people) are infected with TB. Every year, ten million people become sick with TB. Tuberculosis case notification rates have risen up to fourfold in many African countries, since the mid 1980 (Moore et al., 2007, WHO, 2009).

2.12 Impact of tuberculosis in Kenya

Kenya ranks 13th on the list of 22 high-burden tuberculosis (TB) countries in the world and has the fourth highest burden in Africa. According to the World Health Organization’s global TB Report of 2010, Kenya had approximately more than 132,000 new TB cases and an incidence rate of 142 new sputum smear-positive (SS+) cases per 100,000 population. Kenya’s National Division of Leprosy, TB and Lung Disease (DLTLD) began to implement the WHO-recommended DOTS (the
internationally recommended strategy for TB control) strategy in 1993 and reported 100 percent DOTS coverage by 1996 (USAID, 2009). In 2005, the DOTS case detection rate reached WHO’s target of 70 percent and rose to 72 percent in 2007. The DOTS treatment success rate also met WHO’s target of 85 percent in 2007. Data from the national program show that Kenya had met the target for the treatment success rate in 2007. WHO estimates there were around 2,000 cases of multidrug-resistant (MDR) TB in Kenya in 2007, although only 4.1 percent of these cases were diagnosed and notified. There is a policy supporting MDR-TB diagnosis and treatment and a laboratory testing facility, and in 2008, USAID continued to support routine MDR-TB surveillance (USAID, 2009).

Kenya continues to treat more and more TB patients each year. However, widespread co-infection with HIV (close to 48 percent of new TB patients) makes TB treatment difficult. While the number of new cases appears to be declining, the number of patients requiring re-treatment has increased. The government placed the National Leprosy and Tuberculosis Programme (NLTP) (now DLTLD) and the national HIV/AIDS program in the same division in the Ministry of Health (MOH) to better address TB-HIV/AIDS co-infection. This resulted in increased collaborative TB-HIV/AIDS activities across the country (MOH, 2010). In 2007, the government demonstrated increased political commitment by upgrading the then-NLTP to a division within the MOH (DLTLD) and increased funding for TB control. With donor support, a greater proportion of TB patients benefited from improved DOTS services. The DLTLD implements TBHIV/AIDS treatment services, community-based DOTS (C-DOTS), and public-private mix (PPM) DOTS, as well as activities to address MDR-TB.
Tuberculosis treatment results for TB patients started on treatment in 2010 show treatment success rates of 85.86% for new smear-positive pulmonary TB cases (n=37,402), 78% for smear-positive retreatment relapse cases (n=3,643), 79% for smear-positive retreatment failures cases (n=195), 83% for new smear-negative PTB cases (32,786), smear not done 79% (11,7270, other re-treatments 79% (n=5,423) and 83% for Extra-Pulmonary TB cases (WHO, 2010).

The age with the highest TB notification rate in 2010 remained 25-34 years in both males and females as it has been the trend over the last decade. This is the same age category with a high HIV sero-prevalence. Males continue to dominate after the age of 24 over the females who are more below this age group (DLTLD, 2010). In this age-group, male cases were close 380 per 100,000 population and females close to 250 per 100,000 population giving an average of 315 per 100,000 population (DLTLD, 2009-2010).

Mombasa county recorded about 9,500 new cases annually followed by Kilifi (2,617), Kwale (1,362), Taita Taveta (638) while Tana River and Lamu reported 373 and 225 new cases respectively (DLTLD, 2010). Some 600 people in every 100,000 in Mombasa County suffer from multi-drug resistant tuberculosis. Patients develop resistance to drugs for not completing treatment and often after being misled by religious leaders. In the year 2012, Mombasa County recorded close to 14,750 new cases attributed to overcrowding in slum areas and high population.
Figure 2.1 Estimated worldwide TB incidence rates from data collected in 2009 (Adapted from WHO, 2010)
2.13 Laboratory diagnosis and detection of tuberculosis

2.13.1 Microscopy

Microscopic examination of sputum smears for acid-fast bacilli is used throughout the World as a diagnostic test for suspected pulmonary tuberculosis. Bacilli of *Mycobacteria* can be demonstrated by Ziehl-Neelsen (ZN) or fluorochrome staining methods (Gothi *et al*., 2004). The finding of acid-fast bacilli in sputum establishes a presumptive diagnosis of tuberculosis and indicates that the patient is capable of transmitting the infection and appropriate measures must be instituted to prevent infection. In all microscopic diagnostic methods the detection limit is between 104 and 105 bacilli per millilitre of specimen (Pape, 2004). This means those patients with fewer than 104 organisms per ml will be smear-negative and less infectious. Microscopy is rapid, cheap and relatively easy to perform.

Sensitivity of microscopy approaches 60-70%. The sensitivity of acid-fast smears of sputum from HIV positive patients is even lower. Organisms other than *Mycobacteria* may also demonstrate various degrees of acid-fastness (*Nocardia asteroides*) leading to false smear-positive results (Steingert *et al*., 2006; Alisijahana *et al*., 2007). The standard WHO recommendations for TB diagnosis in the DOTS program are the use of direct sputum microscopy on three stained sputum specimens. First and third are on spot while the second is the early morning sample (Gothi *et al*., 2004; Steingert *et al*., 2006). Identification of sputum smear-positive patients is crucial for the control of TB as it is estimated that one undiagnosed case of one smear-positive TB patient infects 10-20 contacts over a year period if untreated (Alisijahana *et al*., 2007). With HIV infection the sensitivity of sputum microscopy is greatly reduced because of the lower rate of caseation necrosis and consequent lower numbers of AFB in the airway (Matee...
et al., 2008). In patients who can produce sputum, several studies have demonstrated that sputum acid-fast stain (AFS) after concentration with sodium hypochlorite restores the already lost sensitivity of sputum direct microscopy (Cain et al., 2010). However, many patients with advanced HIV and suspected PTB do not produce sputum spontaneously making the diagnosis difficult. Induction of sputum is an option in this case (Finch and Beaty, 1997; Mfinanga et al., 2007; Morse et al., 2008).

### 2.13.2 Culture

Culture of sputum samples is more sensitive and can detect 10-100 organisms per ml. The increased sensitivity enables detection of cases earlier, often before they become highly infectious. However, *M. tuberculosis* grows slowly with a doubling time of approximately 18 hours. Because of slow growth, cultures of clinical specimens must be held for over a month before they can be recorded as negative (Della-latta, 2004). Media used for culture include among others Lowenstein-Jensen and the BACTEC liquid medium that contains radioactively marked carbon metabolites like palmitic acid. A definitive diagnosis of tuberculosis depends upon isolating the bacilli from the patient and identifying them as *M. tuberculosis* (Della latta, 2004; Coban et al., 2006).

A patient who cannot produce sputum (in the case of pulmonary tuberculosis), gastric aspiration is a method of choice as a specimen for culture. Specimens used for the diagnosis of TB also include cerebrospinal fluid, bone marrow and liver biopsies. Routine urine cultures may be positive in only 7% to 10% of patients. Species identification is dependent initially on growth rates and pigment production classifying *Mycobacterium* species into four groups. Species within these groups can be further identified by biochemical tests. In the BACTEC liquid-culture system, the
NAP test is used to identify the growing organism as *M. tuberculosis*. Each *Mycobacterium* species can also be identified using high-performance liquid chromatography (HPLC) but this is expensive and seldomly used. Sero-diagnostic test kits for anti-tuberculosis antibodies are available commercially, but serious concerns regarding interpretation of results from these tests in the South African setting have been voiced (Della latta 2004; Coban et al., 2005; Coban et al., 2006). In developing countries, Lowenstein-Jensen (LJ) medium is the most commonly used medium for culture of *M. tuberculosis* (MTB) which is also recommended by WHO (American Thoracic Society, 2000; Marthur et al., 2009). Growth in liquid media is faster (1 to 3 weeks) than growth on solid media (3 to 8 weeks). When sputum is inoculated on Lowenstein-Jensen (LJ), microscopic colonies of *M. tuberculosis* appear in 2-6 weeks and a negative culture report cannot be given before eight weeks. Growth tends to be slightly better on egg-based media but rapid on agar media (American Thoracic Society, 2000; Marthur et al., 2009).

### 2.13.3 Sensitivity testing

The commonly used agar proportion method for *Mycobacterium* antibiotic susceptibility testing requires 8 weeks of incubation before a pattern of susceptibility is established for *M. tuberculosis* (Coban et al., 2006). The duration of the assay can be shortened by the use of the BACTEC radiometric system to around 10 days. However, the BACTEC method is expensive and uses radioactivity, which makes it out reach for endemic countries with limited resources. The ability to detect *Mycobacterium* growth by identification of the colour changes associated with the reduction of nitrate to nitrite is the basis for the microplate Alamar Blue drug susceptibility assay (MABA). MABA has been used previously in drug-susceptibility
testing of *M. tuberculosis* to anti-tuberculosis drugs. Very good correlations with the proportional and BACTEC methods have been described. Alamar blue is a resazurin-based oxidation-reduction indicator which measures colorimetric drug MICs for *M. tuberculosis* for up to 7 days. MICs are determined by using serial dilutions of anti-TB drugs in 96-well microtiter plates, broth, and *M. tuberculosis* isolates. It is also less expensive, simpler to execute than the proportion method, and has a high through-put (Gothi and Joshi, 2004). Tuberculosis drugs sensitivity testing is important during this era of emergence of mycobacterium species which are resistant to the currently used anti tuberculosis drugs. Drugs susceptibility in TB patients’ co-infected with HIV/AIDS has been associated with current emergence of MDR-TB. There is need to establish and strengthen the national surveillance for MDR-tuberculosis (Sande and Rothman, 1999).

### 2.13.4 Diagnosis based on molecular techniques

Variable number of tandem repeat (VNTR) typing is a PCR-based technique identifying alleles of defined regions of DNA that contain a variable number of copies of short sequence stretches. Resolution of the method is cumulative and can be increased by inclusion of additional loci. Tandem repeats are easily identified from genome sequence data, measurement of PCR fragment sizes is relatively straightforward and VNTR typing data can be digitalized and compared between different laboratories. Availability of complete genomic sequences has facilitated identification of repetitive genetic elements of *M. tuberculosis, M. bovis* and *M. avium*, including short tandem repeats, designed exact tandem repeats (ETRs) and *mycobacterial* interspersed repetitive units (MIRUs) (Zink et al., 2003). Strain typing with these sets of polymorphic loci is developing into an important tool in the epidemiological
analysis of tuberculosis and ordinary genetic diversity in *M. tuberculosis* isolates agarose gel electrophoretic separation of PCR products is usually sufficient to estimate the number of repeat units in an allele. In a study of *M. bovis* strains from Chad, VNTR typing of a distinct number of loci was most discriminative for strains of the same clone (Zummaraga *et al*., 1999; Barnes and Cave, D., 2007). More recently, MIRUs and other VNTRs have also been described for *M. tuberculosis* and *M. marinum* typing. Most of the described sequences are orthologues of the *M. tuberculosis* genome database and their resolution seems to be comparable to that of the currently most discriminatory methods, the 2426 PCR analysis and IS2404-Mtb2 PCR which discriminate among isolates from different geographical origin, but not among strains from different endemic regions of Africa (Campos *et al*., 2003; Bernard, 2006; Majid *et al*., 2008).

The Xpert MTB/RIF test for use with the Cepheid GeneXpertR System is a semi-quantitative nested real-time PCR *in-vitro* diagnostic test for detection of *Mycobacterium tuberculosis* complex DNA in the sputum samples or sediments prepared from induced or expectorated sputa that are either acid bacilli (AFB) smear positive or negative. It used in the detection of rifampicin resistance associated mutation of the *rpoB* gene in samples from patients at risk for rifampicin resistance (Lawson *et al*., 2008). The MTB/RIF test is intended for use with specimens from untreated patients for whom there is clinical suspicion of tuberculosis (TB). Use of Xpert MTB/RIF for detection of Mycobacterium tuberculosis (MTB) or determination of rifampicin susceptibility has not been validated for patients who are receiving treatment for tuberculosis (Majid *et al*., 2008).
2.14 Immune response in HIV infection

2.14.1 CD4⁺T lymphocyte

Immunohaematological indices such as leukocyte, lymphocytes and their subsets such as CD4⁺T cells and CD8⁺T cells play a major role in both cellular and humoral types of immunity. CD4⁺T cells are the lymphocytes subsets used for monitoring progression of HIV/AIDS infection and they are also used as a surrogate marker for the improvement of HIV/AIDS patients after initiation of ARV (Jones et al., 1993). Further, CD4⁺T cell levels determine when to start or stop prophylactic drugs for opportunistic infections (Miaini et al., 1996). Management of HIV patients include proper monitoring, irrespective of ARV treatment. This monitoring can be done clinically by means of the WHO clinical staging, but more reliably by measuring CD4⁺T cells and viral load. In resource poor countries like Kenya, viral load determination is not done and the only reliable methods for follow up of HIV infected patients are by CD4⁺T cell counts (Upass et al., 2005).

Immunohaematological variations have been reported in various studies showing association with sex, geographical location, race, altitude and diet (Mientjes et al., 1992). Other reasons for variations are pregnancy, age, exercise, comorbid conditions and diurnal variation. Several studies have shown significant variations of CD4⁺T cells within African populations and in Africans compared with the values established for Europe and North American. The HIV virus targets and destroys CD4⁺T cells responsible for the cellular immunity against infections by intracellular microorganisms like M.tuberculosis. Reduction in CD4+T cells results in immunodeficiency which in turn can lead to reactivation of latent tuberculosis or new tuberculosis infection once exposed to M.tuberculosis (Shahabbuddin 1995; Prins et al., 1999; Jannosy et al., 2000; Lawson et al., 2008).
Cell-mediated immunity contributed by both CD4 and CD8 T lymphocytes, plays an essential role in containing acute *M. tuberculosis* infection. The key role of the CD4+T cell in tuberculosis is thought to be its ability to produce the cytokine, IFNγ, which is essential in the control of experimental tuberculosis in mice and is the first identified human immunologic factor essential to resistance against mycobacterial infection. The IFNγ is a critical factor for inducing macrophage synthesis of the enzyme inducible nitric oxide synthase (NOS2). Upon activation, macrophages generate nitric oxide and other reactive nitrogen intermediates (RNIs), the best characterized antituberculous effector molecules in the mouse (Mientjes *et al*., 1992; Sonnenberg *et al*., 2004; Cailhol *et al*., 2005).

Studies done in Tanzania and Cameroon have reported higher CD4+T cell counts as compared to Ethiopia, Botswana and Uganda (Jones *et al*., 1993; Urasa *et al*., 1999), whereas some studies have demonstrated higher CD4+T cells among Ugandans and Kenyans than the values known for North America, Europe and Asia (Lawson *et al*., 2008). These variations in CD4+T cells have been shown to be associated with ethnicity, gender, diet, geographical area, as well as being dependent of genetic and environmental factors (Miaini *et al*., 1996; Menard *et al*., 2003; Sonnenberg *et al*., 2004; Cailhol *et al*., 2005).

### 2.15.1 CD8+T Lymphocytes

Experiments involving adoptive transfer, *in vitro* cell depletion, and gene knock-out (*e.g.* β2-microglobulin deficient animals), have illustrated the importance of CD8+ cells in the control of tuberculosis in mice (Tsegaye *et al*., 2002). Protection of mice vaccinated with Mycobacterial heat shock protein deoxyribonucleic acid (DNA)
appears to be mediated mainly by CD8+T cells. In an in vitro system, this ability to activate CD8+T cells seemed to involve causing leakiness of the phagosome so that antigens reach the cytoplasm and hence join the conventional pathway for presentation on MHC Class I, though another novel pathway may also be involved. A haemolysin-like molecule is in fact expressed by both *M. tuberculosis* and BCG has been developed in the belief that this will increase the CD8+T response (Badri *et al*., 2000; De Cock *et al*., 2000). These CD8+T cells have been shown to be cytotoxic, though the mechanism of this cell killing has been controversial. It has been thought that most cytotoxic T-lymphocytes (CTLs) act to lyse infected cells and allow the released *mycobacteria* to be taken up by activated, uninfected macrophages that may kill them. However, it now appears that some CTLs directly kill *M. tuberculosis* via a granule-associated protein, granulysin, acting with perforin. On the other hand, lysis by CD4+ cytotoxic T cells does not reduce the viability of the contained bacteria.

Progression of tuberculosis in mice deficient in perforin is not different from progression in the wild-type (Miani *et al*., 1996; Whalen, 1997; Prins *et al*., 1999). The major role of murine CD8+ cells at this stage may be the secretion of IFNγ. Recently tuberculosis-specific CD8+ cells have also been identified in humans but their role in this species is equally uncertain. There are CD8+T cells that will recognise TB-infected cells and secrete IFNγ in blood from individuals with the disease, but these did not appear to contribute to control of intracellular proliferation of *M. tuberculosis* in an in vitro system using human cells. Most tuberculosis-specific CD8+T cells recognise their antigens in association with MHC class I, however some are now known to be restricted by other molecules, such as CD1 (Coban *et al*., 2005). It has been found that there is considerable reduction in cell mediated immunity
during TB infection (Upass et al., 2005). Another study documented significant reduction in CD4+ T cells but it was without notable reduction in CD8+ T cells. However, reduction in CD4+ T cells while increase in CD8+ T cells has also been reported. Less than 300 mm$^3$ CD4+ T cells were found associated with poor prognosis (Mientjes et al., 1992; Sonnenberg et al., 2004; Cailhol et al., 2005). Further, CD4+ and CD8+ T cells did not vary at different times of treatment with ATT and there was no significant change in CD4+ and CD8+ T cells between chronic TB patients and healthy control.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The study was conducted in Mombasa County which has a population of 1,031,266 by the year 2012. The population is steadily growing due to rural-urban migration and immigration from unstable countries. Mombasa County lies between latitudes 3° 08' and 4° 10' S and longitudes 39° 60' and 39° 80' E, with a total land mass of 229.6 km² and Waters covering 65Km². The total area Mombasa is 109 Km² with about 60% of the people living overcrowded informal settlements in the form of shelters. Residents are of mixed ethnicity and are engaged in low-income generating activities, mainly informal sector and small trading. The County has rapid population growth and is characterized by low socio-economic indicator. This creates huge demands on health facilities and inability to keep pace with the environment, continued economic prosperity, public health and quality of life of residents. The County is faced with social problems such as drug abuse and poverty. There are beach hotels which attracts tourists leading to high rate of prostitution and drug abuse.

Tuberculosis and HIVAIDS are the leading causes of deaths in the area representing 50%. Mombasa County recorded close to 100,000 new cases of TB in the year 2012 which was attributed to crowding and poor housing in slum areas. Some 600 people in every 100,000 in the County suffer from multi-drug resistant tuberculosis due to non completion of treatment after being misled by religious leaders. The County has eight TB centre’s; Ganjonii clinic, Coast provincial General hospital (CPGH), Mlaleo, Portreitz, Mikindani, Likoni, Nyali, Kisauni. These hospitals were selected because they serve populations at high risk for TB due to high HIV prevalence or social-
cultural practices that favour TB transmission. These hospitals like all others at levels at their levels have chest clinics where TB patients obtain health care respectively.

3.2 Study population

The study population comprised of adult TB suspects (n=500) eighteen years and above. Different medical examinations were done by the hospital physicians including observation of complex symptoms suggestive of TB such as fever, cough of more than two weeks and not responding to antibiotic treatment, night sweats and weight loss. Mombasa County has the highest TB case load compared to other regions in Kenya. In 2012 there were 18,902 (674/100,000) of all forms of TB cases and 10,570 (535/100,000) of the new smear positive TB cases.

A list of all public diagnostic centers in each district was compiled and ranked according to smear positive TB cases diagnosed in 2009. The diagnostic facility in each of the TB zones with the highest number of patients diagnosed in a quarter of a year was selected. This approach was used because the facilities selected would capture most of the TB patients in that particular health district and reach the required sample size within the intake period. Subjects were recruited from newly diagnosed TB suspects, voluntary counseling and testing (VCT) clinics (Normal subjects-controls) and patients not on treatment. New TB patients are those patients who have not taken any anti-TB drugs before. Different Medical examinations were performed by the hospital clinicians including observation of complex symptoms suggestive of tuberculosis.
3.3 Study Design

This was hospital and laboratory based descriptive cross-sectional study carried out between May 2011 to November 2012. It was carried out to provide a snap-shot (one point measurements) description of prevalence of TB, HIV, TB-HIV and anti-TB drugs susceptibility testing and the significance of immunohaematological cells. All measurements for this design were made at one point of time to describe variables and their distribution patterns. It is the only design which gives the prevalence of TB and HIV-co-infection and its risk factors. This was possible in this study when age, gender and HIV infection was examined as predictor variable in TB and HIV co-infection. The design was used as first step in laboratory experiments involving sputum analysis, HIV testing and CD4+T and CD8+T cell counts. The results obtained defined the demographic and clinical characteristics of the study group at baseline and revealed associations of interest and permitted generation of hypotheses. This study design provided prevalence of tuberculosis and TB/HIV co-infection which are important in public health for assessing the burden in Mombasa County and in planning allocating health resources.

3.4 Study variables

3.4.1 Independent variables

The independent variables included age, marital status, gender, education level, occupation, clinical symptoms (fever, cough, weight loss and hemoptysis), clinical signs (pallor, skin infections, lymphadenopathy, oral thrush and respiratory signs), and CD4 + T and CD8 + T cells, packed blood volume, BMI and drug resistant.
3.4.2 Dependent Variables

The dependent variables included tuberculosis, HIV sero-status (HIV positive and negative)

3.5 Inclusion criteria

This included:

1. Those suspected of having TB and resident in Mombasa County for at least one year and 18 years and above and not on anti-TB chemotherapy.
2. Newly diagnosed pulmonary Tuberculosis (smear positive and negative) and extra pulmonary tuberculosis patients as per the case definitions of the National Tuberculosis control program.
3. Those TB suspects who consented to participated in the study.

3.5.1 Case Definitions

**Pulmonary tuberculosis smear-positive:** TB in a patient with at least 2 initial sputum smear examinations (direct smear microscopy) positive for AFB or TB in a patient with one sputum specimen positive for AFB and culture positive for *Mycobacterium tuberculosis*

**Pulmonary tuberculosis smear-negative:** TB in a patient with symptoms suggestive of TB with at least 3 sputum examinations negative for AFB or diagnosis based on positive culture but negative AFB sputum examinations

**Extra-pulmonary tuberculosis:** TB of organs other than the lungs such as pleura (TB pleurisy), lymph nodes, skin, joints, bones and brain. Participants who met case definitions were referred to

i. Interview on duration of symptoms and care seeking
ii. HIV testing, CD4+T and CD8+T cells count, CTX prophylaxis and referral for HIV treatment

iii. Registration with TB program for treatment

4. Those suspected of having TB and on anti-TB chemotherapy

5. Patients who consented to participate in the study

3.6 Exclusion Criteria

Tuberculosis suspects who had not lived in Mombasa County for at least one year, those who were already on anti-TB chemotherapy and those unwilling to participate in the study and not meeting the above inclusion criteria were excluded.

3.7 Sampling procedures

Mombasa County was purposively sampled because of high cases of TB and HIV co-infection. In the year 2011, Coast region had 100,000 TB suspects were diagnosed and 26,500 turned smear positive of which 35.4% were HIV co-infected. Mombasa County had the highest number of 14,750 newly diagnosed TB patients. The sampling frame consisted of all the public health facilities within the study area. The average monthly attendance of the TB clinics in each facility was obtained. Selection of the study sites was then done through purposive sampling of the facilities with the highest average attendance of TB suspects in each administrative unit. After the selection of the study sites, each was allocated a proportionate number of study subjects based on the level of health care delivery system and the average client attendance in the past one month before embarking on the study. To minimize bias in selecting study subjects, consecutive sampling was used hence every alternate TB suspect who satisfied the inclusion criteria was selected for the study. Immediately after selection, the respondents were referred to the clinicians and nurses running chest clinics for
interview. A semi-structured pretested questionnaire was used to collect information on demographic characteristics and other relevant variables. Data collected included age, gender HIV status and marital status. Six (6) nurses who are trained in counseling filled the questionnaires after doing physical examination and taking medical history of the TB suspects. Each patient was given a code having the first two alphabets from name of their corresponding health facility and a number that goes from 001 to 999. This code was unique for each sampled patient enrolled in the study. Blood and sputum samples were collected from those who consented by qualified laboratory technologists registered by Kenya Medical Laboratory Technicians and Technologists Board (KMLTTB). HIV antibody testing and CD4+T cell counts were also performed for all eligible patients. After post-test counseling, those who tested HIV positive were referred to the hospitals antiretroviral therapy (ART) clinic with their CD4+T cell result count for further management.

### 3.8 Sample size determination

The sample size was arrived at by calculation using *Fisher et al.* (1983) standard formula as shown below.

\[
N = \frac{Z^2pqD}{d^2}
\]

Where

- \(N\) = minimum required sample size when target population was greater than 10,000
- \(Z\) = standard normal deviate (1.96) which corresponds to 95% confidence level.
- \(p\) = proportion of the target population estimated to have TB and HIV/AIDS was assumed to be 50% (0.5) to yield highest sample size.
\[ q = 1 - p \] (proportion in the target population that do not have TB and HIV/AIDS=0.5 (1-p))

\[ d = \text{degree of precision desired} = 0.05 \]

\[ D = \text{design effect} = 1 \]

Thus,

\[ N = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 385 \]

Sample size=384. 30% of this number was added for attrition to make 500.

*Sample size for the healthy subjects was 500 and was chosen conveniently.

### 3.9 Pilot Study

To test the reliability of the questionnaires and validity of results, pre-testing was conducted with thirty (30) TB suspects. Piloting was done in one of the health facilities (Ganjoni Clinic) which was subsequently excluded from the survey. The pre-test yielded a few sequencing issues that were corrected in the final tool. The collected data was checked for completeness, clarity and consistency by supervisors and principal investigator. The pre-test results were analyzed for reliability by using Cronbachs Alpha coefficient (0.587 and 0.631).

#### 3.9.1 Validity

This was established through standardization of conditions under which the study was carried on. Careful planning and execution of the study was done through piloting. Quality control of the smear, microscopy, and culture and drug susceptibility was performed.
3.9.2 Reliability
Reliability is concerned with consistency, accuracy, dependability and comparability of a measuring technique and refers to how consistent or stable the data collection is. In this study, the test-retest assessed the stability and Spearman-Brown’s prophecy formula tested the internal consistency or reliability of tools. Sputum specimen for culture and drug sensitivity testing were sent to Central reference Laboratory (CRL) for analysis. The laboratory is located within the centre for Respiratory Diseases (CDR-KEMRI) at Kenyatta National Hospital. All blood samples for immunohaematological cells analysis (CD4+T and CD+T cells, HB and Total blood count) were done at the University of Washington Research laboratory based at Coast Provincial General Hospital (CPGH). The laboratory has modern BD FACS Count cytometer (Becton Dickson immunocytometry system) and Sysmex Kx-21 (Sysmex corporation; Kobe Japan).

3.10 Data collection procedures
Data collectors were six (6) clinicians, laboratory technologists and nurses who are based in health facilities where the study was done. They were briefed on the aims and objectives of the study and data collection tools.

3.10.1 Collection of demographic data
Counseling and collection of demographic data were done by clinicians/nurses running the adult TB clinics. A structured and pre-tested questionnaire was used to obtain the demographic data. The data collected included age, gender and HIV status.
Quality control: The investigator was responsible for overall co-ordination and conducting of research. The questionnaires were pre-tested and administered by trained research assistants in order to reduce errors. There was regular checking of filled questionnaires for completeness and appropriateness (Appendix 2).

3.10.2 Sputum collection and processing

All tuberculosis suspects attending care and treatment clinics were counseled about the study and those who consent asked to give sputum samples for smear microscopy and culture. The patients were advised to rinse their mouth twice with water before producing the specimen and this helped to remove food and any contaminating bacteria in the mouth. They were instructed to take two breaths, coughed vigorously and expectorated the material in into the sterile 50 ml blue cap screw-capped bottle. This process allowed sputum to be produced from deep in the lungs. The TB suspects were asked to hold the sputum container close to the lips and spit into it gently after a productive cough. Three sputum specimens (spot, early morning, spot) were collected from five hundred TB suspects under the supervision of trained and competent medical staff. At the peripheral laboratory, the standard Acid-fast (AFB) direct smear microscopy using Ziehl-Neelsen (ZN) staining was done on the initial sputum to confirm TB diagnosis of suspected patients. A second sputum specimen was then collected which was refrigerated at 4°C and transported to the Central reference Laboratory (CRL) weekly for culture, identification of isolates and drug sensitivity testing. The central reference laboratory is located within the Centre for Respiratory Diseases Research, Kenya Medical Research Institute (CDR-KEMRI)( at Kenyatta National Hospital. Samples were processed within seven days of collection in order to minimize loss of viability of the mycobacteria. Specimens were processed by a standard procedure using sodium hydroxide (NaOH) N-acetyl-L-Cystine (NALC).
Sputum specimens were processed in batches of 16 and only one tube at a time was uncapped for addiction of solutions. Buffer solution was prepared as individual aliquots in single use tubes and opened five minutes after centrifugation at 3000xg for 15 minutes. After centrifugation, it was examined by using fluorescent microscopy and cultured on slopes of Lowenstein Jensen media (Appendix 4).

Quality control: Patients received instructions from trained laboratory technologists prior to collection of sputum. Sputum was collected in containers that were clean, sterile, screw capped, transparent and labeled with patient’s details. Strict attention was paid to quality specimen-sputum rather than saliva or nasal secretions. This was checked before forwarding specimen to the laboratory accompanied by complete request forms. The samples were refrigerated at 4°C awaiting transportation in cool boxes to the Central reference laboratory (CRL) for culture and drug sensitivity testing on first line anti-TB drugs. This was done to avoid exposure to extreme environmental conditions (heat).

3.10.3 Microscopic examination of sputum specimens

Smear was prepared on clean, unused glass slide labeled with laboratory number. There was only one smear per slide and a swab stick or loop was used to collect representative portion of the sample for smearing. After drying, fixation was done by gently heating. The numbers of slides were placed on staining rack with the smeared side facing up. Finger thick gap was left between sides to prevent further transfer of material from one smear to another and solutions running away from slides. The entire slides were flooded with Ziehl-Neelsen (ZN)1% carbolfuchs in solution. Each slide was heated slowly until steam arises. Steaming was maintained for 5 minutes by
using intermittent heat. Each slide was rinsed individually in a gentle flow of water until all free stain was washed away. Excess rinsed water was drained off by tilting the slide. The slide was then flooded with the decolourizer solution (2% sulphuric acid) for 3 minutes. The slide was thoroughly rinsed with water and excess water drained off from the slide. Back of the slide was wiped off with cotton soaked in decolourizer to clean the dried stains, rinsed with water and excess drained by tilting. The slide was flooded with 0.1% methylene blue and counter stained for 60 seconds. The slide was rinsed with water and excess drained off by placing the slide under gentle stream of running tap water and left standing on holding block to allow smear air dry. Sputum smears were examined for acid-fast bacilli (AFB) after staining following ZN method. The degree of ZN smear positivity was quantified as 1+ for 10-100 AFBs per 100 fields, 2+ for 1-10 AFBs per field (50 fields) and 3+ for >10 AFBs per field (20 fields). For less than 10 AFBs per 100 fields, the exact number of AFBs was indicated. A suspect was considered to be ZN smear positive if at least one specimen was positive (Appendix 4)

**Quality control:** Quality control for AFB-smear microscopy was done in a routine series by using known positive and known negative samples. An experienced microscopist read an arbitrary 10% positive and 10% negative slides randomly selected with concordance of 99% and 97% respectively. This was done by using recommended sputum collection, handling and processing procedure. Validity was also achieved by using newly prepared and pre-tested reagents. All positive and negative smear slides were read blindly by at least two trained laboratory technologists. The smears with discordant results were confirmed as positives or negatives using MGIT cultures.
3.10.4 Isolation of *Mycobacterium tuberculosis*

Central reference laboratory (CRL) is the only public laboratory which does sputum culture. Primary culture of *M. tuberculosis* was performed on egg based solid culture media, Lowenstein-Jensen (LJ). In a class II biological safety cabinet (BSC), 10ml of the specimen was transferred to sterile 50ml conical polypropylene screw-cap centrifuge tube. An equal volume NALC-NaOH solution was also added to decontaminate it to eliminate the associated commensal flora. The caps of the tubes were tightened and mixed well by inverting them to ensure NaOH solution contacts all the sides and inner portions. The decontaminated sputum specimens were then homogenized by vortexing and then centrifuged for 15 minutes at 300 revolutions per minute (rpm). McCartney tubes were carefully removed from the centrifuge without shaking and supernatant fluid discarded slowly into container with 5% phenol solution. The pellet was washed with sterile distilled water at 3000xg for 15 minutes and the supernatant discarded. Using sterile 1ml disposable pipette, 0.5ml of the sediment obtained after centrifuging was inoculated on two Lowenstein Jensen (LJ) slopes/ or MGIT, one with glycerol (0.75%) and one without glycerol, but supplemented with 0.5% pyruvate. The caps were labeled with lab serial number of the specimen and named as 1 and 2. All Lowenstein-Jensen media slopes were incubated at 37°C. The slopes were examined weekly for any visible growth and negative culture tubes discarded after 8 weeks.

**Quality Control:** The quality of the LJ medium to grow *Mycobacteria* was checked by inoculating media with *Mycobacterium gordoneae* a rapidly growing species. Growth of this organism was checked for 3-4 days. The color of the media was checked before using it for culture and only those media with appropriate color (light
green) were chosen. Media with a color between deep green to blue were frequently observed to give negative cultures with further color changes during incubation. This was believed to be due to the acidic pH of the media. Therefore careful adjustment of the inoculum pH (7.2-7.4) was mandatory to avoid overwhelming the already diminished buffering capacity of the media.

3.10.5 HIV diagnosis among newly diagnosed tuberculosis patients

Diagnosis of pulmonary tuberculosis was based on smear positive PTB results before enrolment into the study. Diagnosis was also done according to the national guideline which requires that at least two out of three sputum samples must show acid-fast bacilli by Ziel-Neelsen (ZN) staining. Smear negative cases were based on the clinical and radiological diagnosis according to the algorithm for diagnosis of smear negative tuberculosis. All newly diagnosed tuberculosis patients were counseled about the study and a single sputum sample collected from each patient for microscopy, culture and drugs susceptibility testing. HIV counseling and testing was done for those who consented. Blood were samples was tested for HIV antibodies according to the Kenyan 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 H1/2 (Trinity Biotech, Ireland) rapid test kits. Test was also done according to manufacturer’s instruction. Blood samples were collected in ethylene diamine-tetra-acetic acid (EDTA) tubes and used for HIV test, complete blood cell (CBC) counts and for CD4+T cell counts (Appendix 7).
Quality control: The quality control (QC) for HIV rapid test was performed according to the recommendations of the kit manufacturers (Abott laboratories, Japan co. LTD), Capillus HIV1/2 (The trinity Biotech, Ireland) and Unigold H1/2 (Trinity Biotech, Ireland) rapid test kits. 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.

3.10.6 CD4+T and CD8+T cell counts

CD4+T cells were analyzed using a BD FACSCount flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) with two monoclonal antibodies (aCD4 and aCD8; Becton Dickinson Immunocytometry Systems). The puncture area was identified, swabbed with alcohol and blood sample collected by a well trained phlebotomists. Blood samples were collected by venipuncture using a 10ml syringe and 21g needle which were all sterile. Whole blood samples were collected in 4.5ml BD K2E vacutainer (lavender cap) and mixed adequately with the EDTA by gently inverting up and down for at least ten times to avoid clotting. Blood was processed within 24hrs after collection. The test vial was vortexed upside down and then upright for 5 seconds each to ensure even mixing. Fifty microliter (50ul) of blood was added to the vial using the back pipetting technique with an electronic pipette and vortexed again for 5 seconds before incubating in the dark for a period of 1 hour at room temperature. At the end of the incubation period, the product was fixed with a 50µl fixative solution and vortexed to ensure even mixing. The reagent tubes were placed onto the holder beneath the probe and allowed the machine to read the CD4+ sample. The tube came down automatically after reading is complete. A print
out was automatically issued from FACSCout machine upon completion of sample analysis (Appendix 8).

**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+T result was valid when the sum of CD4+T and CD8+ T cells was within the range of + 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.

### 3.10.7 Complete blood cell counts

Complete blood cell counts were done using Sysmex Kx-21 (Sysmex Corporation; Kobe Japan). The machine automatically dilutes a whole-blood sample, lyses, counts and gives a printout result of absolute numbers of leucocytes (expressed as number of cells × \(10^9\) per litre), erythrocytes (number of cells × \(10^{12}\) per litre), platelets (number of cells × \(10^9\) per litre), lymphocytes (number of cells × \(10^9\) per litre), mononuclear cells (number of cells × \(10^9\) per litre), granulocytes (number of cells × \(10^9\) per litre) and haemoglobin (grams per decilitre). Three (3ml) of venous blood was drawn aseptically from anterior cubital vein and added into two vacutainers BD, each containing ethylene diamine-tetra-acetic acid (EDTA). All samples were processed within four hours and analyzed the same day. Lyse-wash sample preparation method using whole blood was performed. 100µl of whole blood of each
subject was placed into two FACS tubes. 10µl of each of CD4-FTC, CD8-PE and CD45-PercP monoclonal antibodies were added to one tube and isotype control to the other. Tubes were mixed and incubated in dark at room temperature for 15 minutes. Two millimeters (2ml) of BD FACS Lyse (<15% formaldehyde and <50% diethyl glycol was diluted 1:10 in de-ionized water immediately before use) was added to each tube. Tubes were re-incubated in dark for 12 minutes, centrifuged at 250g for 10 minutes and supernatant was discarded. The pellet was broken down and cells were washed twice by adding two (2ml) of sheath fluid, mixed, centrifuged and supernatant discarded. Cells were re-suspended in 0.5ml of the sheath fluid with 2% paraformaldehyde. The cells were analyzed with Sysmex Kx-21 (Sysmex Corporation Kobe Japan). The quality and accuracy of the technique and the machine was assessed every six month by sending the samples to the regional laboratory for the comparison reading. The machine was serviced by the designated engineer from the Sysmex company.

3.10.8 Healthy subjects (controls)

This group was recruited from the HIV voluntary counseling and testing (VCT) clinics. Individuals who tested negative during VCT visits were counseled about the study and those who agreed were included in the study. Subjects were interviewed, using a structured questionnaire, and screened for symptoms such as fever, cough and weight loss to rule out any recent and/or current infections. Blood slide for malaria, blood sugar and rapid plasma regain (RPR) test for syphilis were done for all participants in addition to a physical examination, measurement of height and weight. The following categories were excluded from this group: pregnant women, smokers,
patients receiving medical treatment, chronic alcoholism and moderate and severe malnourished, patients with malaria and those who tested positive for HIV antibody

3.10.9 Drugs sensitivity testing of *Mycobacterium tuberculosis*

Drug susceptibility of all isolates to isoniazid (INH), rifampicin (RIF), streptomycin (STR) and ethambutol (EMB) was performed by standard method. Lowenstein Jensen (LJ) Media with drug incorporated in various concentrations (0.2µg/ml, 5µg/ml, 40µg/ml and 2µg/ml respectively were used. Plain Lowenstein Jensen media for control was also prepared. The sputum specimens were first decontaminated using 4% sodium hydroxide (NAOH) solution to eliminate the associated commensal flora. The decontaminated sputum specimens were then homogenized by vortexing and then centrifuged for 15 minutes at 300 revolutions per minute (rpm). On the drug free medium $10^3$ *Mycobacterium* suspension was inoculated followed by $10^1$ *mycobacterium* into drug containing medium and incubation done at 37°C. The slopes were examined weekly for any visible growth.

A positive culture of *M. tuberculosis* confirmed the diagnosis of active TB disease. Strains of MTB complex from Lowenstein Jensen (LJ) slopes were subjected to drug susceptibility testing (DST) to first line anti-TB drugs using the *Mycobacterium* Growth Indicator tube (MGIT) method. Five MGIT tubes were labeled for each test culture one for growth control without drug, one for Streptomycin, Isoniazid, Rifampicin and Ethambutol each. A loopful of colonies was harvested from the LJ slants, suspended in 1ml sterile saline and vortexes to break the large clumps. The suspension was adjusted to a standard 0.5 McFarland turbidity by visual comparison. 0.5ml of the well-mixed culture suspension (inoculum) was aseptically added into
each drug containing tubes using a pipette. For the control, test culture suspension was first diluted 1:100 by adding 0.1ml of the test culture to 10.0ml of sterile saline and mixed by inverting 5-6 times. This diluted solution was added to 0.5ml of growth control tube. The caps of the tubes were tightened and the inoculated broth mixed well by gently inverting the tube several times. The drug containing and drug-free growth control MGIT tubes which were inoculated with the standardized 0.5 McFarland inoculums of the *M. tuberculosis* isolate were entered into MGIT 960 automated machine in a special rack-carrier with printed barcode. This was read by the machine when entering the tubes to identify the test and apply the adequate algorithm for susceptibility or resistance interpretation. The instrument monitored the entered susceptibility test set and when the test was complete (within 4-21 days), it indicated that the results were ready. All readings were performed inside the machine and results printed as susceptible or resistant for each drug.

**Quality control:** Each new batch of reagents, SIRE drugs or MGIT medium was used. *Mycobacterium tuberculosis* H37RV [ATCC-American Type Culture Collection] number 27294 which is susceptible to all anti-tuberculosis drugs was used as a quality control (QC) strain. The inoculums used in this study were from freshly grown culture in Lowenstein-Jensen slant.

### 3.11 Identification of MTB complex strains by PCR (Xpert) MTB/RIF

Extraction of chromosomal DNA (mini preps) was done in a class II biological safety cabinet (BSC). The primers P1 (forwards 5’) and P2 (reverse 5’) amplify a 123 bp region of the IS6110 was used. *Mycobacterium tuberculosis* DNA (2.5µg/l) was amplified in a 2.5µg/l reaction containing 2.5Mm MgCl₂, 200µM nucleoside
triphosphates, 25pmoles of each primer and 1 unit of Taq polymerase in 1X PCR Buffer II 50mM KCL, 10mM Tris-HCL pH 8.3). The cycling parameters was held 95°C for 4 min, followed by a 30 cycle PCR comprised of denaturation at 94°C for 30s, 68°C for 30s, and 72°C for 30s and 72°C for 10 minutes.

The Gene-expert MTB./RIF cartridge was labeled with sample (sputum) identification number and 0.5ml of the total re-suspension pellet was transferred to a conical screw capped tube for Xpert MTB/RIF using a sterile pipette. This was incubated for 15 minutes at room temperature. Samples were liquefied with non visible clumps of sputum. The cartridge lid was opened and sample transferred into the open port of the Xpert MTB/RIF liquefied cartridge. The cartridge was closed and the remaining liquefied sample kept for not more than 12 hours at 2-8°C for any repeat test.

**Quality Control:** Each test includes a Sample Processing Control (SPC) and probe check (PCC). Sample Processing Control ensures the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB. The SPC verifies lysis of MTB that has occurred if the organisms are present and whether specimen processing is adequate. Additionally, this control detects specimen associated inhibition of the real-time PCR assay. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result is "Invalid" if the SPC is not detected in a negative test.
3.12 Ethical consideration

The proposal for this study was approved by Kenyatta University Ethical Review Committee (No PKU 018/115 of 2011). It was approved by the ministry of education, Science and Technology (MOEST). Clearance was also obtained from respective district health authorities and hospital administrations. Informed consent was obtained from the subjects before they were enrolled into study. The purpose of this study was explained to the subjects in English and Kiswahili. Code numbers rather than names were used to identify them in order to maintain confidentiality. The study did not expose subjects to any unusual risks as competent hospital staff obtained sputum and blood specimens from them using standard procedures. The subjects were free to withdraw at any time without penalty. For those who decline to participate in the study, their views were respected and did not influence the quality of care given to them. The study was conducted in accordance with the declaration of Helsinki.

3.13 Data management and analysis

Demographic data were confidentially obtained from the TB suspects by clinicians / nurses running the chest clinics. Results of ZN smear microscopy, culture, and HIV tests were confidentially sent to the respective clinicians / nurses. Provisions of these data were made available to the clinicians / nurses for the purpose of managing the patients. Data was recorded on questionnaires, register books, ELISA reader printouts, MGIT incubator printouts, and species evaluation sheets. The data was coded, entered into MS Excel 8.0 and processed using a statistical package for social sciences (SPSS) version 16.5 software for windows. Normal distributed values were presented as mean with standard deviation (SD) and others expressed as median with interquartile range (IQR). Chi-square test was used to quantify correlation between
dichotomous variables and Spearman’s correlation coefficient to quantify correlations between continuous variables. Student t-test compared medians and means respectively. P-value < 0.05 was considered as statistically significant. Predictive factors for HIV in TB patients examined by logistic regression. The median CD4+T and CD8+T cells level at which TB disease appear for both HIV negative and positive with different clinical TB type patients were calculated. Univariate odds ratio (OR) with 95% confidence intervals (CI) were calculated to assess risk factors (gender and age-group) with regard to tuberculosis and HIV co-infection. Logistic regression was used to analyze multivariate data.
CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Socio-demographic characteristics of the TB suspects

4.1.1 Age and Gender distribution

The ages of the tuberculosis (TB) suspects enrolled into the study were 18 years and above. In this study, 43.2% of the tuberculosis suspects were in the 25-34 age bracket, followed by those in the 35-44 (24.4%), 45-54 (9.8), 18-24 (19.2%) and 55+ (3.4%) age categories respectively. The combined mean age was 32.79 ± 9.75. The mean age was 32.75 ± 9.64 for males and 32.84 ± 9.90 females (Table 4.1).

4.1.2 Education levels of the respondents

Most respondents (53.2%) had attained some secondary school level education. One hundred and fifty nine (31.8%) had attended college level education, seventy three (14.6%) primary level and two (0.4%) reported never having attended any formal school.

4.1.3 Occupation status of the respondents

Most respondents were self employed (29.2%). One hundred and twenty five (25%) jobless, Government employees 24.2%, others 9.4%, house wife 6.6%, farmers 4.8% and students 0.8%.

*Other occupations include daily laborers, beggars and house hold servants.

4.1.4 Respondents’ marital status

Two hundred and eighty-one (56.2%) of the respondents were married, 38.4% unmarried, 4.2% widowed and 1.2% were divorced.
Table 4.1: Socio-demographic Characteristics of the TB suspects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Groupings</th>
<th>Female (n=229)</th>
<th>Male (n=271)</th>
<th>Total (n=500)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in Years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 – 24</td>
<td></td>
<td>44 (19.2%)</td>
<td>52 (19.2%)</td>
<td>96</td>
</tr>
<tr>
<td>25 – 34</td>
<td></td>
<td>101 (44.1%)</td>
<td>115 (42.4%)</td>
<td>216</td>
</tr>
<tr>
<td>35 – 44</td>
<td></td>
<td>53 (23.1%)</td>
<td>69 (25.5%)</td>
<td>122</td>
</tr>
<tr>
<td>45 – 54</td>
<td></td>
<td>21 (9.2%)</td>
<td>28 (10.3%)</td>
<td>49</td>
</tr>
<tr>
<td>55 +</td>
<td></td>
<td>10 (4.4%)</td>
<td>7 (2.6%)</td>
<td>17</td>
</tr>
<tr>
<td><strong>Mean age</strong></td>
<td></td>
<td>32.84 ± 9.90</td>
<td>32.75 ± 9.64</td>
<td>32.79±9.75</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>College</td>
<td></td>
<td>77 (33.6%)</td>
<td>82 (30.3%)</td>
<td>159</td>
</tr>
<tr>
<td>No Education</td>
<td></td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
<td>2</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>39 (17.0%)</td>
<td>34 (12.6%)</td>
<td>73</td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td>112 (48.9%)</td>
<td>154 (56.8%)</td>
<td>266</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td></td>
<td>2 (0.9%)</td>
<td>4 (1.5%)</td>
<td>6</td>
</tr>
<tr>
<td>Married</td>
<td></td>
<td>122 (53.3%)</td>
<td>159 (58.7%)</td>
<td>281</td>
</tr>
<tr>
<td>Unmarried</td>
<td></td>
<td>89 (38.9%)</td>
<td>103 (38.0%)</td>
<td>192</td>
</tr>
<tr>
<td>Widowed</td>
<td></td>
<td>16 (7.0%)</td>
<td>5 (1.9%)</td>
<td>21</td>
</tr>
<tr>
<td><strong>Employer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government</td>
<td></td>
<td>55 (24.0%)</td>
<td>66 (24.4%)</td>
<td>121</td>
</tr>
<tr>
<td>Jobless</td>
<td></td>
<td>59 (25.8%)</td>
<td>66 (24.4%)</td>
<td>125</td>
</tr>
<tr>
<td>House wife</td>
<td></td>
<td>6 (2.6%)</td>
<td>27 (10.0%)</td>
<td>33</td>
</tr>
<tr>
<td>Others*</td>
<td></td>
<td>13 (5.7%)</td>
<td>34 (12.6%)</td>
<td>47</td>
</tr>
<tr>
<td>Farmer</td>
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<td>15 (6.6%)</td>
<td>9 (3.3%)</td>
<td>24</td>
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<tr>
<td>Self Employed</td>
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<td>79 (34.5%)</td>
<td>67 (24.7%)</td>
<td>146</td>
</tr>
<tr>
<td>Student</td>
<td></td>
<td>2 (0.9%)</td>
<td>2 (0.7%)</td>
<td>4</td>
</tr>
</tbody>
</table>

*Other occupations include daily labourers, commercial sex workers and house hold servants

4.2 Prevalence of tuberculosis in newly diagnosed TB patients.

A total of 500 participants suspected of having tuberculosis (TB) were enrolled into the study at the four study sites. 54.2% males and 45.8% females. The calculated overall TB prevalence in tuberculosis suspects included in the study was 42.0% (210/500) and was higher in females (45.9%) than males (38.7%) which was statistically significant (p<0.05). Higher TB prevalence rate was observed in the age group 45-55 (59.2%) followed by the age group 55 and above (58.8%). Lowest prevalence (30.2%) was observed in the group 18-24 years (p<0.05). A comparison
was made between the educational status of the subjects and outcome of sputum test for AFB. The results indicated that there were more sputum smear positive cases among those with no formal education 100% (OR=2.60; 95% CI: 1.63-4.31, p<0.05), primary level 67% (OR=1.31; 95% CI: 1.51-3.20, P<0.05), College leavers had the least with 32.7% (OR=0.72; 95% CI: 0.25-1.42, p<0.05). This finding was found to be statistically significant ($\chi^2=27.66$, df=3; p< 0.05). Another parameter that was compared was occupational status of the subjects and outcome of sputum examination. High prevalence rate was among self employed 60.3% (OR=2.14; 95% CI: 1.5-4.02, p<0.05), followed by other occupations (42.6%), house wives 33.3% (OR=0.84; 95% CI: 0.45-1.63) and the lowest prevalence (29.2%) was observed among farmers (p<0.05). This finding was also found to be statistically significant ($\chi^2=32.76$, df=6; p< 0.05). Regarding marital status, higher prevalence rate was observed among the widowed patients 61.9%, divorced 50%, married 42.3% and unmarried 39.1%. There was no significant difference between marital status and TB infection ($\chi^2=4.26$, df=3; p>0.05). The unadjusted OR showed that widowed (OR=2.35; 95% CI: 1.15-4.84) and divorced (OR=3.37; 95% CI: 1.57-5.52) were statistically significant risk factors for tuberculosis (p<0.05) (Table 4.2).
Table 4.2: Prevalence of tuberculosis among newly diagnosed TB patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Attribute</th>
<th>Number of TB suspects</th>
<th>TB Cases</th>
<th>TB Prevalence (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in Years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>18 – 24</td>
<td>96 (19.2%)</td>
<td>29</td>
<td>30.2</td>
<td></td>
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<tr>
<td>25 – 34</td>
<td>216 (43.2%)</td>
<td>81</td>
<td>37.5</td>
<td>$\chi^2_{(4)=18.395}$, $\rho=0.001$</td>
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<tr>
<td>35 – 44</td>
<td>122 (24.4%)</td>
<td>61</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 – 54</td>
<td>49 (9.8%)</td>
<td>29</td>
<td>59.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55 +</td>
<td>17 (3.4%)</td>
<td>10</td>
<td>58.8</td>
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<td><strong>Education level</strong></td>
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<tr>
<td>College</td>
<td>159 (31.8%)</td>
<td>52</td>
<td>32.7</td>
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<tr>
<td>No Education</td>
<td>2 (0.4%)</td>
<td>2</td>
<td>100</td>
<td>$\chi^2_{(3)=27.660}$, $\rho=0.003$</td>
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<tr>
<td>Primary</td>
<td>73 (14.6%)</td>
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<td>Secondary</td>
<td>266 (53.2%)</td>
<td>107</td>
<td>40.2</td>
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</tr>
<tr>
<td><strong>Gender</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>271 (54.2%)</td>
<td>105</td>
<td>38.7</td>
<td>$\chi^2_{(1)=2.573}$, $\rho=0.109$</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>229 (45.8%)</td>
<td>105</td>
<td>45.9</td>
<td></td>
<td></td>
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<tr>
<td><strong>Marital Status</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>6 (1.2%)</td>
<td>3</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>281 (56.2%)</td>
<td>119</td>
<td>42.3</td>
<td>$\chi^2_{(3)=4.267}$, $\rho=0.234$</td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>192 (38.4%)</td>
<td>75</td>
<td>39.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>21 (4.2%)</td>
<td>13</td>
<td>61.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Employer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government</td>
<td>121 (24.2%)</td>
<td>40</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jobless</td>
<td>125 (25.0%)</td>
<td>41</td>
<td>32.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>House wife</td>
<td>33 (6.6%)</td>
<td>11</td>
<td>33.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others*</td>
<td>47 (9.4%)</td>
<td>20</td>
<td>42.6</td>
<td>$\chi^2_{(6)=32.764}$, $\rho=0.012$</td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>24 (4.8%)</td>
<td>7</td>
<td>29.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>146 (29.2%)</td>
<td>88</td>
<td>60.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Students</td>
<td>4 (0.8%)</td>
<td>3</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall prevalence (%)</strong></td>
<td>500</td>
<td>210</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Other occupations include daily labourers, commercial sex workers and house hold servants; Yates corrected chi-square test for categorical variables; P-value=level of marginal significance

In this study TB prevalence rate among newly diagnosed TB suspects was 42% and it significantly affected females 45.9% than males 38.7%. Higher TB prevalence was observed in the 45-54 years. These results differ with the division of leprosy, TB and Lung disease (DLTLD) annual reports of 2008, 2009 and 2010, where the 25-34 year age group had the highest tuberculosis infection (DLTLD 2008, 2009 and 2010). Age
has been shown as a risk factor in increasing TB incidence and other studies have shown that females are more susceptible to tuberculosis than men probably due to the effect of female hormones or under-reporting of TB cases (Sonnerberg et al., 2004; Caihol et al., 2005). In this study -lower prevalence rate of 30.2% was observed in the age group of 18-24 years which was statistically sufficient (p<0.05). The Kenya demographic survey and health estimated that fifty percent of adolescents in Kenya are infected with Tuberculosis (ROK 2010). The annual increase in the number of tuberculosis cases in the last 10 years has been above 18% (DLTLD, 2010). These statistics show more males are infected than females in the ratio of 1.6:2 (WHO 2010). High estimates of tuberculosis incidence accounting for up to 34% were reported in South Asia, 31% in Africa, 22% in West Specific, 6% in Eastern Mediterranean 5% in Europe and 4% in America have been reported. (WHO, 2009 and 2010). The significantly high rate of TB (42%) observed in the current study compared to the national standards of 20-25% reported by the DLTLD is worth noting. The difference could be attributed to regional variations of TB disease rate since the national reports cover the whole country with some regions have low disease rate. It may also be attributed to challenges faced by the DLTLD in data collection countrywide which may lead inaccurate data collection.

4.2.1 Prevalence of TB by gender and age

The majority of the TB cases (38.6%) were aged between 25-34 years (OR=58; CI 0.342 – 0994) followed by age group 35-44 years 29.1% (OR=68; CI 0.354 - 1.323), 18-24 and 45-54 (13.8 %). Lowest was 55+ years with 4.8 % and 50% (OR=4.6; CI 0.903 - 23.137). The patients in the age group 35-44 years were 2.68 (OR) times more likely to be sputum positive with p=0.031. Patients in the age bracket 45-55
years are 0.87 (OR) times more likely to contact TB with p=0.794 compared to other age groups. There were no significant differences in TB rate and gender but females in age group 35-44 years being more vulnerable (OR=2.88; CI: 0.354-1.32, p<0.05) as shown in (Table 4.3).

Table 4.3: TB and Gender-Age distribution

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>Female (n=105)</th>
<th>Male (n=105)</th>
<th>Total (n=210)</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 – 24</td>
<td>14 (13.3%)</td>
<td>15 (14.3%)</td>
<td>29 (13.8%)</td>
<td>0.58 (0.342–0.994)</td>
<td>0.048*</td>
</tr>
<tr>
<td>25 – 34</td>
<td>44 (41.9%)</td>
<td>37 (35.2%)</td>
<td>81 (38.6%)</td>
<td>1.00**</td>
<td>-</td>
</tr>
<tr>
<td>35 – 44</td>
<td>30 (28.6%)</td>
<td>31 (29.5%)</td>
<td>61 (29.0%)</td>
<td>2.68 (0.354 - 1.323)</td>
<td>0.031*</td>
</tr>
<tr>
<td>45 – 54</td>
<td>12 (11.4%)</td>
<td>17 (16.2%)</td>
<td>29 (13.8%)</td>
<td>0.87 (0.306 - 2.476)</td>
<td>0.794</td>
</tr>
<tr>
<td>55 +</td>
<td>5 (4.8%)</td>
<td>5 (4.8%)</td>
<td>10 (4.8%)</td>
<td>4.57 (0.903 - 23.137)</td>
<td>0.066</td>
</tr>
</tbody>
</table>

OR=Odds ratio; CI=confidence interval; P-value=level of marginal significance; *statistically significant association; **1.00=Reference exposure

Age was an important factor in this study with 79% of the patients being under 45 years and only 21% above the age of 45 years. In an editorial report, TB incidences were associated with young adult age which creates a main obstacle towards a sound economic and social development of countries where TB is endemic (Cailhol et al., 2005). In south Africa tuberculin skin test increased from 28% in 5-10 years old to 88% in 31-35 years old (Wood et al., 2010), while in Gambia, 90% of the patients were below the age of 49 years (Lienhardt et al., 2005). In Pakistan, only 24% of the prisoners with pulmonary TB were above the age of 42 years (Brouwer et al., 2006).

In this current study, there was no association between TB infection and gender but women were more vulnerable than men (p>0.05). In France, males sex were highly associated with TB infection (Dale et al., 2005) while in Russia 72% of the patients were male (Dye et al., 2009). WHO report on TB incidences and gender show that the rates are higher in males at all ages except in childhood and notes that the sex
differential prevalence begin to appear 10 to 16 years of age but reasons for this difference is poorly understood and need further investigation (WHO, 2010).

4.2.2 Proportions of diagnosed clinical tuberculosis types by age

Two hundred and two patients (96.2%) were suffering from pulmonary tuberculosis while eight (3.8%) had extra pulmonary (EPTB). The majority of the pulmonary tuberculosis cases were in the age group 25-34 years (39.6%) followed by age groups 35-44 (28.2%), 18-24 (14.4%) and 45-54 (13.4 %). Among the extra pulmonary tuberculosis (EPTB) patients 50.0% were in the age group 35-44 years and 25% in 45-54 years (Table 4.4 below).

Table 4.4: Proportions of diagnosed clinical tuberculosis types by age

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>EPTB (n=8)</th>
<th>PTB (n=202)</th>
<th>Total (n=210)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 – 24</td>
<td>0 (0.0%)</td>
<td>29 (14.4%)</td>
<td>29</td>
</tr>
<tr>
<td>25 – 34</td>
<td>1 (12.5%)</td>
<td>80 (39.6%)</td>
<td>81</td>
</tr>
<tr>
<td>35 – 44</td>
<td>4 (50.0%)</td>
<td>57 (28.2%)</td>
<td>61</td>
</tr>
<tr>
<td>45 – 54</td>
<td>2 (25%)</td>
<td>27 (13.4%)</td>
<td>29</td>
</tr>
<tr>
<td>55 +</td>
<td>1 (12.5%)</td>
<td>9 (4.4%)</td>
<td>10</td>
</tr>
</tbody>
</table>

PTB=Pulmonary tuberculosis; EPTB=Extra pulmonary tuberculosis

In this study, the commonest type of TB was pulmonary Tuberculosis (TB) accounting for 96.2% of cases, while 3.8% were Extra Pulmonary type (EXPTB). The majority (39.6%) with pulmonary tuberculosis (TB) cases were in the age group of 25-34 years while 50%. Studies indicate that clinically tuberculosis may manifest as pulmonary tuberculosis when it affects lungs or Extra- pulmonary TB that may affect any other organ of the body (Zugar and lowry 2006). There can be smear positive pulmonary TB which is the most infectious form and is diagnosed by microscopic
examination or culture of sputum. Smear negative pulmonary TB is diagnosed clinically or on chest x-ray findings.

4.2.3 Smear positive and negative distribution by age-gender

The total number of TB cases was two hundred and ten in this study of which 185 (88.1%) were smear positive and 25 (11.9%) smear negative. The proportion of smear positive cases among males and females were 95 (45.2%) and 90 (42.9%) respectively. There was no statistical difference between gender and smear positive ($\chi^2=1.077; \text{df}=4; p>0.05$) and (OR=0.823, 95% CI: 0.476-1.423). The age group 25-34 years showed more smear positivity (36.2%), 35-44 years (26.7%), 18-24 years (12.4%) and 45-54 years 10.9%. There was no statistically significant association between age and smear positive ($\chi^2=40.817, \text{df}=4; p>0.05$). All the two hundred and ten TB patients who were diagnosed with Mycobacterial disease were culture positives (100%) as shown in (Table 4.5).

### Table 4.5: Smear positive and negative distribution by age-gender.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>18-24</td>
<td>14 (15.6%)</td>
<td>12 (12.6%)</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>25-34</td>
<td>40 (44.4%)</td>
<td>36 (37.9%)</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>35-44</td>
<td>21 (23.3%)</td>
<td>35 (36.8%)</td>
<td>3 (20.0%)</td>
</tr>
<tr>
<td>45-54</td>
<td>12 (13.3%)</td>
<td>11 (11.6%)</td>
<td>3 (20.0%)</td>
</tr>
<tr>
<td>≤ 55</td>
<td>3 (3.3%)</td>
<td>1 (1.1%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>95</td>
<td>15</td>
</tr>
</tbody>
</table>

In this study 88.1% of the sputum specimen were smear positive while 11.9% sputum smear-negative. This concurred well with tuberculosis reports of 2010 which showed a treatment success rates of 85.86% for new sputum smear-positive pulmonary TB
cases, 78% for sputum smear-positive retreatment relapse cases, 79% for smear positive retreatment failure cases and 83% for new sputum smear-negative PTB cases (DLTLTD 2011). This figure is much higher than the global target of 70% (WHO, 2011) and that reported from Addis Abba Alisijahbana et al. (2007), where all PTB cases, less than 20% were sputum smear-positive.

4.2.4 Risk factors in the transmission of tuberculosis in Mombasa County

Several demographic factors were analyzed including income, gender and age all of which were found to be significant in transmission PTB (p<0.05). Income emerged as a major significant factor in the spread PTB. Among the patients sampled 61.9% were earning less than 5000 Kshs. 14.3% were earning 5001-10,000 Ksh and only 20 (9.5%) were earning more than 15,000Ksh. Housing conditions were found to have had an effect on TB infection with 132 (62.9%) patients living in single rooms and only 28 (13.3%) patients were living in houses that had more than 5 rooms. Among those living in single rooms 70 were living with more than two people with some households having as many as 6 people. Ninety (42.9%) patients were consuming alcohol while 120 (57.1%). Regarding smoking, ninety (42.9%) were smokers compared to non smokers (57.1%) and this difference was not statistically significant (p<0.05). Seventy eight (37.1%) were HIV co-infected The unadjusted odds ratio (OR) showed that income (OR=2.65; 95% CI: 1.40-6.23), HIV (OR=2.18; 95% CI: 1.03-4.65) and overcrowding (OR=2.71; 95% CI: 1.41-5.62) were statistically significant risk factors for pulmonary tuberculosis. (Table 4.6).
Table 4.6: Risk factors in the transmission of tuberculosis in Mombasa County

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Attribute</th>
<th>PTB- (Suspects)</th>
<th>PTB+ (Cases)</th>
<th>OR (95% C.I)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Income</td>
<td>&lt; 5000</td>
<td>35 (12.1%)</td>
<td>130 (61.9%)</td>
<td>1.00**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5001 - 10000</td>
<td>65 (22.4%)</td>
<td>30 (14.3%)</td>
<td>2.65 (1.40-6.23)</td>
<td>0.041*</td>
</tr>
<tr>
<td></td>
<td>10001 - 15000</td>
<td>50 (17.2%)</td>
<td>30 (14.3%)</td>
<td>0.38(0.12-1.23)</td>
<td>0.042*</td>
</tr>
<tr>
<td></td>
<td>15001 - 20000</td>
<td>140 (48.3%)</td>
<td>20 (9.5%)</td>
<td>0.65 (0.23-0.96)</td>
<td>0.026*</td>
</tr>
<tr>
<td>Housing (No. of rooms)</td>
<td>Single</td>
<td>50 (17.2%)</td>
<td>132 (62.9%)</td>
<td>2.71 (1.41-5.62)</td>
<td>0.051*</td>
</tr>
<tr>
<td></td>
<td>2 - 4</td>
<td>70 (24.1%)</td>
<td>50 (23.8%)</td>
<td>0.12 (0.32-0.81)</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>170 (56.7%)</td>
<td>28 (13.3%)</td>
<td>1.00**</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Smokers</td>
<td>120 (41.4%)</td>
<td>90 (42.9%)</td>
<td>1.00**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non Smokers</td>
<td>170 (58.6%)</td>
<td>120 (57.1%)</td>
<td>0.16 (0.13-0.39)</td>
<td>0.081</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>Consumption</td>
<td>50 (11.3%)</td>
<td>90 (42.9%)</td>
<td>1.00**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non Consumption</td>
<td>250 (89.7%)</td>
<td>120 (57.1%)</td>
<td>0.25 (0.16-0.39)</td>
<td>0.095</td>
</tr>
<tr>
<td>HIV Status (n=78)</td>
<td>HIV+</td>
<td>49 (16.9%)</td>
<td>78 (37.1%)</td>
<td>2.18 (1.03-4.65)</td>
<td>0.034*</td>
</tr>
<tr>
<td></td>
<td>HIV-</td>
<td>241 (83.1%)</td>
<td>132 (83.1%)</td>
<td>1.00**</td>
<td></td>
</tr>
</tbody>
</table>

PTB+=Pulmonary tuberculosis smear positive; PTB-=Pulmonary tuberculosis smear negative; OR=Odds ratio; CI=Confidence interval; P-value=level of marginal significance; *statistically significant association; **Reference exposure

Regarding risk factors for TB infection in this study, 61.9% of the patients who were earning less than ksh 5000 per month were likely to contract the disease. This is similar to other studies which cited low income groups as being most likely to be infected with TB (Grange, 2008). In England and wales, poverty was a strong factor leading to mortality (Elender et al., 1998). A study by Dye et al. (2009) from 134 countries revealed that incidences of tuberculosis were falling more quickly in countries with high income than those in low income. From the data, smoking and alcohol consumption were associated with TB infection with 37.1% of the patients being smokers and 42.9% being alcohol consumers. A study in Russia found that 62% of the TB patients were alcohol abusers which were associated with an increase in
drug resistance and relapse. In the Philippines, TB was attributed to drinking and smoking, and this caused the patients to delay in seeking treatment (Auer, 2000). In India, transmission of TB in children was found to be influenced by among other factors, exposure to environmental tobacco smoke (Singh et al., 2005). Crowding was also found to be associated with incidences of TB with 63.9% of families living in single rooms some as many as eight people. This is because single rooms have less space facilitating the spread of the infection. This is similar to studies in India where 75.6% of the patients lived in overcrowded residences (Singh et al., 2005), and in the United States, crowding was one of the factors contributing to TB infection (Cantwell et al., 1998). In Pakistan, overcrowding was found to affect 75% of the study population (Hill et al., 2006) while in England and Wales there was a strong association between TB mortality and overcrowding at the household level (Dye et al., 2009).

4.3 Prevalence of HIV among newly diagnosed tuberculosis patients

A total of two hundred and ten TB patients were tested for HIV virus and 39.7% (78/210) were sero-positive and the mean age was 35.16 ± 0.71. Mean age for males was 36.12 ± 1.06 and females 34.19 ± 0.94. Overall males constituted 46.2% and females 53.8% of the HIV-cases. There was significant difference between the TB-HIV co-infection rate and age ($\chi^2=3.391$, df=4; p< 0.05). The majority (39.7%) of the TB-HIV co-infection cases were in the 35-44 years age bracket followed by 25-34 (20.5%) and 45-54 (20.5%) and 55+ (17.9%) year’s age brackets. There was no significant difference in the TB-HIV co-infection rate between gender, females being more vulnerable (OR=1.70, 95% CI: 0.347-1.240; p>0.05). Logistic regression
analysis comparing the association of TB with age-group revealed females in the 35-44 and 45-54 age-groups to be significantly affected compared to males (Table 4.7).

Table 4.7: TB-HIV co-infection and gender-age distribution

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>Male (n=36)</th>
<th>Female (n=42)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>0 (0.0%)</td>
<td>1 (1.3%)</td>
<td>1.00 **</td>
</tr>
<tr>
<td>25-34</td>
<td>8 (10.3%)</td>
<td>8 (10.3%)</td>
<td>0.57 (0.26-1.60)</td>
</tr>
<tr>
<td>35-44</td>
<td>14 (17.9%)</td>
<td>17 (21.8%)</td>
<td>2.58 (0.57-3.65)</td>
</tr>
<tr>
<td>45-54</td>
<td>5 (6.4%)</td>
<td>11 (14.1%)</td>
<td>0.3 (0.23-2.56)</td>
</tr>
<tr>
<td>55+</td>
<td>9 (11.5%)</td>
<td>5 (6.4%)</td>
<td>0.92 (0.18-4.57)</td>
</tr>
<tr>
<td>Mean Age</td>
<td>36.12 ± 1.06</td>
<td>34.19 ± 0.94</td>
<td>35.16 ± 0.71</td>
</tr>
</tbody>
</table>

OR=Odds ratio; CI= Confidence interval; **1.00=Reference exposure

The prevalence of TB and HIV co-infection in the current study was 37.1% and it was slightly lower than those reported by the DLTLD 2008, 2009 and 2010. These annual reports gave TB-HIV co-infection prevalence of 48%, 45% and 44% respectively. The discrepancy with the current study could be attributed to decreasing HIV infection rate or some TB cases declining to undergo HIV testing. However, the TB-HIV co-infection rate in this study was higher than the global prevalence of 14.8% during the same period (WHO, 2010). Females dominated males in TB-HIV co-infection with 58.3% of the cases. However, findings of the present study indicate that HIV infection does not alter the prevalence of TB among the gender. The findings of the present study were also in agreement with the DLTLD’s 2008-2010 annual reports (DLTLD 2008, 2009 and 2010), which indicated increasing TB burden in Kenya attributed to the concurrent HIV/AIDS epidemic, which presents special challenges. Women are more likely to have lowered immunity probably because of stress produced by their biological, economic and cultural roles as care givers. The finding of high rate TB-HIV co-infection in females compared to males also reflect the
general notion that females are involved in risky behaviours like prostitution due to poverty in this population. From the data, the most affected age group was 35-44 years (39.7%) which form an important part of the active labour force, and thus the nation's economy will be affected adversely. By affecting this age-group so heavily, HIV/AIDS is not only hitting adults in their most economically productive years but also removing the very people who could be responding to the infection crisis. This means that HIV/AIDS will continue to adversely affect socio-economic development in resource-poor countries for many years to come. Taken together, the HIV-TB co-infection rate reports from different parts of Kenya showed variation 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 Nigeria’s University teaching hospitals showed sero prevalence of 14.9% (Dobler et al., 2008) while an earlier report from Zimbabwe showed a co-infection rate of 6.5% (Banejee, 2008). Likewise in India wide variations in HIV sero-prevalence among tuberculosis patients have been observed.

A study in Tanzania found that HIV prevalence among newly diagnosed tuberculosis patients was 15% (Ngowi et al., 2010). In another study done in Faraja Reserch Clinic in Gambia among tuberculosis patients, 43.2% were diagnosed with HIV. In yet another study done in South Africa among gold miners the point prevalence of HIV among Tuberculosis patients was 3.8%. Similar study conducted in Ukraine observed a prevalence of 6.3%. Among the TB patients, those who divorced and widowed had a higher prevalence of HIV that was statistically significant (p<0.05).
In this present study, the degree of the infection varied significantly with age and the highest number occurred in the age group 35-44 years (OR=2.58, 95% CI:0.57-3.65, p<0.05). The significant association between age and TB-HIV co-infection was also observed by Dobler et al. (2008). Similarly, these findings agree with a study from Ukraine (Vander der Wer et al., 2006) which showed a high frequency of infection in patients with <50 years.

4.3.1 HIV sero prevalence among TB patients by occupation

Among the occupations, sero prevalence was highest in the *other occupations at 50%. This was followed by self employed 47.7%, house wife 42.9%, jobless 26.8%, government employees 25.0% and juakali 18.2%. No seroprevalence was found among students in this study. Self employed TB patients were 2.32 (OR) times more likely to be seropositive with p=0.192 and *other occupations among the TB patients were 3.47 (OR) times more likely to be seropositive with p=0.23. The TB patients who were house wives were 3.21 (OR) times more likely to be seropositive with p=0.015. The association between HIV-seroprevalence among TB patients and occupation was statistically significant ($\chi^2=13.59$, df=6; p<0.05) (Table 4.8)

**Table 4.8: HIV sero prevalence among TB patients by occupation**

<table>
<thead>
<tr>
<th>Occupation</th>
<th>HIV + (n=78)</th>
<th>HIV - (n=132)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Government</td>
<td>10 (25.0%)</td>
<td>30 (75.0%)</td>
<td>40</td>
</tr>
<tr>
<td>Jobless</td>
<td>11 (26.8%)</td>
<td>30 (73.2%)</td>
<td>41</td>
</tr>
<tr>
<td>Juakali</td>
<td>2 (18.2%)</td>
<td>9 (81.8%)</td>
<td>11</td>
</tr>
<tr>
<td>Housewife</td>
<td>3 (42.9%)</td>
<td>4 (57.1%)</td>
<td>7</td>
</tr>
<tr>
<td>Self Employed</td>
<td>42 (47.7%)</td>
<td>46 (52.3%)</td>
<td>88</td>
</tr>
<tr>
<td>Student</td>
<td>-</td>
<td>3 (100.0%)</td>
<td>3</td>
</tr>
<tr>
<td>*Others</td>
<td>10 (50.0%)</td>
<td>10 (50.0%)</td>
<td>20</td>
</tr>
</tbody>
</table>

Inference $\chi^2=13.59$; df=6; p< 0.05
In this study, 87.2% of the patients were unemployed or working in small business ventures and the association between HIV-seroprevalence among TB patients and occupation was statistically significant ($\chi^2 = 13.597$, df=6; p<0.05). Findings in this current study showed that seroprevalence was highest in daily labourers, commercial sex workers and household servants. These patients belong to low income groups which are highly associated with TB and HIV because of poverty (Dye et al., 2009). A study done in Gambia found that only 3 of 100 patients were employed while majority were either unemployed or working in unskilled labour (Hill et al., 2006). In United States unemployment was one of the risk factors responsible for TB-HIV co-infection (Singh et al., 2005). This is similar to a study done in Pakistan where 23% of patients were unemployed while 75% worked on a daily wage basis (Bock et al., 2007). Poor socio-economic status with its attendant poor education is associated with poor knowledge of TB and HIV, risks of infection and dissemination and access to the health care. Silicosis, an occupational disease occurring among mine workers exposed to silica dust, predisposes TB and non-tuberculous mycobacterium (NTM) infections (Sonnenberg et al., 2004). Other researchers reported the occurrence of TB transmission in hospitals in association with healthcare workers born in countries with high TB burden but without further continued spread (Anderson et al., 2007).

**4.3.2 HIV sero prevalence among TB patients by marital status**

Table 4.9 indicates that seroprevalence was higher among the widowed 46.2% and the married 40.3% respectively. The prevalence in divorcees was 33.3% and in single group 30.7% respectively. The association between HIV-TB co-infection and marital status was not statistically significant ($\chi^2 = 2.338$, df=3; p> 0.05). Another predictor of TB and HIV co-infection among this group was contact with commercial sex workers.
Out of ten (10) patients who had history of contact with commercial sex workers, eight (10.2%) were HIV positive and two (2.6%) HIV-negative.

Table 4.9: HIV sero prevalence among TB patients by marital status

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>HIV + (n=78)</th>
<th>HIV - (n=132)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>48 (40.3%)</td>
<td>71 (59.7%)</td>
<td>119</td>
</tr>
<tr>
<td>Single</td>
<td>23 (30.7%)</td>
<td>52 (69.3%)</td>
<td>75</td>
</tr>
<tr>
<td>Widowed</td>
<td>6 (46.2%)</td>
<td>7 (53.8%)</td>
<td>13</td>
</tr>
<tr>
<td>Divorced</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
<td>3</td>
</tr>
</tbody>
</table>

**Inference**

\[ \chi^2 = 2.338, \text{ df}=3, \text{ p}>0.05 \]

Compared to single TB patients, widowed and married TB patients showed high TB-HIV co-infection prevalence in this study and the variation was significant (p<0.05). The odds of HIV infection for widowed and divorced patients was about 5 to 4 times higher than the odds of single patients (OR=5.2, 95% CI; 1.39-15.8) and (OR=4, 95% CI; 1.16-14.5) respectively, showing that being married and widowed are the strongest independent predictors for HIV infection in TB patients. This study indicated that married males and females showed greater HIV prevalence than their married counterparts. Contrary to findings in this study, in Jemikalajah et al. (2009) study, seroprevalence of TB-HIV co-infection was higher among unmarried males and those living singly than male tuberculosis patients living with their spouses.
4.3.3 Tuberculosis recurrences and HIV-infection

A total of 30 (14.3%) of the TB cases were recurrences, having previously been treated for TB and declared cured. No treatment or default failures cases were observed in this study. Males constituted 56.7% (17/30) and females 43.3% (13/30) of the recurrent cases. A total of 8 (26.7%) of the TB recurrent cases were ZN smear positive of which 6 (20%) were culture positive and 22 (73.3%) cases were smear negative and 4 (13.3%) were culture positive. A total of 13 (43.3%) of these cases were HIV sero-positive and 17 (56.7%) were HIV sero-negative. The majority (43.3%) of the recurrences were in the 21-30 age-group followed by the 35-44 age group with 21.9%. There was no statistically differences in TB recurrences between the genders (OR=1.02; 95% CI: 0.66-1.37; p=0.54) and HIV status (OR=0.39; 95% CI: 0.22-1.10; p=0.17) as shown in Figure 4.1 below.

![Figure 4.1: Tuberculosis recurrence and HIV infection](image-url)
In this study, thirty patients (14.3%) had recurrent TB. In the official 2009 DLTLD annual report, retreatment cases contributed 9% of the TB cases of which 3% were classified as PTB relapse category. The rest were recurrent smear negative PTB and EPTB (4%), Treatment failures (0.1%) and return after a default (1.4%). The 2010 annual report published by DLTLD shows a smear positive relapse rate of 3% and a combined recurrent smear negative pulmonary TB and extra pulmonary TB rate of 5%. Many national TB control programmes in sub-Saharan African countries have no capacity to distinguish between relapse (endogenous reactivation) from (exogenous) re-infection TB which constitute recurrent tuberculosis. It is not clear how the DLTLD identified TB relapse case given their annual reports. This is because diagnosis and treatment of new TB cases in most health facilities in Kenya is based on clinical symptom, Ziehl-Neelsen (ZN) smear microscopy and occasionally augmented with chest X-Ray in some health facilities.

In this study males dominated females (56.7%) in the number of recurrent tuberculosis cases. The majority (43.3%) of the recurrences were in the 25-34 year age group which is the most productive segments of society. There was no statistical difference in TB recurrence rates between gender and HIV status (p>0.05). Kenya has been fully under directly observed therapy short counts (DOTs) programme since 1998 (WHO, 2010). However it cannot be concluded categorically that DOTs functions as may be expected on basis of its naming. In this current study, patients in Mombasa County were provided with drugs to take at home for at least two weeks and adherence is not routinely checked. Therefore it is unknown what the degree of compliance is and it can only be determined if differentiation between relapse and re-infection is established. Other studies have reported proportion of re-infection among
recurrent TB cases ranging from 1.2 -7% in low incidence areas (Davis, 2009), 12-75% in medium incidence areas (Jasmer et al., 2004) and 23-75% in high incidence areas which include India, China, Nigeria, South Africa and former Soviet states (Van Rie et al., 2005).

4.4 Clinical features of tuberculosis in HIV sero-positive and sero-negative patients (Clinical outcomes)

4.4.1 Clinical symptoms of TB in HIV sero-positive and sero-negative patients

Regarding clinical symptoms of TB patients enrolled in this study, the majority (50%) were suffering from cough, breathlessness 39 (18.6%), fever 8 (39.5%), constitutional symptoms like fatigue, malaise and anorexia 89 (42.3%) and weight loss 66 (31.4%). In relation to HIV-status, the symptoms among patients were hemoptysis 3.8% (OR=0.14; p=0.001), breathlessness 26.9% (OR=2.33; p=0.017), fever 35.9% (OR=0.78; p=0.409), constitutional symptoms 70.5% (OR=7.17; p=0.001), significant weight loss 44.9% (OR=2.65; p=0.001), seizures 30.8% (OR=0.72; p=0.472), headache 3.8% (OR=0.71; p=0.632). Sero prevalence was 9.0% among patients with symptoms of meningeal irritation (OR=1.09; p=0.873), neck swelling 1.3% (OR=0.42; p=0.422) and gastro-intestinal symptoms (GI) 6.4% (OR=8.97; p=0.018). Patients who had contact with commercial sex workers were 80.0% (OR=215.6; p<0.05). Suggestive symptoms were reported more often with increasing age (p<0.05) and more often by female participants (Table 4.10).
### Table 4.10: Clinical symptoms of TB in HIV positive and negative patients

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>HIV+ (n=78)</th>
<th>HIV- (n=132)</th>
<th>Total</th>
<th>Significance test</th>
<th>Odds ratio (HIV+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>10 (12.8%)</td>
<td>95 (72.0%)</td>
<td>105</td>
<td>$\chi^2=68.61$ p=0.001*</td>
<td>0.057</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>3 (3.8%)</td>
<td>32 (24.2%)</td>
<td>35</td>
<td>$\chi^2=13.20$ p=0.001*</td>
<td>0.136</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>21 (26.9%)</td>
<td>18 (13.6%)</td>
<td>39</td>
<td>$\chi^2=5.72$, p=0.017*</td>
<td>2.333</td>
</tr>
<tr>
<td>Fever</td>
<td>28 (35.9%)</td>
<td>55 (41.7%)</td>
<td>83</td>
<td>$\chi^2=0.68$, p=0.409</td>
<td>0.784</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
<td>55 (70.5%)</td>
<td>34 (25.8%)</td>
<td>89</td>
<td>$\chi^2=41.71$, p=0.001*</td>
<td>7.174</td>
</tr>
<tr>
<td>Significant weight loss</td>
<td>35 (44.9%)</td>
<td>31 (23.5%)</td>
<td>66</td>
<td>$\chi^2=10.406$, p=0.001*</td>
<td>2.652</td>
</tr>
<tr>
<td>Headache</td>
<td>3 (3.8%)</td>
<td>7 (5.3%)</td>
<td>10</td>
<td>$\chi^2=0.229$, p=0.632</td>
<td>0.714</td>
</tr>
<tr>
<td>Symptoms of MI</td>
<td>7 (9.0%)</td>
<td>13 (9.9%)</td>
<td>20</td>
<td>$\chi^2=0.026$, p=0.873</td>
<td>1.085</td>
</tr>
<tr>
<td>Neck swelling</td>
<td>1 (1.3%)</td>
<td>6 (4.5%)</td>
<td>7</td>
<td>$\chi^2=0.645$, p=0.422</td>
<td>0.416</td>
</tr>
<tr>
<td>GI symptoms</td>
<td>5 (6.4%)</td>
<td>5 (2.3%)</td>
<td>8</td>
<td>$\chi^2=5.644$, p=0.018*</td>
<td>8.973</td>
</tr>
</tbody>
</table>

OR=Odds ratio; *statistically significant association; Constitutional symptoms include-malaise, anorexia, fatigue and lack of appetite.

In this study, the most presenting symptoms of TB among those who are HIV negative were cough (72.0%), Fever (41.7%) and weight loss (23.5%). Patients with tuberculosis normally present with fever which develops in the late afternoon or evening (Kumarasamy et al., 1995). The fever is usually low grades at the onset and may become high grade with progression of disease (Bacha et al., 2004). The diagnosis of pulmonary tuberculosis is influenced by the patient's illness, HIV/AIDS status and the level of care services available. In this study, Symptoms like weight loss (53%) and constitutional (62%) were strongly associated TB-HIV co-infection patients. These clinical findings means that screening of tuberculosis in HIV co-infected patients by using symptoms of fever, cough, weight loss and chest
radiography with features suggestive of tuberculosis would detect only 25% of the HIV positive co-infected with tuberculosis. This is expected as TB which is a chronic debilitating disease is coexisting with another debilitating disease (HIV). Studies by Corbett et al., 2009) on HIV-1 co-infection in children hospitalized with tuberculosis in South Africa revealed that clinical features of cough, fever and night sweats or TB contact were similar between HIV-infected and non-infected children. WHO has developed a clinical staging system (originally for prognosis), based on clinical criteria and the definition of symptoms, signs and diseases is according to clinical judgment. Clinical condition or performance score whichever is the higher, determines whether a patient is at clinical stage 1, 2, 3 or 4 which is an important criteria for starting anti-retroviral (ARV) therapy.

4.4.2 Clinical signs of TB in HIV sero-positive and negative patients

Patients had a wide spectrum of clinical signs on examination (Table 4.11 next page). The majority had pallor 105 (50%), 74 (35.2%) night sweats and 62 (29.5%) respiratory signs. In relation to HIV-status, 66.7% of the patients with pallor were seropositive (OR=2.72; p=0.001). Jaundice was reported in 17.9% of the participants (OR=3.90, p=0.003), oedema 9.0% (OR=6.41; p=0.010), lymphadenopathy 26.9% (OR=3.37; p=0.001). Oral thrush was seen in 26.9% of patients (OR=11.04; p=0.001), respiratory signs 25.6% (OR=0.69; p=0.250), per abdomen signs (PA signs) 19.2% (OR=5.00; p=0.001), night sweats 35.9% (OR=1.05; p=0.878), skin infections 6.4% (OR=1.73; p=0.44) and central nervous system (CNS signs) 10.3% (OR=1.56; p=0.377)
Table 4.11: Clinical signs of TB in HIV positive and negative patients

<table>
<thead>
<tr>
<th>Signs</th>
<th>HIV + (n=78)</th>
<th>HIV - (n=132)</th>
<th>TOTAL</th>
<th>Significance (χ² test of difference)</th>
<th>Odds ratio (HIV+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallor</td>
<td>52 (66.7%)</td>
<td>54 (40.9%)</td>
<td>106</td>
<td>χ²=11.74, p=0.001*</td>
<td>2.728</td>
</tr>
<tr>
<td>Jaundice</td>
<td>14 (17.9%)</td>
<td>8 (6.1%)</td>
<td>22</td>
<td>χ²=8.71, p=0.003*</td>
<td>3.906</td>
</tr>
<tr>
<td>Night sweats</td>
<td>28 (35.9%)</td>
<td>46 (34.8%)</td>
<td>74</td>
<td>χ²=0.02, p=0.878</td>
<td>1.047</td>
</tr>
<tr>
<td>Edema</td>
<td>7 (9.0%)</td>
<td>4 (3.0%)</td>
<td>11</td>
<td>χ²=6.65, p=0.010*</td>
<td>6.408</td>
</tr>
<tr>
<td>Skin infections</td>
<td>5 (6.4%)</td>
<td>5 (3.8%)</td>
<td>10</td>
<td>χ²=0.58, p=0.443</td>
<td>1.73</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>21 (26.9%)</td>
<td>14 (10.6%)</td>
<td>35</td>
<td>χ²=10.53, p=0.001*</td>
<td>3.372</td>
</tr>
<tr>
<td>Oral thrush</td>
<td>21 (26.9%)</td>
<td>5 (3.8%)</td>
<td>26</td>
<td>χ²=24.76, p=0.001*</td>
<td>11.034</td>
</tr>
<tr>
<td>Respiratory signs</td>
<td>20 (25.6%)</td>
<td>42 (31.8%)</td>
<td>62</td>
<td>χ²=1.324, p=0.250</td>
<td>0.69</td>
</tr>
<tr>
<td>PA Signs</td>
<td>15 (19.2%)</td>
<td>8 (6.1%)</td>
<td>23</td>
<td>χ²=11.74, p=0.001*</td>
<td>5</td>
</tr>
<tr>
<td>CNS signs</td>
<td>8 (10.3%)</td>
<td>9 (6.8%)</td>
<td>17</td>
<td>χ²=0.77, p=0.37</td>
<td>1.562</td>
</tr>
</tbody>
</table>

T test for numerical variables and Yates corrected chi-square test for categorical variables: OR=Odds ratio; *statistically significant association; CNS=central nervous system

In this study, Patients had a wide spectrum of clinical signs on examination with 66.7% of the patients with pallor being sero-positive compared to 40.9% sero-negative and this was statistically significant (p<0.05). Oral candidiasis was also occurring in 26.9% of the HIV-positive cases. Night sweats, skin infections and respiratory signs did not have any statistically significant association with sero positivity (p>0.05). Signs like pallor and oedema were common in tuberculosis co-infected patients. This study demonstrates that the clinical signs of TB were comparable in HIV sero-positive and sero-negative TB patients, as both groups had similar complaints of pallor, night sweats skin infections and respiratory signs. The
absence of any difference in most of the clinical signs between the HIV sero-positive and sero-negative TB patients might be due to the chronic nature of TB, leading to protracted ill-health and wasting (Majid and Abba, 2008).

**4.4.3 Clinical outcomes observed in TB-HIV co-infection based on CD4+ cells**

Majority of the respondents with CD4+cells<200 presented with night sweats 79.5%, constitutional symptoms 70.5%, seizures 42.3%, weight loss 329.9%, prolonged fever 35.9% and coughs 12.8%. Prolonged fever 23.1% was also common in patients with CD4+T lymphocyte range 201-500 cells/mm$^3$ followed by weight loss 21.8%, constitutional symptoms 15.4%, coughs 12.8% and night sweats 12.8%. Fewer patients 2.6% with >500 CD4+T cells count showed weight loss and night sweats respectively. In a univariate logistic regression analysis, breathlessness, prolonged cough, night sweats and weight loss were associated with CD4+T <200 cells/mm$^3$. The significant symptoms/signs persistently predicting CD4+T count<200 cells/mm$^3$ at a multivariate model were coughs (OR=14.6), prolonged fever (OR=4.5) and weight loss (OR=2, 8). Weight loss was also independently associated with a diagnosis of pulmonary tuberculosis. There was a statistically significant difference between TB-HIV co-infection and the degree of immune suppression-CD4+T cell counts in relation to clinical outcomes (p<0.05) (Table 4.12).
Table 4.12: Clinical features observed in TB-HIV co-infection based on CD4+cells

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Degree of Immune-deficiency (CD4-counts/mm³)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;200</td>
<td>201-500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Weight loss</td>
<td>31 (39.7%)</td>
<td>17 (21.8%)</td>
<td>2 (2.6%)</td>
</tr>
<tr>
<td>Prolonged fever</td>
<td>28 (35.9%)</td>
<td>18 (23.1%)</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Coughs</td>
<td>10 (12.8%)</td>
<td>10 (12.8%)</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
<td>55 (70.5%)</td>
<td>12 (15.4%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Seizures</td>
<td>33 (42.3%)</td>
<td>2 (2.6%)</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>3 (3.8%)</td>
<td>3 (3.8%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>21 (26.9%)</td>
<td>1 (1.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Symptoms of MI</td>
<td>3 (3.8%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Night Sweats</td>
<td>62 (79.5%)</td>
<td>10 (12.8%)</td>
<td>2 (2.6%)</td>
</tr>
<tr>
<td>Swelling</td>
<td>6 (7.7%)</td>
<td>3 (3.8%)</td>
<td>1 (1.3%)</td>
</tr>
</tbody>
</table>

In this study majority of the respondents with CD4+ cells <200 presented with night sweats 79.5% and constitutional symptoms 70.5%. Prolonged fever 23.1% and weight loss 21.8% were also common in patients with CD4+T count range 200-500 cells/mm³. There was a statistically significant difference between TB and HIV co-infection and the degree of immune suppression (p<0.05). It has been observed that when the immune suppression is more marked (CD4 count <200ml³), the features of tuberculosis are a typical with a much greater frequency of extra-pulmonary involvement, especially the lymph nodes (Pape, 2004). A study in Malaysia General Hospital showed that prolonged insidious symptoms found in majority of patients consisting of weight loss, prolonged low grade fever and nocturnal sweat may delay diagnosis. This classical picture of pulmonary TB is seen mainly in less immunocomprised patients with CD4 count > 200 cells/mm³ (Bacha, et al., 2004).
4.5 Immunohaematological markers (CD4, CD8, HB, PCV, ESR and platelets) in tuberculosis, HIV/AIDS, tuberculosis-HIV/AIDS co-infected patients and healthy subjects (controls)

4.5.1 CD4+T cells, CD8+T cells and Haemoglobin levels in tuberculosis patients

The mean CD4+T lymphocyte counts in tuberculosis patients in both males and females combined were 474.52±198.76 cells/mm$^3$. Mean CD4+T cells in males was 474.68±178.18 cells/mm$^3$ and females 484.36±217.83 cells/mm$^3$. The mean CD8+T lymphocyte counts for the participants were 614.78±247.46 cells/mm$^3$. Females had higher CD8+T cells counts than males 647.77±259.45 and 581.78±235.46 cells/mm$^3$ respectively. Females had higher CD4+T cells (t=34.5, df=208, p<0.05) and CD8+T cells (t=35.9, df=208, p=0.001). Haemoglobin mean in males was 11.66±8.12 gm/dl and females 10.66±1.72 gm/dl. Females had lower haemoglobin mean levels than males (t=27.5, df=185, p=.002). The mean BMI for groups was 19.68±2.43, 95% CI: 19.35-20.01. Females had slightly higher mean 19.75±2.48 kg/m$^2$ compared to males 19.61±2.40 kg/m$^2$ (t=117, df=208, p< 0.05). There was no significant association between males and females in nutritional status ($\chi^2$=46.3, df=1, p> 0.05). T-test showed that a significant difference existed between the means of CD4+T count and TB status (p<0.05). (Table 4.13).
Table 4.13: Arithmetic median, 2.5th-97.5th percentile, mean and 95% CI of CD4+ T cells, CD8+ T cells, CD4/CD8 ratio, Haemoglobin and BMI in TB patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Median</th>
<th>2.5th-97.5th</th>
<th>Mean ± SD</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5th-97.5th</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute CD4 T Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>510</td>
<td>350.00-571.00</td>
<td>464.68±178.18</td>
<td>430.19-499.16</td>
<td>t=34.59, df=208, p=0.001</td>
</tr>
<tr>
<td>Female</td>
<td>105</td>
<td>512</td>
<td>291.00-671.00</td>
<td>484.36±217.83</td>
<td>442.21-526.52</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>512</td>
<td>102.55-819.00</td>
<td>474.52±198.76</td>
<td>447.48-501.56</td>
<td></td>
</tr>
<tr>
<td>Absolute CD8 T Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>500</td>
<td>416.00-680.50</td>
<td>581.78±235.46</td>
<td>536.21-627.35</td>
<td>t=35.87, df=208, p=0.001*</td>
</tr>
<tr>
<td>Female</td>
<td>105</td>
<td>547</td>
<td>454.00-790.00</td>
<td>647.77±259.45</td>
<td>597.95-697.59</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>528</td>
<td>330.00-1220.85</td>
<td>614.78±247.46</td>
<td>447.48-501.56</td>
<td></td>
</tr>
<tr>
<td>Absolute CD4/CD8 T Cells Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>1.096</td>
<td>0.455-1.237</td>
<td>0.929±0.454</td>
<td>0.841-1.016</td>
<td>t=26.33, df=208</td>
</tr>
<tr>
<td>Female</td>
<td>105</td>
<td>1.088</td>
<td>0.371-1.369</td>
<td>0.903±0.551</td>
<td>0.796-1.009</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>1.091</td>
<td>0.13-1.67</td>
<td>0.916±0.504</td>
<td>0.847-0.984</td>
<td>p=0.004*</td>
</tr>
<tr>
<td>Absolute CD3 T Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>1210</td>
<td>1016.00-1210.00</td>
<td>1214.71±329.06</td>
<td>1151.03-1278.39</td>
<td>t=52.51, df=208, p=0.009*</td>
</tr>
<tr>
<td>Female</td>
<td>105</td>
<td>1320</td>
<td>1029.00-1560.00</td>
<td>1327.25±363.99</td>
<td>1256.81-1397.69</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>1304</td>
<td>1022.00-1459.25</td>
<td>1270.98±350.70</td>
<td>1223.27-1318.69</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>10.8</td>
<td>9.50-12.50</td>
<td>11.66±8.12</td>
<td>10.09-13.23</td>
<td>t=27.52, df=409, p=0.002*</td>
</tr>
<tr>
<td>Female</td>
<td>105</td>
<td>10.7</td>
<td>9.75-12.10</td>
<td>10.66±1.72</td>
<td>10.33-10.99</td>
<td>df=409, p=0.001</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>10.75</td>
<td>7.20-13.70</td>
<td>11.16±5.88</td>
<td>10.36-11.96</td>
<td></td>
</tr>
<tr>
<td>BMI in Kg/M2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>19.4</td>
<td>17.95-21.50</td>
<td>19.61±2.40</td>
<td>19.14-20.07</td>
<td>t=117.1, df=409, p=0.001</td>
</tr>
<tr>
<td>Female</td>
<td>105</td>
<td>19.5</td>
<td>18.05-21.70</td>
<td>19.75±2.48</td>
<td>19.27-20.23</td>
<td>df=409, p=0.001</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>19.5</td>
<td>14.64-24.00</td>
<td>19.68±2.43</td>
<td>19.35-20.01</td>
<td></td>
</tr>
</tbody>
</table>

CD4+=Cluster differentiation T-lymphocyte 4; CD8+=cluster differentiation T-lymphocyte no.8; BMI=Body mass index; SD=Standard deviation; CI=Confidence interval; P-value=level of marginal significance; *statistically significant association

In this study the mean CD4+T counts for Tuberculosis patients was 474.5±198.8 cells/mm³ and for CD8+T lymphocyte 614.8± 247.5 cells/mm³. Differential Leukocyte count revealed that tuberculosis patients had significantly increased percentage of neutrophils. Lymphocyte percentage was significantly high and there
was no significance difference in monocyte percentages compared to controls. On comparing CD8+T cell mean between newly diagnosed TB patients and recurrent TB patients, it was found that CD8+ T cell mean were high but it was not statistically significant (p>0.05). This finding is in accordance with the studies of Kursar et al., 2007 and Lawson et al., 2008. The results in the current study disagree with the results of Miani et al., 996 and Coban et al., 2005 who reported significant increase in CD8+ T cell values in tuberculosis patients. Probably discrepancy could be due to the difference of study population since they included pulmonary tuberculosis patients but in the current study some of the patients were suffering from extra-pulmonary tuberculosis. Another Study documented significant reduction in CD4+ T cells but it was without notable reduction in CD8+ T cells. However, reduction in CD4+cells while increase in CD8+ T cells has also been reported in other studies (Chenz et al., 2007).

The reduction in CD4+ T cells in peripheral blood in tuberculosis patients is believed to be due to the pulling of CD4+ T cells to the site of infection and incase of pulmonary tuberculosis, these cells are pooled to the lungs (Guyot-Revol et al., 2006). Other studies in HIV negative tuberculosis patients have reported that low peripheral CD4+ T cells restored to normal after successful tuberculosis therapy suggesting that tuberculosis was the course of the low CD4+T cells (Uppas et al., 2005).

In this study the haemoglobin level was lower in tuberculosis patients (11.2 ± 5.9 gm/dl) than the reference values established for this population (12.8 ± 1.3, t = 27.5, df=461, p<0.05) which was statistically significant. Reasons for this could be insufficient dietary intake because of poor appetite or anaemia due to chronic
infections. This is also supported by findings in this current study which showed significantly lower BMI in tuberculosis patients than in healthy subjects and in HIV/AIDS patients without tuberculosis. Patients with tuberculosis were significantly malnourished as compared to the normal values for this population. (BMI 22.7±1.3, t = 8, p<0.05). Malnutrition is normally associated with impaired immunity and it has been reported that malnourished individuals at a risk of acquiring tuberculosis (Patel et al., 2007). This study found no significant difference between males and females with regards to nutritional status (p>0.05).

4.5.2 CD4+T cells, CD8+T cells and Haemoglobin levels in tuberculosis and HIV co-infected patients

The mean absolute CD4+T count in males and females combined was 276.44±142.71 cells/mm$^3$. Mean in males was 265.12±158.35 cells/mm$^3$ and females 289.64±128.67 cells/mm$^3$. Combined CD8+T lymphocyte count in males and females were 796.46±265.69 cells/mm$^3$. The mean CD8+T cells in males were 780.19±288.07 cells/mm$^3$ and females 802.98±247.96 cells/mm$^3$. The mean CD4+/CD8+ ratio in both males and females was 0.387±0.269 cells/mm$^3$. The mean CD4+T cells count in males was lower than for females but the difference was not statistically significant (t=0.754, df=76, p>0.05). The mean haemoglobin levels in males and females combined was 11.09±9.44 gm/dl and the in males was 12.51±13.74gm/dl and females 9.88±1.69gm/dl respectively. Females had significantly lower haemoglobin mean levels than males. The difference was not statistically significant (t=1.23, df=76, p>0.05). On nutritional status assessment using BMI, the mean BMI for the participants was 18.77±2.67 kg/m$^2$. The mean BMI for males was 18.55±2.55kg/m$^2$ and females 18.97±2.76 kg/m$^2$ but it was not statistically significant (p>0.05). Significantly more tuberculosis HIV co-infected patients were malnourished ($\chi^2$=7,
df=1; p<0.05) as compared with HIV positive tuberculosis negative patients (Table 4.14).

Table 4.14: Arithmetic median, 2.5th-97.5th percentile, mean and 95% CI of CD4+ T cells, CD8+ T cells, CD4/CD8 ratio, Haemoglobin and BMI in TB and HIV Co-infected patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Median</th>
<th>2.5th-97.5th</th>
<th>Mean ± SD</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute CD4 TCells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>283.5</td>
<td>169.00 - 368.50</td>
<td>265.12±158.35</td>
<td>236.06 - 343.22</td>
<td>t=0.75</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>249.5</td>
<td>168.00 - 349.50</td>
<td>289.64±128.67</td>
<td>225.02 - 305.22</td>
<td>df=76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>254.5</td>
<td>168.75 - 362.50</td>
<td>276.44±142.71</td>
<td>244.26 - 308.61</td>
<td>p=0.45</td>
</tr>
<tr>
<td>Absolute CD8 T Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>786.5</td>
<td>462.00 - 983.50</td>
<td>780.19±288.07</td>
<td>682.73 - 877.66</td>
<td>t=0.375</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>811.5</td>
<td>699.50 - 972.25</td>
<td>802.98±247.96</td>
<td>725.71 - 880.25</td>
<td>df=76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>786.5</td>
<td>628.75 - 362.50</td>
<td>796.46±265.69</td>
<td>732.56 - 852.36</td>
<td>p=0.708</td>
</tr>
<tr>
<td>Absolute CD4/CD8 T Cells Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>0.365</td>
<td>0.217 - 0.472</td>
<td>0.339±0.320</td>
<td>0.315 - 0.532</td>
<td>t=1.103</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>0.308</td>
<td>0.223 - 0.441</td>
<td>0.356±0.216</td>
<td>0.289 - 0.423</td>
<td>df=76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>0.327</td>
<td>0.223 - 0.468</td>
<td>0.387±0.269</td>
<td>0.326 - 0.448</td>
<td>p=0.273</td>
</tr>
<tr>
<td>Absolute CD3 T Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>1182</td>
<td>975.00 - 1415.25</td>
<td>1187.56±365.68</td>
<td>1063.83 - 1311.28</td>
<td>t=0.529</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>1145</td>
<td>965.25 - 1342.00</td>
<td>1146.12±326.20</td>
<td>1044.47 - 1247.77</td>
<td>df=76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>1166</td>
<td>970.00 - 1348.00</td>
<td>1165.24±343.32</td>
<td>970.00 - 1348.00</td>
<td>p=0.598</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>10.5</td>
<td>9.125 - 11.500</td>
<td>12.51±13.74</td>
<td>7.859 - 17.158</td>
<td>t=1.230</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>10.1</td>
<td>8.725 - 11.125</td>
<td>9.88±1.69</td>
<td>9.355 - 10.407</td>
<td>df=76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>10.2</td>
<td>9.05 - 11.23</td>
<td>11.09±9.44</td>
<td>9.05 - 11.23</td>
<td>p=0.233</td>
</tr>
<tr>
<td>BMI in Kg/M2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>19.4</td>
<td>16.725 - 20.100</td>
<td>18.55±2.55</td>
<td>17.69 - 19.41</td>
<td>t=0.703</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>18.5</td>
<td>17.125 - 20.550</td>
<td>18.97±2.76</td>
<td>18.11 - 19.83</td>
<td>df=76</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>18.5</td>
<td>16.88 - 20.50</td>
<td>18.77±2.67</td>
<td>16.88 - 20.50</td>
<td>p=0.484</td>
</tr>
</tbody>
</table>

CD4+=Cluster differentiation T-lymphocyte no.4; CD8+=Cluster differentiation T-lymphocyte no.8; BMI=Body mass index; SD=Standard deviation; CI=Confidence interval; P-value=level of marginal significance

In this study tuberculosis and HIV co-infected patients had significantly lower CD4+ T-Cells and Leukocytes (276±142 cells/litres) suggesting that a combination of tuberculosis and HIV/AIDS causes a more serious depletion of CD4+T cells.
compared to tuberculosis patients without HIV infection and HIV/AIDS without tuberculosis. The levels of CD4+T cells were significantly low and ratio inverted in comparison to healthy subjects (controls). Mientjes et al. (1992) reported that each person has a unique level of CD4+T cell numbers that is reflective of his/her immunocompetence to protect him/her against the development of clinical symptoms. The mean CD4+ T cells count in males was lower than for females but the difference was not statistically significant (p>0.05).

A study in Haiti indicates that 56% of the tuberculosis patients infected with HIV were diagnosed when CD4+ T cells were >350 cells/microlitre, 23% and 12% of the patients infected with TB had HIV at the CD4+T cell levels of 200-350 cells/microlitre and <200 microlitre respectively (Urasa et al., 1996). Another study in India found a mean CD4+ T cell count of 146.6 ± 88.0 cells/mm³ and it was lower among males (145.6 ± 87.8) compared to females (147.6 ± 87.8 cells/mm³) which was similar to this present study. The very low CD4+ T cell count in their studies was probably the patients they recruited were at advanced stages of infection or there were more pulmonary tuberculosis cases which CD4+ T cell counts were significantly lower than the extra pulmonary cases (Carrol et al., 2001). The findings in this study were also consistent with the report of Tarbarsiet et al. (2008) where CDT+T count was >200 cells/mm³ but contrast with the report of Tegbaruet et al. (2011) and Affusimet et al., 2011). Meanwhile, there were some similarities between the present results and the findings of Yassin et al. (2006) but differed in the sense that the respondents for this study were all 18 years and above while those in the other study were all children.
HIV positive individuals with CD4+ T <200 cells/µl blood are more susceptible to tuberculosis than HIV positive individuals with >500 CD4+T cells/µl/blood, regardless of anti-retroviral therapy (Jones et al., 1993). The combination of the low cellular immunity in tuberculosis and HIV/AIDS has been associated with malnutrition in this current study. The decrease in CD4+ T cells correlate with the severity of both HIV/AIDS and tuberculosis due to the reduction of the cellular immunity against *Mycobacterium tuberculosis* and human immunodeficiency virus (Patel et al., 2007). In this study, combination of tuberculosis and HIV/AIDS co-infection caused serious depletion of both CD4+T cells and leukocytes leading to the rejection of null hypothesis hence there was a significant difference between TB-HIV co-infection and immunohaematological cells (p<0.05).

### 4.5.3 CD4+T cells, CD8+T cells, CD4/CD8 ratio and Haemoglobin levels in HIV without tuberculosis infection.

The mean absolute CD4+T count in males and females combined was 333.27±150.59 cells/mm³. Mean in males was 343.05±149.33 cells/mm³ and females 325.93±150.59 cells/mm³. The mean haemoglobin level in males was 11.47±1.37gm/dl and in females 11.13±1.58gm/dl. Females had lower mean haemoglobin levels than males. There was significant difference between males and females ($\chi^2=1.3$, df=1, p<0.05). The combined BMI in males and females was 19.91±2.16 kg/m². Males had higher BMI 19.97±1.75kg/m² than females 19.87±2.46kg/m² but this was not statistically significant (t=0.157, df=47, p>0.05) (Table 4.15).
Table 4.15: Mean CD4+T cells, CD8+T cells, CD4/CD8 ratio, Haemoglobin and BMI cells in HIV without Tuberculosis

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Median</th>
<th>Mean ± Std. Dev.</th>
<th>95% CI</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute CD4 T Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>339</td>
<td>343.05±149.33</td>
<td>275.07-411.02</td>
<td>t=0.392, df=47,</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>325</td>
<td>325.93±152.92</td>
<td>266.63-385.22</td>
<td>p=0.697</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>339</td>
<td>333.27±150.59</td>
<td>290.16-376.37</td>
<td></td>
</tr>
<tr>
<td><strong>Absolute CD8 T Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>695</td>
<td>742.19±254.69</td>
<td>626.26-858.13</td>
<td>t= -0.174,</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>715.5</td>
<td>756.89±318.27</td>
<td>633.48-880.30</td>
<td>df=47, p=0.863</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>709</td>
<td>750.59±289.93</td>
<td>667.31-833.87</td>
<td></td>
</tr>
<tr>
<td><strong>Absolute CD4/CD8 T Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>0.488</td>
<td>0.51±0.279</td>
<td>0.383-0.637</td>
<td>t=0.655,</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>0.481</td>
<td>0.47±0.258</td>
<td>0.370-0.570</td>
<td>df=47, p=0.604</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>0.488</td>
<td>0.487±0.265</td>
<td>0.411-0.563</td>
<td></td>
</tr>
<tr>
<td><strong>Absolute CD3 T Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>1248</td>
<td>1157.67±370.57</td>
<td>988.98-1326.35</td>
<td>t= -0.551,</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>1225</td>
<td>1213.46±335.76</td>
<td>1083.27-1343.66</td>
<td>df=47, p=0.584</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>1240</td>
<td>1189.55±348.44</td>
<td>1089.47-1289.64</td>
<td></td>
</tr>
<tr>
<td><strong>Absolute Haemoglobin Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>11.6</td>
<td>11.47±1.37</td>
<td>10.843-12.091</td>
<td>t=0.776, df=47,</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>11.05</td>
<td>11.13±1.58</td>
<td>10.520-11.744</td>
<td>p=0.442</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>11.3</td>
<td>11.28±1.49</td>
<td>10.848-11.703</td>
<td></td>
</tr>
<tr>
<td><strong>Absolute BMI Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>19.6</td>
<td>19.97±1.75</td>
<td>19.17-20.76</td>
<td>t=0.157, df=47,</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>19.5</td>
<td>19.87±2.46</td>
<td>18.91-20.82</td>
<td>p=0.876</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>19.5</td>
<td>19.91±2.16</td>
<td>19.29-20.53</td>
<td></td>
</tr>
</tbody>
</table>

CD4+=Cluster differentiation T-lymphocyte no.4; CD8+=Cluster differentiation T lymphocyte no.8; BMI=Body mass index; SD=Standard deviation; CI=Confidence interval

The findings of this study were consistent with the report of Tarbarsiet et al. (2008) and the statistical analysis showed that more men were in the category of >300 CD4+T cell counts. Findings in this current study contrast with the studies done by Tegbaruet et al. (2001) and Affusimet et al.(2011). HIV infects cells that have the CD4+T antigen molecules on their surface. In recent years, it has been discovered that...
HIV needs other molecules called chemokines on the cell surface to gain entry into cell (Affusimet et al., 2011). Patients who do not have of these specific chemokines (CCR5) are more resistant HIV infection. The critical abnormality resulting from HIV infection is progressive decline in the number of CD4+T-lymphocytes. The CD4+T cell count serves as the major laboratory indicator of immune function in patients who have HIV infection. It is one the key factors in deciding whether to initiate ART and prophylaxis for opportunistic infections and it is the strongest predictor of subsequent disease progression and survival (Lawson et al., 2008). In this study, patients had high haemoglobin and BMI levels because of being screened for malnutrition and given regulation health education on diet and opportunistic infections every time they visit the clinic. In this study, HIV/AIDS patients on treatment had significantly higher values of CD4+T cells, leukocytes, lymphocytes and haemoglobin levels HIV/AIDS patients not on treatment. This means that antiretroviral treatment suppresses HIV replication and restores immunity (Zacharia et al., 2008).

4.5.4 Baseline data of immunological markers of the respondents

The mean white blood cell count revealed that TB-HIV co-infected patients had (4.90±2.15) x 10^9 l, PLWHA without TB (5.91±2.43) x 10^9 /l whilst the healthy control subjects reported (6.92±3.12) x 10^9 /l). Mean packed cell volume in TB-HIV co-infected patients was (32.31±4.81%), PLWHA without TB (34.1±4.36%) and the control group (36.31±4.2%). The mean platelets count of the respondents of TB-HIV co-infected patients was 163.29±65.12 x10^9/l, HIV/AIDS without TB 195.16±88.7 x 10^9/l and the healthy control subjects 240.29±99.42) x109/l. The mean ESR findings for HIV+/TB+ was 115.13±11.82 mm/hr, HIV-/TB+115.13±11.82 mm/hr whilst HIV-/TB-controls was 10.03±8.49 mm/hr (Table 4.16).
Table 4.16: Baseline data of immuno hematological markers of respondents

<table>
<thead>
<tr>
<th>Immuno-haematological Variables</th>
<th>HIV+/TB+ (Mean ± SD)</th>
<th>HIV-/TB+ (Mean ± SD)</th>
<th>HIV-/TB- (Mean ± SD)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Blood Cells (x10⁹/L)</td>
<td>4.90 ± 2.15</td>
<td>5.91 ± 2.43</td>
<td>6.92 ± 3.12</td>
<td>0.00</td>
</tr>
<tr>
<td>Packed cells volume (%)</td>
<td>32.31 ± 4.81</td>
<td>34.1 ± 4.34</td>
<td>36.92 ± 4.21</td>
<td>0.00</td>
</tr>
<tr>
<td>Platelets Count (x10⁹/L)</td>
<td>163.29 ± 65.11</td>
<td>195.16 ± 88.71</td>
<td>240.07 ± 99.42</td>
<td>0.00</td>
</tr>
<tr>
<td>Erythrocytes Sedimentation rate (mm/hr)</td>
<td>115.13 ± 11.82</td>
<td>102 ± 17.35</td>
<td>10.03 ± 8.49</td>
<td>0.00</td>
</tr>
</tbody>
</table>

t=7.45, df=521, p<0.05); SD=standard deviation; P-value=level of marginal significance; Yates corrected chi-square test for categorical variables.

Findings from this study indicated that there were significant mean differences between all immunohaematological markers (white blood cell count, packed cell volume and platelets) tested and TB-HIV status of the participants (p<0.05). The findings agreed with Affusiment et al. (2011) and Tarbasiet et al. (2008). The differences in the white blood cells count to the infections according to gender may be hormonal but the end results are often similar. The PCV controls show that females were more prone to blood loss, implying higher strain on their erythropoietic activities, secondary to regulate menstrual.

The reduction in the number of circulatory cells is a common complication of infection with TB and HIV infection and in the course of the disease more than 70% of the patients develop anemia that frequently required transfusion (Onubogu, 2010). Packed cell volume measures the percentage of blood volume taken up by the red blood cells and very low reading can indicate anemia. Platelets stops bleeding by forming clots and scabs which further prevents thrombocytopenia. In this study, erythrocyte sedimentation rate values in TB and TB-HIV co-infection were very high. This may be due to delayed diagnosis of TB in HIV subjects. In view of this, some of
the HIV subjects may have developed Immune Reconstitution Inflammatory Syndrome (IRIS) which is most often times associated TB-HIV co-infection at the onset Anti-Retroviral Therapy (Corbett et al., 2009). This also supports the fact that delayed diagnosis of HIV is a major problem in Kenya particularly in Mombasa County. In clinical practice, ESR test is commonly carried out as a non specific test during the initial diagnostic work-up for TB, which is a chronic bacterial infection. Some studies have documented ESR associated with the infection (Affusimet et al., 2011). T-test analysis showed that there was significant mean difference between TB-HIV status and ESR results (p<0.05).

4.5.5 Immunohaematological reference values in human immunodeficiency virus negative patients (controls).

The mean CD4+T count in males and females combined was 1054.9 ± 156.1 cells/mm³, mean absolute CD4+T lymphocyte in males was 998.7 ± 127.1 cells/mm² and in females 1106.8 ± 162.4 cells/mm³. The Mean absolute CD8+T cells in males and females combined was 688.4 ± 105.9 cells/mm³ and mean absolute CD8+T counts in males were 710.9 ± 113.8 cells/mm³ and in females 667.7± 93.6 cells/mm³. The mean CD4/CD8+T cells ratio in males and females combined was 1.56± 0.39, cells/mm³. Mean CD4/CD8+T cells in males were 1.42± 0.17 while in females’ 1.69 ± 0.49 cells/mm³. Females had significantly higher mean absolute CD4+ cells (t=8.32, df 485.2, p=0.001) and lower mean absolute CD8+T cells ( t=4.62, df=463.8, p< 0.05) than in males. The haemoglobin level in males and females combined was 12.76± 1.28 gram/dl. Mean haemoglobin level in males was 13.06± 1.48 gm/dl and in females 12.48±1.00 gm/dl. Females had significantly lower mean haemoglobin level than males (t=5.06, df, 416, p=0.002) (Table 4.16 below).
Table 4.17: Arithmetic median, 2.5th–97.5th percentile, mean and 95% CI of the mean CD4+T cells, CD8+T cells, CD4/CD8 ratio and haemoglobin level in Normal subjects (Controls)

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Median</th>
<th>2.5th-97.5th percentile</th>
<th>Mean ± SD</th>
<th>95% CI of the Mean</th>
<th>Difference in Means</th>
<th>Difference in Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute CD4 T cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>986</td>
<td>834.0 - 1319.9</td>
<td>998.7 ± 127.1</td>
<td>982.6 - 1014.9</td>
<td>t= - 8.32, df=485.2</td>
<td>F= 19.94, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>1070</td>
<td>843.2 - 1503.9</td>
<td>1106.8 ± 162.4</td>
<td>1087.0 - 1126.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>1011.5</td>
<td>837.6 - 1464.8</td>
<td>1054.9 ± 156.1</td>
<td>1041.2 - 1068.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Absolute CD8 T cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>690</td>
<td>540.1 - 1011.8</td>
<td>710.9 ± 113.8</td>
<td>696.4 - 725.3</td>
<td>t=4.62, df=463.8</td>
<td>F=4.95, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>665</td>
<td>483.7 - 850.5</td>
<td>667.7 ± 93.6</td>
<td>656.3 - 679.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>673</td>
<td>497.5 - 973.8</td>
<td>688.4 ± 105.9</td>
<td>679.1 - 697.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Absolute CD3 T cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>1815</td>
<td>1359.5 - 2929.4</td>
<td>1871.3 ± 355.2</td>
<td>1826.1 - 1916.4</td>
<td>t=-3.79, df=476.5</td>
<td>F=9.708, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>1897.5</td>
<td>1433.0 - 3544.2</td>
<td>2013.2 ± 478.3</td>
<td>1954.8 - 2071.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>1860</td>
<td>1418.6 - 3073.8</td>
<td>1945.1 ± 429.2</td>
<td>1907.4 - 1982.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD4/CD8 T cells ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>1.4</td>
<td>1.03 - 1.80</td>
<td>1.42 ± 0.17</td>
<td>1.40 - 1.44</td>
<td>t=-8.48, df=325.8</td>
<td>F=8.85, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>1.61</td>
<td>1.35 - 2.23</td>
<td>1.69 ± 0.49</td>
<td>1.63 - 1.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>1.51</td>
<td>1.11 - 2.11</td>
<td>1.56 ± 0.39</td>
<td>1.53 - 1.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemoglobin level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>13</td>
<td>10.60 - 15.60</td>
<td>13.06 ± 1.48</td>
<td>12.87 - 13.25</td>
<td>t=5.06, df=416.5</td>
<td>F=19.94, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>12.5</td>
<td>10.20 - 14.50</td>
<td>12.48 ± 1.00</td>
<td>12.36 - 12.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>12.7</td>
<td>10.40 - 15.20</td>
<td>12.76 ± 1.28</td>
<td>12.65 - 12.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD4+=Cluster differentiation T-lymphocyte no.4; CD8+=Cluster differentiation T-lymphocyte no.8; BMI=Body mass index; SD=Standard deviation; CI=Confidence interval; P-value=level of marginal significance; *statistically significant association.

In this study, the mean immunohematological reference values in healthy subjects (controls) for CD4+T and CD8+T counts was 1054.9 ± 156.1 cells/mm³ and 998.7 ± 127.1 cells/mm³ respectively. The study also found that females had significantly higher counts of absolute CD4+T cells and absolute CD8+ T cells than males. Significant differences were observed in the absolute count means of the lymphocytes.
in males and females than in CD3+T cells (p<0.5) and CD4+T cells (P<0.5). Diurnal variation, however, cannot explain the significant gender difference observed in this study, as all the samples were collected between 9.00am to 11.00am. While smoking was also reported to be associated with higher CD4+T count, this could not be the reason for the difference as all female subjects in the present study were nonsmokers. Sex hormone effect could be the possible explanation for the observed gender difference in CD4+T cell count, as the circulating lymphocytes have receptors for androgens and oestrogens (Cailhol et al., 2005). These findings are similar to those reported in other studies in African settings (Prins et al., 1999, Shahabbuddin 1995, Menard et al., 2003). Studies done in Cameroon, Ethiopia and Tanzania have reported higher CD4+T cell counts (Cailhol et al., 2005). There are also several other studies done in Africa and Asia that reported different values for CD4+ T cell levels compared to standard values for western countries (Rob et al., 2005), where as some studies have demonstrated higher CD4+ T cells among Ugandans and Kenyans than the values known for North America, Europe and Asia.

These variations in CD4+T cells have been shown to be associated with ethnicity, gender, diet, geographical area as well as being dependent on genetic and environmental factors. The value of the CD4+ T cell counts obtained in this study is lower than those reported for Ethiopians and the Dutch counterparts (Sonnenberg et al., 2004, Cailhol et al., 2005). In this study, females had significantly lower haemoglobin than males 12.48 ± 1.00 vs. 13.06 ±1.48. This is similar to findings in North America, Europe and Asia. This is attributed by low dietary intake of food rich in iron and vitamins which is the commonest causes of low haemoglobin levels in Kenya. The values obtained for haemoglobin levels in this study are lower than the
current value used in Kenya. This shows that significant proportions of the population are anaemic, though the current study did not explore the causes of anaemia.

4.5.6 Males and females with lower than normal values of CD4 T cells, leukocytes, lymphocytes and body mass index

The mean absolute leukocytes cells in both males and females were 5.15± 0.37 cells/litre. Mean absolute leukocytes cells in males was 4.89± 0.22 cells/litre and in females 5.40± 0.30 cells/litre. Mean absolute lymphocytes cells in males and females combined were 1.85± 0.29 cells/litre. The mean lymphocyte in males was 1.67± 0.20 cells/litre while in females 2.02± 0.26 cells/litre. On nutritional status assessment using BMI, the mean BMI for the participants was 22.6± 95% CI: 22.5-22.7 kg/m². Mean BMI for males was 22.4± 95% CI: 22.3-22.6 kg/m² and for females 22.9± 95% CI: 22.8-23.1 kg/m². There was no significant statistical difference between males and females with regards to the nutritional status ($\chi^2$=76.3, df=1, p>0.05). There was no significant association between nutritional status with any of the immunohaematological parameters like CD4+T cells and CD8+T cells and lymphocytes (p>0.05) but there was association between BMI and leukocytes (p<0.05). Significant statistical differences was observed in the absolute count means of the lymphocytes in males and females (p<0.05). There was association between different age-groups and immunohaematological parameters like CD4+T cells (p<0.05) and CD8+T cells (p<0.05) (Table 4.18).
Table 4.18: Males and females with lower than normal and normal values of CD4 T cells, leukocytes, lymphocytes and body mass index (Controls)

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Median</th>
<th>2.5th-97.5th percentile</th>
<th>Mean ± SD</th>
<th>95% CI of the Mean</th>
<th>Difference in Means</th>
<th>Difference in Variances</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute Leukocytes × 10⁹/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>4.9</td>
<td>4.50 - 5.30</td>
<td>4.89 ± 0.22</td>
<td>4.86 - 4.92</td>
<td>t= -21.98</td>
<td>F=18.97, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>5.4</td>
<td>4.70 - 5.90</td>
<td>5.40 ± 0.30</td>
<td>5.36 - 5.43</td>
<td>df= 471.2, p= 0.003*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>5.1</td>
<td>4.60 - 5.80</td>
<td>5.15 ± 0.37</td>
<td>5.12 - 5.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Absolute Lymphocytes cells/litres</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>1.6</td>
<td>1.40 - 2.20</td>
<td>1.67 ± 0.20</td>
<td>1.64 - 1.70</td>
<td>t= -17.15</td>
<td>F=19.66, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>2</td>
<td>1.60 - 2.50</td>
<td>2.02 ± 0.26</td>
<td>1.99 - 2.06</td>
<td>df= 484.7, p= 0.001*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>1.8</td>
<td>1.40 - 2.50</td>
<td>1.85 ± 0.29</td>
<td>1.83 - 1.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nutritional status given as BMI in kg/m²</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>240</td>
<td>22.75</td>
<td>19.20 - 24.49</td>
<td>22.44 ±1.35</td>
<td>22.27 -22.61</td>
<td>t= -4.26</td>
<td>F=7.32, p&lt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>260</td>
<td>23.1</td>
<td>19.80 -24.60</td>
<td>22.91 ±1.12</td>
<td>22.77 -23.05</td>
<td>df= 465.3, p= 0.001*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>23</td>
<td>19.45 -24.55</td>
<td>22.68 ±1.25</td>
<td>22.57 -22.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI=Body mass index; SD= standard deviation; CI=confidence interval; *statistically significant association

The mean absolute Leukocyte cells was 5.15±0.37 and females had a significantly higher value than males (p<0.05). This finding is similar to a study done in India and Tanzania to determine gender differences in CD4+ T cells which showed that males were more likely to have less CD4+T cells compared to females (Uppas *et al.*, 2005, Sharma *et al.*, 2005). The findings of this study can serve as reference ranges for adults in Kenya. Therefore the current guidelines on the normal CD4 and CD8 in Kenya should be revised to adopt the population specific reference ranges.

4.5.7 Comparison of mean cell types in different study groups

4.5.7.1 Tuberculosis patients and reference values

Tuberculosis patients had statistically significant lower mean CD4+T counts 474.5±198.8 cells/mm³ than the reference value found in this population (1054.9 ± 156.1 cells/mm³, t=34.6, df=485, p<0.05) and lower haemoglobin level (11.2±5.9...
gm/dl) than the reference value for this population (12.8 ±13 g/dl, t=27.5, df=453
p=0.002) (Tables 4.13 and 4.17).

4.5.7.2 Tuberculosis and HIV/AIDS patients
Tuberculosis patients had higher CD4+T cell levels and lower haemoglobin levels than HIV/AIDS patients. HIV/AIDS patients had higher mean lymphocyte cell count (1.8±0.8 gm/dl) than tuberculosis patients (1.4 ±0.5, t=27.5, df=61, p<0.05) as shown in (Tables 4.13 and 4.15).

4.5.7.3 Tuberculosis and HIV/AIDS patients with and without ARVs’ treatment
Tuberculosis patients had statistically significant higher CD4+T counts (474.5±198.8 cells/mm³) than HIV/AIDS patients on treatment (333.3 ± 150.6, t=4.6, df=481, p<0.05) and HIV/AIDS patients not on treatment (290 ± 184, t=7.6, df=58, p<0.05). Tuberculosis patients had significant lower mean haemoglobin level (11.2±5.6 gm/dl) than HIV/AIDS patients on treatment (12.7 ±5.7, t=3.4, df=68, p<0.05)

4.5.7.4 Tuberculosis and tuberculosis HIV co-infected patients
Tuberculosis patients had statistically significant higher mean CD4+T cell counts (t=5.6, df=461, p<0.05) and higher leukocyte counts (t=3.8, df=472, p<0.05) than HIV/AIDS tuberculosis co-infected patients (Tables 4.13 and 4.14).

4.5.7.5 HIV/AIDS and tuberculosis HIV co-infected patients
HIV/AIDS patients had statistically significant higher mean CD4+T cell counts (t=4.9, df=46, p<0.05), higher haemoglobin level (t=4.7, df=41, p<0.05), higher leukocyte counts (t=3.2, df=47, p<0.05) and higher lymphocyte counts (t=2.3, df=44, P=0.02) than tuberculosis co-infected patients (Tables 4.14 and 415).
4.5.7.6 HIV/AIDS treatment naïve and HIV/AIDS patients on treatment

HIV/AIDS patients on treatment had significantly higher mean CD+T cell counts (389.5±330.5) counts than HIV/AIDS patients not on treatment (270.5 ±189, t=3.3, df=69, p=0.01) and higher mean haemoglobin levels (11.7±3.6) than those not on treatment (10.5 ±2.1, t=2.3, df=36, p<0.05). Female HIV/AIDS treatment naïve patients had statistically significant higher mean CD4+T count (310.3±201.4 cells/mm³) than males (207.3 ±135.4, t=2.3, df=56, p<0.05).

4.5.7.7 Nutritional status in different groups

Patients with tuberculosis were significantly malnourished (BMI 19.7±2.4 kg/m²) as compared to the normal values for this population (BMI 22.7 ±1.3, t=8, df=465, p<0.05). HIV/AIDS patients had significantly higher BMI (19.9±2.2 kg/m²) than tuberculosis HIV/AIDS co-infected patients (BMI 18.8±2.7, t=0.70, df=58, p<0.05). There was a significant relationship between low BMI and TB co-infection. This study also found no significant difference between males and females with regards to nutritional status in any of the different groups (p>0.05) (Tables 4.13, 4.14, 4.15 and 4.17).

4.6 The susceptibility and resistance patterns of Mycobacterium tuberculosis isolates to first line anti-tuberculosis drugs among tuberculosis patients

Among the newly diagnosed TB cases resistance to at least one drug was 43 (22.3%). One hundred and fifty (77.7%) of the isolates were susceptible to all four anti-TB drugs tested. Resistance to at least one drug in recurrent TB patients was 12 (70.6%) and sensitive 5(29.6%). Any single drug resistance was most frequent in isoniazid 17.6%, Ethambutol 13.8%, Streptomycin 13.3% and Rifampicin 3.8%. Combined drug resistance in newly diagnosed TB patients was common in isoniazid and
Rifampicin 2.1%, Rifampicin Isoniazid and Ethambutol 0.5%. Other resistance patterns included H+E (5.7%), E+S (5.7%) and H+S (3.6%), R+E (1%) in newly diagnosed patients compared to 17.6%, 5.9%, 17.6% and 11.8% in previously treated cases. Multi drug resistance (MDR) TB was observed in ten isolates (4.8%). One MDR isolate was triple resistant with an additional resistance to Ethambutol (E).

The resistance patterns in recurrent TB cases were Isoniazid 41.2%, streptomycin 58.8%, Ethambutol 52.9% and Rifampicin 23.5%. The most frequent double resistance among this category was Isoniazid and Rifampicin (H+R) 23.5%. Isoniazid (H), streptomycin (S) and Ethambutol (E) represented 5.9% in triple resistance in previously treated patients and 0.5% in newly diagnosed TB patients. Five (2.6%) and five (29.4%) strains from new and previously treated patients were multidrug resistance (MDR) TB defined as resistant to at least both Isoniazid and Rifampicin. Each parameter of drug resistance between previously treated cases and new cases of TB were compared and all parameters were higher in previously treated patients. However among those, statistical difference was observed for Rifampicin and Ethambutol (p<0.05). Otherwise the difference seen for any type of drug resistance (p=0.04), any type of INH resistance (p<0.003), any type of STM resistance (0.05), combined type of STM and INH resistance (p= 0.002) and multi drug resistance (p<0.05) were all higher in the previously treated TB patients (Table 4.19).
4.19: Drug resistance patterns of *Mycobacterium tuberculosis* isolates to first line anti-tuberculosis drugs

<table>
<thead>
<tr>
<th>Item</th>
<th>Newly Diagnosed Patients (95% CI)</th>
<th>Previously Diagnosed Patients (95% CI)</th>
<th>Total (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DST</td>
<td>193 (100%)</td>
<td>17 (100%)</td>
<td>210 (100%)</td>
</tr>
<tr>
<td>Sensitive to all</td>
<td>150 (78%)</td>
<td>5 (29.4%)</td>
<td>155 (73.8%)</td>
</tr>
<tr>
<td><strong>Mono Resistance TB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoniazid (H)</td>
<td>30 (16%)</td>
<td>7 (41.2%)</td>
<td>37 (17.6%)</td>
</tr>
<tr>
<td>Rifampicin (R)</td>
<td>4 (2.1%)</td>
<td>4 (23.5%)</td>
<td>8 (3.8%)</td>
</tr>
<tr>
<td>Ethambutol (E)</td>
<td>20 (10.0%)</td>
<td>9 (52.9%)</td>
<td>29 (13.8%)</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>18 (9.3%)</td>
<td>10 (58.8%)</td>
<td>28 (13.3%)</td>
</tr>
<tr>
<td><strong>Multi-Drug Resistance TB (MDRTB)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H+R</td>
<td>4 (2.1%)</td>
<td>4 (23.5%)</td>
<td>8 (3.8%)</td>
</tr>
<tr>
<td>H+R+E</td>
<td>1 (0.5%)</td>
<td>1 (5.9%)</td>
<td>2 (1.0%)</td>
</tr>
<tr>
<td>H+R+S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H+R+E+S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total MDR -TB</strong></td>
<td>5 (2.6%)</td>
<td>5 (29.4%)</td>
<td>10 (4.8%)</td>
</tr>
<tr>
<td><strong>Other resistance patterns</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H+E</td>
<td>11 (5.7%)</td>
<td>3 (17.6%)</td>
<td>14 (6.7%)</td>
</tr>
<tr>
<td>H+S</td>
<td>7 (3.6%)</td>
<td>3 (17.6%)</td>
<td>10 (4.8%)</td>
</tr>
<tr>
<td>H+E+S</td>
<td>2 (1.0%)</td>
<td>0.98-3.0</td>
<td>2 (1.0%)</td>
</tr>
<tr>
<td>R+E</td>
<td>2 (1.0%)</td>
<td>0.98-3.0</td>
<td>4 (1.9%)</td>
</tr>
<tr>
<td>E+S</td>
<td>11 (5.7%)</td>
<td>1 (5.9%)</td>
<td>12 (5.7%)</td>
</tr>
<tr>
<td>R+S</td>
<td>5 (2.6%)</td>
<td>0.65-4.6</td>
<td>5 (2.4%)</td>
</tr>
<tr>
<td>R+E+S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T test for numerical variables; DST=Drug susceptibility testing; MDR-TB=Multidrug resistant tuberculosis; CI=confidence interval.

Resistance to Isoniazid in this study was 17.6% which was higher than results obtained in earlier studies in Kenya, where resistance to INH was 10.7% (Githui *et al*., 1998). INH resistance in this present study was also much higher than that seen in Ethiopia where one isolate was resistant to INH (Kassu *et al*., 2008) and in Bangladesh and Sri Lanka at 5.4% and 12.2% (Magana *et al*., 2010), respectively. In 2008, the WHO reported a worldwide resistance rate to INH of 5.9% (WHO, 2010). According to WHO, INH resistance rates higher than 10% can predict development of
MDR-TB (Mycal et al., 2005). Isoniazid is given in both the intensive and the continuation phase of TB treatment (Zwareinstein et al., 1998). Isoniazid only given with ethambusol (EMB) in continuation phase but since it is a weak anti-TB drug (Sharma et al 2005), it may not be effective to prevent development of resistance to isoniazid (INH) due to poor compliance by patients.

In this study, resistance to Rifampicin was 3.8% which was higher than that observed in earlier studies in Kenya where resistance was 0.3% and 1.3% (Githui et al., 1998) and in Ethiopia study where one isolate was resistant Rifampicin. Rifampicin has several adverse effects such as nausea, vomiting, rashes, GIT upset, flu-like symptoms, fever and jaundice which could result in patient non-adherence and hence may lead the selection of resistant strains. In the DOTs program, Rifampicin is given in the intensive phase under direct observation together with at least three drugs. Moreover in the continuation phase, Rifampicin is spared. This fact together with relatively lower rate of mutation developed against Rifampicin and the introduction of the drug in Kenya DOTs program could have resulted in a low level of resistance (Sharma et al., 2005).

Resistance to Ethambutol in this study was 13.8% was higher than rates in Ethiopia of 2.7% (Raviglione et al., 2007). However, it was lower than studies conducted in Sri Lanka where 14.5% resistance was reported (Nunes et al., 2008). One possible explanation for increased Ethambusol resistance could be increased defaulting rate from TB treatment in the continuation phase when it is administered. It also enhances the effect of many drugs including beta lactams to different Mycobacteria species and can be used to develop a regimen for MDR-TB (Ziguol et al., 2006). The drug has
been in use since the beginning of tuberculosis (TB) chemotherapy. The treatment regimen used in most countries only adds one extra drug Streptomycin to patients who may have failed in the first treatment.

Resistance to streptomycin (S) in this study was 13.3% which was higher than resistance of 1.8% (Githui et al., 1993) and 10.5% (Ogaro et al., 2011). Furthermore, streptomycin was the first ever TB drug developed and resistance to the drug started to develop almost immediately (Cohen et al., 2006). Streptomycin resistance is common in many African countries with resistance ranging from a high of 28.2% in the Democratic Republic Congo and 24.5% in Ethiopia to a low of 0.8% in Zimbabwe (WHO, 2010).

The findings in this study showed that a high number of patients with TB showed INH resistance yet susceptible to all other drugs. According to WHO guidelines for management of drug-resistant TB, drug-resistant patients can be classified into three groups: those releasing bacilli resistant susceptible to all anti-TB drugs, those releasing bacilli resistant to INH but susceptible to Rifampicin and those releasing bacilli resistant to at least INH and Rif (Kassu et al., 2008). Most of the isolates in this study were resistant to INH and Rif and it is therefore possible for these patients to recover fully if WHO guidelines for retreatment are followed under strict supervision to prevent them from developing MDR-TB. However, the high rate of INH resistance is significant since it is a first-line drug which is used throughout the course of treatment. This indicates a high probability for developing MDR-TB in the future since it has been observed that MDR-TB often develops from initial INH mono resistant strains. Isoniazid (INH) is also the drug of choice for chemoprophylaxis of
TB and is used in developed countries for treating latent TB. The high level of INH resistance among the study population also is an indicator that this drug will be completely useless for both these purposes in Kenya. In this study, eight patients had MDR-TB (4.8%) which is not unusual because in Sub-Saharan Africa countries MDR-TB prevalence is estimated to be 6.3% (WHO, 2010). To account for the high rate of multiple resistance observed in this study, it would be important to look back into the continuation phase where INH and EMB are given for six months. In an area where there is high level of INH resistance, treatment with INH and EMB is not probably effective and will lead to further acquisition of INH resistance and EMB resistance. This is because Ethambutol is a bacteriostatic drug with low efficacy that may not effectively prevent development of resistance to INH.

4.6.1 Drug resistance between HIV-positive and HIV negative newly diagnosed TB patients

Prevalence of any type of drug resistance in TB-HIV co-infection patients was 19.1% and 7.1% in HIV negative. The difference was not statistically significant (P>0.05). In mono drugs, Isoniazid (11.9%) showed the highest resistance pattern in TB-HIV co-infected patients followed by streptomycin 4.8%, Ethambutol 4.3% and Rifampicin 2.4%. Combined resistance to R+E was 1.9% and 0.0% in TB-HIV co-infected and HIV negative patients respectively. These differences was statistically significant (p<0.05). Triple resistance to Isoniazid, Ethambutol and streptomycin was 0.5% in both HIV positive and negative TB patients respectively. These differences was statistically significant (p<0.05). The prevalence of other types of drug resistance was also significantly different in HIV positive and HIV negative TB patients. (Table 4.20)
## Table 4.20: Comparison of drug resistance between HIV-positive and HIV negative newly diagnosed TB patients (n=210)

<table>
<thead>
<tr>
<th></th>
<th>HIV Positive n (%)</th>
<th>HIV Negative n (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any type of drug resistance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>40 (19.1%)</td>
<td>15 (7.1%)</td>
<td>0.379</td>
</tr>
<tr>
<td>Sensitive</td>
<td>38 (18.1%)</td>
<td>117 (55.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Isoniazid (H)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>25 (11.9%)</td>
<td>12 (5.7%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sensitive</td>
<td>53 (25.2%)</td>
<td>120 (57.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Rifampicin (R)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>5 (2.4%)</td>
<td>3 (1.4%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sensitive</td>
<td>73 (34.8%)</td>
<td>129 (61.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethambutol (E)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>9 (4.3%)</td>
<td>20 (9.5%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sensitive</td>
<td>59 (28.1%)</td>
<td>112 (53.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Streptomycin (S)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>10 (4.8%)</td>
<td>18 (8.6%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sensitive</td>
<td>68 (32.4.5%)</td>
<td>114 (54.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>R+E</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>4 (1.9%)</td>
<td>0 (0.0%)</td>
<td>0.009*</td>
</tr>
<tr>
<td>Sensitive</td>
<td>74 (35.2%)</td>
<td>132 (62.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>H+E+S</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>1 (0.5%)</td>
<td>1 (0.5%)</td>
<td>0.705</td>
</tr>
<tr>
<td>Sensitive</td>
<td>77 (36.7%)</td>
<td>131 (62.3%)</td>
<td></td>
</tr>
</tbody>
</table>

P-value=level of marginal significance; *statistically significant association

In this study, drug resistance in TB-HIV co-infection patients was 19.1%. Association was observed between HIV infection and drug resistance in newly diagnosed TB patients. This could suggest that drug resistant TB strains mainly cause TB in immunocomprised not at similar rate with immune competent TB patients. Immunity is not much comprised in early stages of HIV infection. Thus, study may be interpreted to directly argue for the hypothesis that drug resistant TB is virulent and causes disease mainly in immunocompromised TB patients (p<0.05). In this regard, similar findings have been reported by different studies.

Studies in Latvia showed that any resistance and multi-drug resistant tuberculosis (MDR-TB) were significantly associated with HIV infection (Mycal *et al*., 2005). A study done in Northern Tanzania showed that among TB-HIV co-infection, patients’
resistance to at least one drug was 10.8%. Tuberculosis infections in high incidence countries have been shown to be recently transmitted and failure to contain MDR-TB and XDR reflects inability to diagnose the problem early to prevent transmission of the same while continuing to prescribe an ineffective regimen (Millen et al., 2008). Once MDR-TB has developed, further progression to pre-XDR and XDR is only a question of time and will place over few months or even years. The patient remains infectious and transmission of MDR TB and XDR-TB continues particularly in areas with high prevalence of HIV/AIDS and overcrowding. Sarita et al. (2007) found out in a study in Texas that TB patients with HIV were more likely to have rifampicin resistance and less likely to have isoniazid resistance. However a study done in Punai Mahararashtra India on anti-TB drug resistance showed that prevalence of drug resistant isolates among HIV seropositive patients was similar to that of seronegative TB patients indicating that HIV infection may not be associated with drug resistant TB (Sharma et al., 2005).

4.6.2 Association between patients’ demographic characteristics and any type of anti-tuberculosis drug resistance (Rifampicin)

The majority of the respondents (50%) who showed resistance to Rifampicin resistance were in the 31-40 age-group followed by both 21-30 and 41-50 (25.1%) age-groups respectively and statistical significant was noted (p<0.05). Overall females constituted 2.4% and males 1.4% of resistant cases. However there was significant differences in resistance rate between gender, females being more vulnerable (OR=0.735; 95% CI: 0.246-2.196; p<0.05). Eleven (2.4%) of the HIV patients were resistant to Rifampicin. There was a statistically significant difference between Rifampicin and HIV infection ($\chi^2$=11.027, df=1; p<0.05). Indicating a positive
correlation. A multivariate analysis was done by entering the variables that were found to be associated with MDR-TB at \( p<0.05 \) in the bivariate analysis into unconditional logistic regression model. The variables included HIV status and previous treatment with anti-tuberculosis drugs. From the model, these variables were independently associated with MDR-TB in the study. History of previous treatment with anti-TB drugs was the strongest factor associated with MDR-TB (OR=3.09; 95% CI; 0.07-1.57) as shown in table 4.2.

**Table 4.21: Association between patients’ demographic characteristics and any type of anti-TB drug resistance (Rifampicin)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Attribute</th>
<th>Resistant (%)</th>
<th>Sensitive (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly diagnosed TB patients</td>
<td>Yes</td>
<td>8 (100.0%)</td>
<td>202 (100%)</td>
<td>1.00**</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0.17 (0.04 - 0.64).</td>
</tr>
<tr>
<td>Age in Years</td>
<td>18-20</td>
<td>0 (0.0%)</td>
<td>2 (0.95%)</td>
<td>1.00**</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>2 (25.0%)</td>
<td>87 (41.4%)</td>
<td>0.29 (0.11-1.05)</td>
</tr>
<tr>
<td></td>
<td>31-40</td>
<td>4 (50.0%)</td>
<td>54 (25.7%)</td>
<td>0.22 (0.05-0.89)</td>
</tr>
<tr>
<td></td>
<td>41-50</td>
<td>2 (25.0%)</td>
<td>38 (18.1%)</td>
<td>0.43 (0.08-2.23)</td>
</tr>
<tr>
<td></td>
<td>51+</td>
<td>0 (0.0%)</td>
<td>21 (10.0%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>5 (2.4%)</td>
<td>100 (47.6%)</td>
<td>1.00**</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3 (1.4%)</td>
<td>102 (48.6%)</td>
<td>0.73 (0.24 - 2.19)</td>
</tr>
<tr>
<td>HIV +</td>
<td>Yes</td>
<td>5 (2.4%)</td>
<td>200 (95.2%)</td>
<td>0.14 (0.03 - 0.52)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3 (1.4%)</td>
<td>2 (0.95%)</td>
<td>1.00**</td>
</tr>
</tbody>
</table>

OR=Odds ratio; CI=confidence interval; **statistically significant association

In this study, 2.4% females were associated with Rifampicin drug resistant than males (1.4%). This differs with studies in Pakistan where Rifampicin resistant was associated with males 70.9% and females 29.15% (Kassu et al., 2008). The present study showed an increase in resistant to Rifampicin suggesting possible increase in MDR-TB in this population. The high rate of this resistance could be associated with a number of factors like extensive use of the drug for TB, malabsorption,
noncompliance and single drug administration or altered absorption for Rifampicin (Cohen et al., 2006). There was a positive association between this drug resistance and TB-HIV co-infection patients (p<0.05). HIV is a single most important contributor of increasing TB burden in Kenya and it makes MDR-TB challenging both technically and epidemiologically. High prevalence of drug resistance in HIV infected TB patients has been reported. Taylor et al. (2009) found out in a study in Texas that TB patients with HIV were more likely to have Rifampicin resistance.

In this study, fragments of rpoB covering the 511-533 codons were PCR amplified (Gene Expert) using rpoB forwarded and reverse primers to test Rifampicin resistance. Sequencing of 350bp rpoB fragment from both UK and Mombasa County isolates showed absence of mutations in the majority of isolates. Mutation was associated with resistance to Rifampicin as obtained by antibiotic susceptibility testing for these isolates while there was no mutation in the gene sequence of Rifampicin susceptible Mycobacterium tuberculosis strain. It has been noted that different types of mutations to RMP result in different fitness levels. This situation creates differences in probabilities of transmission (Taylor et al., 2009). Probably, the type of mutation frequently observed in Mombasa County could be one that renders strains ineffective in getting transmitted. History with previous treatment with anti-TB drugs was significantly associated with any type of drug resistance and multiple drug resistance (MDR). The association still existed when it was adjusted for HIV, age and occupation. Previously treated TB patients could have drug resistant TB from the outset. These patients have a tendency to have an unsuccessfully treatment out come.
CHAPTER FIVE
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study determined the occurrence of Tuberculosis and HIV co-infect and their influence on immunohaematological markers (CD4+T and CD8+T cells, PCV and ESR) in Mombasa County in Kenya. Of the five hundred (500) subjects who participated in this study, 42% had tuberculosis. Females constituted 45.8% and males 38.7% of cases. The most affected age-group was 25-34 years (38.6%) and 35-44 years (29.1%) but it was not statistically significant (p<0.05). Two hundred and two patients had pulmonary tuberculosis (96.2%) while eight (3.8%) extra pulmonary tuberculosis. Up to 88.1% of TB cases were smear positive and 11.9% smear negative but there was no significant difference between age and smear positive ($\chi^2=40.8; \text{df}=4; p<0.05$). The prevalence of TB-HIV co-infection was 37.1% and it was higher in females (55.1%) than males (49.9%) but there was no significant difference between co-infection rate and gender (p >0.05). Thirty (14.3%) of the TB cases were recurrences and thirteen (43.3%) were HIV co-infected.

Regarding clinical symptoms of TB patients enrolled in this study, the majority (72%) had history of cough, breathlessness (13.6%), fever (41.7%) and weight loss (23.5%). In clinical signs, night sweats and respiratory signs were common in TB and TB-HIV co-infected patients. Logistic regression showed that weight loss and cough remained significantly associated with pulmonary tuberculosis (p<0.05).

Tuberculosis patients had lower mean CD4+T counts (474.5±198.8 cells/mm$^3$) than the reference value in this population (1054.9 ± 156.1 cells/mm$^3$) and lower
haemoglobin level (11.2±5.9 gm/dl). Tuberculosis and HIV co-infected patients had significantly lower CD4+T cell count (276±142 cells/mm$^3$) than people living with HIV/AIDS (PLWHA) without TB (333.27±150.59 cells/mm$^3$). Both TB and TB-HIV co-infected patients were significantly malnourished than the normal subjects in this population. There were significant differences between the mean distribution of haemoglobin levels, white blood cells, packed cell volume, platelets count, sedimentation rate and CD4+T cell counts of the respondents and TB-HIV co-infection (p<0.05).

Prevalence of any type of drug resistance in TB-HIV co-infection patients was 19.1% and 7.1% in HIV negative. The difference was not statistically significant (P>0.05). Among the newly diagnosed TB patients resistance to at least one drug was 22.3% and in recurrent cases 70.6%. Any single drug resistance was most frequent in isoniazid 17.6%, Ethambutol 13.8% and Streptomycin 13.3%. Combined drug resistance was common in isoniazid and Rifampicin 2.1%, Rifampicin, Isoniazid and Ethambutol 0.5%. Multi drug resistance (MDR)-TB was observed in ten isolates (4.8%). There was a statistically significant difference between Rifampicin and TB-HIV infection ($\chi^2=11.027$ df=1; p<0.05) indicating a positive association.

5.3 Conclusions

- The study found high prevalence of tuberculosis (42.0%) among TB suspects in Mombasa County demonstrating a high burden of undetected TB cases.
- The prevalence of HIV in newly diagnosed TB patients was lower (39.7%) compared to current national average of HIV-TB co-infection prevalence (55%).
Clinical features of chronic dry cough, fever, night sweats and weight loss were common in both TB and TB-HIV co-infection patients.

Immunohaematological markers such as haemoglobin, white blood cell count, ESR, packed cell volume, platelets and CDT+T cell counts were significantly elevated in TB and TB-HIV co-infection patients compared to reference (controls) values for this population.

A T-lymphocyte subset reference range (in controls) can be used routinely as normal ranges for grading clinical decisions.

The study observed high resistance to Isoniazid (INH) and Rifampicin (Rif) the first-line drugs suggesting a possible increase in multidrug resistant tuberculosis (MDR-TB). There was a positive association between TB-HIV co-infection and drug resistance.

5.4 Recommendations

High prevalence of tuberculosis and HIV infection Mombasa County underscores the need for more efforts and resources to increase knowledge and poverty eradication.

All newly diagnosed TB and TB-HIV co-infection patients should be exposed to immunohaematological counts to monitor their immune system.

The current guidelines on the normal CD4 and CD8 should be revised to adopt the population specific reference ranges.

There is urgent need to improve drug susceptibility testing which is not routinely performed in Public hospitals in Kenya to monitor drug resistance in all newly diagnosed TB patients to prevent the emergence of multi-drug tuberculosis (MDR-TB).
5.5 Recommendation for further research

- There is need to explore a large case-control studies to identify risk factors and determine the contribution of TB-HIV co-infection to anti-TB first line drug resistance.

- Similar studies need to be replicated in other parts of the country to enable generalization of the results.
REFERENCES


Affussim, C., Kesieme, E. and Abah V. (2011). The Pattern of Presentation of Tuberculosis in HIV seropositive patients seen at Benin City, Nigeria. Ambrose Alli University, Ekpoma.


Onubogu, C., Kunle-Ope, C., Onyejepu, N., Nwokoye, Raheem, et al. (2010). Prevalence of Tuberculosis and Human Immunodeficiency Virus (TB/HIV) co-


**Tegbaru, B., Messele, T., Hailu, E., Girma, M., Demissie, et al. (2001).** Clinical outcomes and Laboratory Results of Tuberculosis Patients with or without HIV Infection in Two Health Institutions in Addis Ababa Ethiopian Health and Nutrition Research Institute.


**USAID. (2009).** Infectious Tuberculosis, countries- Kenya.


**Verwer, S.,Warren, R.M, Bayer, N., Richardson, M., Vander, G.D. and Borgdorff, M.W. (2005).** Rate of re-infection tuberculosis after successful treatment
is higher than rate of New tuberculosis. *American Journal of Respiratory Critical Care Medicine, 171*:1430-1435.


**WHO. (2007).** TB and HIV co-infection, CDC NCHSTP division of tuberculosis elimination. Scaling up prevention and treatment for TB and HIV.


APPENDICES

APPENDIX 1: Consent form

Title: Epidemiology of tuberculosis in HIV-infection, clinical presentation and impact on immunohaematological parameters in Mombasa, Kenya

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Telephone and fax numbers (landline and cell): 8710901 or 0733665099

Purpose of the study
My name is Shadrack Yonge. I am undertaking PhD study at the department of Public, Kenyatta University. You are being kindly asked to participate in a research study about Epidemiology of Tuberculosis and HIV co-infection, Clinical Presentation and Impact on Immunohaematological parameters. 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 TB and HIV infections at the community level. The information on TB levels in HIV 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 will specifically help the participants to know their sero status and status at this comparatively early stage (first TB clinic visit) and take care of themselves according to the counseling give to them to conditions less severe. You are selected as a possible participant because you are receiving care for Tuberculosis and HIV. Any findings from this study will be brought to the attention of your physician for purpose of better client management strategies and guidelines on management of Tuberculosis and HIV/AIDS. Please read this form and ask any questions that you may have before deciding whether or not to be in the research study.

Study procedures:
If you agree to be a participant in this research, you be asked about yourself, HIV and TB care. If you do not wish to answer any of the questions posed during the interview, you may say so and the interviewer will move on to the next question. The interview
will take place in a private room, and no one else but the interviewer will be present. You will also be required to collect only one sputum sample approximately 2ml in the provided specimen container for TB analysis and 1-2ml of blood sample for CD4 T cells count.

**Duration involvement**
The expected duration of the interview is about 10 minutes

**Risks and Benefits to being in the study**
This research has the slight risk that you may share some personal or confidential information by chance or that you may feel uncomfortable about talking some of the topics. However, you may refuse to answer any question or not to take part in a portion of the interview if answering the questions makes you uncomfortable. There will be no direct benefit to you, but your participation is likely to help us find out more about how Tuberculosis and HIV/AIDS. Any positive findings from the study will be brought to the attention of your physician for purpose of better client management strategies. The result will also benefit the policy makers in their provision and distribution of remarks to the affected areas and guidelines on management of TB and HIV.

You will not be given any monetary benefits; neither will you incur any loss regarding services you are getting from the hospital. Your participation is likely to help us find out more about how we can best offer Tuberculosis and HIV/AIDS treatment to people of Coast Province.

**Costs**
There will be no charge to you and you will not be provided with any incentive to take part in the research.

**Confidentiality**
All information collected during this study will be kept strictly confidential to the extent allowed by the law. By joining this study, you will be given a study identification number. All information will be collected on coded forms using this number. Your name will not appear on any of these forms but only on a master list kept separately under lock and key by the primary investigator. Your name will never be used in any of the databases where this information is stored or in connection with any scientific papers or reports published, which may result from the study findings. Information collected during the study will only be viewed by researchers involved in the study. It will only be shared with your medical doctor if it is deemed important in your treatment to improve your health, and upon your authorization.

**Voluntary Nature of the Study**
If you choose not to participate, it will not affect your current or future relations with the health facility here in any way. You will still be offered the standard medical care. If you decide not to answer any questions you will not be penalized by anyone.
Summary of your rights as a participant in this research study
Your participation in this research study is voluntary. Refusing to participate will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled. If you decide to join the study, you may withdraw at any time and for any reason without penalty or loss of benefits. If information generated from this study is published or presented, your identity will not be revealed. In the event new information becomes available that may affect the risks or benefits associated with this study or your willingness to participate in it, you will be notified so that you can decide whether or not to continue participating.

Authorization to Use and Disclose your Information
You are authorizing the Principal Investigators, Coast Provincial General hospital and Kenyatta University to use and disclose information concerning you and your identity, medical history and information collected during this study for the following purposes: Medical evaluation of someone with Tuberculosis and HIV/AIDS. Such information may also be disclosed or used by others involved in overseeing the study including Kenyatta University (KU) ethics review committee and the study sponsor and its agents. You may withdraw authorization to collect additional information about you at any time by writing to the local Principal Investigator, but information already collected may be continue to be used and disclosed. This authorization has no expiration date.

Please understand the following principles apply to all participants of the study.
1) Your participation in the study is voluntary and if you decide to participate, you will not be denied any services that are normally available to you.
2) Your confidentiality will be safeguard and no names of the participant will appear in any final report or publication resulting from the study.
3) No risk will be incurred in participating in the study
4) This study will only require one sputum

CONTACT INFORMATION
If you have concerns now or in the future regarding your rights in this study or research related injury, you may contact me or my supervisor through this number 0733920015 or the Kenyatta University (KU) Ethical Review: P.O Box 43884-00100 Nairobi, Kenya.
You can also contact the investigators of the study: Professor Michael F. Otieno (0720362277) or Dr Rheka R. Sharma (0733665066) both of Kenyatta University

CONSENT FORM TO BE SIGNED BY THE STUDY PARTICIPANTS
Signing below indicates that you have been informed about the research study in which you voluntarily agree to participate; that you have asked any questions about the study that you may have; and that the information given to you has permitted you to make a fully informed and free decision about your participation in the study. By
signing this consent form you do not waive any legal rights and the investigator (s) or sponsor (s) is not relieved of any liability they may have. A copy of this consent form will be provided to you.

The above details about the study and the basis of participation have been explained to me in English/Kiswahili and I agree to take part in the study. I understand that I am free to choose to be part of the study or not. I give consent to be screened for TB or HIV.

Participant signature/Thumb mark………………………….Date……………………
Research assistant: Mary Mwambuaru signature……………Date……………
Qualifications: BSc-Nursing, KRN

Principal investigator: signature……………………………..Date………………
(Affirming subject eligibility for the study and that informed consent has been obtained).
Appendix 2: Questionnaire for tuberculosis patients and PLWHA

File No
1. Age………………………….. Date of Birth……………… 2.Sex 1.M, 2.F
3. Inpatient/Outpatient No.
4. Marital status
6. Level of education
7. Occupation:
8. Socioeconomic status: (Where do you get daily food and shelter)
   1. Self provided 2. Dependent on others
9. How many people are living with you in the same house……………………
10. For women—are you Pregnant? 1. Y 2. N

Medical history
14. What were the results ? 1. Positive 2. Negative
15. Do you have any member of your family with previous (past 5years) or current open tuberculosis? 1.Y 2. N
17. Patient’s Current Pulmonary tuberculosis
20. CXR feature of tuberculosis 1. Y 2. N
21. What did the CXR reveal about the tuberculosis?
   1. Upper lobe infiltration with/without cavity, 2. Bilateral/Unilateral pleural effusions with hilar lymphadenopathy. 3. Others specify 4. N/A
22. Pulmonary tuberculosis 1. Y 2.N
23. State the location………………………………
25. Aspirates or biopsy for tuberculosis culture 1. Positive, 2. Negative 3. N/A
26. Other Radiological features of extra pulmonary tuberculosis 1. Y 2. 3. N/A
27. Biopsy for tuberculosis histology 1. Positive 2.Negative 3.N/A
28. History of extra pulmonary tuberculosis within the last 5yrs
   1. Y 2. N (If possible confirmed by a written records)
29. History of pulmonary tuberculosis within last 5yrs

**Current possibly Tuberculosis/HIV related diseases/symptoms**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>30. Chronic fever and weakness for less than one month</td>
<td>1. Y, 2.N</td>
</tr>
<tr>
<td>31. Weight loss</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>32. &gt; 10% of the body weight</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>33. Chronic and chest pain Cough.</td>
<td>1. Y, 2 N</td>
</tr>
<tr>
<td>34. Chronic intermittent diarrhea &gt; 1 month</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>35. Oral thrush (Candida)</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>36. Kaposi in mouth</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>37. Odynophagia (restrosternal pain on swallowing)</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>38. Recurrent (probable HSV-) sores (Lips)</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>39. Recurrent (probable HSV-) sores Genitalia</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>40. Herpes Zoster currently</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>41. Herpes Zoster Previous</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>42. Recurrent RTIs/ severe infections</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>43. Sexually Transmitted Infection (specify)</td>
<td>1.Y, 2 N</td>
</tr>
<tr>
<td>44. Other Specify</td>
<td>1.Y, 2 N</td>
</tr>
</tbody>
</table>

**Medication**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>45. Have you ever received ARV medication?</td>
<td>1. Y, 2.N</td>
</tr>
</tbody>
</table>
| 46. If previously on ARVs state the regimen.
   1. d4t/3TC/NVCP 2.D4t/3TC/EFZ 3. AZT/3TC/NVP 4. AZT/3TC/EFZ 5. ABC/DDI/Lpr/RT 6.N/A |
| 47. What is the frequency of ART in a month……………………………………………… |
| 48. Have you previously been on TB treatment? | 1. Y, 2.N |
| 49. If yes specify the drugs
   1. INH 2.RH 3.PZA 4.ETH 5.INH/PZA/ETH/RH 6.N/A |
| 50. Have you been on tuberculosis prophylaxis | 1.Y 2.N |
51. If yes specify the drugs used
   1. INH 2. RH 3. PZA 4. ETH 5. Unknown 6. N/A

52. current tuberculosis treatment
   1. Y, 2 N

53. If yes indicate the drugs used.

54. Are you currently on tuberculosis prophylaxis?

55. If yes state the drugs

56. Other OI’s medication taken by the patient
   1. Y, 2 N

57. If yes, specify………………………………………..

Examination
58. Current weight……………………………………………………….kg

59. Previous weight if known…………………………………………….kg

60. Height……………………………………………………………….m

61. BMI………………………………………………………………kg/m2

62. Auxiliary temperature………………………………………………Celsius

63. BP Systolic………………………………………………………diastolic………………

64. Heart rate………………………………………………………………


66. Lymphadenopathy 1. Y 2.N (> 1cm) specify area…….

Laboratory results
67. CD4 T cells count…………………………………..cells/mm³

68. Absolute leucocytes count…………………………x10¹²

69. Absolute lymphocyte count…………………………x10¹²

70. Haemoglobin level……………………………………..g/dl

Appendix 3: Questionnaire for healthy subjects

File No………………………………………………………………

1. Age…………………….. Date of Birth……………………..
2. Sex 1.M, 2.F
3. Address ………………………………………………………
   Ward…………………………………………………………
4. Marital status
5. For women –Pregnant? 1. Y……..weeks 2. N
7. Smoking/chewing local tobacco or Cigarette?
8. (a) Drinking alcohol 1. Y, 2.N
   (b) If yes state 1. Occasionally 2. Chronic alcoholic
9. Any member of the family with recent or current open tuberculosis? 1. Y, 2.N
10. (a) Are you suffering from any kind of illness recently or currently? 1. Y, 2.N
    (b) If yes mention……………………………… (If possible this should be
    confirmed by doctor by doctor or report from the doctor)

Current/recent symptoms of illness diseases

11. Chronic fever and weakness> 1 month 1.Y, 2.N
13. >10% of the body weight 1. Y, 2.N
14. Chest pain and Cough
15. Diarrhea> 1 month 1. Y, 2.N
18. Odynophagia (retrosternal pain on swallowing) 1. Y, 2.N
19. HSV sores on the Lips 1. Y, 2.N
20. HSV sores on the Genitalia 1. Y, 2.N
22. Herpes Zoster Previous 1. Y, 2.N
23. RTIs/Severe infections 1. Y, 2.N
24. Sexually Transmitted Infection (specify) 1. Y, 2.N
25. Other conditions (specify)…………………………………….. 1. Y, 2.N
26. (a) Are you on any kind of medication recently or currently? 1. Y, 2. N
   (b) If on medication mention them…………….. (If possible a doctor should see the Drugs or written document about the drugs)

27. Current weight………………………………………kg

28. Previous weight…………………..kg

29. Height……………………………………………………..m

30. BMI………………………………………………………….kg/m²

31. Auxiliary temperature………………………………..Celsius

32. General state 1. Well  2. Wasted

33. Lymphadenopathy 1. Y, 2. N

**Laboratory test results**

34. Urine for pregnant test or Obstetric ultrasound for suspected pregnancy
   1. Pregnant  2. Not pregnant

35. HIV test 1. Positive  2. Negative


37. RPR for syphilis test 1. Positive  2. Negative

38. Random Blood Sugar (for suspected Diabetic patients)
   1. Proved Diabetes  2. No Diabetes

39. CD4 T cells count…………………………………………….cells/mm³

40. CD3+T cells count cells/mm

41. CD8+T cells count cells/mm

42. Total absolute leucocytes count……………………………x10¹²

43. Absolute lymphocyte count………………………………….x10¹²

44. Haemoglobin level…………………………………………. g/dl
Appendix 4: Sputum sample Collection

Container

Sputum was collected in a robust, leak-proof and clean container. Container must be rigid to avoid crushing in transit and must process a water tight wide mouthed screw top prevent leakage and contamination.

Sputum specimen

Although *M. tuberculosis* is of causing disease in almost any organ of the body, more than 85% of tuberculosis disease in high prevalence countries is pulmonary. Sputum is the specimen of choice in the diagnosis of pulmonary tuberculosis. A good sputum specimen consists of recently discharged material from the bronchial tree, with minimum amount of oral or mucosal material. Satisfactory quality implies the presence of mucoid or mucopurulent material and is of greater significance than volume. Ideally, a sputum specimen has a volume of 3-5 ml, although smaller quantities are acceptable if the quality is satisfactory. It is best obtained a sputum specimen early in the morning before the patient has eaten since food or taken medication (which may interfere with the growth of tubercle bacilli or particles in smear make them difficult to examine). If sputum specimens are collected for diagnostic purposes, specimens were collected before starting tuberculosis chemotherapy (WHO 1998). Because tuberculosis lesions in the lungs may drain intermittently, it is possible for a specimen to be negative on one day and positive the next. For this reason three specimens were collected for diagnosis as follows.

I. One spot specimen when the patient first present to the health service

II. One early morning specimen (preferably the next day)
III. One spot specimen when the early morning specimen is submitted for examination.

IV. These were sent to the laboratory as single specimen. For follow up treatment at regular intervals and to determine outcome at the end of treatment, one specimen should be collected.

**Sputum collection procedure**

The patients were given confidence by explaining to him/her the reason for sputum collection. The patients were instructed to rinse his/her mouth with water before producing the specimen. This helps to remove food and any contaminating bacteria in the mouth. The patients took two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly. Patients repeated this process again and then cough. This process produces a specimen from deep in the lungs. The patients were asked to hold the sputum container close to the lips and to spit into it gently after a productive cough. Specimens were transported to the laboratory as soon as possible after collection. If delay is unavoidable the specimen were refrigerated or kept in cool place as to inhibit the growth of unwanted microorganism.
Appendix 5: Methods of Digestion and Decontamination (Petroff's method)

Reagents

**Digestants:** NaOH solution (2 to 4%). Sterilized by autoclaving.

**2N HCl:** Diluted 33 ml of concentrated HCl to 200 ml with water. Sterilized by autoclaving.

**Phenol red indicator:** Combined 20 ml of phenol red solution (0.4% in 4% NaOH) and 85 ml of concentrated HCl with distilled water to make 1,000 ml.

**Phosphate buffer:** The buffer is 0.067 M and pH 6.8. Mix 50 ml of solution A (0.067 M Na2HPO4; 9.47 g of anhydrous Na2HPO4 in 1 liter of distilled water) and 50 ml of solution B (0.067 M KH2PO4; 9.07 g of KH2PO4 in 1 liter of distilled water). If the final buffer requires adjustment, add solution A to raise pH or solution B to lower it.

Steps:

1. Transferred a maximum volume of 10 ml of specimen to a sterile 50 screw-cap plastic centrifuge tube. Add an equal volume of NaOH.
2. With the cap tightened, the tube was inverted and agitated the mixture vigorously for 15 min on a mechanical mixer, or vortex vigorously and stand for exactly 15 min. If it is necessary to reduced excessive contamination, the NaOH concentration can be increased to 3 to 4%.
3. Phosphate buffer (pH 6.8) was added up to the 50 ml mark on the tube. The tube was recapped and swirled by hand to mix well.
4. The specimen centrifuged at ≥3,000 X g for 15 min, the supernatant decanted and added a few drops of phenol red indicator to the sediment. Neutralized the sediment with HCl. Thoroughly mixed the contents of the tube and stopped acid addition when the solution was persistently yellow.
5. The sediment was resuspended on 1 to 2 ml of phosphate buffer.
6. The resuspended sediment was stored at -20°C for until further use which is appropriate for inoculate in to a culture media, prepare a smear and for extraction of DNA.
Appendix 6: Ziehl - Neelsen Method (ZN)

Reagents

Ziehl-Neelsen carbol fuchsine stain (ZN)

Basic fuchsine 5 gm

Phenol 25 gm

Alcohol (95% of absolute) 50 ml

Distilled water 500 ml

The fuchsine was dissolved in phenol by placing them in a liter of flask over a boiling water bath for about 5 minutes, shaking the contents from time to time when there was complete solution and alcohol added and mixed thoroughly.

Decolorizing agent 25% sulphuric acid

Water 750 ml

Concentrated sulphuric acid 250 ml

The acid should be poured slowly down the side of the flask into the water about 50 ml at a time.

Counter stain: Methylene blue

Methylene blue chloride - 100 µgm

Distilled water - 100 ml

Methylene blue was mixed in distilled water and stored in a amber colored bottle after labeling with name of the reagent and dates of preparation and date of expiration. It can be stored at room temperature for 6-12 months.

According to WHO & National tuberculosis control program interpretation of Ziehl-Neelsen acid-fast staining was be as follows:

<table>
<thead>
<tr>
<th>AFB</th>
<th>RESULT FIELDS</th>
<th>TO BE EXAMINED</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/100Field</td>
<td>Negative</td>
<td>100 fields</td>
</tr>
<tr>
<td>Field</td>
<td>Scanty</td>
<td>200 fields</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>1-9/100 Field</td>
<td>1-9/200 Fields</td>
<td></td>
</tr>
<tr>
<td>10-99/100 Field</td>
<td>1+ 100 fields</td>
<td></td>
</tr>
<tr>
<td>1-10/ Field</td>
<td>2+ 20 fields</td>
<td></td>
</tr>
<tr>
<td>&gt;10/ Field 3+</td>
<td>20 fields</td>
<td></td>
</tr>
</tbody>
</table>

**Sputum smears preparation and Acid Fast Staining procedures**

1. The slide was labeled with patient code/number

2. Appropriate thickness and width sputum smear was made on the slide and air dried when placed horizontally

3. The smear fixed by passing 3 times over the top of Bunsen- burner/ spirit flame

4. Stain the fixed smear with Ziehl-neelsen (ZN) hot method
   a) Slide was placed on the slide rack with the smear upper most, their edges separated and not touching each other.
   b) The whole surface of the slide was covered with filtered Ziehl carbol fuchsin
   c) It was heated very gently until steam appears. Flame of a cotton wool in methylated fixed on end of metal rod was used
   d) The warm stain was left for 5 minutes
   e) The slide was tilted to drain off excess stains and each slide individually in a gentle stream of running water until all the free stain was washed away
   f). This was decolorized by 3% acid alcohol until the solution runs clear and washed with a gentile stream of running water
   g). The smear was counter stained by flooding with 0.3% methylene blue and leaving and leaving it for 1-2 minutes.
   h). Methylene blue stain poured off and the slide washed with gentle stream of running water.
   i). The slide was tilted and placed on the rack to dry in the air
Microscopic slide examination for AFB

1. Well-distributed smear area on the slide was selected using 10 times objective
2. A drop of oil immersion was added and the switched objective to x100
3. The was read slide systematically by looking 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>
Appendix 7: HIV testing procedures

1 Determine HIV 1/2(T1) Procedure

1. The protective foil cover was removed from each test and labeled with client code
2. 50 μl of sample (whole blood) was applied to the sample pad.
3. Left until blood is absorbed in the sample pad, then apply one drop of chase buffer
   applied 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 were allowed to reach room temperature
   (if stored in the refrigerator) before use.
2. Patient sample identification number was labeled on slide.
3. The slide was placed on black interpretation station for both Positive and negative
   internal controls
4. Latex reagent mixed well by gently agitating the bottle to ensure that the Latex
   suspension was homogenous.
5. Latex was drawn to the black calibration mark of the dropper and then dropped the
   latex on the slide at the edge of mixing well.
6. With pre calibrated pipette 10μl of sample or control was added to the latex
   dropped on slide then mixed. A well mixed sample and latex solution was moved to
   the opening of channel untill the capillary flow began using a sample pipette tips.
7. Latex mixture was allowed 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, it was removed and allowed to stand for 20 minutes to reach room temperature.

2. The device were removed from their protective wrappers.

3. Each test device was labeled appropriately.

5. Two drops of sample (approximately 60 μl) added at the sample port carefully.

6. Two drops (approx. 60 μl) of the wash reagent was added to sample port.

7. 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
Appendix 8: CD4+ T cell count determination

(The sample was 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. The reagent pair tube was labeled with the patient’s accession number.
2. The pair was mixed upside down for 5 seconds and then upright for 5 seconds.
3. The reagent was opened tube with the coring station.
4. Patients whole blood mixed by inverting the tube five times.
5. 50 μl of patient’s whole blood to each tube was reversed in pipette and the tubes
capped and mixed upright for 5 seconds.

6. This was incubated for 60 to 120 minutes at room temperature in the dark.

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

8. Tubes were recapped, mixed (vortex) upright for 5 seconds and then incubated for at least 30 minutes at room temperature in the dark.

10. The test was runned on FACS Count instrument with in 24 hrs of preparation.

**Entering patient and reagent information**

1. FACS Count screen or the CONTROL results screen was pressed (sample).

2. The reagent lot code and bead count was entered

3. Pressed (confirm).

4. Patient accession number entered

**Running patient’s sample**

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

2. Pressed (RUN).

3. The reagent pair removed and CD4 tube recapped.

4. CD8 tube uncapped and reagent pair placed in the sample holder so that CD8 tube is in the run position.

5. Pressed (RUN).

6. The reagent pair removed 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, and 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. Reversed pipette 50μl of low control beads in to the CD8 reagent tube labeled low.
3. Medium/high control beads pair and reverse pipette 50μl of medium control beads was vortexed in to the CD4 reagent tube labeled medium.
4. Reversed pipette 50μl of high control beads in to the CD8 reagent tube labeled high and then ran on FACSCCount instrument within 2 hrs after adding the control beads.

**Entering control and reagent information**

The following procedures should be done sequentially. 1. Pressing “control” from the FACSCCount 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. First reagent pair (CD4-zero and CD8-low) vortexed upright for five seconds
2. The CD4-zero tube was uncapped and placed the reagent pair in the sample holder so that CD4-zero tube is in the run position.
3. Pressed (RUN).

4. The reagent pair removed and recapped the CD4–zero tube.

5. The CD8-low tube uncapped and placed the reagent pair in the sample holder so the CD8 low tube is in the run position.

6. Pressed (RUN).

7. The reagent pair removed and recapped 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.
Appendix 9: Map of Mombasa, Kenya
Appendix 10: Letters of Research approval

MINISTRY OF MEDICAL SERVICES
OFFICE OF THE PROVINCIAL DIRECTOR OF MEDICAL SERVICES

Telegrams: "PROVMED", MOMBASA
Telephone Mombasa : 2319819/36
Fax: 2220161
Email: pdmscoast@gmail.com
When replying please quote

Ref. PDMS/MED.1/03/VOL.1(34)

OFFICE OF
THE PROVINCIAL DIRECTOR OF MEDICAL SERVICES
COAST PROVINCE
P. O. BOX 91040 - 80103
MOMBASA

Date: 2nd November, 2011

Shadrack Yonge (MSc-Public Health)
PhD Proposal Kenyatta University
P.O. Box 90420-80100
MOMBASA

Dear Mr. Yonge,

RE: RESEARCH PROPOSAL: EPIDEMIOLOGY OF TUBERCULOSIS AND HIV/AIDS CO-INFECTION AND IMPACT ON IMMUNOHAEMATOLOGICAL PARAMETERS IN MOMBASA KENYA

This is to inform you that we have reviewed and accepted the above cited research proposal.

We are looking forward to a sensitization meeting with the hospital staff prior to initiation of the study and dissemination and a copy of the study findings upon completion of the study. As discussed, to maintain patient confidentiality the patient interviews at the HIV clinic should be conducted by the staff providing services at the clinic.

I wish you a fruitful research process.

Yours sincerely,

Dr. Anisa Baghazal
PROVINCIAL MEDICAL RESEARCH OFFICER

Cc.: The Chief Administrator, CPGH
     The Head, Dept of Medicine, CPGH
     The head, Department of Pathology, CPGH
Our Ref: KU/R/COMM/51/28

Yonge Shadrack Ayieko
School of Public Health,
Kenya University
P.O. Box 43844, Nairobi.

Dear Mr. Yonge,


1. IDENTIFICATION OF PROTOCOL:

The application before the committee is with a research topic ‘A study of Epidemiology of tuberculosis in HIV-infection, clinical presentation and impact on immunohaematological parameters in Mombasa, Kenya’ Version 2, dated 8th March 2012.

2. APPLICANT

Yonge Shadrack Ayieko
School of Public Health,
Kenya University
P.O. Box 43844, Nairobi.

3. SITE

Mombasa District, Kenya.

4. DECISION REACHED:

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

i. Scientific design and conduct of study,

ii. Recruitment of research participant,

iii. Care and protection of research participants,

iv. Protection of research participant’s confidentiality,

v. Informed consent process,

vi. Community considerations,

AND APPROVED that the research may proceed for a period of ONE year from 9th March 2012.
5. **ADVICE/CONDITIONS**

   i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

   ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

   iii. Clearance must be obtained for transportation of any biological material out of the country, Kenya.

   iv. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

   v. Submit a soft copy of the protocol to KU-ERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter

署名

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I, [Name], accept the advice given and will fulfill the conditions therein.

Signature: [Signature] Date: 9/1/2012

cc. Vice- Chancellor
    Director: Institute for Research Science and Technology