IMMUNE RESPONSES IN PATIENTS CO-INFECTED WITH HIV AND *Mycobacterium tuberculosis* ATTENDING TB CLINIC IN KERICHO COUNTY HOSPITAL, KENYA

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DECLARATION

I declare that this thesis is my original work and has not been submitted for the degree or other awards in any other university.

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DEDICATION

I would like to sincerely dedicate this research study to my husband Simion and children Debra and Boni for their love, undivided support and understanding during the long hours spent away from them while carrying out this study. Lastly is to my sisters Betty and Ednah and my brother in law Alphine for their support, they have been a source of constant inspiration to me. I love you all.

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DEFINITION OF TERMS

Acquired immunodeficiency syndrome-is a condition in which a person's immune system is so weakened that she becomes susceptible to conditions that occur rarely in those with intact immune function.

HIV-The virus that causes AIDS; it replicates in and kills the helper T cells.

TB-A bacterial infection caused by *Mycobacterium tuberculosis*.

Statistical significance-The probability that the results observed during the study was not likely due to chance alone. The threshold for statistical significance is an arbitrary value called a p value which is usually set at 0.05 or 5 %. If the probability that the observed results were due to chance is less than the set P value, the results is considered significant.

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
CD8	Cluster of Differentiation 8
CD3	Cluster of Differentiation 3
CD4	Cluster of Differentiation 4
CDC	Center for Disease Control
DNA	Deoxy-Ribonucleic Acid
EIA	Enzyme immune assay
ELISA	Enzyme Linked Immunosorbent Assay
EPT	Extra pulmonary Tuberculosis
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
K3-EDTA	Tripotassium-ethylene diaminetetraacetate
KCH	Kericho County Hospital
NTM	Non-tuberculous mycobacteria
MDR-TB	Multi-Drug Resistant TB
PT	Pulmonary tuberculosis
RNA	Ribonucleic Acid
TB	Tuberculosis
VCT	Voluntary Counseling and Testing
WHO	World Health Organization

ABSTRACT

Tuberculosis, caused by *Mycobacterium tuberculosis* is the most common opportunistic infection in HIV/AIDS patients globally and more cases in sub-Saharan Africa, Kenya included. It is the main cause of morbidity and mortality in adults aged between 18 and 49 years. CD4+ T cell count is a crucial marker of immunologic integrity and therefore clinical signs of TB vary with the CD4+ T cell counts in HIV-TB patients. It has been used with viral load as markers of disease progression. There is therefore a need to establish immune responses in patients co-infected with HIV and *Mycobacterium tuberculosis* specifically using CD4+T cell counts and viral load counts when they are undergoing TB therapy. The study was carried out in Kericho County Hospital with HIV-TB coinfected subjects. Tuberculosis monoinfected patients and the normal standard references were use as controls. Demographic characteristics of patients including age, sex, occupation and marital status were obtained by use of questionnaires. The blood samples were obtained at the start of the TB treatment and after completion of the anti-TB drugs. FACS Calibur flow cytometer was used to count the CD4+ T cells. The viral loads counts were obtained from the hospital patient's records after a period of six months. The data that was obtained was entered in SPSS and analysis was carried out to determine relationship between the coinfection and these various variables. Descriptive statistics such as mean, median, variance, standard deviation and percentages were used to determine socio demographic characteristics, the CD4+ T cells and Viral load counts were compared at the beginning and at the completion of TB chemotherapy using median, range, percentile ranks, quartile ranks and paired sample test. The results were then presented in tables, Charts and graphs. A total of 323 HIV-TB co-infected persons were enrolled in the study. Their age ranged from 18 to 74 years. A total of 54.8% of study participants were females while 45.2% were males, 92.6 % were married. As far as education level is concerned, 44.6 % reported to have achieved primary school education while 39.0 % and 16.4 % had secondary and tertiary education level respectively. Concerning their occupations, 14.9 %, 10.2 % and 9.6 % respondents reported that they were engaged in small business, farming and casual labour respectively. Security personnel and accountants constituted 7.1 % and 4.6 % respectively, 78.0 % and 22.0 % had pulmonary and extrapulmonary tuberculosis respectively. There was a general increase in CD4+ T cell counts after TB chemotherapy irrespective of the type of TB. Statistical significant difference was observed in the median CD4+Tcell counts in patients with any form of TB before and after treatment median (IQR) 88(35-180) and 398(340-490) cells/µl, respectively ($P<0.001$). The median (IQR) viral load was 256,789 (49,000-460,870) and 19 (19-86) copies/µl at pretreatment and post treatment respectively ($P<0.001$).The findings revealed an inverse relation between CD4+ T cell counts and viral copies in the TB-HIV coinfected patients after treatment. The finding of this study are important in informing the ministry of health on the most vulnerable population as well as the importance of TB chemotherapy in strengthening immunity by restoring CD4 T cell counts and reducing replication of virus.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Tuberculosis is caused by *Mycobacterium tuberculosis.* It is the chief cause of death amongst people living with HIV and accounts for one in five HIV related deaths globally (UNAIDS/WHO, 2003). In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive people. An estimated 10.4 million people fell ill with TB in 2016: 90% were adults, 65% were male, 10% were people living with HIV; 74% in Africa and 56% were in five countries: India, Indonesia, China, the Philippines and Pakistan. During the same year (2016), 6.3 million new cases of TB were reported, recording a rise from 6.1 million in 2015 and equivalent to 61% of the estimated incidence of 10.4 million. The latest treatment outcome data showed a global treatment success rate of 83%. There were 476 774 reported cases of HIV-positive TB (46% of the estimated incidence), of whom 85% were on an ART (WHO, 2016).

In the year 2010, about 250 people perished daily in Kenya from HIV/AIDS-related ailments (Azevendo, 2010). Tuberculosis is the commonest opportunistic infection in persons suffering with HIV/AIDS (Mallory *et al*., 2000). The TB-HIV/AIDS problem has been worsened by the emergence of non-tuberculous mycobacteria (NTM) which results as opportunistic infections in the HIV/AIDS patients, and their treatment is not similar to that of TB (Wolinsky, 1992; Johnson, 1999).

The CD4+ T cell count is a key indicator of immunologic integrity and therefore clinical signs of TB vary with the CD4+ cell count in HIV-TB patients (Jones *et al*.,

1993). Cellular immune response, mainly by T lymphocytes has been noted to play an essential role in controlling *M. tuberculosis* replication (Orme *et al*., 1983). CD4 T cell counts are used to assess the central immune defects in HIV disease. Besides, it has been used with viral load as markers of disease progression (Pattanapanyast and Sippy, 2004). A normal CD4+ T cell count is 600 to 1500/ µl of blood. Over 80 % of patients with illnesses that suggest the progression to AIDS occur at a CD4+ T-cell counts of below 200 cells/µl of blood (CDC, 1997).

Measurements of levels of HIV-RNA over time has been of great value in determining the relationship between levels of virus and rates of disease progression, the relationship between immune system activation and viral load replication and the time of antiretroviral drug resistance (Dwyer *et al.*, 1997; Dar *et al.*,1999). Even though CD4+ T count is widely used in most clinics, it is a crude method. A sole abnormal result is not adequate to introduce or change the treatment as there are many physiological variables that may affect the count that includes; time of the day, concurrent infections and recent exercise (Melone *et al*., 1990). A higher viral load count is associated with lower baseline CD4+ T cell counts resulting in a more fast disease progression. The patients who have more than 100,000 copies*/ul* of plasma HIV-RNA within six months of seroconversion have been shown to be 10-fold more likely to progress to AIDS than those with fewer copies except those with advanced disease (Saag *et al*., 1996).

Due to the fact that the clinical signs of tuberculosis changes with the CD4+ cell count in HIV-TB co-infected patients (Jones *et al*., 1993), it is essential to carry out early testing and treatment of TB in order to curb further decline in CD4 counts and HIV-1

virus faster replication which often lead to progression to AIDS. Besides, it is paramount to identify the most infected population as well as monitor the immune response of such patients after introduction and completion of Tb chemotherapy to ascertain whether immunity is restored. Therefore, the objective of this study was to determine immune responses in patients co-infected with HIV and *M. tuberculosis* attending TB clinic in Kericho County Hospital.

1.2 Statement of the problem

Tuberculosis is the main universal public health problem and a leading cause of illness and death in Kenya (WHO, 2016). Control of the infection in this HIV era has been hindered by the advent of multi-drug resistance tuberculosis. Clinical and experimental evidence propose that active TB accelerate the course of HIV disease (Shafer *et al*., 1996). HIV-infected persons are at markedly increased danger of developing primary or reactivated tuberculosis. The HIV/AIDS epidemic has significantly contributed to the upsurge of TB cases and is a major killer of HIV/AIDS patients (WHO, 2009). There is therefore a need to monitor immune responses in patients co-infected with HIV and *M. tuberculosis* when they are undergoing chemotherapy in order to assess whether the treatment assist in improving or boosting the immunity at long run. This will therefore reduce the vulnerability to other opportunistic diseases associated to HIV and also progression to AIDS. In that regard, such a study on immune responses was important because it had not been carried out in Kericho County Hospital.

1.3 Justification of the study

Tuberculosis is still the primary cause of death amongst patients suffering from HIV/AIDS. In 2017, 29,000 deaths occurred due to TB. The case notification rate due to TB in Kericho County per 100,000 people is 257 compared with the national average of 216 per 100,000 people. Prevalence for other counties like Bomet, Nandi, Kisii, Bungoma and Kwale are: 108, 31, 170, 127 and 97 respectively (WHO, 2017). The HIV prevalence in the county was 3.5 % which is higher than for other counties like Bomet, Nandi, Garissa, Nyandarua and Laikipia with 2.5%, 2.4%, 0.4%, 3.2 % and 3.0% respectively (NASCOP, 2016). TB/HIV co-infection proportion was 30 % in 2013 (KAIDS, 2014). These statistics advocate the need for more research on how the immunity of an individual respond after chemotherapy through analysis of CD4+ T cell counts and viral loads in order to identify the best drugs so as to reduce the HIV/TB coinfection. The study was carried out in Kericho County Hospital because of its strategic position. It is located in Kericho town, which has many HIV patients from the tea plantation and the surrounding areas who seek medication in the health facility. The hospital also has an integrated TB/HIV clinic which was established in 2005. Near to the hospital is Kenya Medical Research Institute/Walter Reed Project, therefore its strategic place for vaccines trials.

1.4 Research questions

i. What are the socio demographic characteristics of patients co-infected with HIV and *M. tuberculosis* in Kericho County Hospital?

ii. What are the CD4+T cells counts in patients co infected with HIV and *M. tuberculosis* at the beginning and after completion of anti-TB drugs?

iii. What are the viral load counts in patients suffering from HIV and tuberculosis at the beginning and after completion of anti-TB drugs?

1. 5 Hypothesis

There is no difference in immune responses in patients with HIV/AID and tuberculosis ailments attending TB clinic in Kericho County Hospital before and after completing anti-TB drugs.

1.6 Objectives

1.6.1 General objective

To assess the immune responses in patients co-infected with HIV and *M. tuberculosis* at Kericho County Hospital.

1.6.2 Specific objectives

i. To determine the socio demographic characteristics of patients co-infected with HIV and *M. tuberculosis* in Kericho County Hospital.
ii. To relate CD4+T cell counts at the beginning and after completion of anti-TB drugs in patients with HIV and *M. tuberculosis* co-infection.
iii. To determine the viral load counts in the patients co-infected with HIV and *M. tuberculosis* at the beginning and after completion of anti-TB drugs.

1.7 Significance of the study

The findings of this study are crucial in informing health professionals on the extent of co-infection of HIV and *M. tuberculosis* as well as the importance of TB drugs assisting

to restore the immunity by eliminating *M. tuberculosis* in the system. Besides, it provides awareness on the type of tuberculosis which is common as well as the effect of TB chemotherapy on CD4+ T cell counts and viral load counts on TB monoinfected and TB/HIV coinfected patients with either pulmonary or extrapulmonary tuberculosis. In addition, the study also forms a basis for further research by other researchers.

1.8 Limitations of the study

The scope of the study was limited to information collected in the period under study. The study took much time than expected because, some of the subjects declining in the process, others transferred to other hospitals and unfortunately some died. These entire patient were replaced so as to maintain a sample of 323 subjects. Another challenge was lack of enough funds to carry out the research at the stipulated time.

CHAPTER TWO: LITERATURE REVIEW

2.1 Human Immunodeficiency Virus

2.1.1 History and epidemiology of HIV

The origin and evolutionary mechanisms of human immunodeficiency virus (HIV) remain controversial. The origin and evolutionary relationship between HIV-1, HIV-2 and the simian immunodeficiency virus (SIV) theories are based on seroepidemiological and viral genome molecular data. The theory of transmission of SIV to humans becoming HIV-2, then its rapid evolution to HIV-1 with an explosive escape from an isolated human population, is supported by the existing data (Wolfe *et al.*, 2008).

The beginning of the general awareness of AIDS in the USA in June 1981 was marked by a published report by Centre for Disease Control in Atlanta about the occurrence without identifiable cause of *Pneumonia carinii* in men in Los Angeles (CDC, 1981). The Sub-Saharan Africa still remains the area greatly affected by HIV with 70 % of the new HIV infections globally in the year 2010 (Shaffer *et al*., 2010). Furthermore, 76 % out of the 90 % individuals who tested positive for HIV and gain access to antiretroviral treatment had a lower probability of transmitting the virus to their sexual partners after viral suppression. The viral suppression decreased by 1 % in new HIV infections for each 10 % ART coverage (Shaffer and Maswai, 2010). The Kenya National Aids Control Council has put in more effort in ensuring that the supply of ART exceeds the total number of individuals who are in need of the medications. The ART coverage in Kericho County is 120 % (NACC, 2015).

Kenya is among the six nations in the continent of Africa with highest HIV infections. The country has average prevalence of 6 % and about 1.6 million persons infected with HIV. Regions mostly infected are; Homabay County, Siaya County and Kisumu County indicating proportions of 25.7 %, 23.7 % and 19.3 % correspondingly. The counties having lowest infection proportions are Wajir, Tana River and Marsabit with percentages of 0.2, 1 and 1.2 respectively. Kericho County has a prevalence of 3.4 % (NACC, 2015).

2.1.2 Immunology of HIV

The HIV infection has been known to severely reduce CD4+ T cells in lymphoid tissue with successive reduction in the peripheral blood circulating CD4 lymphocytes. Acute HIV infection results in adverse decline in CD4+ T cells .However, the CD4+ T cells commonly recovers in a few weeks as HIV-specific CD8+ T cells aid to reduce levels of plasma viremia (Butto *et al.*, 2010). In cases of patients not treated, CD4 T cells successively drop over a number of years. Several researches of HIV infections among homosexual men show that the mean CD4+ T cell counts before HIV infection is about 1000 cells/mm^3, it declines to an average of 780 cells/mm^3 at half a year after HIV infection and finally to 670 cells/mm^3 at one year of follow-up (Schmitz *et al.*, 1999). Consequently, the CD4+ T cell counts drop at a mean annual rate of nearly 50 cells/mm^3 .Some cases of variations occurring from one individual to another. Severe depletion in CD 4+ T cell counts can result to opportunistic infections such as TB and also death in case of no medical attention (Schmitz *et al.*, 1999).

The immunity and HIV viremia in persons getting antiretroviral treatment are measured by using the viral load and CD4+ T cell counts (EACS, 2012). However, immunological assessment such as CD4+ T cell counts as well as clinical investigation like the WHO Stage evaluations are mainly used in poor states especially those in sub-Saharan Africa (WHO, 2006). The two methods above are disadvantageous due to their poor predictive value in identifying virological failures (Reynolds *et al.*, 2009). Viral load evaluation after every six months was recommended by 2010 WHO guidelines to evaluate viremia and to monitor suspected treatment failures (WHO, 2010). More recently, the WHO 2013 guidelines recommend viral load as the favorite monitoring method to diagnose as well as confirming ART treatment failure (WHO, 2016). The Ministry of Health in Kenya nowadays recommends clinical and immunological evaluations as well as viral load monitoring in evaluating viremia in instances of either alleged clinical or immunological treatment failure (NASCOP, 2011).

Despite latest recommendations for viral load tracking, records regarding viral load tracking in the appropriate clinical sites are limited (WHO, 2010). Therefore, CD4+T cell counts have been used mainly in poor setting and at times together with viral load as markers of disease progression (Pattanapanyast and Sippy, 2004). A normal CD4+ T cell count is 600 to 1500/ µl of blood and over 80% of patients with AIDS-defining illness occur at a CD4+ T-cell count of less than 200 cells/µl of blood(CDC, 1997).

Anti-HIV antibodies and cytotoxic cells are produced by the immune system against HIV virus. The HIV virus attaches to CD-SIGN which is a glycoprotein expressed on the dendritic cells of the body. The CD4+lymphocytes are infected immediately upon exposure to HIV resulting in destruction of millions of CD4+ lymphocytes finally overwhelming the immune system regenerative capacity. By then, their numbers drop significantly and steadily lose their ability to respond to foreign antigens (WHO, 2007). Apoptosis in CD4+ lymphocytes is also believed to be triggered by HIV (UNAIDS/WHO, 2000).

Reductions in CD4+ T cells may bring about either reduced activation as well as survival of cytotoxic CD8+ T cells or partial activation of CD8+ T cells which are the key in removing HIV infected cells. This results in a lessened capability to kill virally infected cells leading to immunodeficiencies, therefore, vulnerability to opportunistic pathogenic infections such as tuberculosis (Jones *et al*., 1997). The CTL cells have also been involved in the control of HIV replication as suggested by researches on simian immunodeficiency virus infecting non-human primates after *in vivo* CD8+ T cell exhaustion (Schmitz *et al.*, 1999). HIV can as well escape adaptive immune responses due to high mutations rates (Winau *et al*., 2006).

Certain CD4+ T cell thresholds have been identified and are used as standards to either commence medications against opportunistic infections or to start antiretroviral treatment. The CD4+ T cell counts are also a relatively consistent indicator of treatment response (Koethe *et al*., 2010).

2.1.3 Transmission, symptoms and signs of HIV

HIV transmission occurs through several means such as unprotected sex, blood transfusion, sharing of contaminated sharp object such as needles and razor blades, and from the mother to newborn while still in the womb or during childbirth. A mother can also transmit the HIV virus during breastfeeding period (UNAID/WHO, 2003). The HIV virus depletes the CD4+ T cells leading to increased immunodeficiency and increased vulnerability to opportunistic infections and death (Butto *et al.*, 2010). HIV infection is accompanied by the following symptoms; fever, swollen lymph nodes, weight loss, a flu-like illness, diarrhea and cough. Other examples of opportunistic illnesses which occurs due to HIV infection apart from TB are; *Cryptococcus meningitis* and cancers among others. The most progressive phase of HIV infection is AIDS. This occurs after ten to fifteen years of infection with the virus.

2.2 *Mycobacterium tuberculosis*

2.2.1 History and epidemiology of TB

Tuberculosis is still one of the global most fatal communicable diseases, second only to HIV/AIDS and the prominent cause of death in persons infected with HIV/AIDS killing one in every four people co-infected patients. The global HIV/TB confection rate in 2014 was 3.3% (NACK, 2014). In 2013, World Health Organization predicted that 1.5 million individuals out of the 9 million people, who became ill with TB, died from worldwide (WHO, 2013). Tuberculosis incidence on the other hand is falling at about 2% per year and 16% of TB cases die from the disease and the expectation is by 2020, these figures need to improve to 4–5% annually and 10%, respectively, to reach the first (2020) milestones of the End TB Strategy. Most deaths from TB could be prevented

with early diagnosis and appropriate treatment. Millions of people are diagnosed and successfully treated for TB each year, averting millions of deaths (53 million 2000–2016), but there are still large gaps in detection and treatment (WHO, 2017).

Patients with HIV and TB co-infection have also been understood to have a greater lifetime danger of developing active tuberculosis (50 %), as compared to HIV negative persons with latent TB (10 %) in a particular year fuelling an upsurge in the TB epidemic with an estimated annual mortality of 2 million people worldwide (Nullis, 2005).This increases the upsurge in the TB endemic with an annual mortality rate estimate of 2 million people globally (Nullis, 2005).

The HIV infection in TB patients is known to increase mortality up to 4 times (Nunn *et al*., 1992). In addition, TB is the prominent root of respiratory illness (Grant *et al.*, 1998) and death in HIV-infected persons universally accounting for 44 % of all AIDS-related deaths annually (WHO, 1995). Family and community members can be infected faster as a result of its mode of transmission which is airborne if not diagnosed and treated promptly. In fact, the annual infection rate by someone with active TB is an estimate of 10 to 15 people (Shaffer *et al*., 2012).

The Sub-Saharan Africa is still the most horribly hit area in terms of the effects of TB-HIV infections. This is the region where about 33 % of the predicted 40 million people falling sick as a result of HIV/AIDS are also TB patients (WHO, 2002; UNAID, 2009).Kenya was ranked among the twenty two most troubled nations with tuberculosis in the world (WHO, 2005).Tuberculosis killed 29,000 Kenyans in 2017 according to

WHO report during the world tuberculosis day on 24 march 2018. The TB cases diagnosed in Kenya in the year 2013 were 90,000 with approximately 20,000 going undetected (WHO, 2013). *M. tuberculosis* causes infection which has been categorized as the utmost significant cause of ill health and death in humans. Besides, it is a sort of illness that is found among the poor and the urban dwellers who live in crowded households.

Transmission of *M. tuberculosis* occurs when untreated person cough in a confined environment spreading droplets of nuclei which remain airborne for a considerable time. This may be inhaled by a susceptible person (Behr *et al*., 1999). Tuberculosis accelerates HIV infections by increasing viral load by five to seven folds (UNAIDS, 2006).

2.2.3 Immune response to *M. tuberculosis* infection

The infection signals produced by the alveolar macrophages upon entry of *M. tuberculosis* include production of cytokines and chemokines. The bacilli resist the bactericidal responses of the macrophage through prevention of phagosome-lysosome fusion, multiplying in the phagosome causing apoptosis of the macrophage (Chen and Remold, 2006). The free bacilli that multiply outside the cells are then phagocytosed by a different macrophage that also miss to regulate their growth. The dendritic cells which contain engulfed bacilli prime the T cell against mycobacterial antigens after travelling to the regional lymph node (Bodnar *et al*., 2001). The chemokines which are manufactured by these infested cells directs these primed T cells up to the location of contagion whereby they accumulate with other types of cells such as the macrophages,

T cells, and other host cells such as the dendritic cells, fibroblasts cells, endothelial cells, and stromal cells. All these consequentially lead to the development of granuloma (Gonzalez *et al*., 2001).

The condition in the granuloma which includes low pH, low oxygen concentration and presence of nitric oxide and carbon monoxide among other compounds, favours the appearance of numerous *M. tuberculosis* genes concerned with dormancy orientation (Ulrichs, 2006; Rustad *et al.,* 2009). Recent findings suggest sporulation being the general mechanism for mycobacterial dormancy due to prolonged stationary phase or starvation as manifested by the establishment of spore-like structures as confirmed in *Mycobacterium bovis* BCG, *Mycobacterium marinum,* and M*ycobacterium smegmatis* (Shiloh and Digiyseppe; 2010). When the immunity of the host is suppressed as a result of other infection, the dormant bacilli can be activated. This is also indicated by tuberculin skin test (Anuchi *et al.*, 2001).

The CD4+ T cells as well as the cytokine interferon-gamma are the key immune components concerned with the effective immune response produced against *M. tuberculosis* as shown by studies in animals as well as in humans (Chan and Flynn, 2004).

The CD4+ T cells have several paramount roles in controlling infection which occurs within the granuloma. Among these roles include production of IFN-γ, killing infested macrophages, producing cytokines such as interleukin-2 and tumuor necrosis factor, induction of macrophages and dendritic cells to release immunoregulatory cytokines

such as interleukin-10, interleukin-12, and interleukin-15. Other roles include control of intracellular growth of *M. tuberculosis* by a nitric oxide-dependent mechanism that relies on interferon-gamma production and stimulation of macrophages by the direct interaction through CD4 ligand (Oddo *et al.*, 1998). The CD4+ T cells are also important in the cytotoxic role of the CD8+ T cells (Serbina *et al.*, 2001; Cooper, 2009).

The immunity to TB can also result from CD8+ T cells by being concerned with production of interferon-gamma among other cytokines or directly killing *M. tuberculosis* by producing an enzyme known as granulysin assisting to eradicate both the acute and the chronic infection (Grotzke, 2005; Cooper, 2009). The interferon-gamma has been indicated to be the main cytokine as far as the defensive immune response produced against *M. tuberculosis* is concerned. This has been supported by susceptibility to *M. tuberculosis* infection by individuals and mice lacking interferon-gamma or interferon-gamma genes (Flynn, 1993; Cooper, 2009). Among the roles of IFN-γ are: production of CD4+ cytotoxic T cells and the natural killer cells, synergy with tumour necrosis factor-alpha and triggering macrophages to destroy intracellular bacilli, amplifies antigen presentation, resulting in recruitment of CD4+ T cells and cytotoxic T cells, which partake in mycobacterial destruction and preventing weariness of the memory T cells (Scanga *et al*., 2001; Cooper, 2009).

Tumour necrosis factor-alpha functions include; the development of immunopathology associated with TB (Flynn, 2005). It is also concerned with both immune as well as immunomodulatory reactions. Moreover it works together with IFN-γ to increase the manifestation of the iNOS and the antimycobacterial action of macrophages (Scanga,

2001). Tumour necrosis factor-alpha also stimulates movement of cells and development of the microbicidal granulomas. Interruption of TNF-α reaction on the other hand brings about excessive growth in the disease causing bacteria (Chan and Flynn, 2004; Cooper, 2009).

2.2.4 Types of TB and clinical symptoms

Tuberculosis is mainly of two types namely; pulmonary tuberculosis and extrapulmonary tuberculosis. The commonest type is pulmonary tuberculosis which is a contagious infection caused by *M.tuberculosis* mainly affecting the lungs. Extra pulmonary tuberculosis on the other hand occurs when the infection has spread beyond the lungs to other parts of the body such as the bones, pleura, osteoarticular areas, genitourinary system, and the lymph nodes and joints among others (Githui *et al*., 1993; Barnes *et al.*, 1994). The clinical symptoms include fever, sweating during nights, sudden loss of body weight, struggling while breathing, cough, chest pains, absence of expectoration of sputum (WHO, 2002).

2.3 HIV and *M tuberculosis* co infections

Globally, tuberculosis has been discovered to be the foremost cause of the respiratory illness and death in HIV-infected individuals accounting for a total of 44 % of all the AIDS-linked deaths every year (WHO, 1995). Ever since early 1990s, the HIV epidemic has changed the TB illnesses to epidemic amounts. The Sub-Saharan Africa region is one of the most horribly affected region in terms of the effect of TB-HIV, where 33% of the predicted 40 million people suffering from HIV/AIDS are affected by

TB as an opportunistic infection (WHO, 2002; UNAIDS, 2009). Patients who are not HIV victims have a much lower (10 %) lifespan risk of being infected by active tuberculosis as opposed to those with both HIV and TB whose lifetime danger of developing active tuberculosis is higher (50 %) annually (WHO, 2009). This results in the rise in the tuberculosis epidemic with about 2 million people perishing every year globally (Nullis, 2005). The mortality rate in HIV-infected TB patients is reported to increase up to 4-times higher than those with TB alone (Nunn *et al*., 1992).

The HIV infection has been discovered to be the main contributing aspect for new tuberculosis infection in addition to being a possible threat for relapse of the TB illness (Daley *et al*., 1992; Mallory *et al*., 2000). Tuberculosis infection is purported to be an ailment associated with the increase in the multiplication of HIV viral load. This occurs as a result of cytokine expression (Wallis *et al*., 1993) and is also accountable for shortened life span in those individuals who are HIV positive (Ackah *et al*., 1995).

Human Immunodeficiency Virus/AIDS has had a large influence on the tuberculosis epidemic in Kenya with approximately 60 % of people suffering from TB being feared to be HIV positive. The mortality rate as a result of TB in this group is about 130 per every 100, 000 patients (DLTLD, 2009). Nowadays, about 250 patients in Kenya perish daily because of HIV/AIDS-related complications (Azevendo, 2010). Human Immunodeficiency virus and *M. tuberculosis* co-infection has adversely affected the outcome of TB through modification of the natural history and its clinical presentation (Schutz *et al*., 2010).

Acute disseminated tuberculosis in HIV/AIDS circumstances is well-recognized but initial infections may be characterized by slight or no signs at all. The HIV/AIDS compromises the sensitivity of immunodiagnostic methods to confirm latent TB; however, prevention of HIV/AIDS-associated TB using medications is normally effective (Schutz *et al*., 2010). Though advances in microbiological diagnosis have been made, there are still challenges in analysis of HIV/AIDS associated TB due to sputum negative or extra pulmonary disease (WHO, 2003; Schutz *et al*., 2010). These modern and more sophisticated effective TB diagnostics techniques are however, not used in developing countries because they are sophisticated and expensive (Buijtels *et al*., 2009). Another challenge is poor coordination between the TB and the HIV clinic. As a matter of fact, there is little further interaction between the coinfected patient and the HIV/AIDS doctors even in the similar hospital once an HIV positive patient is identified with TB and forwarded to the tuberculosis clinic (Nullis, 2005). Highly skilled personnel are also required to administer medication to TB-HIV coinfected patients so as to curb the challenges of overlapping drug toxicities and severe interactions such as decrease in the effectiveness of the drugs (Schutz *et al*., 2010).

In Kenya TB in many healthcare facilities is managed by clinical officers who lack the necessary skills for specialized management of TB/HIV/AIDS patients, the adoption of the WHO TB management guidelines by the government notwithstanding (WHO, 2009).During the procedure of TB diagnosis, culturing option is rarely done hence treatment of new infection rely on ZN smear microscopy, clinical symptoms and sometimes chest X-ray in selected health amenities (Buijtels *et al*., 2009). Unfortunately, non tuberculous mycobacteria with some other bacterial species give positive results in the ZN smear microscopy (Olson *et al*., 1998) thus, being

misdiagnosed as TB. This results in patients being prescribed with anti-tubercular drugs even though the treatment of Non Tuberculous Mycobacteria (NTM) disease is not the same as for TB treatment (ATS, 2007).

2.3.1 Immunology of HIV and *M. tuberculosis* co infections

Tuberculosis is the prototype of infections that require a cellular immune response their control. The CD4+ T cells serve a very crucial role in protection against *M. tuberculosis* infection. Human immunodeficiency virus on the other hand destroys CD4+ T cells thus have caused a resurgence of tuberculosis, resulting in ill health and death worldwide (Scanga *et al*., 2000). T1 lymphocytes cells which release interferon-gamma have been classified to play fundamental roles in anti-mycobacterial immune resistances, and deadly mycobacterial ailment develops mainly in children running short of interferon-gamma receptor. Tumour necrosis factor produced to control *M. tuberculosis* infection is needed to curb the bacterial growth. However, TNF is well-known to trigger HIV replication which is found in the macrophages. This clearly indicates that immune response produced against individual pathogen can stimulate replication of a different pathogen (Kedzierska *et al*., 2003). The exhaustion of CD4+ T cells, which is one of the chief characteristics of AIDS, is surely a paramount contributor to greater risk of recurrence of dormant TB and vulnerability to the new infection. There is some indication that CD8+ T cells have a role to perform in eradication of latent tuberculosis (Lewinsohn *et al*., 2007).

2.3.2 Pathophysiology of *M. tuberculosis* and HIV

Once they penetrate the respiratory tract, *M. tuberculosis* infect the macrophages.The CD4+ T-lymphocytes as well as Tγδ-lymphocytes releases interferon gamma,

interleukin-2, TNF-α and macrophage colony-stimulating factor. These trigger macrophages and CTLS to hinder the intracellular development of the bacilli. Tuberculosis results when the immune response encouraging growth of granuloma is inadequate to control the development of mycobacteria in the lungs. Interferon gamma plays a crucial role particularly at this point. In fact people with genetic weaknesses that result in less release of either IFN-γ or its cellular receptors result in developing acute and fatal tuberculosis (Ormerod and Horsfield; 1987). At the period of HIV infection, IFN-γ production is reduced intensely in parallel with the decline of CD4+ T cells. All these result in high risk of developing revival or reinfection by *M. tuberculosis* in the infected persons (Marchal, 1997). Increase in HIV viraemia in HIV-TB patients is caused by the release of proinflammatory cytokine by tuberculous granulomas mainly TNF-α which may quicken the progression in the direction of severe immunosuppression (Garrait *et al.*, 1997). The danger of mortality in HIV positive patients with tuberculosis is two times more than HIV positive person without tuberculosis. Indeed, most deaths are due to progressive HIV illness, other than tuberculosis (Whalen *et al.*, 1995).

2.3.3 Diagnosis and treatment of HIV

2.3.3.1 Diagnosis of HIV

Immunological assays mainly antibody screening test such as reactive enzyme immunoassay (EIA) are the methods recommended by Word Health Organization for diagnosis of HIV. Rapid tests are enzyme immunoassay**s** that do not have to be repeated but require a confirmatory test if reactive. The tests above are confirmed by a

positive outcome from a supplementary HIV antibody test like the Western blot or an indirect immunofluorescence (WHO, 2009; Butto *et al*., 2010).

The CD4+ T cell counts are used as indicators for the central immune defects in HIV/AIDS disease. Furthermore, it has also been used with viral load as markers of disease progression (Pattanapanysast and Sippy, 2004). A normal CD4+ T cell count is 600 to 1500 cells/ µl of blood but over 80 % of patients with AIDS-defining illness occur at a CD4+ T-cell count of less than 200 cells/µl of blood (CDC, 1997). Flow cytometry is technique used for enumerating CD4+, CD8+, CD45+ and CD3+ cells in monitoring immune response status due to its accuracy, precision and reproducibility (Pattanapanyasat and Sippy, 2004).

The viral load refers to the amount of HIV in the blood system. Viral load tests measure the quantity of HIV's genetic material (HIV RNA) in a blood sample. The amount of the virus in the blood is described as the number of copies of HIV RNA in a millilitre of blood .Undetectable limits of viral loads is a term us to describe when there is so little HIV in the blood that the HIV virus cannot be transmitted even if the condom is not used. The undetectable limits are copies less than 20 copies per millilitre of blood. Viral load testing is an established surrogate marker for treatment response (Murray *et al*., 1999). Several systematic reviews of data from clinical trials involving thousands of participants have established that decreases in viral load following initiation of ART are associated with reduced risk of progression to AIDS or death (Marschner *et al*., 1998).

Persons who adhere to their ARV regimes and do not harbor resistance mutations to the component drugs can have viral suppression within 8 to 24 weeks after ART initiation.

It may take longer in some patients but this is a rare case. A viral load of 10,000 would be considered low while 100,000 would be considered high. Optimal viral suppression occurs when viral load is persistently below the level of detection (HIV RNA <20 to 75 copies/mL) depending on the assay used (Havlir *et al*., 2001). Instances where viral load testing is recommended is; after initiation of ART or modification of therapy because of virologic failures to confirm an adequate initial virologic response to ART, secondly is in virologically suppressed patients in whom ART was modified because of drug toxicity or for regimen simplification, thirdly is in patients on a stable, suppressive ARTregimen and finally in patients with suboptimal response (Damond *et al*., 2007).

2.3.3.2 Treatment of HIV

Most advances in human immunodeficiency virus treatment occur when the drugs inhibit the activity of enzymes used by the HIV in its life cycle. Antiretroviral drugs are medications for management of HIV. When several such drugs are taken in a combination, the approach is known as highly active antiretroviral therapy (HAART) (Dybul *et al*., 2002). The ARTS have been categorized broadly according to the stage of the HIV life cycle which the ART drug prevents. Zidovudine (ZDV; AZT) was approved in 1987 by the United States Food and Drug Administration as the foremost ART drug to be used in prevention of HIV replication through hindrance of reverse transcriptase enzyme role. This was used as a monotherapy for several years with very limited efficacy and later it was successfully paired with lamivudine (3TC) and used as combination therapy (Montessori *et al*., 2004). The discovery of other classes of antiretroviral drugs and potential development of

resistance and cross-resistance to monotherapy as proved by viral load counts, warranted a switch from monotherapy to combination therapy (HAART) with its dramatic effects since it prevented mutated forms of HIV from evolving (CDC, 2004). Because of the complexity of selecting and following a regime, the severity of the side effects and the importance of compliance to prevent vital resistance, such organization emphasize the importance of involving the patient drug selection (Dybul *et al*., 2012).

Currently, there are six classes of antiretrovirals with over 26 diverse drugs used for HIV cure. These drugs are aimed at stopping HIV in its tracks by stopping the various stages of viral replication.

These categories of drugs include nucleoside reverse transcriptase inhibitors (NRTI's) which reverse transcriptase directly by binding to the enzyme and interfering with its function, non-nucleoside reverse transcriptase inhibitors (NNRTI's), protease inhibitors (PI's) which targets the viral assembly inhibiting the activity of protease, fusion inhibitors that interfere with binding, fusion and entry of HIV, CCR5 antagonists and intergrase inhibitors which inhibit enzyme intergrase which is responsible for integrations of viral DNA into DNA infected cells (Butto *et al*., 2010; WHO, 2011).

Guidelines have been set forth by the WHO in each individual country on how to use and manage patients on HAART (WHO, 2010). WHO currently recommends HAART initiation for all people infected with HIV virus and specifically those whose CD4+ T cell count is equal to or less than 500 cells per microliter of blood or those with WHO

clinical stage three or four in those settings where CD4+ T cell analysis is not available. The second-line of highly active antiretroviral therapy comprises of a ritonavir-boosted protease inhibitor (PI) plus two nucleoside reverse transcriptase inhibitors, one of which should be AZT or TDF, depending on the type of drug which was used initially. Ritonavir-boosted atazanavir (ATV/r) or lopinavir/ritonavir (LPV/r) is mostly preferred (WHO, 2011).

2.3.4 Diagnosis and treatment of tuberculosis

2.3.4.1 Diagnosis of tuberculosis

Diagnosis of TB can be done by sputum microscopy, florescent microscopy and culture.

2.3.4.1.1 Sputum microscopy

This is a diagnostic technique which involves direct examination of sputum for what is called acid-fast bacilli (AFB) by use of a microscope. The only ultimate approach of discovery of pulmonary TB is by direct sputum smear microscopy for AFB. Normally, early morning sputa must be collected before introducing any treatment. This is because the yield is optimal in the morning, as bacilli gather overnight in the lungs. Direct sputum microscopy, using special stains for acid fast bacilli is the cornerstone of diagnosis (WHO, 2006). Microscopy carried out using Ziehl Neelson stain recognizes roughly ten bacilli per ml sputum to attain positive results, with a sensitivity of about 60-70 % with pulmonary tuberculosis. Direct Microscopic examination of sputum for AFB remains the cornerstone for the diagnosis of Pulmonary TB in both industrialized and low income countries. The technique of staining acid- fast bacilli using Ziehl Neelson stain (ZN) has developed with amalgamations from many workers (Bishop *et*

al., 1970) and has kept on being the backbone of TB diagnosis for around hundred years. Even though it is less effective than culture, it is considered to be most consistent, fast, of low- cost and highly specific method for the diagnosis of Pulmonary TB as a microbiological tool in resource poor setting (Githui *et al.*, 2007).

2.3.4.1.1.2 Florescent microscopy

Florescent Microscopy was discovered in mid 1940s, it is a microscopic method of investigating acid fast bacilli by use of aura-mine stains. It is more sensitive than Ziehl Neelson stains (Githui *et al*., 1992). It is extensively used in developed republics but its usage in unindustrialized countries is limited owing to great investment and upkeep costs. Furthermore, it is mainly used in those facilities with more than 50 specimens being tested per day. Fluorescence microscopy has been shown in numerous studies to be at least 10% more effective than old light microscopy (Ebersole, 1992). Thus, fluorescent stains are of paramount importance, not only in confirming the presence of *mycobacterium* in a given specimen, but also in providing an estimated quantification of organisms (Githui *et al*., 1992).

2.3.4.1.1.3 Culture

Culture involves the growing of the bacilli in Lowenstein-Jensen media. This technique is the most effective in the diagnosis of *M. tuberculosis*. Many diverse media have been developed for growing tubercle bacilli. These include; egg-based media, agar-based media and liquid media (Githui, 1994). The sputum specimens submitted for culture must pass through microscopic examination .The best medium for isolation of tubercle bacilli should have the following characteristics; economical, hinder the development of

contaminants, easy to make from r8Ueadily accessible components as well as supporting luxuriant growth of lesser quantities of bacilli. During the culture of sputum specimens, egg-based media should be the first choice. Lowenstein-Jensen medium is most widely used for cultivating *M. tuberculosis* because it contains glycerol which favours the growth of *M. tuberculosis* while Lowenstein-Jensen medium containing pyruvate but without glycerol encourages the growth of *M. bovis*. Both should be used in countries or a region where patients may be infected with either organism. The advantage of culturing as a method of TB diagnosis is that it allows confirmation of cases as well as facilitating species identification (Githui *et al*., 1993; Chaudhary, 2010).

2.3.4.2 Treatment of tuberculosis

Tuberculosis ailment can be easily eradicated when diagnosed early. The first line of drugs during the first two months in the treatment of TB is rifampicin, isoniazid, ethambutol and razinamide. The second line of drugs in the remaining 4 months consists of 6 classes of drugs including aminoglycosides, polypeptides, fluoroquinolones and thioamides (WHO, 2003). Medical or drug prescription should be adhered to otherwise when one defaults this can lead to drug resistance where by the patient has to start the dose afresh. Direct observation and treatment strategy (DOTS) has helped in the management of tuberculosis (Chaudhary *et al*., 2010).

2.4 Gaps in knowledge

The above literature review shows that most of the previous scientific research covered have concentrated mostly on a few areas like on effects of antirectroviral drugs on the

CD4+ T cell counts or effects of HIV on CD4+ T cell counts, for instance a study by Mugwe *et al*., 2013 on the effects of antirectroviral drugs on CD4+ T cells in HIV patients attending Nakuru Hospital. The conclusion from this study was an increase in CD4+ cell counts after using ART as well as an inverse correlation between CD4+ T cells and viral load counts. Another close study on CD4+ and CD8+ lymphocytes counts in HIV negative pulmonary TB patients with those of normal blood donors and effects of antitubeculars was carried out by Uppal *et al.* (2004) in a military hospital in India. The findings were lower CD4+ counts in patients with TB as oppose to the normal donor. The study also concluded TB being a reversible cause of CD4+ lymphocytopenia.

Few studies if any have been done concerning the demographic characteristics patients co-infected with TB-HIV, others have been done in other parts of the world and more so in other hospitals but not in Kericho county hospital. Azerevedo and Hayes (2010) in the study on HIV and TB coinfection in Kenyas: Environment, Resources and Culture, found higher number of coinfected females than males. Besides, illiteracy and poverty are other factors which contribute to high burden of TB /HIV. Not much information has been given on the effects of anti-TB drugs on the CD4+ T cell as well as viral load on HIV/TB coinfected patients counts hence the need for further research on that area. Therefore, this study focuses on determining the demographic characteristic of coinfected patients as well as determining whether TB chemotherapy has an effect on CD4 T cell and viral load counts which is one of the measures of immune response.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Kericho County Hospital (KCH) located in Kericho town 260 kilometers northwest of Nairobi. The integration of HIV services into the tuberculosis clinic was done in the year 2005 as a result of the initiation of HIV clinic services. The clinic offers various services such as medical, counseling, testing, provision of food supplements among other services, to a rural population who's forty six percent of its members living below the poverty line. The tuberculosis and HIV services are facilitated by government's funds besides other donor organization such as the Kenya Medical Research Institute/Walter Reed Project (KEMRI/WRP) HIV Program. Other activities in the TB clinic include testing of the HIV status, guidance and counseling of the patients before and after testing and finally treatment through provision of antiretroviral and anti-TB drugs.

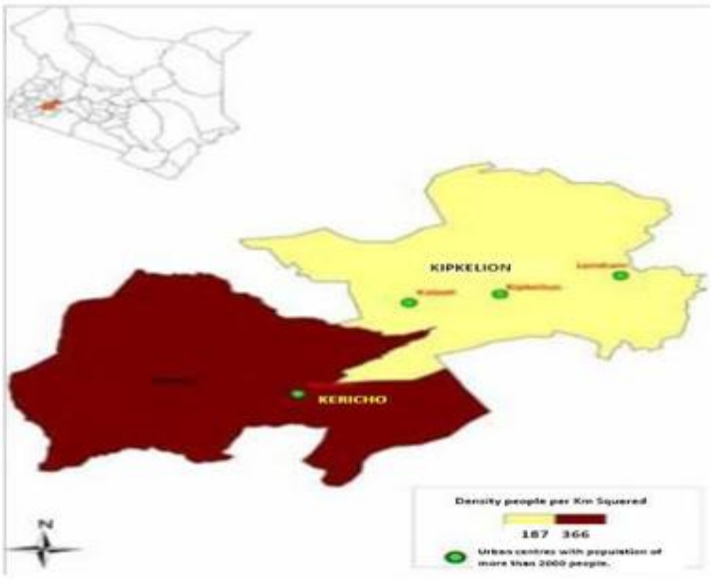

Figure 3.1: Map of study Area

3.2 Study design

A cross sectional study was carried out on HIV individuals co-infected with *M. tuberculosis* visiting or admitted in the Kericho County Hospital. Three experimental groups were used for this study: HIV/TB co-infected as the main study group and TB mono-infected and normal study groups as control groups. Recruited individuals were those identified by the hospital as having these diseases. Blood samples were obtained at the beginning and after completion of TB chemotherapy for analysis of CD4+ T cell counts for patients in the period of November 2015 to December 2016. Data on viral loads matched with recruited persons were obtained from hospital records. Questionnaires were used to collect demographic information from the study population.

3.3 Target population

The target population included all Human Immunodeficiency Virus infected persons with Tuberculosis visiting or admitted in the Kericho County Hospital at the time of the study.

3.4 Patient enrolment

The study subjects were recruited from TB/HIV clinic and were adults over 18 years who visited Kericho County Hospital during the study period and those who had a signed consent form (appendix I). Standardized questionnaires (appendix III) were used to collect social demographic information of the subjects.

3.5 Inclusion criteria and exclusion criteria

Adults over 18 years and those with HIV and *M. tuberculosis* co-infection as well as TB monoinfected who visited Kericho County Hospital during the study and who gave a written informed consent form were included into the study (Appendix II). HIV negative patients and HIV positive patients with TB infections who declined to participate in the study were excluded.

3.6 Sample size determination

The sample size was calculated using similar method by Nyamagoba (2012). In the year 2013, the average tuberculosis and Human Immunodeficiency Virus co-infection proportion in Kericho County was 30 % (NLTP, 2013). Using this as a reference for a single proportion calculation with an error of 5 % at 95 % confidence level, employing Fisher's exact formula as applied by Nyamagoba (2012), the required sample N was:

$$N = \frac{z^2 pq}{\sigma^2}$$

Where:

N = sample size

z = standard normal deviate = 1.96

p = prevalence proportion = 0.3 (30%) (KAIDS 2014)

q = 1-p = 0.7

σ = level of precision = 0.05

$$N = \frac{(1.96)^2\ 0.3x0.7}{(0.05)^2} = 323$$

A total of 323 blood samples were collected through venipuncture by a registered laboratory technologist from the recruited patients attending the hospital for the period of November 2015 to December 2016 before and after TB chemotherapy.

3.7 Sampling technique

Every patient who was declared TB and TB/HIV positive by the hospital and was willing to give a written consent was included in the study. The prescribed treatment for the drug sensitive TB for the recruited patients was the first line of TB drugs during the first two months which included rifampicin, isoniazid, pyrazinamide and ethambutol. The second line of drugs in the remaining 4 months consisted of rifampicin and isoniazid.

3.8 CD4+ and viral load quantification

CD4+ T cells counts were analyzed with a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry). Whole blood was collected in a single K3 EDTA tube for each patient. Twenty microlitres of CD3+/CD4+/ CD45+ monoclonal antibody reagent (BD TriTEST) was introduced to fifty microlitres of whole blood in a tube with a lyophilized pellet having a calibrated quantity of fluorescent beads (BD TruCount). The resulting blood in the tubes were put inside a dark cabinet for incubation for a period of 15 minutes at room temperature (20–25°C) and 450 µl of lysing solution added. The tube was incubated for 15 minutes at room temperature (20–25°C) in a dark place again and then the sample processed in the FACS Calibur system (Wayne, 2007). Results were recorded so as to be used for analysis. Viral load counts were obtained from patients' clinical record since they were done in the Walter Reed Project.

The viral load counts were determined by using polymerase chain reaction (PCR) according to a method described by Murray *et al* (1999). Thirty microlitres of blood sample and five microlitres of nuclease-free water were mixed with 1 mM concentrations of each of the deoxynucleoside triphosphates as well as 25 μM pd (N).The mixture was then incubated at 72°C for 5 min. The denatured RNA was placed on ice for 1 min before the addition of 12 μl of reaction buffer, 10 mM dithiothreitol, 1.5 μl of RNasin and finally 1.5 μl of Moloney murine leukemia virus reverse transcriptase. The reaction mixture was incubated at 37°C for 45 min, and finally, the enzymes were inactivated by heating at 98°C for 3 minutes before processing. The results were recorded in copies/ μl of blood and expressed as powers of ten or log scale (Murray *et al*, 1999).

3.9 Data collection using questionnaires

The study was based on the all adults over 18 years, specifically, HIV - TB co infected and TB monoinfected patients who visited Kericho County Hospital TB clinic during the study period. Standard questionnaires (Appendix III) were used to collect demographic characteristics of the patients and medical history of the participants. The baseline characteristics of patients include: age, gender, sex, type of TB, date when he/she was first diagnosed and occupation.

3.10 Data analysis

Analysis of data was carried out with statistical package for social science (SPSS) software. Descriptive statistics such as count, percent and frequency were used to determine socio demographic characteristics of patients. Viral load counts were

compared at the beginning and at the completion of TB chemotherapy drugs using mean, median, range , variance, standard deviation, percentile ranks and quartile ranks. The results were then presented in tables, Charts, graphs and narratives. Paired t-Test was used to compare pretreatment and post treatment CD4+ counts of the controls. A P value of ≤ 0.05 was considered statistically significant.

3.11 Ethical considerations

After the approval of the research proposal by the Graduate School, research clearance to conduct the study was obtained from Kenyatta University Ethical Review Board and Kericho County Hospital Ethical Board (Appendix II). A permit to conduct research was also obtained from National Commission for Science, Technology and Innovation (Appendix III). Prior to recruitment of the study participants, written informed consent was also obtained from each participant (Appendix I). The study was highly confidential and subjects were not forced to incur any expense during the study. The patients recruited were those declared as having TB and TB/HIV by the hospital. Blood collection and analysis were done by registered laboratory technologist.

CHAPTER FOUR: RESULTS

4.1 Socio-demographic characteristics of study participants

4.1.1 Age distribution

A total of 323 patients coinfected with TB and HIV were recruited at the beginning of the study. The majority of the enrolled subjects were aged between18 and 74 years with the mean age being 35.9±10.0 years. Thirty-six study participants (11.1 %) were between 18 and 24 years of age while 113 (35.0 %) were aged between 25 and 34 years, 66 (20.4 %) study participants were 45 years old or more (Figure 4.1).

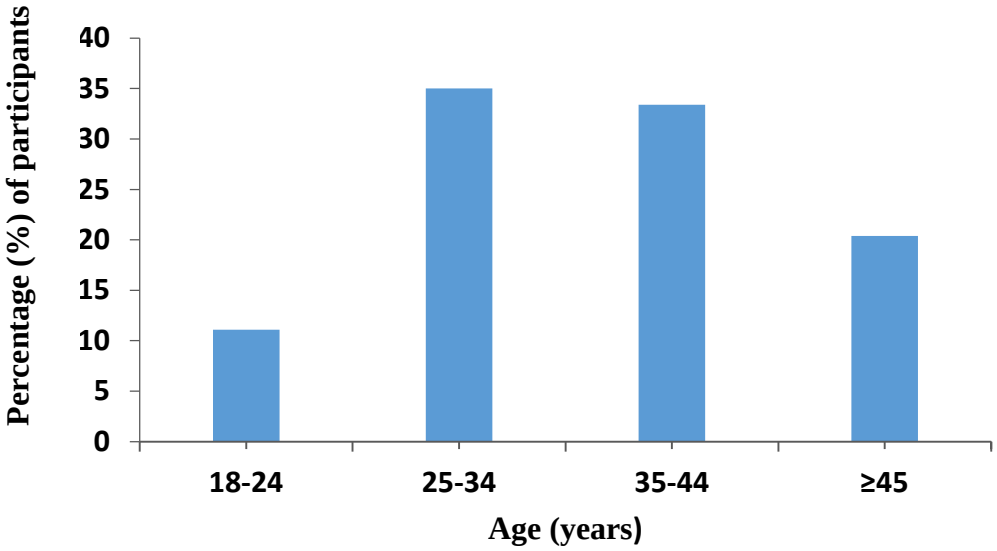

Figure 4.1: Age distribution of the study participants, n=323

4.1.2 Gender

A total of 45.2 % study participants were males while 54.8 % study participants were females. This study reveals that more females were coinfected than males (Figure 4.2).

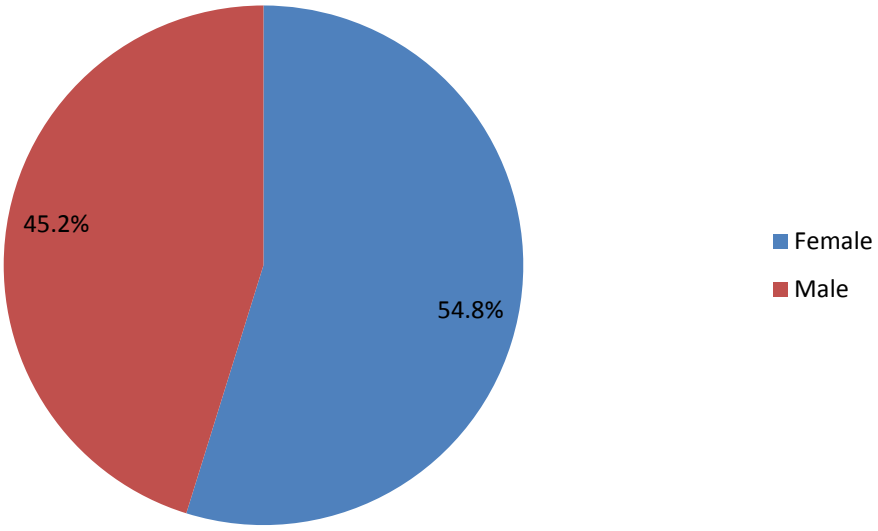

Figure 4.2: Gender of the participants, n=323

4.1.3 Marital status

Assessment of the marital status of the studied patients revealed that majority 229 (92.6 %) were married. A total of 24 respondents (7.4 %) were not married at the time the study was conducted (Figure 4.3).

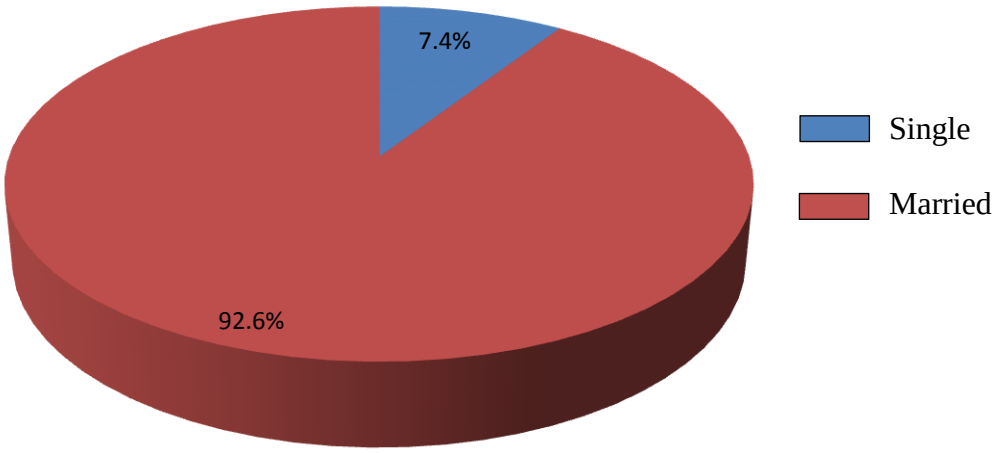

Figure 4.3: Marital status of the study participants, n=323

4.1.4 Level of education

A total of 144 study participants (44.6 %) reported they had attained primary school education as the highest education qualification. Those who had secondary school educational qualifications were 126 participants (39.0 %). The rest (53 or 16.4 %) had tertiary level education as the highest education attained (Figure 4.4).

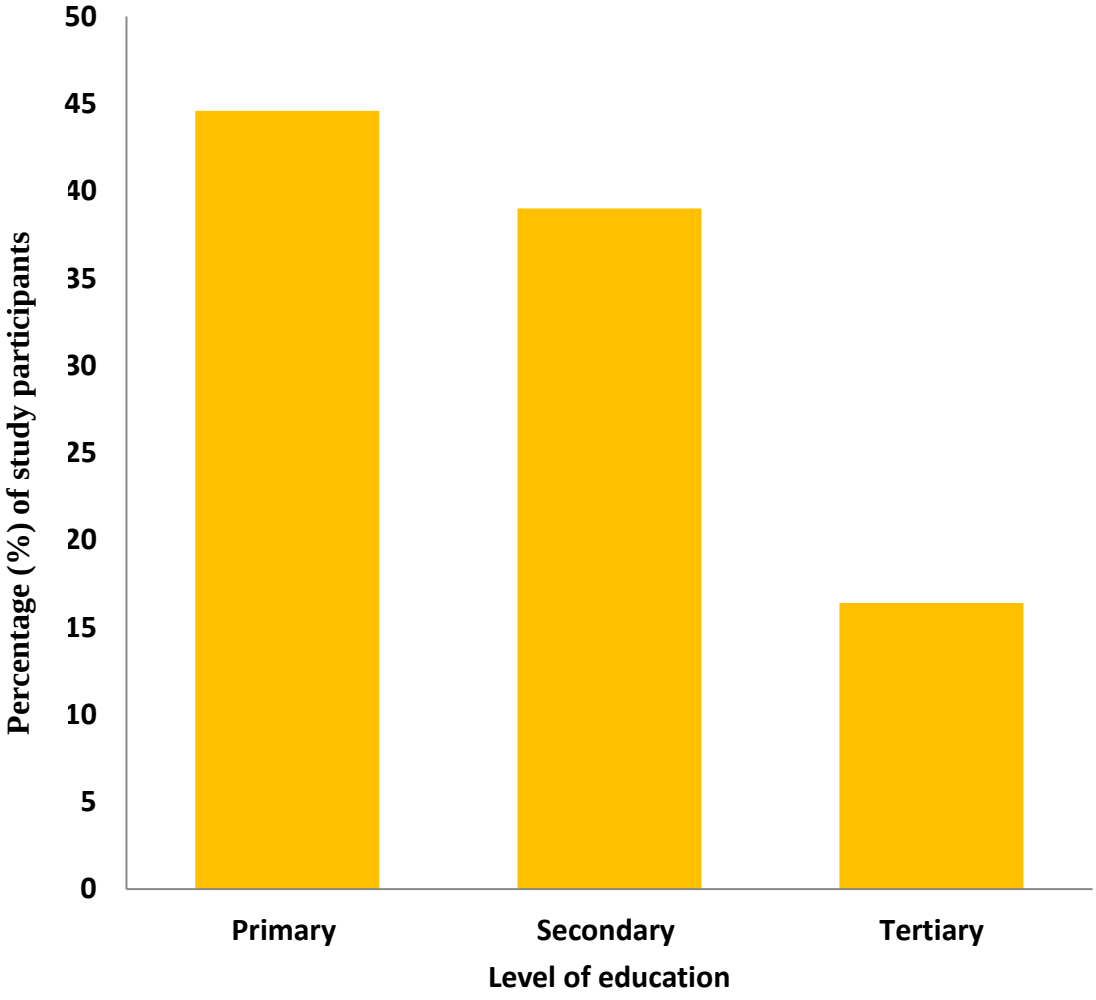

Figure 4.4: Study participants' level of education, n=323

4.1.5 Occupation

Concerning their occupations, 14.9 %, 10.2 % and 9.6% respondents who participated in the survey reported that they were engaged in small business, farming and casual labour (tea plucking) respectively. Security personnel and accountants constituted 7.1 % and 4.6 %respectively of those who participated in the study. Others which include drivers, conductors, teachers, social health workers, bar maids, house wives, house help workers, carpenters and masoners constituted the largest percentage 53.6 % (Figure 4.5).

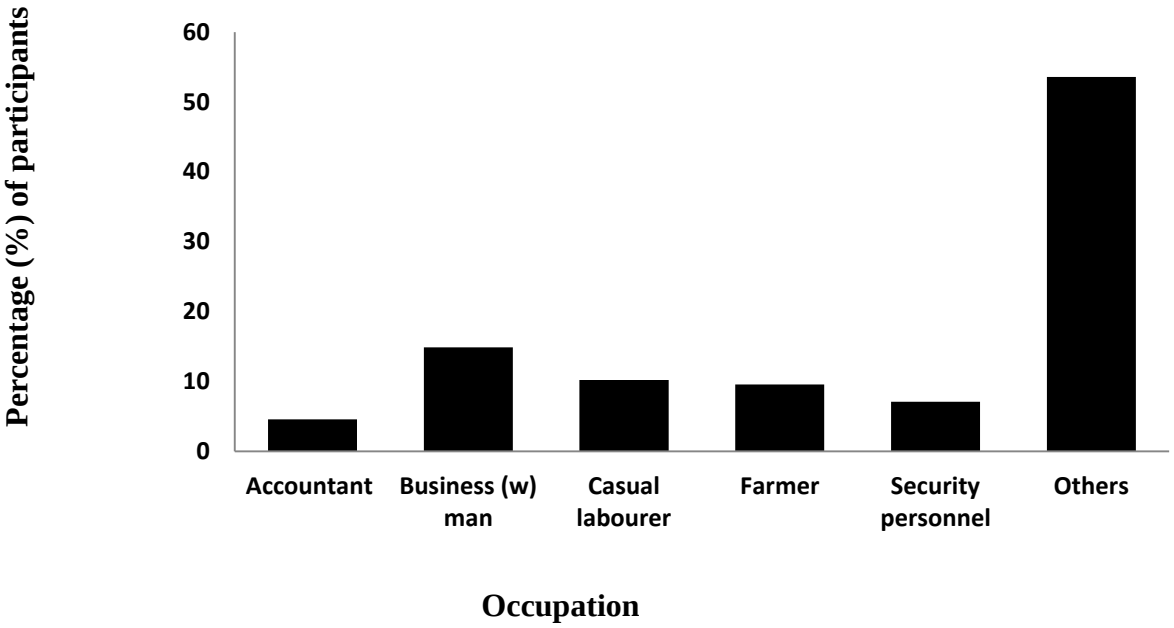

Figure 4.5: Occupations of the study participants, n=323

4.2 Clinical characteristics of study participants

4.2.1 Duration on antiretroviral therapy

The period under which the study subjects had been on antiretroviral therapy was evaluated in years and results indicated that the median (interquartile range) duration for being on antiretroviral therapy (ART) among the participants was 72 (range = 48 - 112) months. Those who had been on ART for two years or less were 9.3% while 37.5 % had been on ART for a period of more than two years and not exceeding five years (Figure 4.6).

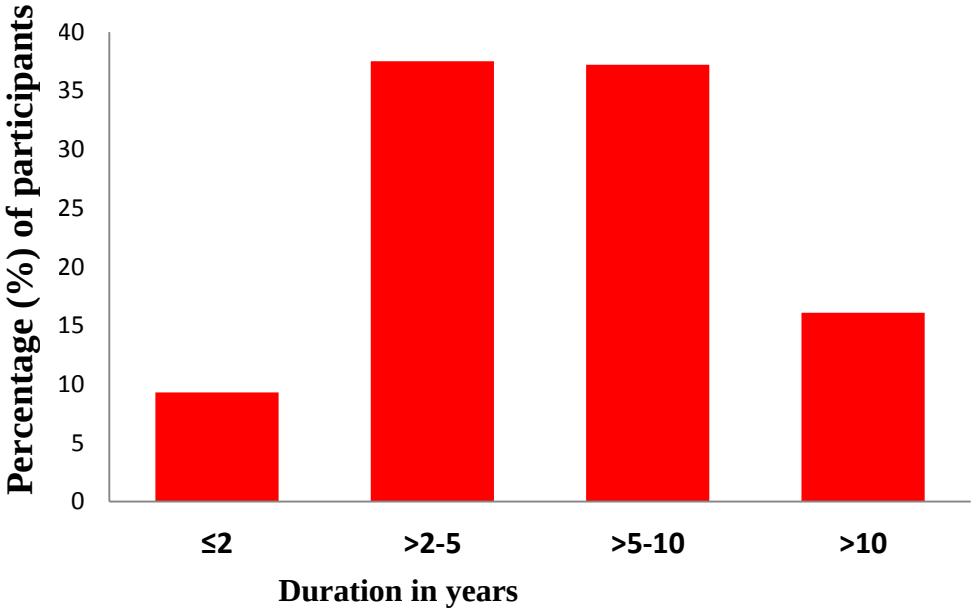

Figure 4.6: Duration on antiretroviral therapy of the study participant, n=323

4.2.2. Types drug sensitive tuberculosis

Most of the study participants had been diagnosed with pulmonary tuberculosis with proportion of 78.0%. Seventy-one participants (22.0 %) had extrapulmonary tuberculosis (Figure 4.7).

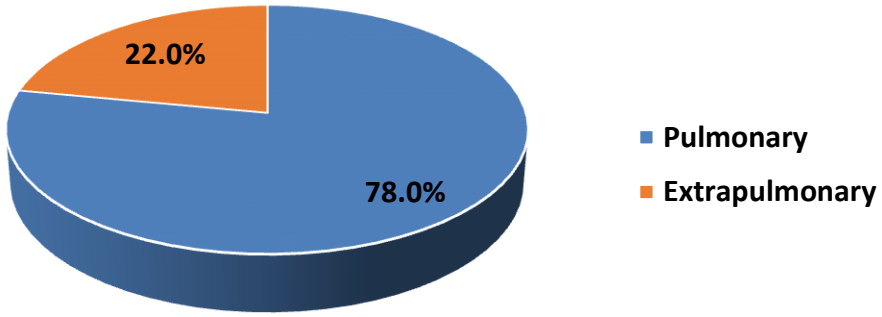

Figure 4.7: Type of tuberculosis the study participant n=323

4.2.3 Pre and post treatment CD4+ counts for HIV-TB (pulmonary)

Patients co-infected with HIV-TB (Pulmonary) were put in various categories of CD4+ T cell counts based on CDC classification (SCA, 1995) before and after anti-TB treatment and the proportion of those having a CD4+ T cell count of between 200 to 499 cells/µl increased from 21 % to 75 % while those whose counts in CD4+ T cells were 500 cells/µl or higher changed from 2 % up to 25 % before and after TB chemotherapy respectively.

4.2.4 Pre and post treatment CD4+ counts for HIV-TB (extra pulmonary)

Among the extra pulmonary tuberculosis group, the proportion of those whose CD4+ T cells counts ranged from 200-499 cells/µl increased from 13 % and 80 % before and after TB chemotherapy respectively. Patients whose CD4+T cell counts were at least 500cells/µl of blood were 1 % before TB chemotherapy treatment and increased to 20 % on completion of TB chemotherapy.

4.2.5 Pre and post treatment CD4+ counts for HIV-TB (irrespective of TB type)

The overall change in the proportion of patients who were classified in the 200 to 500 cells/µl of blood category irrespective of the type of TB increased by 56 % while the percentage of patients in the group of those whose CD4+ T cells count was 500cells/µl or higher increased by 22 %. In all categories of TB, the percentage of persons whose CD4+ T cell counts was lower than 200 cells/µl was 0 % after a period of six month and 8 months for new and relapse cases respectively .(Figure 4.8).

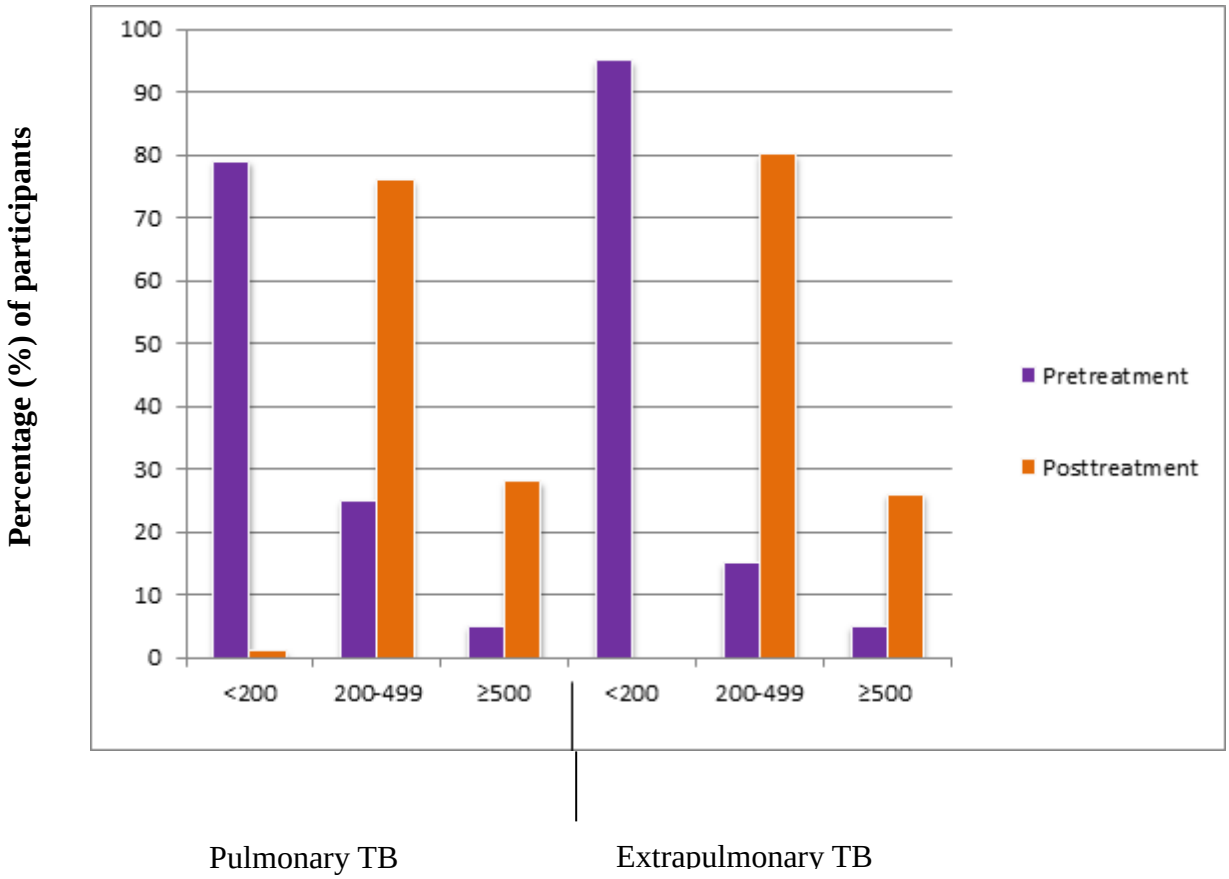

Figure 4.8: CD4+ counts before and after anti- TB treatment, n=323

4.2.6 Comparative analysis of CD4+ change in patients with HIV-TBs

Statistical analysis pointed out a significant difference occurring in the median CD4+ counts in patients with either pulmonary or extrapulmonary TB before and after treatment (median (interquartile range (IQR)) CD4+ T cell counts: 88(35-180) and 398(340-490) cells/µl of blood, respectively, P<0.001. Correspondingly, a significant improvement in CD4+ T cell counts were reported in patients diagnosed with pulmonary and extrapulmonary tuberculosis on completion of the TB chemotherapy, P<0.001 (Table 4.1).

TB Type	Median (IQR) pretreatment	Range (Min-Max) pretreatment	Median (IQR) posttreatment	Range (Min-Max) posttreatment	P-value
Pulmonary	89.5(33.3 - 192.5)	21-750	399(340-499.5)	123 – 960	<0.001
Extrapulmonary	79(38-143)	13 -652	396(337-469)	237 – 950	<0.001
Overall	88(35-180)	13 – 750	398(340-490)	123-960	<0.001

Table 4.1: Comparative analysis of CD4+ counts change in patients with HIV-TB

4.2.7 Pre and post treatment CD4+ counts for the patience with TB only

The TB monoinfected patients were treated with TB chemotherapy for a period of six months. After analysis of pretreatment and post treatment CD4+ T cell counts using paired sample test ,the results showed a significant improvements in CD4+ T cell counts after TB chemotherapy; t=-2.711,p = 0.009 (Table 4.2).

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	TB ONLY Pre and Posttreatment CD4+counts_	-164.60000	429.26354	60.70703	-286.59535	-42.60465	-2.711	49	.009

Table 4.2: pre and post treatment CD4+ cell counts for TB mono infected patients.

4.2.8 Pre and post treatment viral load counts for HIV-TB (pulmonary)

The number of pulmonary TB patients who had a viral load of lower than 1000 copies/µl of blood increased from 10 % to 90 % following completion of TB chemotherapy course. In the same group of pulmonary TB patients, individuals whose viral load counts was 1000 copies/µl of blood and above reduced from 89 % to 10 % at pretreatment and post treatment viral load respectively (Figure 4.9).

4.2.9 Pre and post treatment viral load counts: HIV-TB (Extra pulmonary)

There was a reduction in the number of extra pulmonary TB patients whose viral loads were 1000 copies/µl of blood and above from 92 % to 6% after TB chemotherapy in the cohort that was suffering from extrapulmonary tuberculosis. However, the number of patients whose viral load counts was below 1000 copies/µl of blood increased from 9% to 95% after the tuberculosis treatment (Figure4.9).

4.2.10 Pre and post treatment viral load counts: HIV-TB (combined)

The number of patients whose viral load were lower than 1000 irrespective of the kind of TB increased by about 81% .Correspondingly, the patients with a viral load of 1000 copies/µl of blood or higher reduced in number by eighty one percent (Figure 4.9).

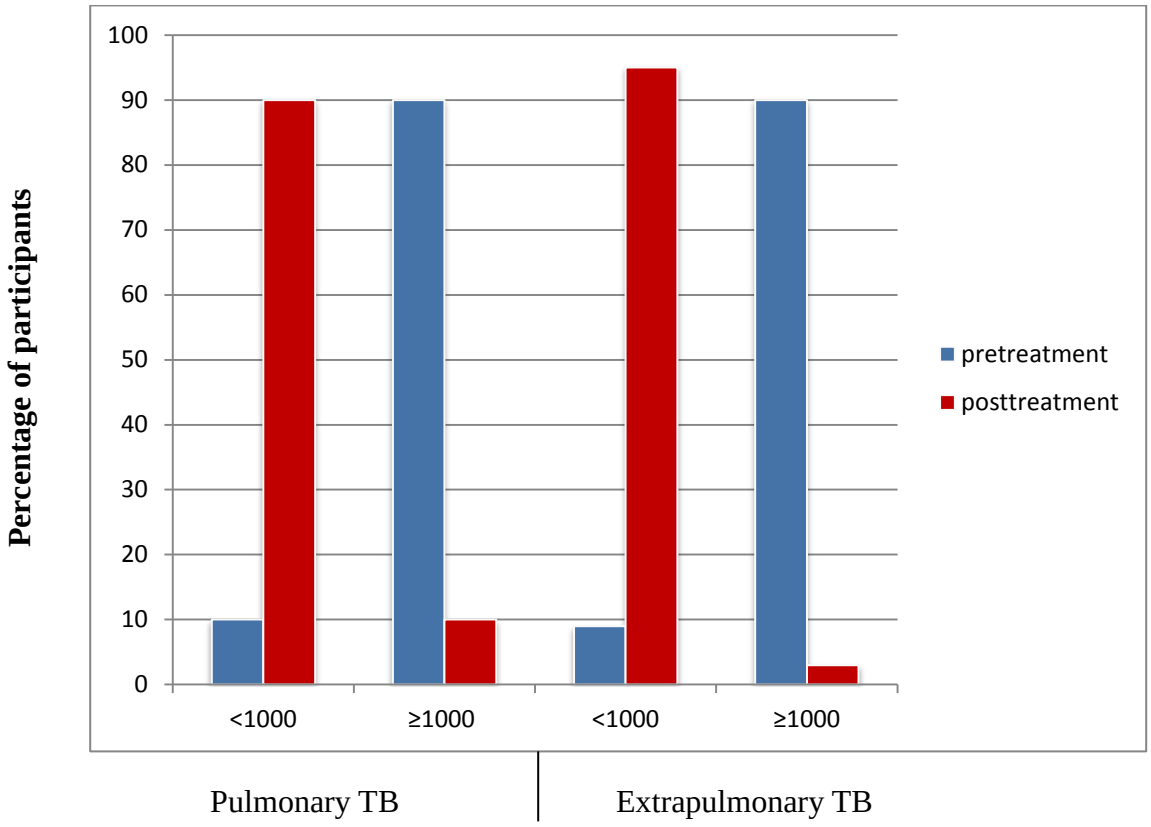

Figure 4.9 HIV viral before and after anti-TB treatment, n =323

4.2.11 Comparative analysis of viral load change in patients with HIV/TBs

The median (IQR) viral load in all the HIV/TB coinfected patients was 256,789 (49,000 – 460,870) copies/ µl of blood at the time of initiation of TB chemotherapy. After a period of 6 months, the median (IQR) viral load was 19 (19-86) copies/µl which was a significant reduction, P<0.001. Likewise, reductions in pretreatment verses post-treatment viral loads were statistically significant in the group of patients who had pulmonary TB (median (IQR) viral load 255,339 (45,821-495,998) versus 19(19-88.5) copies/µl, P<0.001, as well as in the extrapulmonary TB patients (median (IQR) viral

load 267,890 (57,000 – 440,340) versus 19(19-75) copies/µl of blood, P<0.001 (Table 4.3).

TB Type	Median (IQR) pretreatment	Range (Min-Max)pretreatment	Median (IQR) post treatment	Range (Min-Max)posttreatment	P-value
Pulmonary	255,339 (45,821-495,998)	19-2,124,560	19(19-88.5)	19-678,958	<0.001
Extrapulmonary	267,890 (57,000 – 440,340)	19–19,354,657	19(19 – 75)	19-59,403	<0.001
Overall	256,789(49,000 – 460,870)	19-19,354,657	19(19 – 86)	19-678,958	<0.001

Table 4.3: comparative analysis of viral Load change in patients co-infected with HIV-TBs

4.2.12 Comparative analysis of CD4+count and viral load with the normal

According to CDC (1997) normal persons CD4+ T cell counts ranges from 500 to 1600 cells/µl of blood. Another study done by Bosire *et al.,* (2013) in Kenya found the normal CD4+ T cell counts range to be 343-1493 cells/ of blood . A person without HIV has zero viral loads. The standard ranges for CD4+ counts and zero viral loads for normal persons served as reference points for the normal controls. In our study any value below 19 copies/µl of blood was treated as below detectable levels. At pretreatment, patients co-infected with HIV/TB (pulmonary) range and median (IQR) were 21-750 and 89.5(33.3-192.5) respectively, this is lower than the normal reference value (CDC, 1997) thus within the normal reference of 343-1573 cells/ µl of blood as supported by Bosire *et al* (2013) and also closer to the CDC (1997) of 500-1600 cells/

μl of blood. The pretreatment range and median (IQR) for HIV/TB coinfected extra pulmonary patients were 13-652, 79(38-143) and 237-950, 396(337-469) after TB chemotherapy respectively. The range and median (IQR) viral load were: 19-19, 35465, 256789(49000-460870) and 19-678958, 19(19-86) pretreatment and posttreatment respectively irrespective of the type of TB, an indication of reduction in viral load (Havlir *et al*., 2001).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Socio-demographic factors

5.1.1.1 Age distribution

The existence of TB-HIV coinfection in all age groups could be attributed to predisposing factors prevailing in the study area such as ignorance, poverty, lifestyle in town and in tea plantations among others. The most coinfected individuals were those within the sexually active bracket. These findings are in agreement with the findings reported by WHO (2014) that HIV/TB is known to affect reproductive age group of between twenty five to forty five years because these are individuals who are sexually active hence the transmission of HIV is very high. These sexually active brackets don't engage in safe sex or use condoms which are an important protection against HIV (Azerevedo, 2010). The statement mentioned previously as far as condom use is concerned, is supported by other researchers that due to high HIV prevalence in Kenya, it is important to encourage condom use in all types of sexual relationships including consensual and legal unions since both married and unmarried individuals engage in risky sexual behaviours (IFPP, 2006). Moreover, they are likely to encounter sexual partners with high incidences TB and HIV coinfection (Deribe *et al*., 2013).

5.1.1.2 Marital status of the study participants

Marital status is an important risk factor when exploring the patterns of HIV/TB co-infection. These findings of the study indicated that majority of the subjects were married, the reason for such an observation could be the mode of transmission of HIV

virus which is mainly through sexual intercourse thus married couple are at high risk of infection especially if one partner is unfaithful. These results are in agreement with the finding of KDHS (2003) that nearly 2 out of 3 Kenyans coinfected with TB-HIV are aged between 15 to 64 years and are married.

Unfaithfulness within marriage is another underlying factor especially to workers within the tea plantation and the transport sector because most leave their spouses at home and engage in sexual relationship with other workers whose spouses are also far thus transmitting HIV infections to their innocent spouses back home. In addition, they don't engage in safe sex because they cannot afford condoms or fear using them with their couples even after suspecting HIV infection because it's a sign of mistrust. Gathuya (2009) further emphasized in his research on the marital status comparing single parents and those who have been in any type of union, individuals who have ever been married were 3.2 times more likely to be HIV infected.

5.1.1.3 Education level of the study participants

Although patients in all education levels were predisposed to TB-HIV coinfection, higher prevalence were recorded in primary level which was 2.7 times more than those with tertiary education level .This is therefore an indicator that higher education is good for better health. Low level education may be associated with lack of proper knowledge on how best to prevent transmission of HIV virus by using safe methods when engaging in sexual activities or lack of money for purchasing such protective devices (Gathuya, 2009). Others could engage in sexual activities for monetary terms because they cannot afford basic needs due to poverty, mainly seen in commercial sex workers. All these

observations can be summed up by the statement that TB-HIV coinfection increases with decreasing level of education.

The results are in agreement with studies from a nationwide survey in Cameroon which discovered that HIV occurrence is at the peak among individuals with primary or secondary education level mainly those with HIV and TB complications (NIS, 2011). The findings are further supported by the findings on research on association between HIV status and socio-demographic characteristic by Gathuya (2009) that HIV prevalence increases with decreasing level of education. Contrary to the findings of the study are the findings of other researchers that found that patients with high education level, with high chances of being employed in skilled occupations which attracts huge salaries are likely to have multiple partners compared to those with unskilled occupations having attained low education level (Gathuya, 2009). This indicates that availability of disposable income can lead to unhealthy behaviours that result in transmission of HIV which destroy CD4 T cells increasing the susceptibility to TB.

5.1.1.4 Gender of the study participants

Presently, there is no definite indication of sex-based dissimilarities in the incidences of tuberculosis and human immunodeficiency virus co-infection in the third world countries though the study had slightly higher number of co-infected females as opposed to males thus in agreement with the finding of Rwenge (2013) who found greater prevalence of HIV infection amongst females generally in sub-Saharan Africa because they are commonly exposed to sexual actions prior to men.

The present results go in line with a study which was carried out in Kenya which discovered 41.8 % and 58.2 % of HIV/TB cases being males and females respectively. The possible reason for high incidences of coinfected women could be due to socio economic factor and health seeking behaviours of women. As opposed to men, most women have no control of financial resources at the house holds level. Moreover, there is higher rate of transient and permanent immune suppression in women as a result of pregnancy, breastfeeding and HIV in women than male counterparts (WHO, 2014).

Azerevedo *et al.* (2010) on a study of HIV and TB in Kenya found higher number of HIV/TB coinfected females than males comprising of 52% and 48% respectively due to increased sexual violence against women (rape) fueling the spread of HIV, weakening the immunity making one susceptible to HIV. The findings above strongly support the results of the study.

5.1.1.5 Occupations of the study participants

The type of occupations also plays a very important role on HIV-TB confection. People with low income earning casual jobs are highly susceptible to the coinfection than those with high income generating and stable jobs. The reason is because they lack enough disposable income thus tempted to engage in unhealthy behaviors such as prostitution, unfaithfulness among others for more money. All these malpractices lead to HIV transmission which weakens the immunity of an individual making them susceptible to TB infection. The findings of this study concur with the observation that lack of income may lead people to engage in unhealthy commercial or forced sexual practices that are important in the spread of HIV-TB coinfections (Azerevedo *et al.*, 2010). The kind of

occupation is dictated by the education level which in turn dictates the salary earned. Persons with higher education level which attracts huge salaries have better quality live because the can access better health care, food, shelter hence the lesser the coinfection.

As a matter of fact, one of the contributing factors which encourage the spread of tuberculosis which is a contagious infection is overcrowding; the infectious droplet coughed by an infected person can be easily inhaled by those in the surrounding if the place is congested (Behr *et al*., 1999; Shaffer *et al*., 2012). Overcrowding mainly in slums happens because of lack of money to acquire spacious room for the family. Most of the poor families or individuals find themselves living in slums which are congested and with very poor waste disposal system thus higher chances of developing diseases. The unskilled workers in the tea plantations likewise live in small cubes within the tea plantations thus another sign of congestion which encourages the spread of TB. These results are strongly supported by WHO finding which noted TB and HIV accompanying poverty (WHO, 2009). The skilled personnel who are more salaried on the contrary can afford high standard spacious rooms in less congested estates with low TB infection hence low HIV/TB coinfection.

5.1.2 The type of tuberculosis

Pulmonary tuberculosis was found to be the most rampant type, the reason behind could be due to early diagnosis and treatment before spreading from the lungs to other parts of the body resulting to extra pulmonary tuberculosis. These findings concur with previous studies by Rajasekaran *et al. (*2007) who discovered the greatest common type

of tuberculosis in HIV co-infected patients being the smear-positive pulmonary tuberculosis.

Pulmonary tuberculosis was significantly associated with HIV than extra pulmonary in the present study which does not support the findings of WHO (2010) which reported that HIV is associated with extra pulmonary tuberculosis. Such observations could have occurred because of other factors like higher use of ARTS which results in high levels of innate and cell mediated immunity which reduces the progression of pulmonary tuberculosis to extra pulmonary tuberculosis. Other possible reasons for most patients being identified with pulmonary tuberculosis as opposed to extra pulmonary tuberculosis was due to the diagnostic technique which was mainly sputum microscopy as opposed to radiography which is more expensive and requires more skills (Githui *et al*., 2007). Such opinion is supported by the notion by other researchers that direct microscopic examination of sputum for acid fast bacilli remains the cornerstone for the diagnosis of Pulmonary TB in both industrialized and low income countries (Rieder *et al*., 1997). Extra pulmonary tuberculosis which occurs when TB has extended beyond the lungs to other regions like the bones and joint was rare; the disease was salvaged before spreading due to early diagnosis and proper treatment.

5.1.3 CD4 counts of the study participants

The pre-treatment low CD4+ T cell counts levels in HIV/TB coinfected patients is associated with HIV-1 virus which affects CD4+ T cell counts, lowering the individuals capacity to fight diseases therefore, increasing the susceptibility to opportunistic infections. Emergence of TB as an opportunistic infection is mainly as a

result of low CD4+ T cell counts which are significant in defensive response counter to *M. tuberculosis.* These findings concur with studies by Butto *et al.* (2010) that HIV infection leads to severe depletion of CD4+ T cells and further by Scanga *et al.* (2000) that human immunodeficiency virus destroys CD4+ T cells causing reoccurrence of TB resulting to increased cases of illness and death globally.

This is further strengthened by the studies of Ochanda (2003) who discovered HIV weakening the immune system by progressive depletion and dysfunction of the CD4+ T cells, thus affecting monocyte and macrophage function, all of which are the key players in protection against *M. tuberculosis*. Furthermore, there is high immune activation among TB/HIV coinfected individuals indicating the desperate attempts of the immune system to contain *M. tuberculosis* with the attrition of CD4+T cells. Upon initiation of TB chemotherapy the immune system improves steadily to around a normal CD4+ T cell counts of 500/*u*l of blood within six months which is the completion period. Anti-TB drug are taken for duration of six months unless one defaults whereby the dose has to be started afresh. At this point, treatment is expected to have cleared *M. tuberculosis* in the body unless an individual is a defaulter or in cases of drug resistance or relapse whereby the patient is introduced to another type of drugs for the same or longer period. Defaulting can also result in drug resistance therefore strict adherence to the drug is highly recommended (WHO, 2015).

HIV/TB coinfection was associated with a significant decrease in the CD4+ T cell counts and these findings underscore the importance of preventing and promptly treating TB in HIV-infected individuals. The success of the treatment with TB drugs

which resulted in increased number of CD4+ T cell counts in most patients was also contributed by the patients' high drug adherence.

Besides the TB chemotherapy, food supplements were supplied which could have also assisted in boosting the body's immunity. Only a few of the study subjects in this study had negative *improvement* in CD4+ T cell counts. This could be attributed to drug resistance due to default or drug toxicity due to use of anti-TB drugs and ARVS concurrently as supported by the findings of Schutz (2010).

A significant improvement in CD4+ cell counts was also noted on the TB monoinfected patients after TB chemotherapy although the significance was lower than HIV/TB coinfected group's whose pretreatment CD4+ cell counts mean was lower . Studies by Uppal *et al.*, 2004 found significantly lower CD4+ counts in normal blood donors as opposed to TB patients thus supporting the findings. The reason for lowerCD4+ counts in TB/HIV coinfected patients is HIV virus affecting the CD4+ cell depleting them weakening the immunity. Upon completion of TB treatment, CD4+ cells increased to about 500 and above. The study is supported by the findings of Bosire *et al* (2013) having come up with a range CD4+ cell count of 343-1573 cells/*u*l of blood for people living in Kenya and 500-1600 cell/*u*l of blood according to CDC(1997). Moreover, there was higher improvement in CD4+ cell counts after completion of TB chemotherapy because all the patient infected with HIV virus with a CD4+ cell count of 500 cells and below has to be introduced with Antiretroviral drug as per WHO 2013 guidelines and because all HIV/TB patients' CD4+ counts were below 500 cells/ *u*l of blood and were on ARVS.

5.1.4 Viral load counts of the study participants

The viral load counts declined after completion of TB chemotherapy due to greater improvement in CD4+ T cell counts with TB chemotherapy and Anti-retroviral therapy, resulting in reduction in viral replication in the blood. The pretreatment viral load counts was higher than post treatment counts because the immune response generated against *M. tuberculosis* promoted faster replication of HIV virus thus greater copies in the blood. Studies by Kedzierska *et al*. (2003) discovered huge viral load in TB-HIV coinfected patients because immune response produced in response to *M. tuberculosis* infection meant for controlling bacterial growth activated HIV replication. Therefore, the immune response generated against one disease causing organism may promote replication of another one. These findings also correspond to UNAID results that tuberculosis is the primary root of bereavement in HIV patients and resurgence of active tuberculosis in HIV positive persons is likely to be due to intensified HIV replication (Wallis, 1993). Tuberculosis infection is purported to be an ailment associated with the increase in the multiplication of HIV viral load. This occurs as a result of cytokine expression (Wallis *et al*., 1993). Besides, TB also accelerates HIV infections by increasing viral load by five to seven folds (UNAIDS, 2006) and is responsible for reduced lifespan degree in HIV persons (Ackah, 1995).

The post treatment viral load was lower than pretreatment viral load because *M. tuberculosis* had been fully eradicated by TB drugs thus the immune response generated against it promoted HIV virus replication was reduced. In fact most patients

had lower than detectable limits of viral load counts (< 19 copies/µl of blood) after they were cured of tuberculosis (Havlir *et al*., 2001).

The low CD4+ T cell counts in coinfected patients is linked with high levels of HIV viraemia, which may quicken the progression towards severe immunosuppression. The danger of mortality in HIV-positive patients with tuberculosis is twice that of HIV-positive patients without tuberculosis and most deaths is as a result of progressive human immunodeficiency infection, other than TB (Whalen *et al.,* 1995). TB infection was associated with significant increases in the HIV viral load and significant decreases in the CD4+ T cell counts. The findings underscore the importance of preventing and promptly treating TB in HIV-infected individuals. Timely diagnosis and cure of tuberculosis in human immunodeficiency virus and tuberculosis coinfected patients is very paramount in suppressing the viral load to less detectable limits and also improving the immunity of the body.

5.2 Conclusions

i. The current study found that most of the TB-HIV coinfected patients in Kericho County Hospital were married females, aged between 18 to 74 years and with primary school level of education. Most were casual labours employed in various low income earning occupations and mainly affected by pulmonary tuberculosis.

ii. The study found a more significant increase in CD4+ T cell counts in patients coinfected with HIV/TB than the TB monoinfected after completing their TB therapy

iii. The viral load counts decreased significantly after completion of TB chemotherapy due to reduced replication of the HIV virus which was speeded up by *M.tuberculosis.*

5.3 Recommendations

On the basis of the foregoing analysis, discussion, broad observations, issues and perspectives on the study it would be appropriate to make the following recommendations:

i. Since most of the affected population are poor and with low education level, there is need for more sensitization on the importance of higher education since it empowers people with more knowledge on better ways of improving living standards thus eradicating poverty and diseases. There is also an urgent need of educating people on method of reducing HIV transmission since most are married and are sexually active.

ii. Tuberculosis chemotherapy has proved to restore CD4+ cell counts in both HIV/TB coinfected and TB monoinfected patients; therefore there is need for early diagnosis and treatment so as to restore the immunity earlier preventing occurrence of opportunistic infections which goes with lower CD4+ T cell counts.

iii. It is advisable for patients coinfected with HIV/TB to use both TB drugs and antiretroviral drugs concurrently and early so as to reduce the viral load copies whose numbers doubles up faster in the presence of *M.tuberculosis.*

5.3.1 Further research

There is need for further studies on:

i. Why TB drugs and ARVs respond well on most patients and negatively on others and ways of improving immune responses in larger sections of the populations.

ii. The relationship between gender and TB in a larger study.

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APPENDICES

Appendix I

Consent Form

My name is **Eunice Cherono**. I am Master of Science Student from Kenyatta University.

I am conducting a study on 'title of the proposal **Immune responses in patients co-infected with HIV and *Mycobacterium Tuberculosis* attending TB Clinic in Kericho County Hospital'**. The information will by used by the ministry of medical services and ministry of Public health and sanitations to improve access and quality for screening of **Patients co infected with HIV/TB** in the hospitals as well as in other regions of Kenya.

Procedures to be followed:

Participation in this study requires that I asked you some questions and I also examine you in order to screen you for **CD4 counts.**

Some specimen will be taken form you for further tests. I will record the information from you in a questionnaire.

Voluntary participation

You have the right to refuse participation in this study and there is no reward or penalty of any kind if you decide to or not to participate, You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time. Please remember the participation in this study is voluntarily. You may ask questions related to the study at any time. You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive form this clinic or any other organization now or in the future.

Discomforts and Risks:

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable, If this happens, you many refuse to answer these questions if you so choose. You will also experience some pain and discomfort during collection of blood. Some of your time will be used before receiving your normal treatment.

Benefits:

If you participate in this study you will help us to learn how to provide effective screening services that can improve the health of HIV/TB coinfected patients and reduce the risk of **HIV/TB coinfection.** You will also benefit from being screened for… and if you are found to have a problem you will be advised on the treatment.

Reward:

There is no immediate individual benefit at now for those who will participate in this Study other additional laboratory investigation on the C4+ counts .0therwise your assistance in this research will aid in improving control and treatment of TB and HIV

Confidentiality:
All The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. All the samples taken will be labeled with code link to your name. Your name will not appear when reporting the findings .The questionnaires will be kept in a locked cabinet for safe keeping at the Hospital. Everything will be kept private.

Contact information:
If you have any questions you may contact(1)Principal researcher Eunice Cherono on 0722493354 (2). Prof Gicheru on 0722609565 or (3) Dr Mutiso on 0722492228 or the Kenyatta university ethical Review committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, Secretariat.kuerc@ku.ac.ke

Participants statement:

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care that I will receive from the clinic today or that I will get from any other clinic at any other time.

Name of Participant …….

_____ _____

Signature or Thumprint **Date**

Investigators statement
I, the undersigned, have explained to the volunteer in a language she understands the procedures to be followed in the study and the risks and benefits involved.
Name
Interviewer:…….

_____ _____

Signature or Thumb print **Date.**

Appendix II (a)

Ethical approval

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

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Our Ref: **KU/ERC/APPROVAL/VOL.1** (111) Date: 23rd October, 2017

Eunice Cherono
Kenyatta University
P.O. Box 43844-0100
NAIROBI.

Dear Eunice,

APPLICATION NUMBER- PKU/669/I747 "IMMUNE RESPONSES IN PATIENTS CO-INFECTED WITH HIV AND MYCOBACTERIUM TUBERCULOSIS ATTENDING TB CLINIC IN KERICHO COUNTY HOSPITAL, KENYA."

1. **IDENTIFICATION OF PROTOCOL**

The application before the Committee is with a research topic Application Number PKU/669/I747 "Immune Responses in Patients Co-Infected with HIV and Mycobacterium Tuberculosis attending TB Clinic in Kericho County Hospital, Kenya." received on 24th April 2017 and discussed on 9th May 2017.

2. **APPLICANT**

Eunice Cherono

3. **SITE**

Kericho County, Kenya

4. **DECISION**

The Committee has considered the research protocol in accordance with the Kenyatta University Research Policy (Section 7.2.1.3) and the Kenyatta University Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 23rd October 2017.**

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 committee immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

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.

DR. TITUS KAHIGA.
CHAIRMAN ETHICS REVIEW COMMITTEE

I ..... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of... .....18-12-2017.....2017.

C.c. DVC Research Innovation and Outreach

Appendix II (b)

COUNTY GOVERNMENT OF KERICHO

KERICHO DISTRICT HOSPITAL

Telegrams: "MEDICAL", Kericho
Telephone: Kericho (0734) 758102
e-mail: kerichodistricthospital@yahoo.com
When replying please quote
Ref: ER/010/15

Medical Superintendent
Kericho District Hospital
P. O Box 11
KERICHO

Date 27th October 2015

Dear Cherono Eunice,

RE: RESEARCH APPROVAL

Reference is made in regard to your letter dated 13th October 2015 requesting for permission to carry out a research in our Health facility.

The Ethics & Research committee through the office of the Medical Superintendent is therefore pleased to inform you that, your request to carry out a research entitled ***"Immune Response in Patients Co- infected with HIV and Mycobacterium Tuberculosis attending TB Clinic in Kericho County Referral Hospital "*** has been approved.

You are therefore expected to continuously observe Ethical considerations and ensure informed consent is sought from all clients willing to participate in this study.

Feedback and sharing of the findings of the study with the committee is **mandatory** upon completion of the data collection exercise. All concerned departments in this facility are requested to facilitate you in this process.

Yours Sincerely,

DAVID K.LIMO

MEDICAL SUPERINTENDENT
KERICHO DISTRICT HOSPITAL
P. O. Box 11, KERICHO - 20200
TEL: 052 - 31177 / 31191

For, CHAIRMAN

ETHICS & RESEARCH COMMITTEE

Appendix III

(a) LETTER FROM NACOSTI

NATIONAL COMMISSION FORSCIENCE, TECHNOLOGY ANDINNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/17/63127/17038** Date: **19th June, 2017**

Eunice Cherono
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Immune response in patients coinfected with HIV and mycobacterium tuberculosi attending TB clinic in Kericho Couny Hospital,"*** I am pleased to inform you that you have been authorized to undertake research in **Kericho County** for the period ending **19th June, 2018.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Kericho County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kericho County.

The County Director of Education
Kericho County.

Appendix III

(b) NACOSTI CERTIFICATE

THIS IS TO CERTIFY THAT:
MS. EUNICE CHERONO
of KENYATTA UNIVERSITY, 0-0
SOSIOT,has been permitted to conduct
research in *Kericho County*

on the topic: *IMMUNE RESPONSE IN PATIENTS COINFECTED WITH HIV AND MYCOBACTERIUM TUBERCULOSI ATTENDING TB CLINIC IN KERICHO COUNY HOSPITAL*

for the period ending:
19th June,2018

.....
Applicant's Signature

Permit No : NACOSTI/P/17/63127/17038
Date Of Issue : 19th June,2017
Fee Recieved :Ksh 1000

.....
Director General
National Commission for Science, Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officer will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEACH CLEARANCE PERMIT

Serial No.A 14446

CONDITIONS: see back page

Appendix IV

Questionnaire

I humbly request you to answer this short questionnaire which is purely designed for academic purposes. Mark (X) where applicable. Thank you in advance for your participation.

ID codes …….

1. Age……

2. Sex

Male [ ] Female [ ]

3. Marital Status

Married [ ] Single [ ]

4. Education level

Primary [ ] Secondary [ ] College [ ] University [ ]

5. Occupation……

6. When were you first diagnosed with TB? ……

7. a) Which type of TB do you have?

Pulmonary [ ] Extra pulmonary [ ]

b) Are you on TB treatment?

Yes [ ] No [ ]

c) If yes in (7b) above, for how long? ……

8. a) Are you on ARVS?

Yes [ ] No [ ]

b) If the answer in (8a) above is yes, for how long have you used? ……

Appendix V

CD4+counts and viral loads pre and post-treatment

Type	Median (IQR) pretreatment	Range (Min-Max)	Median (IQR) post treatment	Range (Min-Max)	P-value
CD4 counts (cells/µl)					
Pulmonary	89.5(33.3 - 192.5)	1-750	399(340-499.5)	123 - 960	<0.001
Extrapulmonary	79(38-143)	5 -652	396(337-469)	237 - 950	<0.001
Overall	88(35-180)	1 – 750	398(340-490)	123-960	<0.001
Viral load (copies/µl)					
Pulmonary	255,339 (45,821-495,998)	19-2,124,560	19(19-88.5)	9-678,958	<0.001
Extrapulmonary	267,890 (57,000 – 440,340)	19–19,354,657	19(19 – 75)	19-59,403	<0.001
Overall	256,789(49,000 – 460,870)	19-19,354,657	19(19 – 86)	9-678,958	<0.001

Appendix VI

Change in CD4+counts and viral loadspre- and post-treatment

Variable	Type of TB (n, %)			x^2, df,p-value
	All	Pulmonary	Extrapulmonary	
Percentage increment in CD4+ counts				
No change (0%)	2(0.6)	2(0.8)	0(0.0)	x^2=2.814, df=3, p=0.421
1% - 100%	40(12.4)	33(13.1)	7(9.8)	
>100% - 500%	156(48.3)	116(46.0)	40(56.3)	
>500%	125(38.7)	101(40.1)	24(33.8)	
Percentage reduction in viral load				
No change (0%)	23(7.1)	20(7.9)	3(4.2)	x^2=3.301, df=2, p=0.192
1% - 50%	7(2.2)	7(2.8)	0(0.0)	
>50% - 100%	293(90.7)	225(89.3)	68(95.8)	