HIV/AIDS and Tuberculosis Coinfection in Rural Northern Tanzania

Epidemiology, clinical presentation and impact on CD4 T cell counts

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Dedicated

To my wife Agatha Elinisa Mushi and
Our daughter Joan for your great care, love and prayers

To my parents James Ngowi and Eliaikesa Ngowi for your struggle to give me best education.

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TO OUR ALMIGHTY GOD; WHAT SHALL I RENDER TO THE LORD FOR ALL HIS BOUNTY TO ME? I WILL LIFT UP THE CUP OF SALVATION AND CALL ON THE NAME OF THE LORD. MY MOUTH WILL SPEAK THE PRAISE OF THE LORD, AND ALL FLESH WILL BLESS HIS HOLY NAME FOREVER AND EVER.

Original papers

1. B J Ngowi, SG Mfinanga, JN Bruun, O Morkve: **Pulmonary tuberculosis among people living with HIV/AIDS attending care and treatment in rural northern Tanzania**. *BMC Public Health* 2008, **8**:341.
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Abbreviations

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
ARV	Anti retroviral drugs
BMI	Body Mass Index
CBC	Complete Blood Cell Count
CPT	Cotrimoxazole Preventive Therapy
CTRL	Central Tuberculosis Reference Laboratory
CXR	Chest X Ray
E	Ethambutol
ELISA	Enzyme Linked Immuno Absorbent Assay
EPTB	Extrapulmonary tuberculosis
BD FACS	Becton Dickinson Fluorescent Activated Cell Sorter
HLH	Haydom Lutheran Hospital
HIV	Human immunodeficiency virus
INH	Isoniazid
IPT	Isoniazid Preventive Therapy
LJ Media	Lowenstein Jensen Media
MDR TB	Multi-Drug Resistant Tuberculosis
MOHSW	Ministry of Health and Social Welfare
MRCC	Medical Research Coordinating Committee
NACP	National AIDS Control Programme
NIMR	National Institute for Medical Research
NTLP	National Tuberculosis and Leprosy Programme
OI's	Opportunistic Infections
PTB	Pulmonary Tuberculosis
R	Rifampicin
S	Streptomycin
SSA	Sub Saharan Africa
TB	Tuberculosis
TACAIDS	Tanzania Commission for AIDS
PEPFAR	President's Emergency Plan for AIDS Relief
USA	United States of America

VCT	Voluntary Counselling and Testing
WHO	World Health Organisation
Z	Pyrazinamide

Executive summary

Tuberculosis (TB) and HIV/AIDS are the main causes of morbidity and mortality in adults aged 15-49 years in Sub Saharan Africa (SSA). The interaction between tuberculosis and HIV/AIDS makes the diagnosis and management of the coinfection difficult. A cross sectional hospital based study was conducted at Haydom Lutheran Hospital (HLH) to assess the interaction between tuberculosis, HIV/AIDS and tuberculosis HIV/AIDS coinfection in relation to the CD4 T cells. Furthermore, CD4 T cell counts in healthy subjects in different age groups were determined for the purpose of establishing reference values.

Study subjects were recruited from

- People living with HIV/AIDS
- Tuberculosis clinic
- HIV Voluntary counselling and testing clinic (VCT).

Physical examination and investigation including sputum for fluorescence microscopy and culture, tuberculosis drugs susceptibility testing and Chest X-Ray (CXR) were done for all tuberculosis and HIV/AIDS patients.

Sputum samples were stained using auramine and examined by fluorescence microscopy. Sputum culture was done using Lowenstein Jensen media and sensitivity to the first line TB drugs was tested.

HIV test was done using 2 different rapid antibody tests, Determine HIV-1/2 (Abbott laboratories, Abbott Park, IL, USA) and Capillus HIV-1/2 (Trinity Biotech, Bray, Co Wicklow, Ireland). Discordant samples were sent to the regional hospital for confirmatory test using ELISA; Enzygnost anti-HIV 1+2 Plus ELISA (Behring, Marburg, Germany) and Well-coenzyme HIV recombinant ELISA (Murex, Dartford, England).

Complete blood cells (CBC) count was done using Sysmex Kx-21 (Sysmex Corporation; Kobe Japan). CD4 T cells were analyzed using a FACSCount flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.)

We enrolled 440 subjects (102 healthy subjects, 105 newly diagnosed tuberculosis patients with unknown HIV status, and 233 people living with HIV/AIDS). Males were 158 (35.9 %) and 282 (64.1 %) were females. The overall HIV/AIDS and tuberculosis coinfection prevalence was 34/338 (10.1 %). For the newly diagnosed tuberculosis patients 14/105 (13.3%) were HIV/AIDS coinfected; and for the people living with HIV/AIDS 20/233 (8.5 %) were coinfected with tuberculosis.

Sixty three out of 92 (68.5%) sputum specimens from newly diagnosed tuberculosis patients were culture positive and 66/92 (71.7%) were smear positive for acid fast bacilli (AFB). Out of 66 culture positive specimens, 58 (92.1%) isolates were susceptible to the first line tuberculosis drugs.

Twenty (8.5%) sputum samples from people living with HIV/AIDS were culture positive. Eight of the culture positive samples (40%) were smear positive AFB. Fifteen (75%) of these patients neither had clinical symptoms nor chest X-ray findings suggestive of tuberculosis. Nineteen isolates (95%) were susceptible to the first line tuberculosis drugs. In groups, (newly diagnosed tuberculosis and PLWHA coinfected with tuberculosis) there were no cases of multi-drug resistant tuberculosis.

For the healthy subjects recruited for the establishment of reference values, the mean absolute CD4 T cells was 745.9 $\pm$ 256.6, and the mean absolute CD8 T cells was 504 $\pm$ 218.4. Females had significantly higher mean CD4 T cells (802 $\pm$ 250) than males (665 $\pm$ 247, t=2.7, df =89, p=0.007) and higher mean absolute CD8 T cells (551.0 $\pm$ 215.4) than males (438.2$\pm$208.4, t=2.7, df =90, p=0.009). The mean haemoglobin level was 13.6$\pm$ 2.4 (males 14.1$\pm$ 2.7, females 12.6$\pm$1.9. Females had significantly lower mean haemoglobin level than males, (t=3.2, df =68, p=0.03)

For the newly diagnosed tuberculosis patients; tuberculosis patients had statistically significant lower mean CD4 T cells (559 $\pm$ 238) than healthy subjects (746 $\pm$ 257, t=5.3, df =190, p<0.01). Tuberculosis patients had significantly lower haemoglobin level (10.9 $\pm$ 2.4) than healthy subjects (13.2 $\pm$ 2.4, t=6.5, df =188, p<0.01) and HIV/AIDS patients (11.6 $\pm$ 2.2, t=2.4, df =152, p=0.02). Tuberculosis patients were significantly more malnourished (BMI 15.6 $\pm$ 2.3) than healthy subjects (BMI 23.4 $\pm$ 3.4, t=18.8, df=178, p<0.01), HIV/AIDS patients (BMI 19.4 $\pm$ 3.4, t=11.5, df=241, p<0.01) and HIV/AIDS tuberculosis coinfected patients (BMI 16.7 $\pm$ 2.8, t=2 df =50, p=0.04).

We found high prevalence of HIV/AIDS and tuberculosis coinfection as compared to the HIV prevalence in the general population in this setting. Half (17/34) of the sputum samples from HIV/AIDS coinfected patients were smear positive AFB by fluorescence microscopy. About 90% of the mycobacteria isolated from these patients were sensitive to the first line tuberculosis

drugs. Chest radiograph suggestive of tuberculosis and clinical symptoms of fever and cough were uncommon findings in HIV/AIDS and tuberculosis coinfected patients.

Irrespective of the HIV status tuberculosis was significantly associated with lower mean CD4 T cells, malnutrition and anaemia when compared to healthy subjects, indicating that tuberculosis may cause a reduction in CD4 T cells independent of HIV infection.

The immunohaematological values we found were different from standard values for western countries. Females had significantly higher mean CD4 T cell counts and lower mean haemoglobin levels than males. This raises the issue of the appropriateness of the present reference values and guidelines for monitoring HIV/AIDS patients in Tanzania.

We recommend active detection of tuberculosis among PLWHA patients and also HIV infection among tuberculosis patients; this will improve clinical staging of HIV and AIDS disease and also help to scale up anti-retroviral therapy (ARV), cotrimoxazole preventive therapy (CPT) and isoniazid preventive therapy (IPT) to those in need, provided that there is no contraindications to ARV, CPT and IPT. The screening for tuberculosis among PLWHA by smear microscopy and/or culture (if available) can be done at inclusion of the patient to care and treatment and during the routine follow up. Screening for HIV among tuberculosis patients is recommended to be done on a routine basis once the diagnosis of TB is made.

We also recommend reviewing published reports to establish local immunohaematological reference value for the Tanzanian population.

1.0 Introduction

1.1.0 General

Globally tuberculosis (TB) is the leading cause of morbidity and mortality among HIV/AIDS patients accounting for about 30% of all death of HIV/AIDS patients [1-4]. Tuberculosis in HIV/AIDS patients is curable provided it is diagnosed accurately and treated promptly, but this need special attention due to the complexity of the diagnosis and treatment involved in tuberculosis and HIV/AIDS coinfection. In Africa and in Tanzania tuberculosis is spreading rapidly due to the high prevalence of HIV infection signifying the need of steps to be taken urgently to stop this spread. Globally about 11% of new adult cases of tuberculosis are also HIV/AIDS coinfected and in Sub Saharan Africa 31% of new tuberculosis cases are also HIV/AIDS coinfected[5, 6]. HIV/AIDS fuels the tuberculosis epidemics in many ways, such as promoting progression to active tuberculosis, increasing the risk of reactivation of latent tuberculosis infection, as well as increasing chance of tuberculosis infection once exposed to tubercle bacilli. The other way round tuberculosis increases the risk of progression from HIV to AIDS [4, 7]. World Health Organisation (WHO) recommends inclusion of HIV testing in the algorithm for diagnosis of tuberculosis in countries with adult HIV prevalence rate of $\geq$ 1% or in settings where the HIV prevalence rate in tuberculosis patients is $\geq$ 5%[8-10]

1.1.2 Tuberculosis epidemiology

Worldwide tuberculosis prevalence has declined by more than 20 per cent but Africa's rates have tripled since 1990 in countries with high HIV prevalence like Tanzania, and are still rising across the continent at 3-4 per cent per year[11]. Between 1998 and 1999, a 20% increase of TB cases was reported in countries severely affected by HIV/AIDS in Africa[12]. This contributed much to the increase of the TB burden globally. About 7-12% of all new tuberculosis cases in adult 15-49 years are also coinfected with HIV worldwide while in WHO African region 31% of all new tuberculosis cases are also HIV/AIDS coinfected[4, 5]; and in Tanzania 50% of all tuberculosis patients are coinfected with tuberculosis [8].

In Tanzania during the year 2001, 61,603 tuberculosis cases were reported, with a treatment success rate of about 75% and death rate of about 10 %[8]. Treatment success depends on completion of treatment according to national guidelines once a diagnosis of tuberculosis is made. Proper diagnosis and correct treatment of tuberculosis will result in reduction of prevalence, provided that the infectious cases are detected and brought to treatment. However, there are difficulties in achieving the goal of reducing tuberculosis in Tanzania due to a number of challenges, in addition to prevailing problems in the control program, the difficulties in diagnosing tuberculosis in HIV/AIDS patients due to unusual clinical picture, increase in extrapulmonary (EPTB) and acid fast bacilli (AFB) smear negative pulmonary disease and atypical findings in chest x ray, all these complicate the tuberculosis diagnosis[13, 14]. To date, no simple test, apart from smear microscopy that can be used to diagnose these cases. Moreover, maintaining quality of smear microscopy in Tanzania still need emphasis [15]

The stigma associated with tuberculosis with its link to HIV/AIDS, poor adherence associated with high pill burden in case of coinfection, high mortality in HIV/AIDS and tuberculosis coinfected patients and difficulties in integrating tuberculosis and HIV/AIDS in one control program complicate the whole tuberculosis, HIV/AIDS management

1.1.3 HIV/AIDS epidemiology

About 1.8 million people in Tanzania are living with HIV/AIDS[16]. The epidemic is spreading fast in rural areas with less health facilities compared with the urban areas. The spread in rural area is accelerated by poverty, ignorance and lack of information about proper methods of prevention. Although the epidemic is reported to decrease or stabilise in some areas of Tanzania like Kagera with a prevalence 4.8%, in other regions like Mbeya prevalence (15.3%) it is still high and the overall country prevalence is 6.9%[16]. WHO estimates show that where the HIV prevalence in the general population is high, the prevalence of HIV in tuberculosis patients is also relatively high and *vice versa* .For example, the 1999 World Health Organisation estimates show that in Botswana, with HIV prevalence of 36% in the general population, the prevalence of HIV in tuberculosis patients was 77%. In Sub Saharan Africa with HIV prevalence of 8.7% in the general population, the prevalence in tuberculosis patients was 37% [6]

Efforts to control HIV/AIDS are in progress countrywide through the National AIDS Control Program (NACP) and Tanzania Commission for AIDS (TACAIDS).The control program

includes information, education and communication (IEC) about the prevention of HIV/AIDS and behaviour change and communication (BCC), Emphasis is on abstinence, faithfulness and promotion of safer sex through condom use in high risk groups. Prevention of mother to child transmission of HIV/AIDS (PMTCT) is also promoted by administering anti-retro viral drugs (ARV) during the third trimester or at onset of labour, and by education about breast feeding options. The Government of Tanzania initiated the roll out ARV program in October 2004 which aims at scaling up ARV to reach those in need in resource constrained areas. In the roll out ARV program all patients with medical eligibility for ARV are treated free of charge according to Tanzanian national policy for HIV/AIDS managements. In order for the HIV/AIDS patients to start ARV treatment among other screening they should also be screened for tuberculosis before initiation and on the course of treatment with ARV. The World Health Organisation recommends screening of HIV-infected person for TB diseases after HIV diagnosis, before initiation of ARV and during routine follow up care. In this strategy TB, if diagnosed, is treated promptly before starting ARV or for a few days before introduction of ARV to minimise overlapping of the drugs side effects[17]. To achieve the target of treating many HIV/AIDS patients in need of ARV we need to screen those with features or diseases suggesting HIV infection such as tuberculosis and this will help as the entry point to HIV/AIDS care and treatment since tuberculosis patients coinfected with HIV/AIDS are eligible for ARV [8, 17-19]. The available drugs for treating HIV/AIDS patients can suppress the viral replication, which results in increase in cellular immunity (CD4 T lymphocytes) and improved response/fight against opportunistic infections including tuberculosis. However the drugs do not eradicate the virus from the body of an infected individual. HIV/AIDS patients need tuberculosis prophylaxis using isoniazid (IPT). However due to difficulties in diagnosing active tuberculosis in HIV/AIDS patients, starting isoniazid preventive therapy may be challenging. Isoniazid, if given to HIV/AIDS patients reduces the increased risk of these patients to develop active tuberculosis [20-24]. If isoniazid prophylaxis is given to patients with active TB there is a risk of developing resistance due to anti TB mono-therapy in patients with active tuberculosis.

1.1.4 HIV/AIDS and tuberculosis coinfection

In Sub Saharan Africa, including Tanzania, the HIV/AIDS infection contributed significantly to the rising in the tuberculosis incidence. Tanzania's Ministry of Health figures show that the

TB incidence has increased tremendously since 1981 and has intensified because of the HIV/AIDS infection. Whereas the country had 11,753 reported cases of TB in 1983, when the first case of HIV/AIDS was reported in the country that figure had increased to 51,000 in 1998, 61,603 in 2001, 66,665 in 2004 and declined to 62,000 in 2006 [8]

People with HIV infection are increasingly infected with TB because HIV weakens their immune system. HIV/AIDS is the most risk factors for the development of tuberculosis [25-31]. Patients with TB infection, coinfected with HIV, have a 20-30 times higher risk of developing tuberculosis diseases during their lives, than TB infected person without HIV infection[4, 8]. In immunocompetent individuals with TB infection the lifetime risk of developing active TB disease is 10% in contrast with TB infected patient coinfected with HIV where the annual risk of developing TB disease is 5-8% [4, 5, 8, 32-35]. TB is the commonest opportunistic infection (OI) in HIV/AIDS patients in developing countries [4, 36-40]. Autopsy studies have found disseminated TB in 40-54% of HIV infected people in HIV prevalent countries, many of whom were undiagnosed prior death[9, 10].

Tuberculosis is the common pre AIDS opportunistic infection and accounts for about 40% of all presentations seen in HIV patients in Haiti [41]. Other common presentations are the wasting syndrome, which includes weight loss of more than 10% of normal weight and prolonged fever or diarrhoea (appendix III). The wasting syndrome is also associated with TB and more often the symptoms of TB are misattributed to HIV/AIDS [42]. TB can occur at any stage of CD4 T cells depletion but it is common during the early stage when the CD4 T cells is relatively normal [4, 43]. In Haiti 56% of the TB patients infected with HIV were diagnosed when the CD4 T cells were $\geq$ 350/microlitre, 23% and 12% of the patients infected with HIV has TB at the CD4 T cell levels of 200 - 350 /micro litre and < 200/microlitre, respectively [41]. The pattern of chest radiography in TB patients coinfected with HIV/AIDS varies diversely, the typical upper-lobe cavitatory picture usually seen in reactivated adult pulmonary tuberculosis (PTB) occurs when the CD4 T cells are still relatively normal. As the CD4 T cells continue to fall with the progression of HIV to AIDS atypical presentations such as pleural effusion, mediastinal and lower lobe consolidation, milliary pattern and hilar lymph node enlargement become more common. Some of these changes are similar to presentations of other opportunistic infections affecting the lungs in HIV/AIDS patients [4, 43] and, therefore, making interpretation of radiography for assisting diagnosis of TB difficult.

1.1.5 Sputum smear microscopy and culture

Atypical presentation of pulmonary tuberculosis patients coinfected with HIV/AIDS includes smear negative AFB pulmonary tuberculosis. In Tanzania, the smear positivity is about 40% and in the rest of the cases, the diagnosis is made clinically with the assistance of the chest radiography. In the area where the study was done, the total tuberculosis cases in 2003 were 501, PTB were 291 (58.1%), Smear positive PTB were 116 (39.8%), Smear negative PTB were 175 (60.2%), and EPTB were 210 (41.9%) [44]. In the year 2005, total tuberculosis were 962, PTB cases were 346 (35.9%), Smear positive were 140 (40.4%), Smear negative PTB were 206 (59.5%), EPTB were 616 (64.1%) [45], and TB among HIV/AIDS patients registered for care and treatment between October 2003 to Dec 2004 was 10% [46]. However the diagnosis of EPTB was mainly clinical and may not be correct. The diagnosis of extrapulmonary tuberculosis is made histologically in areas where this facilities is available; however in Tanzania histology for TB diagnosis is not done routinely, it is done in zonal referral hospitals and at Universities for research purposes. Other author has found that histological evidence of mycobacterial disease was only found in three quarters of patients that were clinically diagnosed and started on empirical treatment for tuberculous adenitis [47]. If culture was available in this setting the diagnosis of tuberculosis might be more reliable than using only smear microscopy. Sputum culture is a more reliable means of diagnosing tuberculosis; however in resource poor setting like Tanzania sputum culture for tuberculosis is not done routinely in district and regional Hospitals. In referral hospitals and in Universities *mycobacterium* culture is done mainly for teaching and research purposes. In resource constrained countries the available culture media is Lowenstein Jensen media (LJ media); this media lack sensitivity as compared to the liquid media, though liquid culture media has disadvantages such as high rates of contamination. Also, more complicated logistics are involved during drug susceptibility testing using liquid media as compared to LJ media.

1.1.6 Tuberculosis drugs susceptibility

Tuberculosis drugs susceptibility testing is important during this era of emergence of mycobacterium species which are resistant to the currently used anti tuberculosis drugs. Drugs susceptibility in TB patients' coinfected with HIV/AIDS is important since HIV/AIDS has been associated with the current emergence of MDR-TB [48]. There is a need to establish and strengthen the national surveillance for MDR-tuberculosis. However, in Tanzania TB drugs

susceptibility testing is not done routinely even for the patients referred to the National Referral Hospital for tuberculosis. The reason for this is lack of resources including enough staff, lack of funds for the procurements of reagents and culture media used for the isolation and drugs susceptibility testing.

1.1.7 CD4 T cells count

Immunohaematological indices such as leukocytes, lymphocytes and their subsets such as CD4 T cells and CD8 T cells play a major role in both cellular and humoral types of immunity. CD4 T cells are the lymphocytes sub sets used for monitoring progression of HIV infection, and they are also used as a surrogate marker for the improvement of HIV/AIDS patients after initiation of ARV [17, 49-52]. Furthermore, CD4 T cell levels determine when to start or stop prophylactic drugs for opportunistic infections [53]. Management of HIV patients include proper monitoring, irrespective of ARV treatment. This monitoring can be done clinically by means of the WHO clinical staging, but more reliably by measuring CD4 T cells and viral load. In resource poor countries like Tanzania, viral load is not done and the only reliable methods for follow up of HIV infected patients are by CD4 T cell counts. Immunohaematological variations have been reported in various studies, showing association with sex, geographical location, race [54-58], altitude and diet [55, 59-62]. Other reasons for variations are pregnancy, age [63, 64], exercise, cormobid conditions and diurnal variation, in addition to variations caused by methodological differences [65-67]. Several studies have shown significant variations of CD4 T cells within African populations and in Africans compared with the values established for Europe and North America [62, 68, 69]. The HIV virus targets and destroys CD4 T cells responsible for the cellular immunity against infections by intracellular microorganisms like *Mycobacterium tuberculosis*. In patients with HIV/AIDS, the CD4 T cells decrease as the HIV viruses targets the CD4 T cells; this results in immunodeficiency which in turn can lead to reactivation of latent tuberculosis or new tuberculosis infection once exposed to *Mycobacterium tuberculosis* [4].

1.2.0 Challenges in tuberculosis and HIV/AIDS coinfection in Tanzania.

1.2.1 Heath care system over occupied by tuberculosis and HIV/AIDS coinfected patients.

With the current increase in tuberculosis and HIV/AIDS patients, the health facilities in Tanzania are over-occupied by these patients. This accounts for the shortage of staff we have compared with the increasing number of these patients. Most of inpatients hospital beds in Africa are occupied by tuberculosis and or HIV/AIDS patients. HIV infected patients occupy approximately 60% of all beds in urban hospitals in Africa [70].

1.2.2 Overlapping of signs and symptoms between HIV/AIDS and tuberculosis.

Clinical features of HIV/AIDS and tuberculosis are difficult to separate, both diseases present with wasting and persistent fever. In cases of tuberculosis patients coinfected with HIV/AIDS the physician tends to attribute the signs and symptoms of tuberculosis to HIV/AIDS, hence under-diagnosing tuberculosis in HIV patients. The none-specific signs and symptoms of HIV/AIDS and its coinfection with tuberculosis make the clinical diagnosis difficult in most cases. The fact that HIV/AIDS also makes the patient susceptible to other opportunistic infections with symptoms similar to tuberculosis are among the difficulties encountered in diagnosing tuberculosis in HIV/AIDS patients.

1.2.3 Difficult to diagnose tuberculosis in HIV infected patient.

In advanced cases of HIV/AIDS, the sputum samples are often AFB negative, yet does not rule out tuberculosis. In early stages of HIV infection the sputum may be AFB positive but the proportion of these smear positive cases is small. Chest x-ray in HIV/AIDS coinfection may be atypical and not specific for tuberculosis. It may present with only pleural effusion or other atypical radiological findings similar to *Pneumocystic jirovecii* pneumonia and other opportunistic lung infections in individuals with immunodeficiency.

1.2.4 Both tuberculosis and HIV/AIDS are stigmatizing diseases.

The link between tuberculosis and HIV/AIDS may make people equating tuberculosis with HIV/AIDS. This may lead to increasing stigma and discrimination and delay tuberculosis

patients in seeking care and treatment, and also be an obstacle to HIV/AIDS patients to care and treatment.

1.2.5 Complexity of treatment of tuberculosis and HIV/AIDS coinfection

Two different diseases, two different modalities of diagnosis and treatment, do exist in one patient. Both diseases involve the combination of more than one drug. Treatment is for life in HIV/AIDS and for a minimum of six month in tuberculosis patients, resulting in high pill burden, many side effects and interaction of drugs. The consequence may be poor adherence to treatment and loss to follow up.

1.2.6 Difficulties in fitting the HIV/AIDS and tuberculosis programmes together.

The National Tuberculosis and Leprosy Programme (NTLP) and the National AIDS Control Programme (NACP) are coordinated differently. There is a need to have one national program coordinating these activities since the diseases have common problems which need to be tackled uniformly. However there are difficulties in coordinating these control programs due to different modes of operation and different policies for the two diseases. This results into a referral of patients from one programme to another within the same hospital even in a health centres. This may creates delays in treatment and loss from follow up since patients find it difficult to move from one clinician to another.

2.0 Rationale of the present study

The difficulties in diagnosing tuberculosis in HIV infected patients are among the challenges which are facing the national tuberculosis control programmes. Lack of rapid and effective methods for TB diagnosis is a major problem in developing countries. These makes difficult to address the prevalence of HIV/AIDS and tuberculosis coinfection in resource constrained area like the setting where we did our study. The current study explores the prevalence of tuberculosis among PWLHA and also HIV/AIDS among tuberculosis patients. The study was done when Tanzania is scaling up ARV to those PLWHA with medical eligibility and also planning to offer isoniazid prophylaxis to PLWHA. Active screening of tuberculosis for HIV infection and PLWHA for tuberculosis help to make decision about starting the patient on IPT or not also CPT and ARV. Tuberculosis diagnosis among PLWHA help to avoid the possibility of offering INH monotherapy to patients with active TB. Screening for active tuberculosis among PLWHA using clinical symptoms is proposed by the NTLP as a measure to rule out active TB. The study addresses the magnitude of the tuberculosis among PLWHA and the needs for routine screening for tuberculosis among PLWHA. The study also reports on the sputum smear and culture positivity among newly diagnosed tuberculosis patients. We address the need of establishment of the routine TB culture and TB drugs resistance testing among the HIV/AIDS and tuberculosis coinfected patients. This is due to the emergency of multi-drug resistance tuberculosis which has been associated with HIV/AIDS.

The level of CD4 T cells count in a selected group of healthy subjects provided reference values for CD4 T cells count in the area. These values will be used as the reference normal value for healthy people in this setting.

2.1 Study aims

The aim of this thesis is to improve HIV/AIDS, tuberculosis and HIV/AIDS and tuberculosis coinfection care and treatment in Tanzania.

2.2 Specific objectives

1. To determine the prevalence of pulmonary tuberculosis among PLWHA.

2. To determine the prevalence of HIV/AIDS among newly diagnosed tuberculosis patients

3. To determine the CD4 T cells count of all tuberculosis, HIV/AIDS, and tuberculosis HIV/AIDS coinfected patients.

4. To determine the CD4 T cells count in healthy subjects and establish reference values.

3.0 Methods

3.1 Study area and population

The Haydom Lutheran Hospital, with bed capacity of 400 patients, is owned by the Evangelical Lutheran Church in Tanzania, Mbulu Diocese. It is incorporated fully into the national health plan under the Ministry of Health and Social Welfare (MOHSW). It is situated in Mbulu District, Manyara Region in Northern and Central part of Tanzania. It is about 300 Km from Arusha City in the area with predominantly poor, rural population. Its catchment area covers Dongobesh Division in Mbulu District and Basotu Division in Hanang District Manyara region and Nduguti Division in Iramba District of Singida region with a population of about 250,000 according to 2002 census [71]. The area is unique ethnically as it is occupied by the four main language groups of Sub Saharan Africa; these are the Hadzabe (Khoisan speaking people), Iraqwi (Cushitic), Datoga (Nilotic) and Nyiramba, Nyaturu, Nyisanzu and Sukuma (Bantu speaking groups). It is situated approximately 1700 metres above sea level on a highland plateau between two branches of the Great Rift Valley.

3.2 Study design

A cross sectional study conducted from September 2006 to March 2007. The hospital has facilities for tuberculosis and HIV/AIDS diagnosis, treatment and monitoring.

3.3 Sample size

Sample size was calculated using a Statistical computer package for descriptive studies STATA version 9. A total number of 304 TB, HIV/AIDS or TB and HIV/AIDS coinfected subject was adequate to detect the prevalence of TB in HIV/AIDS and HIV/AIDS in TB patients and the coinfection, with the precision of 3% at 95% confidence level. The calculation was based on the average national HIV prevalence of 7.0% and a population size of 300,000 (the population within Haydom Hospital catchments area). The crude prevalence of HIV among TB patients in this setting was taken as 10% and the prevalence of TB among PLWHA was taken as 10% for convenience since this prevalence is not known. We decided to use the national prevalence of HIV in general population and calculated the sample size

using the formula for single proportion sample size calculation. The crude prevalence of HIV among TB and TB among PLWHA patients was considered and the sample size was smaller than that calculated using HIV/AIDS prevalence. We therefore decided to use the larger sample size which is based on HIV/AIDS prevalence. For the sample size for the healthy subjects we chose 100 subjects for convenience. The total sample size for the whole study, therefore, was 440.

3.4 Study subjects

Subjects were recruited from;

1. Newly diagnosed tuberculosis patients
2. People living with HIV/AIDS (PLWHA)
3. HIV voluntary counselling and testing (VCT) clinic.

Eligible subjects were those aged 10 years and above and who agreed to participate in the study.

3.5 Data collection

3.5.0 Tuberculosis diagnosis among PLWHA[1]

All PLWHA attending care and treatment clinic at Haydom hospital were counselled about the study. Those who agreed were asked to give sputum samples which were sent to the laboratory for smear microscopy and culture. All patients were able to provide sputum samples, but for those who could not produce sputum spontaneously (43 patients); we used induced sputum by inhalation of nebulised hypertonic saline. A single sputum sample was obtained from each patient. Tuberculosis diagnosis was made based on the finding of AFB by fluorescence microscopy and/or culture [8]. Blood was collected in ethylene diamine-tetra-acetic acid (EDTA) tubes for complete blood cell (CBC) counts and for CD4 T cell counts.

[1] In Tanzania screening for tuberculosis among PLWHA by using sputum microscopy and culture is not done unless there are clinical and radiological features suggesting tuberculosis in these patients.

3.5.1 HIV diagnosis among newly diagnosed tuberculosis patients[2]

The diagnosis of pulmonary tuberculosis at the hospital before enrolment into the study was made as smear positive PTB and was diagnosed according to national guidelines being at least two out of three sputum samples showing acid-fast bacilli by Ziehl Neelsen (ZN) staining. For the smear negative cases the diagnosis was based on the clinical and radiological diagnosis according to the algorithm for diagnosing smear negative tuberculosis [8]. All newly diagnosed tuberculosis patients during the study period were counselled about the study and those who agreed were asked for sputum samples for repeat microscopy prior to initiation of anti-tuberculosis drugs. A single sputum sample was collected from each patient for fluorescence microscopy, culture and drugs susceptibility testing. HIV counselling and testing was also done for those who consented. Blood was collected in ethylene diamine-tetra-acetic acid (EDTA) tubes. The same sample was used for HIV test, complete blood cell (CBC) counts and for CD4 T cell counts.

3.5.3 Healthy subjects

This group was recruited from the HIV voluntary counselling and testing (VCT) clinics. Individuals who tested negative during HIV VCT were counselled about the study and those who agreed were included in the study. Subjects were interviewed, using a structured questionnaire, and screened for symptoms such as fever, cough and weight loss to rule out any recent and/or current infections. Blood slide for malaria; blood sugar and rapid plasma reagin (RPR) test for syphilis were done for all participants, in addition to a physical examination, including measurement of height and weight.

The following categories were excluded from this group: pregnant women (1), smokers (8), patients receiving medical treatment and those with history of recent or current cormobid conditions, chronic alcoholism and moderate and severe malnourishment (9), patients with malaria (7), subjects testing positive for HIV antibody (2).

3.6.0 Radiological examination

Chest X-Ray (CXR) was taken for all patients irrespective of their HIV status. For the tuberculosis patients the X-ray was taken before commencing anti TB drugs and for the

[2] We repeated collecting sputum for microscopy and we used fluorescent microscopy technique for TB diagnosis while at he hospital were TB was diagnosed they used standard microscopy, also we did culture which are not done at the regional and district hospitals in Tanzania.

PLWHA the x-ray was taken when the patient was included in the study. The chest X-rays were taken in anterior-posterior view and were read and reported by the doctor who went on the radiology training and also discussed by all doctors during the routine morning X-ray session. The X-ray was reported as unilateral/bilateral infiltration with/without cavities, infiltrations with hilar lymph node enlargement and unilateral/bilateral pleural effusion. The radiological findings were used together with clinical information such as chronic cough for more than 2 weeks, weight loss and chronic fever to make the diagnosis of smear/culture negative pulmonary tuberculosis. For the smear/culture positive pulmonary cases chest X-ray were not considered in making the diagnosis.

3.7.0 Laboratory procedure for sputum microscopy, culture, drugs susceptibility testing, HIV test, CD4 T cell counts and complete blood cell counts

3.7.1 Sputum microscopy and culture

At the CTRL the sputum specimens were decontaminated by modified Petroff's methods using 4% sodium hydroxide (NaOH) and then concentrated by centrifugation at 3000g. After centrifugation it was examined by fluorescence microscopy and also cultured onto slopes of Lowenstein Jensen (LJ) medium with glycerol (GLJ) and pyruvate (PLJ), and incubated at 37^{o}C for 8 weeks. Culture slopes were inspected after 48 hours to detect contamination and thereafter weekly to observe growth. Identified contamination of the culture was removed by sub-culturing the specimen. All positive culture slopes were assessed for M. tuberculosis by growth rate, acid fastness, colony morphology, pigment production, rate of growth at 25^{o}C, growth onto 500 mg/ml PNB, and sensitivity or resistance to the thiophen 2-carboxylic acid hydrazide (TCH 2mg/ml).

Smear microscopy were read as follows; 1-9 AFB per 100 scanned fields were recorded in absolute number, 10-99 AFB per 100 scanned fields were reported as (1+), 1-10 AFB per field scanned were reported as (2+) and >10 AFB per field scanned as (3+) [8]. Positive culture was quantified as the total number of colony forming units.

3.7.2 Drugs susceptibility test

Tuberculosis drug susceptibility testing was done on culture positive specimens. Different LJ media was prepared, two drug free LJ media, one containing thiophenecarboxylic acid hydrazide (TCH), one containing para-benzoic acid (PNB) and four containing one of the following drugs: 40microgram/ml of rifampicin, 5microgram/ml of dihydrostreptomycin sulphate, 0.2microgram/ml and 1.0microgram/ml of isoniazid and 2 microgram/ml of ethambutol. On the drug free medium 10^{-3}*mycobacterium* suspension was inoculated followed by 10^{-1} *mycobacterium* suspension into the drug containing media. The media containing *mycobacterium* were incubated at 37^{0}C for 4 weeks. The culture was read after 3 and 4 weeks. The results were recorded as follows; Colony growth of 1-20, the colonies were recoded in absolute number, 20-100 colonies were recorded as (1+), 100-200 as (2+) and >200 colonies as (3+). The proportion of bacilli in inoculums that were resistant to the drug used were calculated as the ratio of the number of colonies in a drug media to number of colonies in control medium, multiplied by 100. While susceptible isolates were interpreted as colony growth of less than 1% in a drug medium compared to the control tube, resistant isolates were 1% or more colony growth in a drug medium [72]

3.7.3 Quality control for the smear microscopy culture and drug susceptibility test.

Laboratory staff received proficiency testing in the performance of culture and sensitivity procedures though the Supranational Reference Laboratory (SNRL) in Antwerp, Belgium prior to the survey.

3.7.4 HIV test

HIV test was done using 2 different rapid antibody tests, Determine HIV-1/2 (Abbott laboratories, Abbott Park, IL, USA) and Capillus HIV-1/2 (Trinity Biotech, Bray, Co Wicklow, Ireland) Discordant samples were sent to the regional hospital for confirmatory test using both ELISA; Enzygnost anti-HIV 1+2 Plus ELISA (Behring, Marburg, Germany) and Well-coenzyme HIV recombinant ELISA (Murex, Dartford, England) [73, 74].

3.7.5 Complete blood cell counts.

Complete blood cell counts were done using Sysmex Kx-21 (Sysmex Corporation; Kobe Japan). The machine automatically dilutes a whole-blood sample, lyses, counts and gives a printout result of absolute numbers of leucocytes (expressed as number of cells × [10^9] per litre), erythrocytes (number of cells × [10^{12}] per litre), platelets (number of cells × [$10^{9]}$ per litre), lymphocytes (number of cells × [10^9] per litre), mononuclear cells (number of cells × [10^9] per litre), granulocytes (number of cells × [10^9] per litre) and haemoglobin (grams per decilitre).The quality and accuracy of the technique and the machine was assessed every six month by sending the samples to the regional laboratory for the comparison reading and servicing the machine by the designated engineer from the Sysmex company.

3.7.6 CD4 T cell counts

CD4 T cells were analyzed using a BD FACSCount flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) with two monoclonal antibodies (aCD4 and aCD8; Becton Dickinson Immunocytometry Systems). In brief, 100 µl of whole blood was mixed and incubated at room temperature for 20 min with 10 µl of aCD4 and aCD8. Red blood cells were then lysed by adding 2 ml of fluorescence-activated cell sorter lysing solution (Becton Dickinson Immunocytometry Systems). The sample was then analyzed with the FACSCounts's Cell Quest software (Becton Dickinson Immunocytometry Systems). The FACSCount was calibrated with fluorescent beads (CaliBrite; Becton Dickinson Immunocytometry Systems) and Auto-Comp software (Becton Dickinson Immunocytometry Systems) weekly. By using quality control (Multicheck; Becton Dickinson Immunocytometry Systems), the accuracy of the technique was assessed every 6 months.

3.8 Definition of cases

Tuberculosis included cases positive for acid fast bacilli by smear microscopy and/or culture and those smear/culture negative patients with clinical and radiological features suggestive of pulmonary tuberculosis and failure to respond to a course of broad spectrum antibiotics[8]. Anaemia was classified as haemoglobin level < 10g/dl [75]. Immunological status was assessed using CD4 T cells count and immunodeficiency was defined as CD4 T cells count < 500 cells/mm^3[17]. Nutritional status was assessed using body mass index (BMI). Normal nutritional status was defined as BMI of $\geq$18.4 Kg/m^2, mild malnutrition was defined as BMI of 17-18.4kg/m^2, moderate malnutrition as BMI of 16-16.9kg/m^2, severe malnutrition as BMI of <16kg/$m^2$and malnutrition as BMI $\leq$ 18.3 Kg/m^2 [76].

3.9 Statistical analysis

Completed questionnaires were coded by numbers and double entered in a computer software Epi-data version 13.1. Cross-checking and data cleaning was done. The data was then transferred to Statistical Package for Social Sciences version 15 (SPSS Inc, Chicago, USA) for analysis. Chi square test was used to test for differences in proportions (paper 1 and 3). Student t test was used to test for differences in means between 2 or more groups (paper 2 and 4). Where appropriate, logistic regression was used to explore the association of social demographics and other variables with CD4 T cells (paper 2). All statistical tests were considered significant if the two sided P-value (p) was <0.05.

3.10 Data quality and assurance

The investigator interviewed and examined the patients and filled the information obtained from the patients to the questionnaire. Completed questionnaires were coded by numbers and double entered in a computer software Epi-data version 13.1. Cross-checking and data cleaning was done. During data cleaning and cross checking missing information were obtained by going back to the questionnaire and when necessary reviewing the patients on the next visit to the clinics. The data was then transferred to SPSS version 13 for analysis. The data were also stored in a non recordable CD as a back up.

All information obtained from the patients were recorded in questionnaire and kept in a hard cover file. The files were stored into a medical record room in lockable shelves. Only the investigator and staffs working with HIV/AIDS and tuberculosis care and treatment had an access to the files.

3.11 Ethical consideration

The protocol was approved by the Medical Research Coordinating Committee of the Ministry of Health and Social Welfare, Tanzania. Oral informed consent was obtained from the patients prior to enrolment. For those below the age of 18 years permission was sought from parents or caretakers. For the newly diagnosed tuberculosis patients counselling for HIV test was done and those who gave consent were tested for HIV. Tuberculosis, and other opportunistic infections in HIV/AIDS patients and HIV/AIDS diagnosis and treatment are given free of charge according to Tanzanian national policy for HIV/AIDS and tuberculosis management.

3.12 Table 1 Summary of the study population, design, data collection tools and statistical analysis

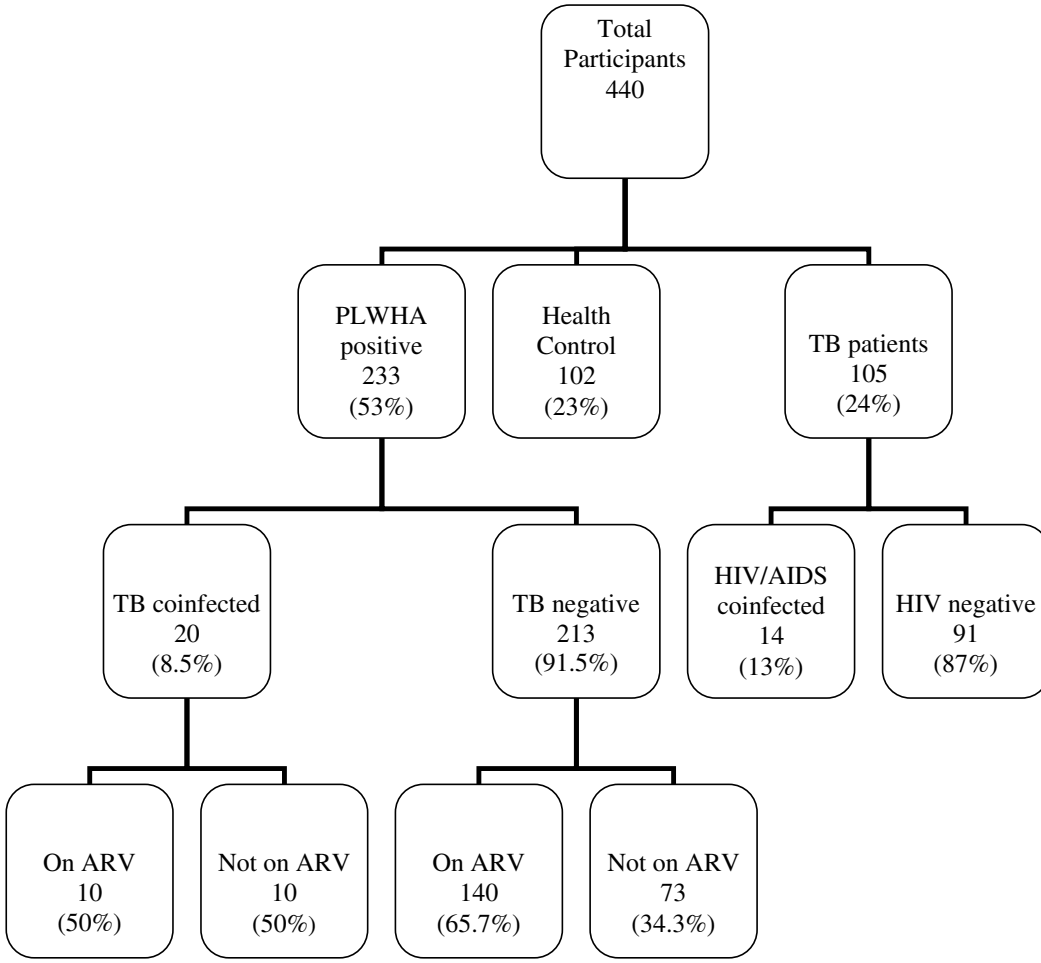
Paper	Study population	Study design	Data collection tools	Statistical methods
I	233	Crossectional	Clinical and laboratory records	Descriptive Logistic regression
II	102	Crossectional	VCT visit, clinical and laboratory records	Descriptive Student t test Logistic regression
III	105	Crossectional	Clinical and laboratory records	Descriptive
IV	420	Crossectional	Clinical and laboratory records	Descriptive Student t test

4.0 Results

4.1 General

We studied 440 patients, 102 health subjects for reference values, 105 newly diagnosed tuberculosis patients with unknown HIV status, and 233 PLWHA (Figure 1). Males were 158 (35.9 %) and 282 (64.1 %) were females. Among healthy subjects 42 (41%) were males and 60 (59 %) were females; the mean age for the healthy subjects was 32 $\pm$ 12.5. For the newly diagnosed tuberculosis patients males were 58 (55.3%) and females were 47 (44.7%); the mean age for the newly diagnosed tuberculosis patients was 39.4 $\pm$ 17. Among PLWHA 58 (24.9 %) were male and 175 (75.1%) were females. The mean age for the PLWHA was 37.0 $\pm$ 10.2. The overall HIV/AIDS and tuberculosis coinfection prevalence was 34/338 (10.1 %).

4.2 Figure1. Flow pattern of the study participants



4.3 Pulmonary tuberculosis prevalence among people living with HIV/AIDS

Pulmonary tuberculosis prevalence among people living with HIV/AIDS was 20/233 (8.5%). Five (25%) of the HIV/AIDS tuberculosis coinfected patients had features suggestive of tuberculosis on chest radiography; these 5 patients were also sputum smear microscopy and culture positive for AFB. Fifteen (75%) of the PLWHA coinfected with tuberculosis had neither symptoms nor chest x-ray suggestive of tuberculosis but the culture was positive for AFB. One (12.5%) smear positive AFB patient had CD4 T cells of $<200/mm^3$, 3 (37.5%) had a CD4 T cells of 200-349/mm^3 and 4 (50%) had CD4 T cells of $\geq 350/mm^3$.Patients who were HIV positive without tuberculosis had higher CD4 T cells count than the HIV/AIDS patients coinfected with tuberculosis; the difference was not statistically significant. Nineteen (95%) of the isolates from these patients were sensitive to rifampicin, isoniazid, streptomycin and ethambutol (the first line tuberculosis drugs). One isolate (5%) was resistant to isoniazid, representing a patient with a history of past tuberculosis treatment, where isoniazid was one of the drugs used. Ten (6.7%) HIV/AIDS and tuberculosis coinfected patients were on ARV. Five (15%) of the HIV/AIDS and tuberculosis coinfected patients were anaemic and 52 (24.4%) of HIV/AIDS, TB negative were anaemic. Significantly more HIV tuberculosis coinfected patients were malnourished (14/20, 70%, $X^2= 7.2$, p=0.007) as compared with HIV/AIDS TB negative patients 83/213 (39%). Six (30%) of HIV/AIDS and tuberculosis coinfected patients were in WHO clinical stage III and 14 (70 %,) in WHO clinical stage IV.

4.4 Immunohaematological reference values in human immunodeficiency virus-negative adolescent and adults

The mean absolute CD4 T cells in males and females combined was 745.9 $\pm$ 256.6, mean absolute CD4 T cells in males was 665.6 $\pm$ 246.8, and mean absolute CD4 T cells in females was 802 $\pm$ 250.2. Mean absolute CD8 T cells in males and females combined was 504 $\pm$ 218.4, mean absolute CD8 T cells in males 438.2 $\pm$ 208.4, mean absolute CD8 T cells in females 551.0 $\pm$ 215.4. Mean CD4/CD8 T cells ratio in males and females combined was 1.6 $\pm$ 0.5, mean CD4/CD8 cells ratio in males 1.6 $\pm$ 0.5, mean CD4/CD8 T cells ratio in females 1.5 $\pm$ 0.3. We found that females had significantly higher mean absolute CD4 T cells, (t=2.7 df =89, p= 0.008) and higher mean absolute CD8 T cells (t=2.7, df =90, p=0.009) than males.

The mean haemoglobin level in males and females combined was 13.6± 2.4, mean haemoglobin level in males was 14.1± 2.7, and mean haemoglobin level in females was 12.6 ± 1.9. Females had significantly lower mean haemoglobin level than males, (t=3.2, df =68, p=0.03). Mean absolute leukocytes cells in males and females combined were 5.1 ± 1.6, mean absolute leukocytes cells in males were 4.9 ± 1.6, mean absolute leukocytes in females were 5.3 ± 1.7. Mean absolute lymphocytes cells in males and females combined were 1.8 ± 5.1, mean absolute lymphocytes cells in males were 1.6 ± 4.9, mean absolute lymphocytes in females were 1.9 ± 5.3. For those with CD4 T cells lower than normal (<500cells/mm^3) males had significantly lower CD4 T cells than females (p=0.01, OR 4.4, 95% CI 1.3-15). There was no statistically significant difference between males and females having lower than normal haemoglobin levels (X^2=1.1, df =1, p= 0.2), lower than normal leucocytes (X^2=0.1, df =1, p=0.7) and lower than normal lymphocytes (X^2=2.5, df =1, p=0.1).

On nutritional status assessment using BMI we found that mean BMI for the participants was 23.4 ± 95% CI 22.8-24.1; Mean BMI for males was 23.3 ± 95% CI 22.0-24.5 and for females 23.6 ± 95% CI 22.8-24.3. There was no statistically significant difference between males and females with regards to the nutritional status (X^2=0.07, df =1, p=0.8). There was no significant association between nutritional status and any of the immunohaematological parameters, neither by bivariate nor by multivariate analysis. There was no statistically significant association between age and any of the immunohaematological parameters.

4.5 HIV prevalence, smear microscopy, culture and drug susceptibility among newly diagnosed tuberculosis patients

The prevalence of HIV/AIDS among newly diagnosed tuberculosis patients was 13.3% (14/105) Sixty three out of 92 sputum samples (68.5%, 95% CI 60-80) were culture positive AFB and 66/92 (71.7%, 95% CI 61-80) were smear positive AFB. Among the specimens from tuberculosis and HIV/AIDS coinfected patients, 9/14 (64%) were smear positive and 5/14 (36%) were smear negative for AFB. All smears from tuberculosis and HIV/AIDS coinfected patients were culture positive for AFB. All isolates from tuberculosis and HIV/AIDS coinfected patients were susceptible to rifampicin, streptomycin, isoniazid and ethambutol (the first line tuberculosis drugs). Forty four out of 49 (89.8%) of the isolates from HIV negative tuberculosis patients were susceptible to the first line tuberculosis drugs.

Three (4.8%) patients were resistant to isoniazid, 1 (1.6%) patient was resistant to ethambutol and 1 (1.6%) patient was resistant to streptomycin and isoniazid.

For the tuberculosis and HIV/AIDS coinfected patients, 10/14 (71.4%) had severe malnutrition, 2/14 (14.3%) patients had moderate and mild malnutrition. The HIV negative tuberculosis patients, 57/92 (63%) had severe malnutrition, 9/92 (10 %) had moderate malnutrition, 12/92 (13%) had mild malnutrition. Of the tuberculosis and HIV/AIDS coinfected patients 8/14 (57%) were anaemic (HB <10g/dl) and of the HIV negative tuberculosis patients 30/92 (33%) were anaemic. Five (36%) of the tuberculosis and HIV/AIDS coinfected patients had unilateral and/or bilateral pleural effusion with/without infiltrations, 6/14 (43%) had infiltrations without effusion and 3/14 (21%) had normal chest X-ray but they had a sputum culture positive for AFB. HIV negative TB patients 87/92 (96%) had unilateral/bilateral infiltrations with cavities and 5/92 (4%) had bilateral/unilateral pleural effusion.

Seven (50%) of tuberculosis HIV/AIDS patients were in WHO clinical stage III and 7 (50%) patients were in WHO clinical stage IV. Five tuberculosis and HIV/AIDS coinfected patients were using cotrimoxazole tablets. This was prescribed because of cough and fever and not as prophylaxis, since their HIV status was unknown to the clinician who prescribed it.

4.6 Peripheral blood CD4 T lymphocytes, leucocytes and haemoglobin level in HIV/AIDS, tuberculosis and HIV/AIDS tuberculosis coinfected patients

In this section, we report on immunohaematlogical parameters in different groups of patients. Tuberculosis female patients tended to have higher mean CD4 T cell counts and lower haemoglobin levels than males, but the difference was not statistically significant.

In HIV/AIDS patients; males had significantly higher mean haemoglobin level (12.3 $\pm$ 2.4) than females (11.4 $\pm$ 2.1, t = 2.5, df =79, p=0.01). The mean CD4 T cells count in males was lower than for females, but the difference was not statistically significant. In tuberculosis HIV/AIDS coinfected patients; the mean haemoglobin level in males was 10.0 $\pm$ 3.2, and in females 10.6 $\pm$ 2.1. Females had higher mean CD4 T cell counts (282 $\pm$ 202) than males (209 $\pm$ 179). The differences were not statistically significant.

4.6.1 Comparison of mean cell types in different groups.

4.6.1.1 Tuberculosis patients and reference values

Tuberculosis patients had statistically significant lower mean CD4 T cell counts (559 $\pm$ 238) than the reference value we found in this population, (746 $\pm$ 257, t= 5.2, df = 190, p<0.01), and lower haemoglobin level (10.9 $\pm$ 2.4) than the reference value for this population (13.2 $\pm$ 2.4), t=6.7, df =188, p<0.01). [77].

4.6.1.2 Tuberculosis and HIV/AIDS patients

Tuberculosis patients had higher CD4 T cell levels and lower haemoglobin levels than HIV/AIDS patients. HIV/AIDS patients had higher mean lymphocyte cell counts (1.7 $\pm$ 0.7) than tuberculosis patients (1.3 $\pm$ 0.6, t= 5.1, df =190, p=0.01).

4.6.1.3 Tuberculosis and HIV/AIDS patients with and without ARV treatment

Tuberculosis patients had statistically significant higher mean CD4 T cells count (559 $\pm$ 238) than HIV/AIDS patients on treatment (339 $\pm$ 344, t=4.4, df =241, p<0.01), and HIV/AIDS patients not on treatment (280 $\pm$ 193, t=8.6, df =171, p<0.01). Tuberculosis patients had significant lower mean haemoglobin level (10.9 + 2.4) than HIV/AIDS patients on treatment (11.9 $\pm$ 2.0, t=3.4, df =159, p<0.01).

4.6.1.4 Tuberculosis and tuberculosis HIV/AIDS coinfected patients

Tuberculosis patients had statistically significant higher mean CD4 T cell counts (t=7.4, df =72, p=0.01) and higher mean leukocyte counts (t=2.8, df =70, p=0.01) than HIV/AIDS tuberculosis coinfected patients.

4.6.1.5 HIV/AIDS and HIV/AIDS tuberculosis coinfected patients

HIV patients had statistically significant higher mean CD4 T cell counts (t=2.8, df =60, p=0.01), higher haemoglobin level (t=2.6, df =40, p=0.01), higher mean leukocyte counts (t=2.2 df =44, p=0.04) and higher mean lymphocyte counts (t=1.4, df=40, p=0.01) than HIV/AIDS and tuberculosis coinfected patients

4.6.1.6 HIV/AIDS treatment naive and HIV/AIDS patients on treatment

HIV patients on treatment had significantly higher mean CD4 T cell (399 $\pm$ 344) counts than HIV patients not on treatment (280.0 $\pm$ 193, t=3.4, df =224 p=0.01), and higher mean haemoglobin levels (11.9 $\pm$ 2.0) than those not on treatment (11.1 $\pm$ 2.4, t=2.5, df =127, p=0.01). Female HIV treatment naïve patients had

statistically significant higher mean CD4 T cells (309.1 $\pm$ 205.7) than males (209.4 $\pm$ 136.6, t=2.5, df =58, p=0.02).

4.6.2 Nutritional status in different groups

Patients with tuberculosis were significantly malnourished (BMI 15.6 $\pm$ 2.3) as compared to normal values for this population (BMI 23.4 $\pm$ 3.4, t=18.8, df =178, p<0.01)[77]. We also found that HIV/AIDS patients had significantly higher BMI (19.4 $\pm$ 3.4) than HIV/AIDS tuberculosis coinfected patients (BMI 16.7 $\pm$ 2.8, t=5.1, df =48, p<0.01). No significant difference between males and females with regards to nutritional status in any of the different groups

5.0 Discussion

5.1.0 Discussion of the methods

5.1.1 Study design

The study was a cross sectional hospital based study. The study design is appropriate and has strength in assessing disease prevalence. Cross sectional studies may not demonstrate the temporal relationship between exposure and outcome but are important for health planning and resource allocation in resource constrained countries. There was a potential increase in the number of tuberculosis patients coinfected with HIV/AIDS in this setting and we conducted our study to gather information about the magnitude of the tuberculosis and HIV/AIDS coinfection and also to generate immunological reference values for the population living in this setting.

5.1.2 Validity

The validity (absence of systematic error) of a study refers to the adequacy with which the study measured its findings. Validity includes internal and external validity.

5.1.3 Internal validity

Internal validity for our study refers to the extent to which the tools we used to estimate the magnitude of tuberculosis, HIV/AIDS and HIV/AIDS coinfection were accurate. Bias (deviation from the truth) is a systematic error in the design conduct or analysis of the study that results in a mistake in estimating the exposure[78].

5.1.4 External validity

External validity refers to the extent to which our findings can be applied to general population. The finding from hospital based study can not apply for the general population but can be generalised to other hospital findings. The community survey for the immunological reference values can apply for the general population in this setting; though this is limited with our small sample size for the healthy subjects.

5.1.5 Bias

5.1.6 Selection Bias

Selection bias results from the distortion that results from the procedures used to select study subjects and other factors that may influence study participation [79]. The interpretation and the accuracy of the prevalence estimates depend on the selection and participation biases. Our study was hospital based and is subjected to selection bias since those who come to hospital are those who perceive themselves sick, and we might have missed those who were not very sick and opted not to come to the hospital; This is reflected by our results whereby we have more HIV/AIDS patients in WHO stage III and IV and also the tuberculosis patients were malnourished and anaemic. WHO clinical stage III and IV, malnutrition and anaemia indicates the severity of the disease. However our study subjects were tuberculosis patients and PLWHA and the only place to get them was in the hospital.

Also for the healthy subjects we recruited those who tested HIV negative during their visit to VCT, this means that we included only those who come to VCT because they want to know their HIV status and we missed those who perceive themselves not at risk of acquiring HIV and therefore did not visit the VCT services.

5.1.5 Information bias

Information bias is a distortion in the measure of association caused by inaccurate information that may result from poor interviewing techniques and level of recall by the respondent [78]. Our study was prone to recall biases, since we used some questions reflecting the past five years. The recall bias was minimised by confirming the response from the patients by reviewing the hospital records. As an example, a history of tuberculosis and previous HIV test was confirmed by reviewing the patient's file from the medical records at the hospital.

5.2 Discussion of the main findings

Tuberculosis is a common opportunistic infection among PLWHA in developing countries. Active detection of tuberculosis among PLWHA and also detection of HIV among tuberculosis patients is important in order to scale up ARV, IPT and CPT for PLWHA. In this study the overall prevalence of HIV/AIDS and tuberculosis coinfection is 10.1%. This prevalence is low compared to the prevalence of 50% of tuberculosis patients coinfected with HIV in Tanzania [8]; however there is no available data addressing the overall tuberculosis HIV/AIDS coinfection in Tanzania. Our study recruited PLWHA with no complaints suggestive of tuberculosis, and we actively looked for tuberculosis among these patients. In addition, we included newly diagnosed tuberculosis patients and tested them for HIV infection. Thus, we were able to give an estimate of the overall prevalence, and we believe this to be the first study to address the overall coinfection in Tanzania. The prevalence of tuberculosis disease among PLWHA in this setting was 8.5%. A study done in Dar es Salaam found a tuberculosis prevalence of 15% among HIV infected ambulatory subjects [80]. Our prevalence of 8.5% is low when compared with the study done in Dar es Salaam. The difference between these two studies can be explained by the difference in study settings; the fact that Dar es Salaam is urban and contributes about 24% of all tuberculosis cases in Tanzania, while the rural area where we did our study contributes less than 2% [8]. Patients with low immunity due to HIV are more likely to acquire tuberculosis in an area with high tuberculosis prevalence. Also, the prevalence of HIV in Dar es Salaam is higher than the national average of 7% [81, 82] and higher than the prevalence of 2% [83] for the area where we did our study. This means that there are relatively more HIV patients who are susceptible to tuberculosis in Dar es Salaam than in the area of our study. The prevalence of 8.5% is lower than 40-54% found in autopsy studies done in HIV infected people who were undiagnosed prior to death [84]. In another study done in Fajara Research clinic in Gambia among HIV patients who were followed at the clinic, 43.2% were diagnosed to have tuberculosis after 28 days of follow up, and 66% of them had pulmonary tuberculosis confirmed by microscopy and/or culture [85]. In yet another study done in South Africa among African gold miners the point prevalence of undiagnosed tuberculosis among HIV positive participants was 3.8% [86]. Our prevalence of 8.5% is close to 9% found in a study done in Cambodia [87]. Part of the differences between the prevalence in our study and the other studies may be due to differences in study design and setting, including inclusion and exclusion criteria. As patients already diagnosed to have tuberculosis and started tuberculosis

treatments and patients with extrapulmonary tuberculosis (EPTB) were excluded in our study, this may have given lower rates than in some of the other studies.

Out of 20 tuberculosis patients in our study, smear was positive in 8 (40%) patients; this is close to the figure of 42% reported in Ethiopia [88]. Other authors have reported smear positivity of 48% [89] and 45% [90] among HIV/AIDS patients coinfected with tuberculosis. Most of the mycobacteria isolated from these patients were susceptible to the first line tuberculosis drugs (rifampicin, streptomycin, isoniazid and ethambutol). Resistance to isoniazid was found in only one patient (5%), and no cases of multi-drug resistant tuberculosis was identified; this is similar to the report from the Tanzanian National tuberculosis programme, where 90% of all isolates were sensitive to these drugs and resistance to isoniazid was 5% [8]. However, another study done in northern Tanzania showed that among HIV/AIDS tuberculosis coinfected patients resistance to at least one drug was 10.8% [91]. A study done in Mwanza Tanzania and published 10 years ago demonstrated that among HIV/AIDS tuberculosis coinfected patients 13% had strains resistant to isoniazid, rifampicin, thiacetazone and /or streptomycin.[1] . A study done in Dar es Salaam showed low level of drug resistance [92]. A recent study from Cambodia shows that the resistance to anti tuberculosis drugs is decreasing since the introduction of antiretroviral drugs and there was a decrease of resistance from 48% in 1999 to 7.9% in 2004 [93].

Symptoms like fever, cough and weight loss were uncommon among PLWHA coinfected with tuberculosis. This unusual presentation means that the screening of tuberculosis among HIV patients by using symptoms of fever, cough, and weight loss and chest radiography with features suggestive of tuberculosis would detect only 25% of the HIV positive coinfected with tuberculosis. Pulmonary tuberculosis in PLWHA with normal chest radiograph findings has been reported by other authors [89, 94-97]; however the proportion of the tuberculosis HIV/AIDS coinfected patients with normal chest radiography reported by these authors is small compared to our findings. The difference between our findings and that reported by these authors may be due to inclusion and exclusion criteria, since the referred studies included patients with symptoms suggestive of TB, while our study included ambulatory PLWHA with no symptoms. Also, these studies had small sample size of less than 100 as

compared to our study with sample size of 233. Our findings suggests that chest radiography and clinical symptoms, as recommended in Tanzania for follow up screening of HIV patients for tuberculosis, may miss up to 75% of tuberculosis cases in people living with HIV. Recently, Tanzanian National Tuberculosis Control Programme has developed a screening tool for PLWHA in order to exclude those suspected to have active tuberculosis from IPT programme; this screening tool uses symptoms like chronic cough, fever, weight loss and chest radiography suggestive of tuberculosis to identify suspects for further investigation like smear microscopy so as to exclude patients from IPT programme. However, our study indicates that these symptoms and radiological findings are not enough to rule out active tuberculosis. Even sputum smear microscopy cannot exclude active tuberculosis with certainty, and there is a need to consider *mycobacterium* culture in order to be sure to treat correctly. Twelve out of twenty HIV/AIDS and tuberculosis coinfected patients in our study had no symptoms and also no radiological features suggestive of tuberculosis, and if we use the current guidelines we may miss the diagnosis of active tuberculosis. Since these patients otherwise are eligible for IPT, there is a potential for the emergence of INH resistance due to monotherapy to patients with active tuberculosis. Smear was positive for 8/20 (40%) of HIV/AIDS and tuberculosis coinfected patients, 12/20 (60%) of them were smear negative, culture positive; therefore if we rely on smear microscopy to rule out active TB among PLWHA we will also offer IPT to 60% of patients with active TB, which could have serious consequences for development of drug resistance.

Eight patients with tuberculosis were on antiretroviral drugs for more than six months and yet developed tuberculosis; this shows that there is a need to do regular tuberculosis screening by sputum microscopy and culture to diagnose tuberculosis in HIV/AIDS patients on treatment. Ten HIV/AIDS patients found to have tuberculosis were not yet eligible for antiretroviral drugs. This is because tuberculosis was not diagnosed at a right time; if tuberculosis were

diagnosed in these patients and correctly staged; they would be eligible for antiretroviral drugs and cotrimoxazole prophylaxis (CP) [8, 17-19].

Anaemia was uncommon among the PLWHA, 75% of them had normal haemoglobin level. This could be due to the regular check up of haemoglobin every time the patients come to the care and treatment clinic, whereby those who are found to be anaemic are treated free of charge.

Malnutrition was found in 70% of the coinfected patients. Malnutrition and tuberculosis increases morbidity and mortality in HIV patients [18, 19]. Most of the HIV patients, irrespective of their tuberculosis status, were in WHO stage III and IV. This means that they present at hospital in late stage with advanced disease. Overall, females were more affected with HIV (71.1%) than males, may be due to the fact that females are more vulnerable to HIV than males because of social-economic and cultural factors which drives HIV epidemics in Africa, and more females are tested for HIV in VCT as compared to males [98, 99]. Also, females are tested for HIV when they become pregnant and those found to be HIV positive are refered to HIV care and treatment clinics.

Among the newly diagnosed tuberculosis patients the prevalence of HIV was 13.3%. This prevalence is low when compared to the national prevalence of 50% [8], and that for Sub Saharan Africa of 31%[4, 5]. However when compared to the HIV prevalence of 2% in the general population in this setting [83] the prevalence of 13.3% in our study is high probably due to the fact that our study was hospital based and we included those patients who were sick and likely to have HIV/AIDS compared to general population.

We found that a significant number (64.3%) of tuberculosis HIV coinfected patients had smear microscopy positive for AFB. The smear positivity among tuberculosis HIV patients ranges from 40% to 75% according to different studies. For example smear positivity in tuberculosis and HIV/AIDS coinfected patients in Nigeria was 48%,[100] in Ethiopia 52%[88] and in Cambodia 71%[101]. Smear positivity irrespective of the HIV status in this study was 71.7%.

Although this is high compared with the national average of 40%, our 95% CI is broad and our finding might therefore not be significantly different from the national average.

Culture results were positive in 63 (68.5%) patients irrespective of the HIV status. Our 95% CI is broad (60-80) and the findings might therefore not be significantly different from other studies done in Nigeria, where culture positivity was 62%,[100] and in Kenya, where it was 56% [102].

Our results show higher sensitivity for smears than for cultures; this is contrary to the literature, which states higher sensitivity for culture than stains. The reason for this can partly be explained by the type of culture media that we used (Lowenstein Jensen media, which has a low sensitivity); also the specimens were collected in the rural area and transported to the Central Tuberculosis Research Laboratory in Dar es Salaam, a process which took an average of three to four days. Therefore, some of the mycobacteria might have died during this time of specimen collection and transportation.

All isolates from tuberculosis and HIV/AIDS coinfected patients were susceptible to the first line tuberculosis drugs. This could be explained by low levels of all type of drug resistance in the country [8]. This is contrary to a report published in 2003, which states concomitant emergence of Multi-Drug Resistant (MDR) strains of *Mycobacterium tuberculosis* among tuberculosis patients coinfected with HIV/AIDS [42]. However other authors have found that in Africa, MDR is not associated with HIV-1 [6, 103-105]. In Tanzania, 90% of the tuberculosis isolates are sensitive to rifampicin, isoniazid, streptomycin and ethambutol [8]. Mono-resistance to isoniazid is 5%, which is close to the 4.8% we found in our study. Resistance to ethambutol is 0.2%, and dual resistance to rifampicin and isoniazid is 1%; in our study dual resistance of 1.6% was found in case of streptomycin and isoniazid [8]. We found that 3 out of 4 mono-resistant isolates were resistant to isoniazid, and this is similar to a study done in northern Tanzania where they found resistance to isoniazid in 7 out of 8 mono-resistant isolates [91] .Our findings differ from a study done ten years ago in Mwanza, Tanzania, which showed that among tuberculosis HIV coinfected patients 13% had strains resistant to isoniazid, rifampicin, thiacetazone and/or streptomycin. For HIV negative tuberculosis patients, 9% had resistant strains[1]. The difference between our study and the study done in Mwanza could be due to the study setting, whereby Mwanza is an urban setting and our study was restricted to a rural setting in a different region. However, we do not have data to explain the difference.

In our study, no patient with past history of tuberculosis treatment was found to have resistant strains.

In both groups; newly diagnosed tuberculosis coinfected with HIV/AIDS and PLWHA coinfected with tuberculosis there were no cases of MDR-TB was isolated. These encouraging drug susceptibility results could be explained by several factors. Of great importance is the national policy regarding tuberculosis drugs, whereby drugs are owned by the government and are given free of charge to tuberculosis patients, the WHO strategy of directly observed therapy (DOT), the uninterrupted supply of anti-tuberculosis drugs to the clinics, and correct prescription of the drugs , as recommended by the National Tuberculosis and Leprosy Programme [8]

Tuberculosis occurs at any level of CD4 T cells depletion. In our study we found that half of the PLWHA infected with tuberculosis had CD4 T cells <350 cells/mm^3. This is due to the fact that as HIV progresses to AIDS, the CD4 T cells also decreases, resulting in poor or little cytokine secretion by the CD4 T cells. This in turn leads to poor ability to kill ingested mycobacteria by the macrophages, therefore failure to contain the infection, rendering the patient at risk of reactivation or new tuberculosis infection [42].

Close to 90% of the newly diagnosed tuberculosis and HIV/AIDS coinfected patients and 70% of PLWHA coinfected with tuberculosis were malnourished. This may be explained by the fact that they present to the clinic with advanced disease and both HIV and tuberculosis present with wasting. Contrary to PLWHA coinfected with tuberculosis in which 25% of them were anaemic, more than half of the newly diagnosed tuberculosis and HIV/AIDS coinfected patients were anaemic. Anaemia and malnutrition increase mortality in these patients [18, 106]. All the tuberculosis HIV/AIDS coinfected patients were in WHO clinical stage III or IV. Current pulmonary tuberculosis or pulmonary tuberculosis during the last year qualifies for WHO stage III, extrapulmonary tuberculosis qualifies for WHO stage IV [107]. Other symptoms such as wasting, diarrhoea, and fever qualify for WHO stage IV [107] though these symptoms may be misattributed to HIV rather than tuberculosis, as both diseases present with wasting and fever [42] .

Lymphocytes and lymphocyte subsets are important for the immunity against intracellular microorganisms such as virus and mycobacteria. Individuals with deficiency in cellular immunity are at risk of developing tuberculosis once exposed to *Mycobacterium tuberculosis,* or reactivation of tuberculosis for those with latent infection [4]. Absolute CD4 T cell counts and haemoglobin level are the most important parameters for monitoring progression of HIV to AIDS and also the improvement after initiation of antiretroviral therapy (ARV). Tanzania is

scaling up ARV for people living with HIV/AIDS, and CD4 T cell counts and haemoglobin level are the recommended parameters for monitoring these patients[17, 49, 50].

For immunohaematological reference values in healthy subjects we found that females have significantly higher counts of absolute CD4 T cells and absolute CD8 T cells than healthy males, and also higher mean absolute leukocyte and lymphocyte counts. Our results are similar to those reported in other studies in African settings [54, 55, 62, 108]. More important, our findings are different from the standard values established for Europe and Northern America [109]. There are also several other studies done in Africa and Asia that report different values for CD4 T cell levels compared to standard values for Western countries [62, 66, 110]. Studies done in Tanzania and Cameroon have reported higher CD4 T cell counts as compared to Ethiopia, Botswana and Uganda [62, 111-114], whereas some studies have demonstrated higher CD4 T cells among Ugandans and Kenyans than the values known for North America, Europe and Asia [108]. These variations in CD4 T cells have been shown to be associated with ethnicity, gender, diet, geographical area, as well as being dependent of genetic and environmental factors [55, 59-62]. The value of the CD4 T cell counts obtained in our study is lower than those reported for Ethiopian and the Dutch counterparts [111, 115]

In our study a significant proportion of males had lower mean absolute CD4 T cell and absolute CD8 T cell counts than females. This finding is similar to a study done in Tanzania to determine gender difference in CD4 T cells, which showed that males were more likely to have CD4 T cells <500cells/mm^3 as compared to females [112]. Although the risk of acquiring OI's due to low CD4 T cells once the patients with immunodeficiency is exposed to pathogens responsible for causing OI's is the same for Africans and those living in Western countries; there is a little chance for the people living in Western countries to be exposed to OI's as compared to those living in Africa; example tuberculosis is common in African setting as compared to Western countries. It might be of importance to start patients with HIV/AIDS in developing countries on ARV at early stages when the CD4 T cells is relatively normal in order to prevent further depletion of the CD4 T cells and rendering them susceptible to OI's. The risk of acquiring OI's in patients with immunodeficiency does not vary with sex, therefore females and males have the same risk of developing OI's once they are exposed. The CD4 T cells variation with sex might complicate the decision to start ARV and prophylactic drugs for OI's by considering the level of CD4 T cells since males have lower CD4 T cells count than females; therefore if we use the same cut off point to make decision to start ARV and prophylactic drugs for OI's we might denies females the opportunity to start ARV and OI drugs on early stages of CD4 T cells depletion.

Females had significantly lower haemoglobin levels than males in our study. This is similar to findings in North America, Europe and Asia. Overall we found low haemoglobin levels compared to European values and the standard reference haemoglobin values. This can be partly explained by low dietary intake of food rich in iron and vitamins, which is the commonest causes of low haemoglobin levels in Tanzania; In addition infections such as malaria and worm infestations are well known cause of low haemoglobin levels [116]. The values obtained for haemoglobin levels in this study is lower than the currently value used in Tanzania. This shows that a significant proportion of this population is anaemic, though we did not explore the causes of anaemia in our study. Overall the mean absolute leukocyte, lymphocyte and haemoglobin levels are lower than the standard reference values used, similar to other studies in Africa [62, 108] The causes of these variations are unknown, though genetic factors, diet, altitude and environmental factors such as infections can partly explain the differences[62, 108]

In countries with high prevalence of HIV the most common cause of immunodeficiency in adults is Acquired Immune Deficiency Syndrome (AIDS), which is caused by the Human Immunodeficiency Virus (HIV). This virus attacks and destroys CD4 T cells, rendering the patients vulnerable to infections like tuberculosis. The most striking finding in our study is that tuberculosis patients had lower mean CD4 T cell counts compared to the normal values for healthy individuals in this population, irrespective of their HIV status[77]. Our study did not explore the reasons for these low CD4 T cell levels, but it is in line with findings reported by other authors [27, 117, 118]. The reduction in CD4 T cells in peripheral blood in tuberculosis patients is believed to be due to the pulling of CD4 T cells to the site of infection, and in case of pulmonary tuberculosis these cells are pooled to the lungs [119]. Other studies in HIV negative tuberculosis patients have reported that low peripheral CD4 T cells have been restored to normal after successful tuberculosis therapy, suggesting that tuberculosis was the cause of the low CD4 T cells [117, 120-122]. The haemoglobin level we found was significantly lower in tuberculosis patients than the reference values established for this population[77], though these values were found to be lower than the currently used cut off point for the Tanzanian population[77]. Reasons for this could be insufficient dietary intake because of poor appetite, or anaemia due to chronic infection. This is also supported by our finding of significantly lower BMI in tuberculosis patients than in healthy subjects [77], and in HIV/AIDS patients without

tuberculosis. Malnutrition is also associated with impaired immunity and it has been reported that malnourished individuals are at risk of acquiring tuberculosis [123, 124].

HIV/AIDS and tuberculosis coinfected patients had significantly lower CD4 T cells and leukocytes, suggesting that a combination of tuberculosis and HIV/AIDS causes a more serious depletion of CD4 T cells compared to tuberculosis patients without HIV infection and HIVAIDS patients without tuberculosis. The combination of the low cellular immunity, tuberculosis and HIV/AIDS has been associated with malnutrition [125]. The decrease in CD4 T cells correlate with the severity of both HIV/AIDS and tuberculosis due to the reduction in the cellular immunity against *mycobacterium* by the human immunodeficiency virus [118]. PLWHA on treatment had significantly higher values of CD4 T cells, leukocytes, lymphocytes and haemoglobin levels than HIV/AIDS patients not on treatment. This may be explained by the antiretroviral treatment, which suppresses HIV replication and restores the immunity [126]. Also, patients on anti-retroviral therapy in this setting attend the HIV/AIDS clinic regularly for monitoring, and during their visits they are screened for anaemia and, if necessary, treated promptly free of charge according to the Tanzanian national policy on HIV/AIDS management. PLWHA are also screened for malnutrition and are given regular education about diet and opportunistic infections. This education is not given regularly to tuberculosis patients and may therefore explain why more tuberculosis patients are anaemic and malnourished as compared to HIV/AIDS, tuberculosis negative patients. HIV negative tuberculosis patients and, HIV/AIDS and tuberculosis coinfected patients were malnourished, anaemic and had lower CD4 T cells count than HIV positive tuberculosis negative patients. Anaemia, malnutrition and low cellular immunity increase morbidity and mortality in HIV/AIDS and tuberculosis coinfected patients.

6.0 Limitations of our study

The study design had some limitations/weakness related to selection bias. Our study was hospital based and is subjected to selection bias as it is shown by more HIV/AIDS patients in WHO stage III and IV and this could limit our interpretation to moderate and severe disease conditions for tuberculosis and HIV/AIDS. Also, for tuberculosis patients we have patients who were anaemic and malnourished, this reflects the severity of the disease and can be explained by the fact that those who opt to come to hospital perceived themselves as sick, while those with less severe disease did not come to the hospital.

Recall bias is also a limitation of our study. However, we tried to limit the interview questions for the past five years and also tried to confirm the response from the patients by going back to the hospital records for confirmation

Study design; the design was cross sectional study and for the tuberculosis patients coinfected with HIV/AIDS it was difficult to determine whether tuberculosis preceded HIV infection or *vice versa.*

Culture media used; The Lowenstein Jensen (LJ) media we used to culture the *mycobacterium* lacks sensitivity. Thus, we might have missed some cases of tuberculosis. However, in a resource poor setting the only available culture media is Lowenstein-Jensen, and we used it to reflect the actual situation in this poor setting

Data collection; we collected only single sputum samples from each patient. This might have led to underestimation of the proportion of smear positivity, since the recommendation is at least two samples.

7.0 Conclusions

1. i) The prevalence of undiagnosed pulmonary tuberculosis among PLWHA was 8.5%.

ii) Chest X-ray (abnormal) suggestive of tuberculosis and clinical presentations of chronic cough, chronic fever and weight loss, were uncommon findings in PLWHA coinfected with tuberculosis.

iii) No case of multi-drug resistant tuberculosis was identified among PLWHA coinfected with tuberculosis.

2. i) The prevalence of HIV infection among newly diagnosed tuberculosis patients were high compared to prevalence of HIV in general population.

ii) Ninety percent of the mycobacteria isolated from these patients were sensitive to the first line tuberculosis drugs

iii) No case of multi-drugs resistant tuberculosis was identified

3. i) Immunohaematological values in healthy subjects are different from the standard reference values for Western Europe and America

ii) Females has significantly higher CD4 T cells and CD8 T cells counts than males

4. Irrespective of the HIV status tuberculosis patients had lower CD4 T cells than the reference value for this population

8.0 Recommendations

i) Active detection of tuberculosis should be done among HIV/AIDS patients, and all tuberculosis patients should be screened for HIV infection.

ii) Screening of PLWHA may be done at inclusion to care and treatment, and during follow up visits by smear microscopy and/or culture according to the availability of the facility at the care and treatment clinic

iii) We also recommend reviewing published reports to establish local immunohaematological reference value for the Tanzanian population.

iv) Isoniazid prophylaxis should not be used until the patients is proved tuberculosis negative by smear microscopy and/or culture

v) Cotrimoxazole and ARV for all HIV/AIDS and tuberculosis coinfected patients with no contraindication to CPT and/or ARV.

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PAPERS I-IV

Appendices

Appendix I:

Questionnaire for tuberculosis patients and PLWHA

File No----------

1. Age---------- Date of Birth ----------2.Sex 1. M, 2.F

3. Address Village ----------

Ward----------

Division----------

4. Ten Cell Leader----------

5. Marital status

1. Single 2.Married 3.Cohabiting 4.Separated 5. Divorced 6.Widowed

6. Religion 1. Christian 2. Muslim 3.Traditional 4.Hindu 5. Other

7. Ethnic -tribe/race

1. Datooga 2.Iraqw 3.Nyiramba 4.Nyaturu 5. Nyisanzu 6.Sukuma 7.Hadzabe 8.Others

8 .Level of education

1. No education 2 .Primary schools less than 7yrs 3. Primary 7yrs

4. Secondary 1-4yrs 5. Secondary 5-6yrs 6. Post secondary education

9. Occupation:

1. Farmer/pastoralist 2.Business 3.Civil servant 4.Self employment

10. Socioeconomic situation (Where do you get daily food and shelter even before illness):

1.Self provided. 2. Dependent to others

11. How many people living with you in the same house----------

12. Sexual partner(s): No. present ----------

13. For women - Pregnant? 1. Y ------weeks 2.N

14. Breastfeeding 1. Y---------months 2.N

Medical history

15. Any other known PLWHA in the family 1.Y, 2.N

16. Other members of your family died from HIV/AIDS 1Y 2.N

17. HIV-test result 1.Positive 2.Negative

18. Any member of the family with previous (past 5years) or current open tuberculosis

1. Y 2.N

19. Patients Current Pulmonary tuberculosis 1. Y 2.N

20. Sputum for AFB Standard Microscopy Positive 1.Y 2.N 3.Not done

21. Sputum culture Positive 1.Y 2. N 3. Not done

22. CXR features of tuberculosis 1.Y 2.N

23. If tuberculosis by CXR

1. Upper lobe infiltration with/without cavity, 2 Bilateral/Unilateral pleural effusions with hilar lymhadenopathy, 3.Others specify_____ 4. N/A

24. Extra pulmonary tuberculosis 1.Y 2.N

25. State the location----------

26. Aspirates for AFB microscopy 1.Positive 2.Negative 3.N/A

27. Aspirates or biopsy for tuberculosis culture 1.Positive 2.Negative 3.N/A

28. Other Radiological features of extra pulmonary tuberculosis 1.Y 2.N 3.N/A

29. Biopsy for tuberculosis histology 1.Positive 2.Negative 3.N/A

30. History of extrapulmonary tuberculosis within last 5 years 1.Y 2. N
(If possible confirmed by a written records)

31. History of pulmonary tuberculosis within last 5 years

1. Y, AFB Positive 2.Y, AFB Negative 3.No

Current possibly HIV/tuberculosis related diseases/symptoms

32. 1.Y, 2.N chronic fever and weakness > 1 month
33. 1.Y, 2.N Weight loss.
34. 1.Y, 2.N > 10 %of the body weight
35. 1.Y, 2.N Chronic and chest pain Cough.
36. 1.Y, 2.N chronic intermittent diarrhea > 1 month
37. 1.Y, 2.N Oral thrush (Candida)
38. 1.Y, 2.N Kaposi in mouth
39. 1.Y, 2.N Odynophagia (retrosternal pain on swallowing)
40. 1.Y, 2.N Recurrent (probable HSV-) sores (Lips)
41. 1.Y, 2.N Recurrent (probable HSV-) sores Genitalia
42. 1.Y, 2.N Herpes Zoster currently
43. 1.Y, 2.N Herpes Zoster Previous
44. 1.Y, 2.N Recurrent RTIs/ severe infections
45. 1.Y, 2.N Sexually Transmitted Infection (specify)
46. 1.Y, 2.N Other Specify

Medication

47. Have you ever got ARV medication 1. Y, 2.N

48. If previous ARV state the regimen.

1. d4t/3TC/NVP 2. D4t/3TC/EFZ 3.AZT/3TC/NVP 4.AZT/3TC/EFZ 5.ABC/DDI/Lpr/RT 6.N/A

49. Duration of ART in month ----------

50. Previous tuberculosis treatment 1.Y, 2.N

51. If previous tuberculosis treatment specify the drugs

1. INH 2.RH 3.PZA 4.ETH 5.INH/PZA/ETH/RH 6.N/A

52. Previous tuberculosis prophylaxis 1.Y 2.N

53. If previous tuberculosis prophylaxis specify the drugs used

1. INH 2. RH 3. PZA 4. ETH 5.Unknown, 6.N/A

54. Current tuberculosis treatment 1.Y, 2N

55. If current tuberculosis treatments indicate the drugs used

1. INH 2. RH 3. PZA 4. ETH 5.INH/PZA/ETH/RH 6.Unknown, 7.N/A

56. Current tuberculosis prophylaxis 1.Y 2.N

57. If current tuberculosis prophylaxis state the drugs

1. INH 2.RH 3.PZA 4. ETH 5.Unknown 6.N/A

58. Other OI's medication taken by the patient Specify----------

Examination

59. Current weight ----------kg

60. Previous weight if known----------kg

61. Height----------m

62. BMI----------kg/m2

63. Auxiliary temperature----------Celsius

64. BP Systolic---------- diastolic----------

65. Heart rate----------

66. General state 1. Well 2.Wasted

67. Lymphadenopathy 1.Y 2.N (>1cm) specify area----------

Laboratory results

68. CD4 T cells count----------cells/mm^3

69. Absolute leucocytes count----------$x10^{12}$

70. Absolute lymphocyte count----------$x10^{12}$

71. Hemoglobin level----------g/dl

72. QuantiFERON test 1. Positive 2. Negative 3.Not done

73. WHO clinical stage for HIV patient ----------

Appendix II:

Questionnaire for health subjects

File No----------

1. Age---------- Date of Birth ----------

2. Sex 1. M, 2.F

3. Address Village ----------

Ward----------

Division----------

4. Ten Cell Leader----------

5. Marital status

1. Single 2.Married 3.Cohabiting 4.Separated 5. Divorced 6.Widowed

6. Ethnic -tribe/race:

1. Datooga 2.Iraqw 3.Nyiramba 4.Nyaturu 5. Nyisanzu 6.Sukuma 7.Hadzabe 8.Others

7. For women - Pregnant? 1. Y ------weeks 2.N

8. Breastfeeding 1.Y---------months 2.N

9. Smoking/chewing local tobacco or Cigarette

10. (a) Drinking alcohol 1.Yes 2.No

10. (b) If yes 1. Occasionally 2. Chronic alcoholic

11. Any member of the family with recent/current open tuberculosis 1.Y 2.N

12. (a) Are you suffering from any kind of illness recently or currently 1.Y 2.N

(b) If yes mention_____ (if possible this should be confirmed by doctor or report from the doctor)

Current/recent symptoms of illness diseases

13. 1.Y, 2.N chronic fever and weakness > 1 month

14. 1.Y, 2.N Weight loss.

15. 1.Y, 2.N > 10 %of the body weight

16. 1.Y, 2.N Chest pain and Cough

17. 1.Y, 2.N Diarrhea > 1 month

18. 1.Y, 2.N Oral thrush (Candida)

19. 1.Y, 2.N Kaposi in mouth

20. 1.Y, 2.N Odynophagia (retrosternal pain on swallowing)

21. 1.Y, 2.N HSV sores on the Lips

22. 1.Y, 2.N HSV sores on the Genitalia

23. 1.Y, 2.N Herpes Zoster currently

24. 1.Y, 2.N Herpes Zoster Previous

25. 1.Y, 2.N RTIs/ severe infections

26. 1.Y, 2.N Sexually Transmitted Infection (specify)

27. 1.Y, 2.N other conditions Specify _____

28. (a) Are you on any kind of medication recently or currently 1. Y, 2.N

(b) If on medication mention them_____ (If possible a doctor should see the Drugs or written document about the drugs)

29. Current weight ----------kg

30. Previous weight if known----------kg

31. Height---------- m

32. BMI----------kg/m^2

33. Auxiliary temperature----------Celsius

34. General state 1. Well 2.Wasted

35. Lymphadenopathy 1.Y 2.N

Laboratory test results

36. Urine for pregnant test or Obstetric ultrasound for suspected pregnancy

1. Pregnant 2. Not pregnant

37. HIV test 1.positive 2.Negative

38. B/s for malaria parasites 1.Positive 2.negative

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

40. Random Blood sugar (for suspected Diabetic patients)

1. Proved Diabetes 2.No Diabetes

41. CD4 T cells count----------$cells/mm^3$

42. Total absolute leucocytes count----------$x10^{12}$

43. Absolute lymphocyte count----------$x10^{12}$

44. Haemoglobin level----------g/dl

Appendix III

WHO Clinical staging of HIV/AIDS in adolescents and adult

Clinical Stage I:

1. Asymptomatic

2. Generalized lymphadenopathy

Performance scale 1: asymptomatic, normal activity

Clinical Stage II:

3. Weight loss <10% of body weight

4. Minor mucocutaneous manifestations (seborrhea dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis)

5. Herpes zoster within the last five years

6. Recurrent upper respiratory tract infections (i.e. bacterial sinusitis)

And/or performance scale 2: symptomatic, normal activity

Clinical Stage III:

7. Weight loss >10% of body weight

8. Unexplained chronic diarrhea, >1 month

9. Unexplained prolonged fever (intermittent or constant), >1 month

10. Oral candidiasis (thrush)

11. Oral hairy leucoplakia

12. Pulmonary tuberculosis

13. Severe bacterial infections (i.e. pneumonia, pyomyositis)

And/or performance scale 3: bedridden <50% of the day during last month

Clinical Stage IV:

14. HIV wasting syndrome [i]

15. Pneumocystic carinii pneumonia

16. Toxoplasmosis of the brain

17. Cryptosporidiosis with diarrhea >1 month

18. Cryptococcosis, extra pulmonary

19. Cytomegalovirus disease of an organ other than liver, spleen or lymph node (e.g. retinitis)

20. Herpes simplex virus infection, mucocutaneous (>1 month) or visceral

21. Progressive multifocal leucoencephalopathy

22. Any disseminated endemic mycosis

23. Candidiasis of esophagus, trachea, bronchi

24. Atypical mycobacteriosis, disseminated or pulmonary

25. Non-typhoid Salmonella septicemia

26. Extrapulmonary tuberculosis

27. Lymphoma

28. Kaposi's sarcoma

29. HIV encephalopathy [ii]

And/or performance scale 4: bedridden >50% of the day during last month

[i] HIV wasting syndrome: weight loss of >10% of body weight, plus either unexplained chronic diarrhea (>1 month) or chronic weakness and unexplained prolonged fever (>1 month).

[ii] HIV encephalopathy: clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition, other than HIV infection, which could explain the findings.

The WHO clinical staging of HIV/AIDS is adopted from Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance African Region; Reference number: WHO/HIV/2005.02; WHO 3X5; 2005

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Dr Bernard J Ngowi
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CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: HIV AIDS Epidemiology and its impact on Tuberculosis and CD4 counts (*Ngowi B J et al)* whose Principal Investigator is Bernard J Ngowi, has been granted ethics clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is made available to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine.

Name: Dr Andrew Y Kitua

Signature

CHAIRMAN
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Gabriel L Upunda

Signature

CHIEF MEDICA L OFFICER
MINISTRY OF HEALTH