Vitamin D status among HIV infected and HIV/TB co-infected patients attending Haydom Lutheran Hospital, rural Tanzania.

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Philosophy in International Community Health at the University of Oslo.

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**Acronyms and abbreviations:**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>ARV</td>
<td>Antiretroviral drugs</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette Guerin</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CTC</td>
<td>Care and Treatment Clinic</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>EPI</td>
<td>Expanded programme for immunisation</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly active antiretroviral treatment</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immune Deficiency Virus</td>
</tr>
<tr>
<td>HLH</td>
<td>Haydom Lutheran Hospital</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressured liquid chromatography</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>IRIS</td>
<td>Immune reactive inflammatory syndrome</td>
</tr>
<tr>
<td>MoHSW</td>
<td>Ministry of Health and Social Welfare</td>
</tr>
<tr>
<td>MTB</td>
<td>Mycobacterium tuberculosis</td>
</tr>
<tr>
<td>NNRTIs</td>
<td>Non-Nucleoside Reverse Transcriptase Inhibitors</td>
</tr>
<tr>
<td>NRTIs</td>
<td>Nucleoside Reverse Transcriptase Inhibitors</td>
</tr>
<tr>
<td>OI</td>
<td>Opportunistic Infection</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PLWHA</td>
<td>Persons Living with HIV/AIDS</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard operating procedures</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
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</tbody>
</table>
VCT  Voluntary counselling and testing
WHO  World Health Organization
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Abstract

Background, Rationale and aims of the study

Human immune deficiency virus type 1 (HIV-1) infection remains the most common risk factor for the development of active and reactivation of latent tuberculosis (TB); on the other hand, TB is commonly known to accelerate the progression of HIV disease. Vitamin D has been shown to provide protection against tuberculosis, and its supplementation has also been shown to increase immunity.

The currently available data from studies in humans regarding the potential value of vitamin D as adjunctive therapy in mycobacterial infection (TB) remain conflicting. There is also limited and contradictory evidence about the effect of vitamin D on the immune system. Furthermore, many of these studies were done in the developed world.

Tanzania is faced with high burden of HIV and tuberculosis. Assessment data on vitamin D status in HIV and HIVTB were in short supply, especially the rural settings. When programmes are well planned, using locally relevant, up to date data, implementation is likely to be more effective than when international or national guidelines are followed without appropriate adaptation. We found reasonable and ethical to conduct this study investigating the interaction existed between vitamin D, calcium, parathyroid hormone, and CD4 cells count in HIV-1 monoinfected and HIV/TB coinfected patients.

Methods: A cross sectional study using quantitative methods was conducted from July 2010 to January 2011 at Haydom Lutheran Hospital in rural Tanzania. A purposive sampling technique was used to recruit 159 subjects aged 5 years and above. A standard data abstraction tool was used to obtain required information from the patients’ files/database. Serum vitamin D concentrations were measured by competitive Radioimmunoassay (RIA).

Main findings

The subjects mean (SD) age was 35.5 (11.3) years; 85 (53.5%) were female, and 71(44.7%), 41(25.8%), and 47(29.6%) were HIV, HIVTB and healthy controls respectively. Subjects enrolled during postharvest (Aug-Oct) season had significantly higher serum 25(OH)D compared to short rain season (Nov-Jan); (75.9 ± 20.8 vs. 64.6 ± 22.2, P = 0.034. Similarly subjects aged less than 25 yrs had higher level of serum 1, 25-(OH)2D than age groups 35 – 45 yrs
and > 45 yrs; P = 0.043, 0.002 respectively. Overall hypovitaminosis D (serum 25-(OH)-D < 75nmol/l) was found in 60.4%.

Subjects with HIV infection had higher serum 25-(OH)-D concentration than HIVTB-coinfected subjects (77.2 ± 20.8 vs 63.2 ± 23.8); P = 0.003. We observed hypovitaminosis D in 78.6% (11/14) of the HIVTB coinfected patients and in 52.1% (37/71) of the HIV monoinfected patients; the odd ratio (OR) of hypovitaminosis D for HIVTB coinfected patients was 3.4 (95% CI: 0.9, 13.1), P = 0.08.

Advanced Clinical HIV stage (three and four) was significantly associated with lower serum 1,25-(OH)2D concentration, P = 0.021 and 0.0013 respectively. Subjects with CD4 cells count less 200 had lower level of serum 1,25(OH)2D than subjects with CD4 200 – 500 cells/mm³, 137.6 ± 63.9 vs 199.7 ± 58.1, P = 0.05.

Both serum 25 (OH)D and 1,25 (OH)2D levels were higher in HIV positive patients not on ART compared with those on ART, but the difference did not reach statistical significance; 81.4 (20.5) vs 74.7 (20.7) nmol/l and 182.8 (67.1) vs 165.1 (75.5) pmol/l, P = 0.190, 0.323 respectively.

However in both univariate and multivariate logistic regression analysis, hypovitaminosis D remained significantly higher among HIV patients on antiretroviral therapy (ART) compared to HIV patients not on ART, OR = 3.5 (95% CI; 1.1, 12.3). However this may be due to advanced disease.

**Conclusion;**

Advanced HIV stage was associated with lower serum 1,25(OH)2D concentrations, possibly due to reduced hydroxylation of 25(OH)D to 1,25(OH)2D in the macrophages. Serum 25-(OH)-D concentration was higher in HIV monoinfected than HIVTB coinfected patients, and hence hypovitaminosis D was more common among HIVTB coinfected than HIV monoinfected but the difference was not statistically significant due to small sample size. Hypovitaminosis D was higher in HIV patients on antiretroviral therapy compared with patients not on ART.

In conclusion, hypovitaminosis D was more common in HIV and HIVTB patients. Both health professionals and policy makers should be aware of this common co-morbidity and act accordingly.
1. Background.

1. 1. Introduction to geography and economy of study area

1.1.1. Country location
Tanzania is the largest country in East Africa (i.e. Kenya, Uganda and Tanzania), covering 945,000 square kilometres. It is located in Eastern Africa between longitude 29° and 41° East, and Latitude 1° and 12° South and shares borders with eight countries: Kenya and Uganda to the north; Rwanda, Burundi, Democratic Republic of Congo to the west; and Zambia Malawi and Mozambique to the south (1) as shown in figure1.

![Tanzania Map](image-url)

Figure 1: Location of Tanzania within Africa and its administrative regions.(1)
1.1.2. Geographical Features
Tanzania has a spectacular landscape of mainly three physiographic regions namely the Islands and the coastal plains to the east; the inland saucer-shaped plateau; and the highlands. The Great Rift Valley that runs from north east of Africa through central Tanzania is another landmark that adds to the scenic view of the country. The rift valley runs to south of Tanzania splitting at Lake Nyasa; one branch runs down beyond Lake Nyasa to Mozambique; and another branch to north-west alongside Burundi, Rwanda, Tanzania and western part of Uganda. The valley is dotted with unique lakes which include Lakes Rukwa, Tanganyika, Nyasa, Kitangiri, Eyasi and Manyara. The uplands include the famous Kipengere, Udzungwa, Matogoro, Livingstone, and the Fipa plateau forming the southern highlands. The Usambara, Pare, Meru, Kilimanjaro, the Ngorongoro Crater and the Oldonyo Lengai, all form the northern highlands. From these highlands and the central saucer plateau flow the drainage system to the Indian Ocean, and the inland drainage system.

1.1.3. Climate
Tanzania has a tropical type of climate. In the highlands, temperatures range between 10° C and 20° C during cold and hot seasons respectively. The rest of the country has temperatures never falling lower than 20° C. The hottest period spreads between November and February (25° C - 31° C) while the coldest period occurs between May and August (15° - 20° C). Two rainfall regimes exist over Tanzania. One is unimodal (December - April) and the other is bimodal (October -December and March - May). The former is experienced in southern, south-west, central and western parts of the country, and the latter is found to the north and northern coast. In the bimodal regime the March - May rains are referred to as the long rains or Masika, whereas the October - December rains are generally known as short rains or Vuli.

1.1.4. Economy
Tanzania is an agrarian country. Agriculture accounts for 50% of the gross domestic product and accounts for about 56% of the exports. Agriculture also employs about 80% of the population mainly from the rural area. Major agricultural exports are coffee, cotton, tea, tobacco, cashew nuts, and sisal. Industrial exports have been on the rise following adoption of trade liberalization, and privatization of public enterprises.

Tanzania remains one of the least developed countries in the world. Its Gross Domestic Product (GDP) at constant 1992 prices recorded an average of real growth rate of 4.0 percent per annum during 1996-1999. Given the annual population growth rate of 2.8 percent, per capita real growth rate was around 1.2 percent. The composition of GDP is such that, agricultural sector accounts for
around 50.0 percent, followed by trade sector which accounts for around 16.0 percent. Financial and business services rank third at the tune of 10 percent, followed by the industrial sector by around 8.0 percent. The mining sector has been contributing around 2.0 percent, but there is a bright future for the sector as foreign investments continue to flowing in. It is apparent that in the near future the sector will record a significant proportion of GDP.

1.1.5. Health profile
The health system in Tanzania remains underdeveloped. The majority of the population resides in the rural areas and has limited access to modern health facilities. The health service coverage remains low and the quality of services available, especially in rural areas, is variable, aggravated by poor transportation system. There is a heavy burden of disease with a growing prevalence of communicable infections in the country. Many Tanzanians face disease morbidity and mortality largely attributable to potentially preventable infectious diseases and nutritional deficiencies.

Tanzania is among the countries with a high burden of disease caused by HIV/AIDS Tuberculosis and malaria. For many years, malaria has been a major cause of morbidity and mortality, particularly among children under age five. Furthermore, HIV/AIDS is increasingly seen among children under five. Neonatal, postneonatal, infant, child, and under-5 mortality rates for three successive five-year periods before the 2010 survey are presented in Table 1. For the five years immediately preceding the survey (approximately calendar years 2006-2010), the infant mortality rate was 51 per 1,000 live births and the under-five mortality rate for the period is 81 per 1,000. These data showed that childhood mortality has declined considerably over the past 10-15 years (2).

The percentage of the Tanzanian population with sustainable access to improved sanitation was estimated to be 33% in 2006, rural children estimated to be stunted, underweight for age group less than 5 years of age in 2010 was 44.5%, 17% respectively and the percentage of children sleeping under insecticide treated nets in 2010 was 73% (2).
The estimated prevalence of HIV/AIDS in Tanzania as a whole in adults aged 15 - 49 years was 5.8% in 2007 (3). This prevalence is 10\textsuperscript{th} highest of 41 African nations listed by the WHO (3). Manyara Region had HIV prevalence of approximately 2% in 2002 (4). The prevalence of HIV among TB is 13.3% and that of TB among HIV/AIDS patients is 8.5% at Haydom Lutheran Hospital (HLH) in 2007 (5).

Table 2. Basic health indicators for Tanzania, Tanzania Ministry of Health (2)

<table>
<thead>
<tr>
<th>Life expectancy</th>
<th>Under 5 mortality rate</th>
<th>EPI *coverage</th>
<th>Maternal mortality rate</th>
<th>ANC coverage</th>
<th>Attended Delivery</th>
<th>Contraceptive Prevalence rate</th>
<th>Use of insecticide treated net</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.4 and 52.8 yrs for female and male respectively.</td>
<td>81/1000 live births</td>
<td>75%</td>
<td>454 per 100,000 live births</td>
<td>96%</td>
<td>51%</td>
<td>36%</td>
<td>75%</td>
</tr>
</tbody>
</table>

*Expanded programme for immunization.
1.2. HIV, TB/HIV: pathogenesis, global and national burden

1.2.1. HIV-Pathogenesis

The first case of HIV was diagnosed in 1981 and since that period, researchers have identified two serotypes of HIV: HIV-1, the commonest worldwide, and HIV-2, most common in West Africa. Both have the same routes of transmission, but HIV-2 is believed to be more easily transmitted, and progression to AIDS might be slower in those infected with HIV-2 (6).

The main route of transmission worldwide remains unprotected sexual intercourse although the routes of transmission vary greatly between regions. In Sub-Saharan Africa, sexual intercourse, contaminated blood and mother to child transmission play the major role in transmission whereas in the fast growing epidemics in Russian federation and the Ukraine, the commonest route of transmission is injecting drug use (6). HIV infects cells which have the CD4 antigen on their surface that is principally the helper T-lymphocytes, which are critical for cell mediated immunity. Thus the principal abnormality in patients with HIV infection results from the fact that the number of CD4+ T-lymphocytes is decreased and the remaining lymphocytes also have an alteration in function, resulting in progressive immune suppression, which will result in increased susceptibility to opportunistic infections in those infected.

1.2.2. Global burden

In 2009, 33.3 million people were living with HIV, with 2.6 million (19% fewer than 1999) new infections and 1.8 million deaths in that same year. Of these, 22.5 million (68%) were estimated to be living in Sub-Saharan Africa and 4.1 million in South and South-East Asia. Of the 7,400 infections occurring every day in year 2007, more than 96% were in low and middle income countries. Among infections occurring in the age group of 15 and older, 45% occur among young people hence affecting largely the economically productive group (7). In the most affected countries, HIV has decreased life expectancy by more than 29 years, has aggravated household poverty and slowed economic growth (8).

1.2.3 National burden

HIV/AIDS is a major development crisis that affects all sectors. Results from the 2007-08 Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS) show that, 6 percent of Tanzanian adults are infected with HIV(9). The prevalence is higher among women than men (7 percent and 5 percent, respectively). During the last two decades the HIV/AIDS epidemic has spread relentlessly
affecting people in all walks of life and devastating the most productive segments of the population particularly women and men between the ages of 15 and 49 years.

The increasing number of AIDS related absenteeism from workplaces and deaths reflects the early manifestation of the epidemic leaving behind suffering and grief. Others include lowering of life expectancy, increasing the dependency ratio, reducing growth in GDP, reduction in productivity increasing poverty, raising infant and child mortality as well as the growing numbers of orphans (10). The epidemic is a serious threat to the country’s social and economic development and has serious and direct implications on the social services and welfare. Given the high HIV prevalence in the society, and in the absence of cure, the devastating impact of the epidemic is incomprehensible.

1.3. HIV/TB-Pathogenesis

Human immunodeficiency virus type 1 (HIV-1) infection remain the most common risk factor for the development of active tuberculosis (TB), reactivation of a latent tuberculosis, and new TB infection once HIV infected patients are exposed to tubercle bacilli (11). The lifetime risk of developing TB from an infection with *Mycobacterium Tuberculosis* is 5-10% in an HIV negative person versus 50% in an HIV positive individual. The risk of death in a patient with HIV who has TB is twice that of an HIV patient without TB, adjusted for CD4 count, the death resulting not from TB but from HIV disease progression (12).

Attack of macrophage is the crucial step in the pathogenesis of both TB and HIV-1 infection. They serve as the principal effectors cells against *Mycobacterium tuberculosis* and as reservoirs for the intracellular growth of both organisms. The infection with HIV-1 impairs intrinsic macrophage-mediated defenses against a variety of intracellular pathogens such as decrease chemotaxis, binding of microorganisms, phagocytosis, antigen processing, microbicidal activity and increase of intracellular growth of microorganisms. HIV also impair the T-lymphocyte/macrophage immune axis, hence, HIV-1-infected individuals have increased susceptibility to opportunistic infections (12).

The development of the tuberculous granuloma is of paramount important as far it protect the spread of microorganisms in the body. The three steps of development are: (1) the development of a monocytic infiltrate; (2) the aggregation, maturation and organization of mononuclear cells into a granuloma; and (3) the further maturation and evolution of an epithelioid granuloma. HIV induce defect in granuloma assembly and function through impaired chemotaxis (13) and bactericidal functioning (14) of macrophages and decreased proliferation of IFN-γ-secreting clones of MTB.
specific TH lymphocytes. As a consequence, progressive immunosuppression associated with the development of AIDS results in failure of epithelioid differentiation of macrophages, no formation of Langhans giant cells, and no caseous necrosis.

Similarly, tuberculosis is commonly known to accelerate the progression of HIV disease (15; 16). Proinflammatory cytokine production by tuberculous granulomas has been associated with increased HIV viraemia, accelerating disease progression. The activation of HIV-1 replication in coinfected cells by TB, or by the immune activation that accompanies TB infection, has been well documented (17) (18; 19). Thus, if an HIV-subtype of a particular strain was particularly responsive to activation during TB infection or reactivation, it could in theory impact outcome during coinfection. The net effect is increased rates of both virus-induced and immune mediated loss of CD4+ cells.

The impact of distinct TB strains and their variation on HIV replication and AIDS progression in coinfection is unknown, and is probably another area that deserves further study. However different TB strains have been shown to have distinct immunomodulatory properties upon host cells (20; 21). This led to the hypothesis that different TB strains may influence HIV replication via the manipulation of cytokine networks, and thus influence disease progression in coinfection in a strain specific manner. In-vitro studies on TB/HIV coinfection showed that, a distinct activation of the host immune response by phenotypically distinct M. tuberculosis clinical strains directly regulates HIV-1 replication in a strain-specific manner.

1.3.1. Global Burden

With about 11% of the global population, Africa has the greatest regional burden of the global HIV/TB epidemic, with about 68% of the estimated burden of HIV in 2007 and 85% of all cases of HIV/TB (22). Africa’s weak health systems have resulted in very slow progress towards TB control even before HIV (23). The increasing rate of HIV infection in this region has had an impact on TB epidemiology (24). About one-quarter of deaths in people with HIV worldwide was caused by TB in 2007. Around 450,000 people with HIV died of TB in 2007, and there were 1.4 million HIV-positive TB cases (25).

1.3.2. National burden

In Tanzania between 1977 and 1984, the National Tuberculosis and Leprosy Programme (NTLP) cut the number of tuberculosis (TB) cases by two-thirds, earning a reputation as one of the best performing disease-control programs in the world. However, the emergence of the HIV/AIDS
pandemic reversed this successful trend, by increasing the number of TB cases six times in the year between 1983 and 2003.

1.3.3. National measures to control TB and HIV

1.3.3.1. National Tuberculosis and Leprosy Programme (NTLP)

The National Tuberculosis and Leprosy Programme (NTLP) was launched as a single combined programme in 1977 by the Ministry of Health and Social Welfare (MoHSW). NTLP is within the department of Preventive Services in the MoHSW under the Epidemiology and Disease Control section (26). NTLP works administratively at three levels namely, national, regional and district levels. At the national level there is Tuberculosis and Leprosy Central Unit (TLCU) which is responsible in coordinating all activities pertaining to tuberculosis and Leprosy in the country. Also, the unit is responsible for planning, policy formulation, monitoring, evaluation, resource mobilization and coordination of drugs and supplies procurement and distribution.

At the regional level there is Regional Tuberculosis and Leprosy Coordinator (RTLC) who works closely with TLCU and the districts. Their responsibility is to interpret the policy guidelines and monitor their implementation at the district level. RTLC is answerable to the Regional Medical Officer (RMO). At the district level there is a District Tuberculosis and Leprosy Coordinator (DTLC) who works under the District Medical Officer (DMO). The DTLC is the main link between health units and community on one hand and TLCU through the region on the other hand (26).

1.3.3.2. National Aids Control programme

In response to the epidemic, the Tanzanian’s Government with technical support from the World Health Organization Global Programme on AIDS (WHO-GPA) formed the National HIV/AIDS Control Programme (NACP) under the Ministry of Health and Social Welfare in 1988. The national response consisted on developing strategies to prevent, control and mitigate the impact of HIV/AIDS epidemic, through health education, decentralization, multi-sectoral response and community participation. However the response has not had much impact on the progression of the epidemic as expected. The national response initiatives were constrained by a number of factors; inadequate human and financial resources, ineffective co-ordination mechanisms and inadequate political commitment and leadership. Some of these constraints are now being addressed.
There is strong political commitment and leadership from the highest level. HIV/AIDS has been declared a National crisis and is now one of the top priority development agenda in the Government, along with poverty alleviation, improvement of the social sector services. The Tanzania Commission for AIDS (TACAIDS) has been established in 2001 to provide leadership and coordination of multisectoral responses. The Multisectoral Policy Guidelines on HIV/AIDS is now in place. Decentralisation facilitates people’s participation in decision making in issues that affect their lives, including HIV AIDS. As HIV/AIDS epidemic affects all sectors, its control demands a well coordinated response.

Currently NACP is operating under Directorate of Preventive Services of the Ministry of Health and Social Welfare (MOHSW) despite the fact that its activities cut across various departments of the Ministry. At the national level NACP is a vertical programme with 7 Units i.e. Programme Management, Information, Education & Communication (IEC), Counseling and Social Support, Laboratory, Clinical and STD Control, Epidemiology and Research Coordination, Care and Treatment (27).

1.4. Vitamin D
Vitamin D is a fat soluble vitamin important for serum calcium and phosphorus homeostasis for proper neuromuscular function and optimal skeletal health (28;29). The predominant source of vitamin D is sun light exposure: pre-vitamin D3 (7-dehydrocholesterol) is converted by solar ultraviolet B radiation in the skin into vitamin D3 (cholecalciferol). Alternatively, vitamin D, in the form of either vitamin D2 (derived mainly from irradiated plants) or D3, (from dietary sources). Skin pigmentation is a known risk factor for hypovitaminosis D since melanin, responsible for the skin pigmentation, filters UV radiation (28;29). Food uptake is limited to vitamin D supplementation or consumption of oily fish (28).

Vitamin D is transported in the blood by vitamin D-binding protein (VDBP) and is then converted in the liver by 25-hydroxylase enzyme into 25-hydroxyvitamin D (25-(OH)-D). The plasma concentration of 25-(OH)-D is considered the best indicator of vitamin D status, (28;30) with half-life 2-3 weeks (31) and normal levels ranging between 30 and 50 ng/ml (32). The concentrations of 1, 25-(OH)2D provide an indication of renal 1α 25 hydroxylation and also of defects in disposal by the cytochrome P-450 enzyme CYP24. Serum phosphorus, calcium, fibroblast growth factor 23, and other factors can either increase or decrease the renal production of 1,25(OH)2D. The active form of vitamin D (1,25[OH]2D) can decreases its own synthesis through negative feedback and decreases the synthesis and secretion of parathyroid hormone by the parathyroid glands (28).
Vitamin D status can be defined as hypovitaminosis D; vitamin D deficiency and insufficiency, adequacy, or toxicity. The worldwide prevalence of suboptimal vitamin D status is estimated to be high (28;33). Vitamin D deficiency is defined as 25-(OH)-D below 10 ng/m or 25 nmol/l (28) and its major causes are insufficient exposure to sunlight, decreased dietary intake, skin pigmentation, obesity, and advanced age (29).

The cytochrome CYP34A, present in liver, intestine, kidney and leukocytes (34) is also a key enzyme in P450 cytochrome-mediated drug metabolism such as anti-retroviral (non nucleoside reverse transcriptase inhibitor and Protease Inhibitors) as well as certain anti-tuberculous drugs (rifampicin and Isoniazid) (30;34;35)

The lesser active form of vitamin D, 25-(OH)-D, is converted in the kidney cells into its circulating active form of 1, 25-(OH)_{2}D by the enzyme 1-α-hydroxylase (CYP27B1). Other cells in the body such as macrophages also express CYP27B1(30). In the late phase of macrophage activation, macrophage-CYP27B1 produces 1,25-(OH)_{2}D which apparently has a local rather than a systemic effect on immune cells (36). Although the macrophage-CYP27B1 is identical to the renal CYP27B1, its expression is not down-regulated by the parathyroid hormone (PTH) neither the active vitamin D and is mainly up-regulated by inflammatory cytokines such as interferon gamma (IFN-γ) and by lipopolysaccharides (LPS) (30;36).

1.5. Literature Review

MEDLINE was searched in order to find relevant review articles and originals reports on the topics of this study. In addition textbooks and readers in vitamin D, nutrition and health care in developing countries were read.

1.5.1. Studies worldwide

Effective medication can slow the progress of HIV, reduce opportunistic infections, and ease symptoms; however food can interact with drugs and affect the drugs’ efficacy. Drugs can also interact with foods and nutrients and negatively affect nutritional status. And ultimately, drug and food interactions can result in poorer health and nutritional status of an individual. Optimal nutrition can help boost immune body function, maximizing the effectiveness of drugs, reduce the risk of opportunistic infections, and contribute to a better overall quality of life (37).
1.5.2. Vitamin D and Tuberculosis

The active form of vitamin D modulates monocyte–macrophage activity in the body and plays a role of innate immunity to certain infectious agent. This role is important in the body’s defence against TB in which the attack of macrophage is the crucial step in pathogenesis. Therefore its lower level or abnormality in receptor structure and function may result in impairment in immunity against infectious agents.

A number of human trials on vitamin D replacement as treatment or prevention of tuberculosis has been attempted to translate the mechanism of vitamin D–mediated macrophage activation to human host, the outcome yield mixed results. Martineau et al (38) reported encouraging results of improved immunologic control of Bacille Calmette-Guerin (a M. tuberculosis surrogate) in the peripheral blood following administration of a single dose of 100,000 IU of ergocalciferol to purified protein derivative-positive (PPD) contacts of active TB cases.

In a trial by Morcos et al (39) the findings showed a benefit of increased weight gain and faster resolution of TB symptoms in children treated with 1,000 IU of vitamin D daily as an adjunct to standard TB therapy while a trial by Nursyam et al (40) demonstrated a significant higher rates of sputum conversion to culture negativity in the group treated with 10,000 IU of vitamin D daily for 6 weeks in comparison with placebo. Both of the two trials, (Morcos and Nursyam) however, failed to report baseline or follow-up serum 25-OH-D levels for either the intervention or the control group, leaving uncertainty about the adequacy of repletion in each case.

In contrast, a trial by Wejse et al (41) on vitamin D therapy in patients with TB reported a significant increase in serum 25-OHD levels in the intervention group receiving 100,000 IU of vitamin D at baseline, 5 months, and 8 months of TB therapy. However variables, such as increased exogenous intake of vitamin D irrespective of group assignment or an independent effect of improving nutritional status with TB therapy, may also be confounding the results of this study, which found no difference in TB-related clinical outcomes between the 2 study groups. Recruitment and follow-up for the study took place during the course of 24 months, and it is unclear whether seasonal variations in vitamin D status affected any study outcomes (41).

In a systematic reviews and Meta analysis of observational studies, Nnoaham and his colleagues (2008) concluded, low serum vitamin D levels are associated with higher risk of active tuberculosis. In this review, two studies, one done in indigenous Indonesians showed no association due to insufficient information about the controls and the controls on the second study.
were having hypertension or Diabetic conditions that might have interfered with vitamin D metabolism (42).

Sunlight exposure and dietary intake has been shown to be the main sources of body vitamin D however much remain to be known of their relative contribution. In a review by Nnoaham and his colleges on the observational studies, a study done in Asians people with good year-round sunshine, people maintained adequate serum levels of vitamin D inspite of poor dietary intake. Another similar study in India found low vitamin D levels in the study population despite adequate sunlight exposure, concluding that diet was the more important factor (42), however the latter study did not take into account the actual time spent outdoors, extent of body exposed to the sun or level of cutaneous pigmentation.

In a recent open and controlled clinical trial done in Denmark which included 182 participants divided into three different groups. The findings in each group were as follow: When 50 participants with all baseline levels of 25(OH)D due to previous different sun exposures were exposed to UVB radiation, the 25(OH)D mean (SD) level increased by 23.3 nmol/l (10.6) in response to the UVB treatments, with a strong negative correlation between the increase in 25(OH)D and baseline 25(OH)D levels, furthermore the findings showed a strong positive and negative correlation between baseline 25(OH)D and the number of fish meals per week and PTH respectively. However no significant correlation found between baseline 25-(OH)-D levels and body mass index.

The influence of skin pigmentation and baseline total cholesterol were analysed in a homogeneous group of 28 non-sun worshippers with limited past sun exposure: The findings showed a significant different in baseline 25-(OH)-D level between the 28 non-sun worshippers and the 22 sun worshippers due to different amounts of previous sun exposure. In this group of 28 non sun worshipers the 25-(OH)-D levels increased by (mean (SD)) 25.3 nmol /l (10.5) in response to the UVB treatments. Furthermore the findings showed no correlation between change in baseline 25(OH) D and skin pigmentation, however significant correlation was found in baseline total cholesterol, the precursor of vitamin D.

In confirming whether skin pigmentation has a role for change in baseline 25-(OH)-D after UVB exposure, a total of 18 participants consisting of 9 pairs of dark and fair skinned participants were matched according to “identical” baseline 25-(OH) D levels. The findings showed no significant
differences in change 25-(OH)-D between the dark- and the fair-skinned group despite their significant difference in constitutive and facultative skin pigmentation showing that the change in 25-(OH)-D is unrelated to skin pigmentation (43).

African Americans have been known to have significantly lower serum levels of vitamin D because melanin filters out ultraviolet light. The macrophages cultured in blood provided by African Americans produced 63% less defensins antimicrobial peptides (cathelicidin) than when cultured in serum from whites and this might explain why African Americans and perhaps dark-skinned Indians have higher incidence rates of TB (44). In another study done in Australia among African immigrants, the mean serum level of 25(OH)D was lower among immigrant with latent tuberculosis than those with no *Mycobacterium tuberculosis* infections (45).

In another review of three randomised control trials and ten prospective studies by Martineau et al, examining the role of vitamin D supplementation to pulmonary tuberculosis patients who are on anti TB, the findings showed a growing evidence to suggest that active form of vitamin D modulates antimicrobial activity in vitro; however the existing studies investigating the effect of vitamin D supplementation on treatment of TB are methodologically inconsistent (46).

The occurrence of tuberculosis in different countries has been associated with the seasonality of vitamin-D status. Among immigrants from tropics to Europe, vitamin D deficiency have been suggested to contribute to increased risk of TB reactivation (45). Asians living in London have a high prevalence of both vitamin D deficiency and tuberculosis, partly due to a decline in immunity secondary to reduced vitamin-D status on passing from a country where sunlight is plentiful, to one where sunlight is sparse (47). The persons with the lowest levels of vitamin D have the highest incidence of tuberculosis, and patients with no detectable value for vitamin D had a tenfold higher incidence of tuberculosis than patients with low levels of vitamin D.A higher incidences of TB has been reported in Central Africa, S. Africa and Russia during the winter and early spring (47).

### 1.5.3. Vitamin D and HIV infection

Research done suggests that vitamin D enhances innate immunity. The active form of Vitamin D acts by binding to nuclei receptors on target cells. Therefore both low levels of the vitamin and abnormalities in receptor structure and function may result in impairments in host immunity to the infectious agent such as tubercle bacillus (48) and also augment the progression of disease such as HIV/AIDS. More recent research has found evidence that one of the most potent metabolites of
vitamin D, 1, 25-dihydroxyvitamin D3, is often deficient in people with advanced HIV infection, low CD4 T cells count and high blood levels of TNF alpha (49). Deficiency of this particular vitamin D metabolite is a feature of other immunological disorders.

The knowledge on the potential role for Vitamin D on HIV infection is important. For example, Micronutrients supplementation has been shown to improve CD4T-cell counts in HIV-positive individuals (50). The review of observational studies by Villamor et al showed an inverse association between 1,25(OH)₂D concentrations and mortality has been reported from a small cohort of HIV-infected adults, and some cross-sectional studies have indicated positive correlations between 1,25(OH)₂D and CD4 T cell counts (51). More contradictory findings have also been seen in bimonthly administration of 100,000IU of cholecalciferol to 64 HIV infected children and adolescents where no group differences were seen in the change in CD4 count or CD4% or viral load during 12 months, however the overall mean monthly serum 25-hydroxyvitamin D concentrations were higher in the group that received vitamin D than in the placebo group (52).

The effects of vitamin D on HIV infection in vitro have been examined in terms of its potential role on monocyte/macrophage function and on HIV expression and replication in monocytes/macrophages. Starting with monocyte/macrophage function, one study of monocytes from 10 AIDS patients showed that incubation with 1,25(OH)₂D resulted in significant increases in chemotaxis (51). In another experiment, 1, 25-(OH)₂D tended to decrease the number of M. avium in macrophages from HIV-positive patients, whereas the opposite effect was seen in macrophages of HIV-negative controls (53). These findings suggest that 1,25-(OH)₂D could enhance some macrophage functions such as the respiratory burst in cells from HIV-positive persons, or it may have a direct effect against M. avium replication in these subjects.

In another study on the effect of active form of vitamin D (1,25(OH)₂D) on monocyte function showed that vitamin D improved growth and maturation of monocyte from both HIV-infected and uninfected patients, but among the former, the improvements in vitro were greater in patients with low CD4 cell counts or symptomatic disease compared with those at less-advanced stages (54). No effects were observed in monocytes from the sickest subjects, suggesting that very severe monocytic dysfunction may not be responsive to 1, 25 (OH)₂ D.

Active human tuberculosis is associated both with HIV infection and vitamin D deficiency (55). Vitamin D obtained from food supplements and skin is hydroxylated to 25-(OH)-D in the liver and
further hydroxylated to the more active metabolite 1,25(OH)$_2$D in the kidney. Studies have also shown low levels of 1, 25(OH)$_2$D in patients with HIV infection (49) however, the level of 25(OH)D was normal in this study. Hydroxylation 25-(OH)-D to 1,25(OH)$_2$D also occurred in macrophages and it has been suggested that the low level of 1,25(OH)$_2$D found in HIV patients with serious immunodeficiency may be due to deficient macrophage function also manifesting itself as lack of granuloma formation in patients with HIV/AIDS and tuberculosis co-infection (56). The active form of vitamin D is a key player in the clearance of pathogens and influences the level of inflammation and macrophage activation (57).

The genetic predisposition of the host and the virus is the most important determinant for prediction and understanding the course of HIV-1 viral infection and AIDS progression. Transcription from the HIV-1 long terminal repeat (LTR) is a crucial step for viral replication. The findings from the study done in Spain showed that vitamin D binding receptors can activates the HIV-1 LTR through different mechanisms, including non-classical nuclear receptor transcriptional actions that may ensure viral transcription under different physiological scenarios (58). These findings suggest that the vitamin D receptor may play a role during the infectious process and in the progress of AIDS in patients.

Studies reporting the concentration of vitamin D metabolites among HIV infected subjects offer an opportunity to examine vitamin D deficiency in the course of HIV disease. In a study of 22 HIV infected individuals (not on ARV) conducted in Spain, half of them had AIDS, the average 25-(OH)-D and 1,25-(OH)$_2$D concentrations were 16.5 ug/L and 35.8 ug/L respectively (59). In another study done in German the mean concentration of 1,25-(OH)$_2$D was significantly lower among both men and women infected with HIV compared with men and women who were uninfected. However, 25-(OH)-D was nonsignificantly lower in HIV infected men and women compared with HIV negative controls (60). Similar findings of low 1,25-(OH)$_2$D levels were also observed in a study conducted in Norway among 54 HIV infected patients compared with healthy control however serum level of 25-(OH)-D and VDR were normal in this study (49).

The possible explanations for the above discrepancy; Since the 25-(OH)-D level, an indicator from diet and skin was not affected by HIV status, the low concentrations of 1, 25-(OH)$_2$D in these studies could have been due to a defect in the 1-alpha hydroxylation of 25-(OH)-D into 1,25(OH)$_2$D which normally occurred in the kidney in response to low 1,25-(OH)$_2$D or PTH.
Alternatively, the low 1, 25-(OH)_2D could be due to increased utilization for maturation and proliferation of T-lymphocytes during HIV infection.

The efficacy of highly active antiretroviral therapy (HAART) in inhibiting HIV replication and improving morbidity and mortality of HIV infection is unquestionable. This progress in therapy, however, is not without problems. All components of HAART regimens can have major acute and long-term toxicities. A high prevalence of bone demineralization occurs in people living with HIV/AIDS. Protease inhibitors (PIs) are potent inhibitors of the cytochrome P450 enzyme system. Reported studies conducted in the human hepatocyte and monocyte, showed that PIs impair hepatocyte 25-(OH)-D3 (61;62) and macrophage 1, 25-(OH)_2D3 synthesis in a reversible, dose-dependent manner. And furthermore, PIs inhibit 1, 25-(OH)_2D3- degradation in macrophages with lower potency than that elicited on 1-hydroxylase(61).

Vitamin D and calcium play important roles in the proper mineralization of bone for optimal skeletal health and immune function (63). In patients with immune reconstitution inflammatory syndrome (IRIS) hypercalcemia has been found and thought to be due to improvement of macrophage function, granulomatous reaction and increased hydroxylation of 25-(OH)-D to 1,25-(OH)_2D (56).

Osteoporosis is most commonly associated with inadequate calcium intakes, but insufficient vitamin D contributes to osteoporosis by reducing calcium absorption (64). Although rickets and osteomalacia are extreme examples of the effects of vitamin D deficiency, osteoporosis is an example of a long-term effect of calcium and vitamin D insufficiency. Adequate storage levels of vitamin D maintain bone strength and might help prevent osteoporosis in older adults, non-ambulatory individuals who have difficulty exercising, postmenopausal women, and individuals on chronic steroid therapy (65). A study conducted in Netherland found the use of both NNRTI and PIs in HIV patients is associated with higher levels of PTH suggesting that it might be a risk factor for bone problem (66).

1.5.4. Studies in Tanzania

Not much has been done on vitamin D deficiency among TB/HIV patients in Tanzania and again these few studies were done in the urban settings. In randomized clinical trial done in Tanzania on multivitamin and micronutrient formulations containing doses of vitamin D, have demonstrated declines in morbidity and mortality when given to HIV-positive patients, especially those with co infected with M. tuberculosis (67), however it was difficult to know the specific effect of vitamin D.
In another study done in Northern part of the country (Mwanza city) found low concentration levels of vitamin D in PTB positive patient as compared with PTB negative and no association on vitamin D level found among HIV positive patients. However higher concentration of vitamin D for both conditions was found during the harvest seasons (68) showing that there is seasonal variation of serum vitamin D levels.

1.5.5. Contradictory research findings

The currently available data from studies in humans regarding the potential value of vitamin D as adjunctive therapy in bacterial infection (TB) remain conflicting. Three of the 4 TB trials (38-40), demonstrated positive outcomes, although these studies were hampered by major limitations, such as poor sample size and limited information regarding the effectiveness of the repletion strategy. The most recent (2009) and the most rigorously designed trial of the series, reported by Wejse et al (41) demonstrated no clear benefit of adjunctive vitamin D therapy in TB treatment.

There is limited and contradictory evidence about the effect of vitamin D on the immune system. Some studies findings suggest that high levels of vitamin D may actually have an immune suppressive effect and that it may stimulate HIV replication and AIDS progression (58). On the other hand, the active form of vitamin D has also been shown to stimulate macrophages, white blood cells which combat opportunistic infection such as *Mycobacterium tuberculosis* (29,69).

More recent research has found evidence that one of the most potent metabolites of vitamin D, (1, 25-dihydroxyvitamin D3), is often deficient in people with HIV, especially those with advanced disease (49). Deficiency of this particular vitamin D metabolite is a feature of other immunological disorders, and some experts argue that it is an important part of a fully functioning immune system.

CD4 T cells are most important in immune response against *Mycobacterium tuberculosis* (70) as well as monitoring HIV disease progression and response to anti- retroviral therapy (ART) (71,72). Some studies done showed low 1,25(OH)D3 levels were associated with low CD4 counts, immunological hyperactivity and AIDS progression (49,60), while other studies did not showed the effects (59,66). For example a cross-sectional study done in 13 USA Cities found no association between HIV infection and vitamin D status among HIV positive and negative individuals (73). And a more recent study done in Switzerland found low 1,25(OH)2D level is correlated with higher CD4 and Previous AIDS (62).
Many of the above studies were done in the Western world. It is reasonable and ethical to do this study under controlled conditions in Tanzania where the ecology of diseases (HIV/TB), vitamin D deficiency and the genetic of the population are different.

1.6. Problem statement
There was a probability to suspect high prevalence of Hypovitaminosis D in Haydom, since it is situated in an economically deprived area with problems of recurrent drought and land displacement, within an economically deprived country. Nationwide, the number of TB/HIV cases is rising. Both conditions contribute 17.5% of the entire disease burden in Tanzania affecting mainly the age group 15-45 years. The two conditions account for about 64% of the estimated years lost to those who require long term care and management (74). Half of the TB patients are co-infected with HIV accounting for 60 -70% of the increase TB cases in the country. Agriculture is the backbone of the Tanzanian economy, and most agricultural workers are in the same age group affected by both diseases, leading to negative effect on the growth of National economy due to absenteeism and reduced productivity (7).

The successful use of combined antiretroviral therapy (cART) has led to a decrease in HIV related mortality, noninfectious diseases associated with older age, such as cardiovascular and neoplastic diseases (62). Because many of these diseases are modulated by vitamin D, we believe that the minimal vitamin D target levels suggested for the general population should also be met by HIV-positive patients, although the effect of vitamin D has been poorly evaluated in HIV-infected individuals and no target level has been validated.

Current anti-TB/ARV chemotherapies, although effective, are associated with many side effects and are limited in treating drug-resistant strands. Treatment of HIV-TB co-infection is complex and associated with high pill burden, overlapping drug toxicities, risk of immune reconstitution inflammatory syndrome (IRIS) and challenges related to adherence (24). The burden of TB might further increase by reactivation threats hovering over millions harboring latent infection, thus, calling for novel approaches for this terrible disease. In recent years, the non-calcemic physiological actions of vitamin D have drawn a great deal of attention.

Although many studies have been conducted in the western world, to what extent the findings of such studies can be applicable in the third world context such as Tanzania is questionable, given the fact that the ecology of the two diseases, Vitamin D deficiency, and population genetic are different. Review of the available numerous observational studies on the potential role of vitamin
D deficiency have shown contradictory findings. These findings suggest the need for more observational studies to confirm the associations between vitamin D status, TB and HIV disease progression. More research will provide useful insights on the potential role of vitamin D supplementation to HIV-infected persons and better planning of intervention trials (51).

1.7. Rationale.

1.7.1. Reason for conducting the study
Vitamin D is essential for body immune function. Assessment data are needed to plan appropriate programme. Evidence based findings are essential for effective programming, but reliable data on the level of vitamin D status among HIV/TB patients are currently limited in Tanzania especially the rural settings.

1.7.2. Significance of the study
This study assessed the level of vitamin D status in Haydom from a local/ rural point of view. A locally appropriate operational definition for the term “Vitamin D status” is of value to all individuals and groups working with HIV/TB patients in this area, for example, MoHSW, social welfare officers, teachers and health care workers. The findings help them to identify patients with Vitamin D deficiency and to plan appropriate action. When programmes are well planned, using locally relevant, up to date data, implementation is likely to be more effective than when international or national guidelines are followed without appropriate adaptation.

If HIV/TB patients are properly cared for and their health is promoted in all aspects, including physical, psychological, social health and Vitamin D supplementation, this may help to improve the treatment outcome, and increase the chances of these people becoming physically and psychologically fit individuals able to contribute to society e.g. economic growth of the country.

1.8. Objectives

1.8.1 Broad Objective
Assessment of interaction between vitamin D, calcium metabolism and parathyroid hormone in HIV-1 monoinfected and HIV/TB co infection patients in Tanzania rural settings.

1.8.2 Specific Objectives

1) To determine the mean serum levels of vitamin D among HIV/AIDS, and HIV/TB co infection patients.

2) Determine the risk factors associated with Vitamin D status among HIV infected patients
3) To determine the association between vitamin D, serum calcium, and PTH among the following groups
   i) HIV on ARV
   ii) HIV pre ARV
   iii) HIVTB co infection
   iv) Healthy subjects

4. Study the association between vitamin D status and CD4 cell counts among HIV and HIV/TB patients.

5. Study the association between ARV and Vitamin D metabolism/status

1.8.3. Research questions
1. How common is vitamin D deficiency in HIV positive individuals in rural Tanzania?

2. Is there a difference of vitamin D deficiency between treated and untreated HIV-infected individuals?

3. Is vitamin D level lower in HIV/TB co infected patients than HIV without TB?

4. What is the consequence of vitamin D on calcium metabolism?

2. Methodology

2.1. Study Area and population
Tanzania is divided into 29 administrative regions and 130 administrative districts. Each region is composed of districts. Manyara region lies between latitudes 3° 40 min and 6° 0 min S and longitude 33° and 38° E. It is in the north-east of the country bisected into two by the Great Rift Valley, and is composed of Babati, Mbulu, Hanang, Simanjiro and Kiteto districts, (figure 1). Simanjiro, Kiteto and part of Babati districts lie east of the great rift wall while Mbulu and another part of Babati District remain bracketed between two great walls. The remaining part of Babati District and the whole of Hanang District lie west of the two great walls. The town of Babati is the regional centre for Manyara. Haydom, where this proposed study is conducted, is situated in Mbulu district.

We conducted this cross-sectional study in a rural church owned hospital practice (Haydom Lutheran Hospital) at a Latitude 5°,5min, and longitude 32°, 50min; at an altitude of 1700m in Manyara Region. The Region has a tropical climate with an average annual relative humidity of 44.6%. There are four distinct seasons; the long (Feb-Apr) and short (Nov-Jan) rainy seasons with an average rainfall of about 400 and 150 millimetres annually respectively. The harvest season
occur from May to July and Postharvest season start in August and last in October. The average temperature is 23°C (73°F), with the highest temperature sometimes reaches 32°C (90°F) around noon in October, while the lowest may fall below 15°C (59°F) in the early morning of late June. The average sunlight hours in Manyara region range between 6.9 hours per day in January and 10.0 hours per day in July and August. In general, the study population was unveiled, with their faces and often lower arms or lower legs exposed to the sun.

Manyara region is not only the home of the Great Rift Valley and numerous livestock; it also contains Tanzania’s most interesting and distinctive indigenous ethnic groups. The Iraqw of Mbulu and their cousins the Gorrowa of Babati as well as the Alawa and Burunge of Kondoa are a unique group in Tanzania. The only similar ethnic groups are found far away in Ethiopia among the Oromo. The Hadzabe of Yaeda Valley in Mbulu District who number only about 1,500 are also distinctive in that as a group, they have the smallest stature in Tanzania. They still live on hunting and the collection of wild honey, fruits and roots. The only similar ethnic group is that of the Bushmen of the Kalahari Desert in Namibia. The region is also home to Mainland’s greatest concentration of the Barbaig, the Ndorobo/Akea and the Maasai who are historically, the most warlike ethnic groups.

The Region had a population of 1,040,461 (534,565 males & 505,896 females), with a growth rate of 3.8 and an average household size of 5.2 persons, and a relatively low population density of 23 persons per square kilometre. Mbulu district had a population of 237,882, of whom only 19,121 were living in urban areas (http://www.tanzania.go.tz/census/). In 2002, Haydom ward had an average household size of 6.2. In 2002, the area designated as Haydom rural area had a total population of 18,362, while Haydom urban area had a total population of 4,551, giving a total of 22,913 in Haydom ward as a whole. The median age of the population of all of Haydom ward was found to be 17.5 years.

Haydom village is about 80 km from Mbulu, the district administrative centre, and about 300 km from the nearest urban centre of Arusha. It is in a rural situation, reached by poor quality roads (figure 2), and has suffered from drought and famine in recent years. It is situated on a ridge between two rift valleys at an altitude of over 1700m above the sea level, in an area that only 50 years ago was populated by game animals, and was largely uninhabited by humans because of Tsetse fly. Settlement in the area was encouraged by the British Colonial Rule of that time. Many of the villagers of Haydom survive solely by subsistence farming (maize & wheat); some also work in
small retail outlets, small grain mills, primary and secondary schools, and the hospital, but there are no industries. This reflects the occupational patterns of Mbulu district, in which 76,343 out of a population of 82,950 were farmers or livestock keepers according to the 2002 Tanzania census (http://www.tanzania.go.tz/census/regions/htm). Most people in the study population ate several types of staple foods such as maize, cassava, potato, rice, millet, white barley and cow meat with sunflower oil being mostly used for cooking. Fish was also the animal food that was eaten occasionally. Rural area was defined as an isolated area of the country with limited access to social services and poor standard of living conditions.

2.2. Haydom Lutheran Hospital

Haydom Hospital is a 400-beds capacity hospital in Mbulu District, owned by the Evangelical Lutheran Church in Tanzania. The hospital serves a population of about 250,000 from the surrounding three divisions. The hospital has facilities for tuberculosis and HIV/AIDS diagnosis, treatment and monitoring.

![Road through Magara Mountain to Haydom Lutheran Hospital](image)

Figure 2: Road through Magara Mountain to Haydom Lutheran Hospital.
2.3. Treatment and monitoring

2.3.1 Treatment of HIV
It was estimated in 2000 that 400,000 Tanzanians have advanced HIV infection and would benefited from antiretroviral therapy (ART). The Tanzania National HIV/AIDS Five-Year Strategic Plan of 2001-2006 called for the development of a national antiretroviral therapy (ART) program for HIV-infected people in need of HIV treatment. Scale up of care and treatment services continued throughout 2006 and 2007 and by September 30, 2007, 119,302 HIV-infected people had been initiated on ART at 210 Care & Treatment Clinics (CTCs) located in referral, regional, district, private and mission hospitals.

In response to the Government strategies, Haydom Lutheran Hospital established HIV Care and Treatment Centre adjacent to the hospital in October 2003. Majority of the patients enrolled were detected through VCT services in the villages, OPD or in patients department (IPD) tested on clinical suspicion e.g. the tuberculosis patients. Patients were considered eligible for ART if they were in WHO stage IV irrespective of CD4 cell count, WHO stage III with CD4 ≤ 350 cells/ìL, or had CD4 ≤ 200 cells/mm$^3$ regardless of clinical stage, in accordance with WHO and Tanzanian guidelines (10;75).
HIV patients eligible to ART were put on first-line treatment comprised of two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI). The combination include; stavudine (d4T) or zidovudine (ZDV), combined with lamivudine (3TC), and either nevirapine (NVP) or efavirenz (EFV). Second-line treatment in case of treatment failure was abacavir (ABC), didanosine (ddl) and ritonavir-boosted lopinavir (lpv/r). Patients with CD4 < 200 cells/mm³ irrespective of HIV disease stage were given co-trimoxazole prophylaxis 960 mg thrice weekly or 480 mg daily.
2.3.2. Treatment of tuberculosis

Direct Observed Treatment Short Course (DOTS) strategy with the aim of; cure TB patients, preventing death from active TB, preventing further transmission of TB to the community, and preventing the development of drug resistance due to inadequate drug therapy was been used. Four combination of anti tuberculosis drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) were prescribed. The treatment regimen has an initial phase which is also called the intensive phase, and a continuation phase. The initial phase (facility DOTS, which lasts for two months, patients were hospitalized while the continuation phase (community DOTS), which last for four months, patients took drugs while observed with one member of the community. Co-trimoxazol preventive therapy (CPT) was as provided as trimethoprim 80 mg/ sulfamethoxazole 400 mg two times per day to TB patients infected with HIV.

After the initial 2 weeks and 2 months of daily ARV and anti-TB drug administration respectively, both antiretroviral and anti-TB drugs were dispensed on a monthly basis.

2.4. Study subjects

We carried out this study within the framework of the National Tuberculosis and Leprosy programme (NTLP) (76) and National Aids Control programme (NACP) (10) with diagnosis and treatment of HIV (75) and TB (77) patients conducted in accordance with recommended procedures. Diagnosis of HIV infection was established using 2 different rapid antibody tests. And diagnosis of pulmonary tuberculosis (PTB) was established using sputum smear microscopy and chest x-ray.

In brief patients suspected to have TB from in and out patients department were asked to submit 3 sputum (spot - morning – spot) for examination of acid fast bacilli (AFB) using Zielh-Neelsen staining. A definitive diagnosis of PTB was made when patients met the following criteria; (a) at least two positive AFB smears from the two different sputum smears; and (b) One positive AFB smears and typical results of lung infection on chest X-ray.

HIV test was done using 2 different rapid antibody tests, among the following: Determine HIV-1/2 (Abbott laboratories, Abbott Park, IL, USA), Capillus HIV-1/2 (Trinity Biotech, Bray, Co Wicklow, Ireland)(78), SD bioline (Standard Diagnostic, INC, Hagal-Dong, Korea), and Unigold (Trinity Biotech, PLC, Bray, Ireland) (79). Concordant positive results were interpreted as positive for HIV antibody. Discordant results were interpreted as inconclusive. However the discordant results were solved with a third tie-breaker rapid test, usually Unigold.
The healthy controls were randomly recruited from the individuals attending VCT and relatives accompanied the patients. Subjects were excluded from the study if they had diseases deemed to affect vitamin D metabolism such as, Diabetic, Hypertension, liver and renal failure. Subjects were also excluded if found using drugs that might affect serum vitamin D, such as ketoconazole and anticonvulsants. In addition, individual with prolonged immobility (over one month) were not recruited to the study. The recruitment of cases and healthy control group was taken place at approximately during the same period.

2.5. Sampling
Sample size calculation

From the review of studies on serum vitamin D level by Nnoaham and his colleges, a study done in Kenya showed TB cases and controls had median vitamin D level 40 nmol/l, and 66 nmol/l respectively, while that of Thailand TB cases had a mean vitamin D level of 70 nmol/l (SD 25) and controls 96 nmol/l (SD 29) (80). One more study from Thailand among adolescent and young adults showed the mean plasma 25(OH)D did not differ significantly between the HIV-positive 20.3 nmol/L (SD 1.1) and control (19.3 nmol/L (SD 1.7) subject (73). For comparison of mean vitamin D levels between groups using computer software Russ lenth’s power and sample size calculator: 26 in each group gave 81% power (Alfa=0.05) to detect a difference of 20 nmol/l (50 compared to 30) with a SD of 25. An additional 20% was included to account for attrition (the non-response) rate rendering minimum 31 in each group. Physical and laboratory blood samples were done to;

All randomly selected HIV-infected patients attended Care and Treatment Clinic during the study period, including at least 31 on ARV, 31 pre-ARV and 31 HIV/TB co-infected patients making 93 of the total sample size required. In some patients with TB, blood sample was drawn before/after start of anti-TB therapy.

At least 50 healthy individuals from normal population were recruited from the same site by using selection criteria to make HIV positive and HIV negative group comparable with regards to risks behavior profile such as age, sex, and tribe/ethnicity. Finally our study included a total of 159 subjects, among these 71, 41 and 47 were HIV, HIVTB and healthy controls respectively.
However, due to limited time, we only managed to include 14 HIVTB coinfected patients not on anti-TB drugs not reaching the minimum number of 26 required.

2.6. Inclusion and exclusion criteria
The present study included all HIV positive and Pulmonary Tuberculosis’ patients who attended Haydom Lutheran Hospital between July 20, 2010, and January 11, 2011. HIV positive women who were pregnant at the time of recruitment were excluded from the study, as were lactating mothers in WHO stage I or II, who started ART exclusively to prevent vertical transmission. Subjects with diabetes, hypertension, chronic liver (AST>200, or ALAT>225 IU/liter) and kidney (Creatinine>220 µmol/liter) disorders (66) as well as patients who refused consent and lacked blood sample for vitamin D test were also excluded from this study. Extra pulmonary TB as well as patients found using anticonvulsants were also excluded.

2.7 Data Collection

2.7.1. Participants Recruitment
Patients attending Care and Treatment Centre (CTC) department at Haydom Lutheran Hospital (HLH) were seen on a first come first serve basis. An estimate of more than 800 patients attended the CTC clinic during the study period. These include patients who come three monthly for laboratory checkup, drugs collection, and newly HIV, TB diagnosed. Each person was given a CTC/VCT number and a patient card, which then waits until their number was called to be seen by the clinician. A purposive sampling method meeting the criteria during the data collection period was applied. Due to the fact that HIV/TB coinfected patients were relatively few, a roaster list of patient planned to visit the clinic the day after was used to identify patients with coinfection (HIV/TB). To every HIV/TB patient identified, two HIV patients were then selected randomly. The list (ID numbers) of the patients selected was then handled to the clinician on duty the day after.

When the potential patient arrived, the examining clinician referred him/her to the researcher. The researcher queried their interest in participating in this study. If interested, the researcher reviewed eligibility criteria and explained the purpose of the study, method which included free viral loads, vitamin D and parathyroid hormone test, duration, benefits and risks as part of the informed consent. Adequate time was given for the participant to consider participation and asking questions if any. A written informed consent was obtained from all individuals agreed to participate. A study ID number was then allocated.
2.7.2. Abstraction of medical records

A standard data abstraction tool relating to anthropometric and clinical history was developed and used to abstract required information from the patients’ files/charts and HIV/AIDS treatment and monitoring database. The following data were recorded: duration and kind of antiretroviral/ Anti-TB therapy, CD4 / CD8 T cell counts, Viral load, Complete Blood Cells count (CBC) and blood chemistries (Creatinine, albumin, AST and ALT.), demographic data such as (age, sex, height and weight), duration and stage of HIV infection [according to WHO clinical criteria], HIV and ARV duration were calculated from the date confirmed HIV positive and date started ARV respectively to the date when blood sample for vitamin D analysis was taken. And age was calculated from the date of birth to the date when blood taken for vitamin D and PTH. Height without shoes (in centimeters) was measured to the nearest 0.1 cm by a mounted stadiometer. Weight without shoes and light sleeveless clothing was measured (to the nearest 0.1kg) on a beam balance scale with built in stadiometer. Body mass index (BMI) was then derived as the ratio of weight in (kg) over height square in (m²).

2.7.3. Specimen collection & Processing

Under aseptic techniques, we performed routine venous puncture. Using a serum separator tube (SST; Becton Dickinson, Franklin Lakes, NJ, USA), 7mls non fasting whole blood was drawn and left to clot for about 30 minutes, followed by centrifugation for 12-15 minutes to obtain 4mls of serum required divided into two and kept separately. The first 2ml was needed for analysis of vitamin D 25-(OH)-D, 1,25-(OH)₂D and intact parathyroid hormone. The second 2ml was used for locally test of calcium, phosphorus, and albumin. We used a sterile pipette to transfer serum to plastic tube. Immediately, the transferred serum was kept in - 60⁰c freezer for about six months and was then transported to Norway.

Viral load and blood chemistry (calcium, albumin and phosphorus) were tested locally at Muhimbili National referral Hospital (MNH). Since calcium is unstable and sensitive to changes in PH; we kept it air-tight and frozen upside down. Other laboratory measurements that we checked locally at Haydom Lutheran Hospital include liver function enzymes (Aspartate & Alanine transaminases), serum Creatinine, absolute CD4 & CD8 cells count, and Complete Blood Cells counts (CBC).
2.7.4. Specimen storage
We stored our samples at -60°C freezer for about six months. This is supported by the literature which showed serum samples can be stored in freezer -20 or -80 degrees up to 6 months for calcium, and more than one year for vitamin D (81) and PTH (82).

2.7.5. Specimen transport
Using cool box (2 – 8°C), the second 2mils serum was transported to Muhimbili National referral Hospital (MNH) in Dar es Salaam for calcium, albumin and phosphorus testing. Samples arrived MNH 12hrs later the same day. The samples were then re-frozen at -20°C for two weeks.

Since there was no capacity to do laboratory analysis of Vitamin D and PTH in Tanzania, a material transfer agreement was signed to allow transport of blood samples (2mils serum) from Tanzania to Norway. We organized transport in order to avoid unnecessary freeze-thaw (but ok for vitamin D (81), and for PTH (82). We kept the serum for both vitamin D and PTH in a cool box (2 – 8 degrees) for 8 hours, then room temperature for 11 hours. The samples arrived Norway the next day, (more than 8 hours at room temperature recommended for PTH, but according to reference above, arrival on the next day was ok however we couldn’t test all the samples for PTH the same day. The samples were then refrozen in -20°C freezer for one month.

2.8. Laboratory analysis

2.8.1. Hematological and Chemistry analysis
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 × [10⁹] per liter), erythrocytes (number of cells × [10¹²] per liter), platelets (number of cells × [10⁹] per liter), lymphocytes (number of cells × [10⁹] per liter), mononuclear cells (number of cells × [10⁹] per liter), granulocytes (number of cells × [10⁹] per liter) and hemoglobin (grams per deciliter). The quality and accuracy of the technique and the machine was assessed every six months.

Serum calcium, serum phosphorus and serum albumin were quantified by standard methods using automated Architect c800 System (Abbot Diagnostic, USA). And serum creatinine, serum aspartate and alanine transaminasases were measured using Roche COBAS c 111 clinical analyzer (Roche diagnostic, Rotkreuz, Switzerland). The machine analyses and automatically calculates the analyte concentrations in each sample. Corrected calcium was calculated as follows; (Calcium) – [0.025 × (albumin)] + 1.
2.8.2. CD4/CD8 T cell counts analysis
Cluster differential cells (CD4 and CD8 T cells) were analyzed using a FACSCount flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). In brief, 50 μl of whole blood was mixed and incubated at room temperature for 20 min with 20 μl of aCD4 and aCD8. Red blood cells were then lysed by adding 450 μl of fluorescence-activated cell sorter lysing solution (Becton Dickinson Immunocytometry Systems). The tubes were incubated at room temperature for 10 min, and then analyzed with the FACSCount’s Cell Quest software (Becton Dickinson Immunocytometry Systems) within six hours. By using quality control (Multicheck; Becton Dickinson Immunocytometry Systems), the accuracy of the technique was assessed every 6 months.

2.8.3. HIV-1 RNA viral load
HIV-1 RNA viral load was not a routine test in Haydom Lutheran Hospital, but we performed as part of this study in all HIV-1 positive patients. We collected blood on plasma preparation tubes (PPT; Becton Dickinson, Franklin Lakes, NJ, USA) by venous puncture and centrifuge within 30 minutes. Plasma was immediately transferred to sterile plastic tubes and stored at -60°C. Manufacture’s instructions were followed with regards to sample collection, storage and transport. Viral load was measured at Muhimbili National Hospital, Tanzania, using the Cobas Amplicor HIV-1 Monitor v 1.5 (Roche Diagnostics) with a detection limit at 400 copies/ml.

2.8.4. Vitamin D and parathyroid hormone analysis
These analyses for vitamin D and PTH were performed at Hormone Laboratory of Endocrinology, Oslo University Hospital, which is a reference laboratory in Norway for hormone analyses. We measured total serum 25-(OH)-D and 1,25-(OH)_{2}D by Diasorin competitive radioimmunoassay (RIA) (Diasorin, Stillwater, MN, USA). Prior to analysis, the serum samples were precipitated and extracted with acetonitrile. 1,25-(OH)_{2}D was further purified by C18OH chromatography. After centrifugation, an aliquot of the supernatants were incubated with specific antibodies against 25(OH)D and 1,25(OH)_{2}D for 90mins and 2hours respectively at an ambient temperature. After centrifugation, the supernatants were decanted and counted. The 25-(OH)-D interassay and intrassay coefficients of variation (CV) were 13% at 38 nmol/l and 8% at 53 nmol/l respectively, and for 1,25-(OH)_{2}D the interassay and intrassay coefficients of variation (CV) were 17% at 73 pmol/l and 11% at 57 nmol/l respectively.
We measured serum intact parathyroid hormone (PTH) by automated clinical chemistry analyser (Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA). The intra-assay coefficient of variation (CV) was 9% at 98pmol/l and the inter-assay (CV) was 5% at 4.4pmol/l.

Definition of Vitamin D and elevated PTH levels

Vitamin D was defined as; serum 25(OH)D levels ≤ 75 nmol/l was used to define hypovitaminosis, and serum 25(OH)D ≤ 25, 25 – 50, and 50 – 75 nmol/l defined severe vitamin deficiency, mild vitamin D deficiency and vitamin D insufficiency, respectively(83). Elevated PTH levels were defined as serum intact PTH more than 6.5 pmol/liter.

2.9. Ethical consideration

The Ethics Committee of the National Institute for Medical Research in Tanzania granted permission to conduct the study and the Regional Committee for Medical Research Ethics (REK) in Norway recommended it. Written informed consent was obtained from all study participants before inclusion. Patients diagnosed with liver and kidney disorders were referred for further evaluation. Pre-HIV test counseling was given to all volunteers and post-test counseling was offered to those who wanted to know their HIV test results. Those who tested HIV positive were referred to Treatment and Care Clinic for further management. The free viral load test results for HIV patients helped the clinicians in decision making regarding the management of these patients, whether to start or change ARV regimen. All information obtained from the patients were recorded in abstraction form 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 accessed the files.

2.10. Data management

The principle investigator abstracted and filled the information obtained from the patients file to the abstraction forms. Immediately following completion of abstraction form, the researcher double checked the instruments for completeness and consistency of answers. Completed abstraction forms were then coded by numbers and entered in Microsoft excel sheet version 2003. Cross-checking and data cleaning was done. During data cleaning and cross checking missing information were obtained by going back to the abstraction form, HIV treatment and monitoring database and when necessary reviewing the patients on the next visit to the clinics. The data were then transferred to PAWS version 18 for analysis.
2.11. Statistical analysis

All analyses were performed with PAWS software for Windows, version 18 (PAWS Inc., Chicago, IL, version 18.0, USA). Normal probability plots were used to assess the distribution of continuous variables. Descriptive statistics were computed. Values in the text are means ± SD or means (95% CI) unless otherwise indicated and statistical significant was considered when P < 0.05. The 2-sample t test or 1-way ANOVA was used to test for differences in means between 2 or more groups and the chi-square test was used to test for differences in proportions.

When distributions were not normal, continuous variables were compared between 2 groups by the Mann - Whitney rank-sum test or between 3 or more groups with a nonparametric Kruskull-Wallis test.

Calculation of Pearson/ spearman’s rank correlation coefficients and linear regression were performed to asess the relationships between key variables. Because of skewness (that is, the lognormal nature) of PTH, we transformed PTH by \( \log_{10} \) its scores and assessed the correlation of continuous predictors of it with the parametric (Pearson) correlation.

Regression Analyses:

Logistic regression analyses were used to study the associations with hypovitaminosis D; and linear regression analysis was used to study differences in mean serum vitamin D concentrations.
3. Results


The skewness values for HIV, HIV/TB and healthy groups are positive and close to zero indicating that very few of the patients’ serum vitamin D scores were clustered to the left at the low values. The kurtosis values in HIV positive and healthy group was negative and that of HIV/TB group was positive and both were close from zero indicating the distribution of HIV and health group were relatively flat (many cases in the extremes) and HIV/TB group distribution was rather peaked. The Kolmogorov-Smirnov statistic test in both cases were insignificant indicating no violation of the assumptions of normality, hence the distribution was normal in HIV, HIV/TB patients and healthy individuals, figure 4.

**Figure 4.** Box and whiskers plot showing the distribution of serum 25(OH)D scores for HIV, HIV/TB and healthy groups. Box plots show mean (horizontal line in center of each box), 25th and 75th percentiles (bottom and top of each box), and 10th and 90th percentiles (bottom and top of error bars). Data are shown below separately for HIV (n = 71), HIV/TB (n = 41) and healthy (n = 47).
3.2. Demographic and biochemical characteristics

A total of 175 participants were available for the study; ten HIV-1 monoinfected patients didn’t supply blood for vitamin D and parathyroid hormone, three healthy controls had their blood samples contaminated, and two HIVTB coinfected patients had their serum sample tubes broken during transport. The final study sample included 159 (53.5% were females) subjects of whom HIV, HIV/TB coinfected, and healthy controls were 71 (44.7%), 41 (25.8%), and 47 (29.5%) respectively. The average age was 35.5 ± 11.3 years and their BMI was 20.9 ± 4.0. Other biochemical measures with their means (standard deviation) or medians (interquartile range) includes: serum calcium (sCa) 1.7 ± 0.4, albumin corrected calcium (scCa) 2.7 ± 0.4, serum phosphorus (sPsi) 1.3 ± 0.4, serum albumin (sAlb) 26.7 ± 0.2, serum parathyroid hormone (sPTH) 3.1 (1.5 – 5.3), and serum Creatinine (sCr) 62.6 ± 21.6.

3.3. Serum vitamin 1, 25-(OH)₂D

The mean serum 1, 25-(OH)₂D was 166.6 (67.4) pmol/l for all subjects. There was a considerable variation by age group and marital status in serum 1, 25-(OH)₂D concentration Table 3. Post hoc comparisons using the Tukey HSD test revealed subjects group with age less than 25 yrs had higher level of serum 1,25-(OH)₂D than age group 35 – 45 and more than 45 yrs; P = 0.043, 0.002 respectively. Similarly age group two (25 – 35 yrs) subjects had higher level of serum 1, 25-(OH)D than age group four ( > 45 yrs). The difference was leaning towards statistical significance (P = 0.062). Post hoc analysis also revealed single individuals had higher level of serum 1,25-(OH)D than married and separated individuals; P value = 0.002 and 0.008 respectively. Categories of sex, BMI and season did not differ, Table 3.
Table 3. Serum 1,25 (OH)\textsubscript{2}D by category of demographic variables and season among all subjects (n = 159).\textsuperscript{1}

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>%</th>
<th>Serum 1,25(OH)\textsubscript{2}D nmol/l</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td>0.481</td>
</tr>
<tr>
<td>Males</td>
<td>74</td>
<td>46.8</td>
<td>162.7 (61.1)</td>
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<tr>
<td>Females</td>
<td>84</td>
<td>53.2</td>
<td>170.9 (68.2)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<td>0.002</td>
</tr>
<tr>
<td>&lt; 25</td>
<td>29</td>
<td>18.4</td>
<td>199.0 (83.9)</td>
<td></td>
</tr>
<tr>
<td>25-35</td>
<td>52</td>
<td>32.9</td>
<td>173.8 (53.6)</td>
<td></td>
</tr>
<tr>
<td>35-45</td>
<td>46</td>
<td>29.1</td>
<td>158.1 (72.3)</td>
<td></td>
</tr>
<tr>
<td>&gt;45</td>
<td>31</td>
<td>19.6</td>
<td>136.6 (48.6)</td>
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</tr>
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<td></td>
<td></td>
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<td>63.3</td>
<td>158 (63.9)</td>
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<tr>
<td>Single</td>
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<td>22.2</td>
<td>202.3 (74.7)</td>
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<tr>
<td>Separated/Widowed</td>
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<td>14.5</td>
<td>149.3 (52.4)</td>
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<td>BMI(kg/m\textsuperscript{2})</td>
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<tr>
<td>Normal (18.5 – 25)</td>
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<td>63.9</td>
<td>160.5 (72.4)</td>
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<tr>
<td>Overweight (bmi &gt; 25)</td>
<td>17</td>
<td>11.8</td>
<td>165.5 (59.4)</td>
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<tr>
<td>Seasons (Months)</td>
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<td></td>
<td></td>
<td>0.699</td>
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<tr>
<td>Long rain(Feb-Apr)</td>
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<tr>
<td>Harvest(May-July)</td>
<td>47</td>
<td>29.7</td>
<td>166.5 (70.3)</td>
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<tr>
<td>Postharvest(Aug-Oct)</td>
<td>84</td>
<td>53.2</td>
<td>163.5 (61.5)</td>
<td></td>
</tr>
<tr>
<td>Short rain(Nov-Jan)</td>
<td>27</td>
<td>17.1</td>
<td>176.2 (74.4)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} Data are n (%) and means (standard deviation) for each category and P-value based on t test or 1-way ANOVA, one patient had undetectable serum 1,25-(OH)\textsubscript{2}D.

\textsuperscript{2} Married includes cohabiting, and single were never married.

3.4. Serum vitamin 25-(OH)-D

The mean serum 25-(OH)-D was 72.4 ± 20.7 nmol/l for all subjects. Overall hypovitaminosis D was present in 60.4%; 13.2% had deficiency (< 50 nmol/l) and 47.2% had insufficiency (50-75 nmol/l). Serum 25-(OH)-D concentration did not differ between men (73.1 ± 20.7) and women (71.8 ± 20.9); P = 0.695 nor did the proportion with hypovitaminosis D which was 63.7% and 62% respectively; P = 0.922. Serum 25-(OH)-D concentrations vary by season and are typically greater in the summer and fall than in the winter and spring due to variation in sun exposure (73). This seasonal effect was also observed in the present study. There was a considerable variation by season in serum concentration of 25-(OH)-D; P = 0.037. Post hoc analysis revealed that subjects
enrolled during the postharvest (Aug-Oct) season differ significantly with short rain season (Nov-Jan); \( P = 0.034 \). Categories of sex, age, marital status and BMI did not differ; Table 4.

Table 4. Serum 25-(OH)-D by category of demographic variables and season among subjects (\( n = 159 \)).

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
<th>Serum25(OH)D nmol/l</th>
<th>P</th>
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<td>72.7 (20.6)</td>
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<tr>
<td>25-35</td>
<td>52</td>
<td>32.7</td>
<td>72.8 (21.4)</td>
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<td>35-45</td>
<td>46</td>
<td>28.9</td>
<td>72.6 (18.3)</td>
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<td>&gt;45</td>
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<td>71.8 (20.9)</td>
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<td>11.7</td>
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<td><strong>Seasons (Months)</strong></td>
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<td>29.6</td>
<td>70.9 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Post-harvest (Aug-Oct)</td>
<td>84</td>
<td>52.8</td>
<td>75.9 (20.8)</td>
<td></td>
</tr>
<tr>
<td>Short rain (Nov-Jan)</td>
<td>28</td>
<td>17.6</td>
<td>64.6 (22.2)</td>
<td></td>
</tr>
</tbody>
</table>

1. Data are \( n \) (\%) and means (standard deviation) and \( P \)-value based on t test or 1-way ANOVA.

2. Married includes cohabiting, and single were never married.
Post harvest (Aug-Oct) differ with Short rain (Nov-Jan), $P = 0.034$

Demographic and biochemical variables of the three distinct groups

The demographic data for the three distinct groups are given in table 5, and that there was no statistically significant between groups with regards to sex and BMI distribution. Significant variation of mean serum 25-(OH)-D levels had also been observed in different type of infections; Table 5. Post hoc comparison test revealed that, subjects with HIV infection differed significantly with HIV/TB-coinfected ($77.2 \pm 20.8$ vs $63.2 \pm 23.8$); $P = 0.003$. However post hoc analysis revealed no significant difference noted between healthy controls and HIV group ($P = 0.734$) and HIV/TB coinfected and health controls, $P = 0.095$. We investigated whether the duration of TB treatment was important to vitamin D status, and found positive and negative average correlation between serum 25-(OH)-D, 1,25-(OH)$_2$D and the weeks passed since the start of the four-drug treatment regimen (Spearman’s $r = 0.34$, $P = 0.048$; $r = -0.40$, $P = 0.018$) respectively.
Table 5. Anthropometric and biochemical variables in HIV HIV/TB patients and healthy subjects (n = 159)\textsuperscript{1}

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV (n = 71)</th>
<th>HIV/TB (n = 41)</th>
<th>Healthy (n = 47)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>35.8 (12.2)</td>
<td>38.9 (9.7)</td>
<td>32.1 (10.4)</td>
<td>0.042\textsuperscript{a}</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>20.9 (4.1)</td>
<td>19.9 (3.4)</td>
<td>20.1 (3.2)</td>
<td>0.137</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>77.2 (20.8)</td>
<td>63.2 (23.8)</td>
<td>73.3 (14.8)</td>
<td>&lt;0.001\textsuperscript{b}</td>
</tr>
<tr>
<td>1,25(OH)D (pmol/l)</td>
<td>171.9 (72.4)</td>
<td>131.7 (53.5)</td>
<td>189.0 (59.3)</td>
<td>&lt;0.001\textsuperscript{c}</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>3.4 (1.8 – 5.3)</td>
<td>2.4 (1.1 – 5.1)</td>
<td>3.5 (1.8 – 5.7)</td>
<td>0.146</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>1.9 (0.4)</td>
<td>1.6 (0.5)</td>
<td>1.7 (0.4)</td>
<td>0.006\textsuperscript{d}</td>
</tr>
<tr>
<td>Albumin correct Ca</td>
<td>2.8 (0.4)</td>
<td>2.5 (0.5)</td>
<td>2.7 (0.3)</td>
<td>0.009\textsuperscript{e}</td>
</tr>
<tr>
<td>Phosphorus (mmol/l)</td>
<td>1.1 (0.2)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>0.095</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>31.3 (10.0)</td>
<td>23.5 (10.4)</td>
<td>25.8 (10.0)</td>
<td>&lt;0.001\textsuperscript{f}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are mean, standard deviation except PTH, median (Interquartile range) and P value based on 1-way ANOVA or Kruskal-Wallis test.

\textsuperscript{a}Healthy controls differ with HIV/TB , P =0.042

\textsuperscript{b}HIV group differ with HIV/TB P = 0.003

\textsuperscript{c}HIV group differ with HIV/TB P = 0.006

\textsuperscript{d}HIV group differ with HIV/TB group, P = 0.007

\textsuperscript{e}HIV group differ with HIV/TB group, P = 0.008,

\textsuperscript{f}HIV group differ with HIV/TB, & Healthy controls, P = 0.003, & 0.04 respectively

3.5. Anthropometrics and biochemical variables in HIV and HIV/TB groups not on anti TB drugs.

Most of the subjects in HIV/TB group were on anti-TB drugs and rifampicin and isoniazid has already been documented to have a decreasing effect on serum vitamin D (84). To avoid the
interference of anti-TB drugs in our analysis, we selected 14 HIV/TB co-infected subjects who had not yet started anti-TB drugs and compared with 71 HIV-1 mono-infected patients. The demographic and biochemical characteristics for the two distinct groups are given, Table 6, and that there was no statistically significant difference between groups with regards to age and BMI. In the HIV group, serum 25-(OH)-D was significantly higher than the group with coinfection (HIV/TB); Table 6. Using multiple linear regression, adjusting for sex, age, BMI and season, the overall equation explained approximately 13% of the variation in serum 25-(OH)-D concentrations ($R^2 = 0.129; n = 85$), the coefficient for HIV and HIV/TB status was significantly different from zero ($P = 0.015$), which indicated that mean serum 25-(OH)-D for HIV/TB was significantly lower than HIV group. Other biochemical variables did not differ between groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD) HIV(n = 71)</th>
<th>HIV/TB(n = 14)</th>
<th>MD (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(yrs)</td>
<td>35.8(12.2)</td>
<td>39.4 (10.2)</td>
<td>-3.5 (-10.4 – 3.4)</td>
<td>0.313</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>21.3(4.5)</td>
<td>19.8(2.6)</td>
<td>1.5 (1.2 – 1.0)</td>
<td>0.239</td>
</tr>
<tr>
<td>25(OH)D(nmol/l)</td>
<td>77.2 (20.8)</td>
<td>62.2 (14.3)</td>
<td>15.0 (5.6– 24.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>1,25(OH)₂D(pmol/l)</td>
<td>171.9 (72.4)</td>
<td>164.6 (61.8)</td>
<td>7.3 (-33.9 – 48.6)</td>
<td>0.723</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>3.4 (1.8 – 5.3)</td>
<td>3.5 (1.8 – 5.7)</td>
<td>-</td>
<td>0.728</td>
</tr>
<tr>
<td>Calcium(mmol/l)</td>
<td>1.9(0.4)</td>
<td>1.7 (0.7)</td>
<td>0.2 (-0.3 – 0.6)</td>
<td>0.429</td>
</tr>
<tr>
<td>Corr Cal(mmol/l)b</td>
<td>2.8(0.4)</td>
<td>2.7 (0.6)</td>
<td>0.1 (-0.3 – 0.6)</td>
<td>0.425</td>
</tr>
<tr>
<td>Phosphorus(mmol/l)</td>
<td>1.1 (0.2)</td>
<td>1.1 (0.2)</td>
<td>0.0 (-0.1 – 0.2)</td>
<td>0.717</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>31.3 (10)</td>
<td>30.1 (7.3)</td>
<td>1.2 (-5.7 – 6.9)</td>
<td>0.853</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.2 (2.4)</td>
<td>13.1 (1.7)</td>
<td>0.1 (-1.4 – 1.5)</td>
<td>0.915</td>
</tr>
<tr>
<td>Creatinine (umol/l)</td>
<td>62.3 (17.9)</td>
<td>54.7 (18.2)</td>
<td>7.6 (-3.1 – 18.2)</td>
<td>0.163</td>
</tr>
</tbody>
</table>

1Data are means (standard deviation) or median (Q3 – Q5) for each category P-value based on Independent samples t-test or Mann-Whitney U test. MD, mean difference.

3.6. Comparison of hypovitaminosis D in HIV and HIV/TB not on anti TB

We observed hypovitaminosis D in 78.6% (11/14) of the HIV/TB coinfect patients and in 52.1% (37/71) of the HIV monoinfected patients; the odd ratio (OR) of hypovitaminosis D was 3.4 (95%
CI: 0.9, 13.1, P = 0.08, in HIV/TB coinfected patients compared with HIV monoinfected patients. In both HIV/TB co-infected and HIV monoinfected patients, the majority of those with hypovitaminosis fell into the insufficiency category (25(OH)D 50-75 nmol/l). We observed vitamin D deficiency (25(OH)D ≤ 50 nmol/l) in only 2 of the 14 HIV/TB coinfected patients (14.3%) and in 9 of 71 HIV patients (12.7%), Table 9. The proportions of various degree of lack of vitamin D in the two groups are displayed in Figure 5.

**Figure 5.** Distribution of the degree of Hypovitaminosis D among HIV-1 and HIV-1 coinfected with tuberculosis patients.
3.7. Vitamin D in HIV patients

3.7.1. Clinical manifestation

We found a statistically significant association between clinical stage of HIV disease and serum 1,25-(OH)\(_2\)D, however no significant association found in serum 25-(OH)-D; Table 7. Post hoc analysis revealed that, HIV-1 infected patients with clinical stage (WHO) four had significantly lower serum levels of 1,25-(OH)\(_2\)D than stage one individuals, \(P = 0.023\), and stage three was marginally significant different with HIV stage one, \(P = 0.056\). We examined the question of whether HIV stage affected/predicted serum 1,25-(OH)\(_2\)D concentrations in this study using multiple regression analysis, controlling for sex, age, CD4 cells count, use of HAART and season. The overall equation explained approximately 15% of the variation in serum 1, 25(OH)\(_2\)D concentrations (\(R^2 = 0.145\); \(n =74\)), the coefficient for HIV stage was statistically significantly different from zero (\(P= 0.013\)), which indicated that HIV stage was significantly associated with serum 1,25-(OH)\(_2\)D concentrations. In both analyses, HIV/TB patients on anti-TB drugs were excluded.

Table 7. Clinical manifestation of HIV-1 infection on serum vitamin D\(^1\).

<table>
<thead>
<tr>
<th>Clinical stage (WHO 2007)</th>
<th>N (%)</th>
<th>Mean 25-(OH)-D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage one</td>
<td>12 (16.0)</td>
<td>73.2 (17.5)</td>
<td>0.638</td>
</tr>
<tr>
<td>Stage two</td>
<td>16 (21.3)</td>
<td>78.6 (16.2)</td>
<td></td>
</tr>
<tr>
<td>Stage three</td>
<td>13 (17.3)</td>
<td>73.5 (21.5)</td>
<td></td>
</tr>
<tr>
<td>Stage four</td>
<td>34 (45.3)</td>
<td>73.3 (19.6)</td>
<td></td>
</tr>
</tbody>
</table>

**Mean 1,25(OH)D*\**

<table>
<thead>
<tr>
<th>Clinical stage (WHO 2007)</th>
<th>N (%)</th>
<th>Mean 1,25(OH)D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage one</td>
<td>12 (16.2)</td>
<td>222.8 (104.2)</td>
<td>0.021</td>
</tr>
<tr>
<td>Stage two</td>
<td>16 (21.6)</td>
<td>185.9 (55.7)</td>
<td></td>
</tr>
<tr>
<td>Stage three</td>
<td>13 (17.6)</td>
<td>152.4 (60.0)</td>
<td></td>
</tr>
<tr>
<td>Stage four</td>
<td>33 (44.6)</td>
<td>143.7 (59.9)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Data are n (%), mean (standard deviation) and P-value based on one way ANOVA, ten subjects had their clinical stages missing.

*one subject had undetectable serum 1,25(OH)\(_2\)D
Because the increased immune activation of HIV infection can increase requirements for some nutrients (85), we examined the question of whether HIV status affected serum 25-(OH)-D and 1,25 (OH)D concentrations in this study. Using multiple regression analysis, we adjusted for sex, season, and age to determine whether HIV status was a significant predictor of serum 25(OH)D and 1,25(OH)D concentrations.

Although the overall equation explained approximately 10% of the variation in serum 1, 25(OH)D concentrations ($R^2 = 0.103; n = 85$), the coefficient for HIV status was marginally significantly different from zero ($P = 0.054$), which indicated that HIV status was marginally significantly associated with serum 1,25-(OH)$_2$D concentrations. For serum 25-(OH)-D concentration, the overall equation explained 1.4% of the variation in serum 25-(OH)-D concentrations ($R^2 = 0.014; n = 84$), the coefficient for HIV status was not significantly different from zero ($P = 0.576$), which indicated that HIV status had no significant association with serum 25-(OH)-D concentrations.

There were clinically significant difference in median duration of known HIV infection, duration of known using Antiretroviral therapy (ART), and platelet cells count among patients who were vitamin D (25[OH]D) sufficient, insufficient and deficient, ($P = 0.009, 0.03$ and $0.022$) respectively. Median HIV duration 28.4, 141.7 and 48 weeks, ARV duration 49.4, 122.8 and 150.9 weeks, platelet cell count 313.6, 229.9 and 243.1 ($* 10^3/µl$) in deficiency, insufficiency and sufficiency respectively. Post hoc analysis revealed higher platelet cell count in vitamin D deficiency group than insufficient and sufficient groups, $P = 0.017, 0.047$ respectively.

3.7.2. Vitamin D and the immune stage

A one way between groups analysis of variance was conducted to explore the impact of immune stages (1993 CDC criteria) on the levels of serum 25-(OH)-D and 1,25-(OH)$_2$D. In this analysis all patients including those on HAART and without were included. There was no statistical significant difference at the $P < 0.05$ level in serum 25-(OH)-D and 1,25-(OH)$_2$D for the three immune stages; $P = 0.681$ and $0.335$ respectively.

When patients on HAART were excluded in the analysis, There was a marginally statistically significance difference at a $P < 0.05$ in serum 1,25-(OH)$_2$D for the three immune stages, $P = 0.062$ Table 8. However, we found no statistical significant difference between immune stages and serum 25-(OH)-D. The post hoc comparisons using the Tukey HSD test revealed that, subjects with CD4 cells count between 200 – 500 cells/mm$^3$ had higher serum 1,25-(OH)$_2$D$_3$ concentrations than subjects with CD4 cells count less than 200 cell/mm$^3$. Subjects with CD4 cells $> 500$ cells/mm$^3$
did not differ significantly from either group with CD4 < 200 or CD4 200 – 500 cells/mm³, P = 0.800, 0.342 respectively.

Table 8. Immune stage of ART-naïve HIV-1 infected patients by serum vitamin D¹.

<table>
<thead>
<tr>
<th>Immune stage (1993 CDC)</th>
<th>N (%)</th>
<th>Mean 25(OH)D (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 &lt; 200 cell/mm³</td>
<td>19 (50.0)</td>
<td>74.2 (26.7) 0.242</td>
</tr>
<tr>
<td>CD4 200 - 500 cell/mm³</td>
<td>11 (28.9)</td>
<td>86.3 (15.4)</td>
</tr>
<tr>
<td>CD4 &gt; 500 cell/mm³</td>
<td>8 (21.1)</td>
<td>70.4 (16.6)</td>
</tr>
</tbody>
</table>

Mean 1,25(OH)D

<table>
<thead>
<tr>
<th>Immune stage (1993 CDC)</th>
<th>N (%)</th>
<th>Mean 1,25(OH)D (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 &lt; 200 cell/mm³</td>
<td>19 (50.0)</td>
<td>137.6 (63.9) 0.062</td>
</tr>
<tr>
<td>CD4 200 - 500 cell/mm³</td>
<td>11 (28.9)</td>
<td>199.7 (58.1)</td>
</tr>
<tr>
<td>CD4 &gt; 500 cell/mm³</td>
<td>8 (21.1)</td>
<td>155.6 (84.0)</td>
</tr>
</tbody>
</table>

¹Data are n (%), mean (standard deviation) and P-value based on one way ANOVA,

3.8. Effect of HAART on Vitamin D

Of all 71 HIV-1 monoinfected subjects, 62% (44/71) were on antiretroviral treatment. Use of HAART has been documented to have a decreasing effect on hepatocyte serum 25-(OH)-D and macrophage 1,25-(OH)₂D levels (66;86). We found higher mean serum levels of both 25 (OH)D and 1,25 (OH)₂D among HIV positive patients not on HAART compared with those on HAART, but the difference did not reach statistical significant; 81.4 (20.5) vs 74.7 (20.7) nmol/l and 182.8 (67.1) vs 165.1 (75.5) pmol/l, P = 0.190, 0.323 respectively. We also investigated whether the duration of HAART treatment was important to vitamin D status, and found weak positive association between serum 25(OH)D, 1,25(OH)₂D and the weeks passed since the start of the HAART regimen (Pearson’s r = 0.27, P = 0.033; r = 0.12, P = 0.368) respectively.

3.8.1. Comparison of Hypovitaminosis D in HIV-1 infected patients on ARV and without ARV

In a univariate logistic regression analysis, we observed hypovitaminosis D in 63.6% (28/44) of the HIV patients on treatment and in 33.3% (9/27) of HIV patients not on antiretroviral therapy. The odd ratio (OR) of hypovitaminosis D was 3.5 (95% CI: 1.3 – 9.6), P = 0.015 in HIV patients on treatment compared with HIV patients not on treatment, Table 9. We observed serum vitamin D deficiency (25-(OH)-D ≤ 50nmol/l in 6 of 44 HIV patients (13.6%) on treatment and in 3 of 27
HIV patients (11.1%) not on treatment. Furthermore, we observed higher vitamin D insufficiency (25-(OH)-D 50 - 75nmol/l) in half (50%) of HIV patients on treatment compared with 6 of 27 HIV patients (22.2%) not on treatment; Table 9. The proportions of various degrees of lack of vitamin D in the two groups are displayed in **Figure 6**

In multivariate logistic regression analysis, controlling for age, sex, BMI and season, hypovitaminosis D remained significantly higher among HIV patients on treatment compared to HIV patients not on treatment, Table 9.

**Figure 6.** Distribution of the degree of Hypovitaminosis D among HIV infected patients on and without ARV.
Table 9. Crude and adjusted odds ratio of various subject groups for different vitamin D categories.

<table>
<thead>
<tr>
<th>Subject groups</th>
<th>Overall N</th>
<th>n (%)</th>
<th>OR² (95%CI)</th>
<th>OR³ (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypovitaminosis (25(OH)D ≤75 nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>71</td>
<td>37 (52,1)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HIV/TB</td>
<td>14</td>
<td>11 (78,6)</td>
<td>3,4 (0,9 - 13,1)**</td>
<td>2,6 (0,6 - 10,7)</td>
</tr>
<tr>
<td>HIV</td>
<td>71</td>
<td>37 (52,1)</td>
<td>0,7 (0,3 – 1,4)</td>
<td>0,3 (0,1 – 1,0)*</td>
</tr>
<tr>
<td>Healthy</td>
<td>47</td>
<td>29 (65,9)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HIV/TB</td>
<td>14</td>
<td>11 (78,6)</td>
<td>3,5 (0,2 – 60,5)**</td>
<td>2,4 (0,2 – 27,2)</td>
</tr>
<tr>
<td>Healthy</td>
<td>47</td>
<td>29 (65,9)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HIV without ARV</td>
<td>27</td>
<td>9 (33,3)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HIV with ARV</td>
<td>44</td>
<td>28 (63,6)</td>
<td>3,5 (1,3 - 9,6)</td>
<td>3,7 (1,1 - 12,3)*</td>
</tr>
</tbody>
</table>

| Deficiency (25[OH] ≤ 50nmol/l) |           |           |                 |                 |
| HIV                     | 71        | 9 (12,7)  | 1               | 1               |
| HIV/TB                  | 14        | 2 (14,3)  | 1,1 (0,2 - 6,0) | 1,1 (0,2 - 6,4) |
| HIV                     | 71        | 9 (12,7)  | 6,7 (0,8 – 54,6)** | 2,5 (0,2 – 34,7) |
| Healthy                 | 47        | 1 (2,1)   | 1               | 1               |
| HIV/TB                  | 14        | 2 (14,3)  | 7,7 (0,4 – 91,8) | -               |
| Healthy                 | 47        | 1 (2,1)   | 1               | -               |
| HIV without ARV         | 27        | 3 (11,1)  | 1               | 1               |
| HIV with ARV            | 44        | 6 (13,6)  | 1,3 (0,3 - 5,5) | 1,1 (0,2 - 7,5) |

1OR, odds ratio, ARV, antiretroviral drugs, HIV, human immune virus; HIVTB, HIV coinfected with tuberculosis.
2 Univariate logistic regression analysis of variables against various levels of 25 (OH)D.
3 Logistic regression analysis with variables adjusted for age, sex, BMI, and season.
*Significant, **marginal significant.

Our subjects were on first line treatment which comprised of two nucleoside reverse transcriptase inhibitors (NRTIs) stavudine (d4T) or zidovudine (ZDV), combined with lamivudine (3TC), and one non-nucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine (NVP) or efavirenz.
(EFV). In univariate analysis based on either nevirapine or efavirenz and stavudine or zidovudine regimens, we found a statistically significant association of zidovudine with lower serum level of the most potent metabolite of vitamin D (1,25-(OH)₂D) compared with stavudine, P = 0.007. However there was no significant different based on either Nevirapine or Efavirenz regimen. Both groups had no effect on serum 25-(OH)-D concentration.

**Hypovitaminosis D**

Using the criteria of 25-(OH)-D < 75 nmol/l, there was no significant difference of hypovitaminosis D among nevirapine and efavirenz as well as among stavudine and zidovudine based regimens, OR = 1.6 (95% CI, 0.5 – 5.0) and 1.1 (95% CI, 0.4 – 3.5) respectively. However, vitamin D deficiency (25(OH)D < 50 nmol/l) was significantly common among zidovudine based regimen than stavudine based regimen, and this remained significant following adjustment with sex, Table 10.
Table 10. Crude and adjusted odds ratio for hypovitaminosis D in 62 HIV patients infected on highly active antiretroviral therapy

<table>
<thead>
<tr>
<th></th>
<th>Overall N</th>
<th>N with vit D &lt; 50 nmol/l</th>
<th>OR(^2)(95% CI)</th>
<th>OR(^3)(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26</td>
<td>6 (23.1)**</td>
<td>5.0 (0.9 – 26.9)</td>
<td>5.2 (0.9 – 31.2)**</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>2 (5.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>4</td>
<td>1 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 - 35</td>
<td>15</td>
<td>1 (6.7)</td>
<td>0.2 (0.0 – 4.5)</td>
<td></td>
</tr>
<tr>
<td>35 - 45</td>
<td>29</td>
<td>4 (13.8)</td>
<td>0.5 (0.0 – 5.8)</td>
<td></td>
</tr>
<tr>
<td>&gt; 45</td>
<td>13</td>
<td>2 (15.4)</td>
<td>0.5 (0.0 – 8.3)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI(kg/M2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18.5</td>
<td>11</td>
<td>1 (9.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 - 25</td>
<td>40</td>
<td>7 (17.5)</td>
<td>2.1 (0.3 – 19.4)</td>
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</tr>
<tr>
<td>&gt; 25</td>
<td>8</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TB infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>5 (11.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>3 (16.7)</td>
<td>1.5 (0.3 – 7.2)</td>
<td></td>
</tr>
<tr>
<td><strong>TB treatment</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11</td>
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<td>0.3 (0.0 – 3.5)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>2 (28.6)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Season</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>39</td>
<td>5 (12.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postharvest</td>
<td>20</td>
<td>3 (15.0)</td>
<td>1.2 (0.3 – 5.6)</td>
<td></td>
</tr>
<tr>
<td>Short rain</td>
<td>2</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD4 cells count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>11</td>
<td>2 (22.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 - 500</td>
<td>33</td>
<td>4 (11.4)</td>
<td>0.5 (0.1 – 3.1)</td>
<td></td>
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<tr>
<td>&gt; 500</td>
<td>18</td>
<td>2 (11.1)</td>
<td>0.4 (0.1 – 3.8)</td>
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<tr>
<td><strong>HAART regimen</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nevirapine based</td>
<td>37</td>
<td>5 (13.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efavirenz based</td>
<td>25</td>
<td>3 (12.0)</td>
<td>0.9 (0.2 - 4,3)</td>
<td></td>
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<tr>
<td>Stavudine based</td>
<td>37</td>
<td>2 (5.4)</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Zidovudine based</td>
<td>25</td>
<td>6 (24.0)</td>
<td>6.0 (1.1 – 33.1)*</td>
<td>6.7 (1.1 – 39.8)*</td>
</tr>
</tbody>
</table>

\(^{1}\) OR, odds ratio, HAART, highly active antiretroviral therapy, HIV, human immune virus; TB, tuberculosis.\(^*\)Significant, \(^**\)marginally significant.

\(^{2}\) Univariate logistic regression analysis of variables against hypovitaminosis D.

\(^{3}\) Logistic regression analysis adjusted for variables with P-value less than 0.100, (sex)
3.8.2. Elevated PTH levels and effect of ARV use in HIV-1 patients

For one patient (with normal 25-(OH)-D) the PTH level was missing. Overall, 18 patients (18/111, 16.2%) had elevated PTH levels. Among patients with hypovitaminosis, 14 patients (14/67, 20.9%) had elevated PTH levels. Two patients (2/27, 7.4%) without treatment, and twelve patients (12/40, 30%) received anti retroviral therapy. Patients treated with ARV had a significantly higher risk of developing elevated PTH levels in addition to hypovitaminosis D (OR 0.756, 95%CI (0.601 – 0.951), P = 0.026).

Among the patients with normal vitamin D levels, 4 patients (4/44, 9.1%) developed elevated PTH levels; no patient (0/23, 0%) without treatment, four patients (4/21, 19.0%) received ARV. There was no significant difference in prevalence of elevated PHT levels among these treatment groups (OR 4.05, 95%CI (0.984 – 16.674), P = 0.095).

3.9. Predictors of hypovitaminosis (serum 25[OH]D) < 75nmol/l) in 85 HIV-1 infected subjects

Sex, age, BMI, viral load, CD4 cells counts and clinical stage of the disease had no effect on hypovitaminosis D, Table 11. In univariate logistic regression analysis, subjects recruited during postharvest (Aug - Oct) and short rain (Nov - Jan) seasons had lower and higher risk of hypovitaminosis respectively compared with harvest (May - July) season. Tuberculosis infection, use of highly active antiretroviral drugs, and elevated parathyroid hormone were found to predict hypovitaminosis in a univariate logistic regression analysis; however when variables with a P-value < 0.1 in univariate analysis were advanced into a multivariable model using the forward stepwise method, none of them remained significant predictor of hypovitaminosis. In both analysis, HIV/TB coinfected patients on anti-TB drugs were excluded.
Table 11. Crude and adjusted odds ratio for hypovitaminosis D in 85 patients infected with HIV

<table>
<thead>
<tr>
<th></th>
<th>Overall N</th>
<th>N with vit D &lt; 75nmol/l</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
<td>19 (54.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>29 (58.0)</td>
<td>1.2 (0.3 - 4.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>11</td>
<td>5 (45.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>25 - 35</td>
<td>26</td>
<td>13 (50.0)</td>
<td>1.2 (0.3 - 4.9)</td>
<td></td>
</tr>
<tr>
<td>35 - 45</td>
<td>33</td>
<td>22 (66.7)</td>
<td>2.4 (0.6 - 9.6)</td>
<td></td>
</tr>
<tr>
<td>&gt; 45</td>
<td>15</td>
<td>8 (53.3)</td>
<td>1.4 (0.3 - 6.5)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/M²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>&lt; 18.5</td>
<td>16</td>
<td>9 (56.3)</td>
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<td></td>
</tr>
<tr>
<td>18.5 - 25</td>
<td>55</td>
<td>31 (56.4)</td>
<td>1.0 (0.3 - 3.1)</td>
<td></td>
</tr>
<tr>
<td>&gt; 25</td>
<td>10</td>
<td>6 (60.0)</td>
<td>1.2 (0.2 - 5.8)</td>
<td></td>
</tr>
<tr>
<td><strong>TB infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>71</td>
<td>37 (52.1)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>11 (78.6)</td>
<td>3.4 (0.9 - 13.1)**</td>
<td>3.4 (0.9 – 12.0)**</td>
</tr>
<tr>
<td><strong>Use of ARV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>12 (40.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>55</td>
<td>36 (65.5)</td>
<td>2.8 (1.1 - 7.1)*</td>
<td>2.1 (0.5 – 9.6)</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>43</td>
<td>28 (65.1)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Postharvest</td>
<td>36</td>
<td>15 (41.7)</td>
<td>0.4 (0.2 - 0.9)*</td>
<td>1.5 (0.4 – 6.3)</td>
</tr>
<tr>
<td>Short rain</td>
<td>6</td>
<td>5 (83.3)</td>
<td>2.7 (0.3 - 25.1)</td>
<td>0.6 (0.0 – 8.1)</td>
</tr>
<tr>
<td><strong>CD4 cells count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>15</td>
<td>8 (53.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>200 - 500</td>
<td>42</td>
<td>23 (54.8)</td>
<td>1.1 (0.3 - 3.5)</td>
<td></td>
</tr>
<tr>
<td>&gt; 500</td>
<td>22</td>
<td>13 (59.1)</td>
<td>1.3 (0.3 - 4.7)</td>
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</tr>
<tr>
<td><strong>WHO HIV stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>12</td>
<td>6 (50.0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Two</td>
<td>16</td>
<td>8 (50.0)</td>
<td>1.0 (0.2 - 4.5)</td>
<td></td>
</tr>
<tr>
<td>Three</td>
<td>13</td>
<td>8 (51.5)</td>
<td>1.6 (0.3 - 7.8)</td>
<td></td>
</tr>
<tr>
<td>Four</td>
<td>34</td>
<td>23 (67.6)</td>
<td>2.1 (0.5 - 8.0)</td>
<td></td>
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<tr>
<td><strong>Viral Load (copies/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 400</td>
<td>49</td>
<td>32 (65.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt; 400</td>
<td>28</td>
<td>14 (50.0)</td>
<td>0.5 (0.2 - 1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>PTH (pmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt; 6.5</td>
<td>66</td>
<td>36 (52.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt; 6.5</td>
<td>18</td>
<td>12 (80.0)</td>
<td>3.7 (1.0 - 14.2)*</td>
<td></td>
</tr>
<tr>
<td><strong>HB (g/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 12</td>
<td>56</td>
<td>30 (53.6)</td>
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<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>26</td>
<td>16 (61.5)</td>
<td>1.4 (0.5 - 3.6)</td>
<td></td>
</tr>
</tbody>
</table>

¹OR, odds ratio, ARV, antiretroviral drugs, HIV, human immune virus; HIV/TB, HIV coininfected with tuberculosis.*Significant, **marginally significant.
Univariate logistic regression analysis of variables against hypovitaminosis D.

Logistic regression analysis adjusted for variables with P-value less than 0.1 (TB infection, ARV and season)
4. Discussion

This study describes the serum levels of vitamin D status among HIV-1 monoinfected and HIV/TB-coinfected patients in the Northern part of the United Republic of Tanzania. Serum 25-(OH)-D and 1,25-(OH)₂D associations with infections and antiretroviral therapy were determined.

4.1 Methodological discussion

4.1.1. Discussion of the study design

This study was a cross sectional study, and the design should be appropriate for the study questions, because we set out to determine serum vitamin D levels among HIV-1 positive and HIV -1 coinfected with pulmonary tuberculosis (88). With regards to the sample size, we calculated based on the study design to minimise the chance of committing a type two error (89). Assessment of normality of the distribution of scores for continuous variables such as age, BMI, serum 25(OH)D and 1,25(OH)₂D showed that the samples were normally distributed for both groups.

4.1.2. Discussion on bias

Bias is a systematic error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure/disease association(90). Information bias is a distortion in the measure of association caused by inaccurate information or measurement that can result from things such as poor interviewing techniques or differing level of recall by respondents (90). Although using a structured face to face interview is the most effective method in gathering high quality information, our study adopted a retrospective design, and hence, was subject to weaknesses inherent to retrospective studies namely that some important information such as for example occupation, education were not available in the charts we reviewed.

Selection bias is a distortion that results from procedures used to select subjects and from factors that influence study participation (90). These results were obtained from a non randomized sample of individuals attending Haydom Lutheran Hospital; selection bias may thus be present.

Furthermore the distribution of characteristics of the patients reported to hospital during the study period could be different from those who did not. Therefore, generalization of the finding could be inappropriate. To reduce selection bias introduced by staffs in the clinics, we enrolled all tuberculosis patients who were found to be pulmonary tuberculosis during the study period. However, this was not possible for the HIV-1 monoinfected patients.
Furthermore, bias due to selection may thus have been introduced in this study because we invited some of the HIV/TB relatives to participate as healthy controls. This group was at high risk of being infected compared with the individual from the general population, though they didn’t have symptoms of these diseases during the enrolment period. This problem may thus be overcome by random selection of individuals from the general population.

4.1.3. Strengths

Researcher as enumerator

All abstraction of information from the patients’ charts/files, data entry and cleaning was conducted by the researcher himself. This has had a positive impact bearing on the reliability of the results in that, the researcher was well familiar with the study objectives/questions and ensured that correct information in the light of the questions/objectives of the study, was obtained. This also provided the researcher with some qualitative insights through observations; which have been useful in interpreting the findings in the discussion.

The inclusion of healthy controls which provided us with the reference serum level of vitamin D was another added strength in this study. Furthermore, serum vitamin D levels were determined in an international reference recognized laboratory (Hormone Laboratory department of Endocrinology, Oslo University), ensuring high precision and reliability.

4.1.4. Limitation

A limitation of our study is that, though we included healthy controls for reference vitamin D status which increased the strength of our study, ruling out obvious co-morbid conditions by clinical screening and laboratory investigations such as HIV rapid test and smear microscopy, we might had missed conditions such as latent tuberculosis infection and also HIV infection during the window period because our HIV rapid tests can not pick up HIV antibodies before 2 weeks to 3 months following infection. We might also erroneously have excluded some of the participants because of over-reporting of clinical symptoms used as screening tool for inclusion.

4.1.5. Sample and representativeness

Haydom Lutheran Hospital is an old mission which has benefited from supports from partners such as Norway, and as such might not be representative of all rural hospitals in Tanzania. Certain advanced equipment available in Haydom, such as computer tomography (CT scan) is definitely unavailable in most rural hospital in the country.
Although we only included a total of seventy one HIV patients and forty one HIV coinfected with tuberculosis patients in our study, we believe that we have, through our sampling, achieved a fairly adequate diversity among subjects; in addition, the results we found were largely similar and repeatable among subjects. On other hand, our study included patients from an area where the majority rely on subsistence farming and pastoralism, a typical of rural Africa.

Further more Haydom Lutheran Hospital is faced with some logistical challenges as many rural hospitals in the country due to its remote location and high patients load. Although the hospital is a church owned, both TB and HIV programmes are fully integrated in National Tuberculosis and Leprosy programme (NTLP) and National AIDS Control Programme (NACP) respectively under the Ministry of Health and Social Welfare. Moreover, approximately more than half of the hospital beds and rural health services in Tanzania are operated by church and thus, being a church owned is not atypical.

Clearly, hospitals in Tanzania differ hugely, and our findings are not necessarily applicable to all settings. However we believe our findings may better represent the situation in rural Africa than studies carried out in larger urban cities.

4.2 Results discussion

4.2.1. Summary of the main findings
Our results show four major findings: First, Hypovitaminosis D was common in all subjects. HIV/TB coinfected patients had lower serum 25(OH)D compared with HIV monoinfected and healthy controls. Second, Mean serum 25(OH)D did not differ significantly between HIV positive and HIV negative subjects nor was HIV stage a significant predictor of serum 25(OH)D when multivariate regression analysis was used to adjust for other variables, however the lower level of the most potent metabolite of Vitamin D (1,25[OH]$_2$D) was associated with advanced stage of HIV disease, may be due to reduced hydroxylation of 25(OH)D to 1,25(OH)$_2$D in the macrophages. Third, HIV patients on treatment had higher prevalent of hypovitaminosis compared with HIV naïve, and fourth, subjects enrolled during post harvest season had low risk of hypovitaminosis compared with others.
Hypovitaminosis (VDI and VDD) occurred frequently in healthy individuals, HIV and HIV/TB patients from rural Tanzania as studies in Sub-Saharan African populations also have shown (68;91-93). We hypothesized hypovitaminosis D among HIV, HIV/TB, and patients on ARV. We found that hypovitaminosis D was indeed common among patients on ARV compared to those not on ARV because of more frequent vitamin D insufficient and deficient in this group. We also found a high frequency of hypovitaminosis D among HIV infected group compared to healthy individuals, because of high frequency of vitamin D deficiency in this group.

Furthermore we found that hypovitaminosis was common among HIV/TB group compared to healthy controls because of high frequent of vitamin D deficiency. HIV/TB-coinfected group failed to differ significantly from that of HIV and healthy groups in the final model. This may have been due to relatively small sample size however, and the difference approached significance. Future studies should explore this question with a larger sample size.

The nutritional status of HIV/TB did not explain the lower serum vitamin D because, mean serum 25(OH)D concentration remained lower even after adjusting for BMI, and dietary intake is usually not considered sufficient to maintain optimal vitamin D status if sunlight is avoided (29;94)

Although several lines of in-vitro evidence suggest a link between vitamin D and immunity, the in-vivo association between vitamin D status and tuberculosis is still a controversial issue. In this study we found that hypovitaminosis D, as assessed by 25-(OH)-D, was higher among HIV-coinfected with tuberculosis patients than in healthy controls as well as than HIV monoinfected patients. These findings add to the growing evidence that vitamin D plays a role in the regulation of *Mycobacterium tuberculosis*.

The possible association between vitamin D and tuberculosis was first reported more than 20 years ago (95), but subsequent studies have yielded contradictory findings. A number of studies in Gujarati Indian (96), Indian, Somali, Pakistani, Afghan, Sri Lankan and African populations in London (97), and African immigrants living in Australia (45) all have shown that tuberculosis infected individuals had lower serum levels of 25(OH)D and higher prevalence of vitamin D deficiency than non-TB individuals.

Other studies in Kenya and West Africa (98) have also reported that TB patients had lower 25-(OH)-D levels compared to non-TB individuals. However, there was no significant difference in 25-(OH)-D levels between TB patients and controls in Indonesia (99) and Hong Kong (100).
studies. Nevertheless, a recent meta-analysis concluded that TB patients had about 0.70 standard deviation (95% CI 0.42, 0.93) lower 25(OH)D concentration than non-TB individuals (80). In our study, HIV-TB coinfected patient had 0.50 standard deviation (95% CI: -20.0, -2.2) lower serum 25(OH)D concentrations than in the healthy controls which was approximately the same range of the effect size found in the meta-analysis. Thus, overall, our finding is consistent with most previous studies.

The observation of low serum 25-(OH)-D in HIV-1 patients coinfected with TB could be due to nutritional factors, however, this is unlikely. It is well known that about 90% of vitamin D is synthesized in the skin under the influence of ultraviolet B radiation from sunlight, only about 10% of vitamin D is obtained from food, (mainly salmon and cod fish, egg yolks, or liver) or from supplements. We did not assess dietary intake of calcium nor vitamin D supplementation but the typical Tanzanian diet generally contain little salt and largely based on starches such as millet, sorghum, beans, pilaf, and cornmeal. The meal that could be considered the country's national dish is ugali; stiff dough made of cassava flour, cornmeal (maize), millet, or sorghum, and usually served with a sauce containing either meat, fish, beans, or cooked vegetables.

The present study was, however limited by the lack of detailed dietary information. Fish is consumed occasionally in the region of the study, both freshwater and saltwater fish, but intake varied considerably within the different groups of the population (P Sinyaw, personal communication, 2010). We have no data on the vitamin D content of these fish or individualized information on frequency of intake, but only fatty fish have considerable amount of vitamin D (101). We suggest future studies to explore the dietary information including vitamin D content and intake frequency of these fish in this community.

Although low 25-(OH)-D and calcium levels in HIV/TB patients could be a consequence of the disease, it is highly possible that vitamin D is antecedent risk factors for tuberculosis. The relation between vitamin D and protection against tuberculosis may be mediated through two mechanisms; increased production of cathelicidin and enhancement of macrophage ability. The active form of vitamin D [1, 25-(OH)2-D] may enhance production of IL-37, one of class of defensins-antimicrobial peptides of the cathelicidin family, culminating in TB destruction (46;102). Moreover, Vitamin D also restricts intracellular growth of Mycobacterium tuberculosis via enhancement of macrophage ability. Avoidance of phagolysosome fusion within the macrophage constitutes a key survival mechanism of Mycobacterium tuberculosis. The ability of
Mycobacterium to obstruct phagosomal progression, also known as phagosome maturation arrest, is partly achieved via its retention of the host’s tryptophan-aspartate-containing coat protection (103).

4.2.2. Vitamin D and HIV-1 infection

Very few small studies have reported data on the possible relationship between vitamin D status and HIV infection, and many are from high-income countries (104). A study from Germany reported lower serum 25-(OH)-D and 1,25(OH)₂D among asymptomatic HIV patients receiving treatment compared with uninfected controls (105), where as a similar study from Norway found lower serum 1,25(OH)₂D, but not serum 25(OH)D, among HIV infected patients (49). One similar study done in the northern City of Tanzania found no difference in serum 25-(OH)-D with HIV coinfected among TB patients (68).

HIV infection was not significantly associated with vitamin D status among HIV subjects in this study. This is consistent with the limited data available from previous studies (49). However, HIV affects serum concentrations of the active metabolite of vitamin D, 1,25-dihydroxyvitamin D (49). Thus, an apparent lack of effect of HIV infection on serum 25-(OH)-D concentrations does not mean that HIV infection has no effect on vitamin D metabolism and, consequently, on vitamin D–related physiologic functions, including the immune response. We found the lower level of the most potent metabolite of vitamin D (serum 1,25-hydroxyvitamin D) and not serum 25(OH)D was significantly associated with advanced stage of HIV disease (WHO stage three and four). Our findings are consistent with the previous studies (49) and contradict with the recent study done in Tanzania urban, where they found low vitamin D status (serum 25-hydroxyvitamin D < 32 ng/ml) was significantly associated with HIV progression (WHO HIV disease stage III) (106).

Under normal physiological condition, vitamin D from sunlight/food is converted in the liver to 25(OH)D and is further hydroxylated in the kidneys to the active metabolite 1,25-(OH)₂D, which is immediately metabolized to less metabolites. The enzymes that generate 25-(OH)-D from vitamin D in the liver and the enzymes that generate 1,25-(OH)₂D in tissues operate below Michaelis-Menten constant throughout; i.e. the reaction follow 1st order mass action kinetic (107). The more vitamin D is available, the more is converted to 25-(OH)-D, and the more is converted to 1, 25-(OH)₂D in the tissues. As long as sunlight exposure is adequate, 1, 25-(OH)₂D can be produced by the body without the requirement for ingestion in the diet. The explanations of the low serum 1,25-(OH)D found in advanced stage of HIV infected individuals would have been due to lack of precursor, reduced kidney or macrophages 25(OH)D hydroxylation to 1,25-(OH)₂D. However,
likes other hormones, 1,25-(OH)₂D circulate at a pictogram concentrations that are 1000 times less than serum 25(OH)D, therefore serum 25 (OH)D would have been virtually absent in the circulation in order to have effect on serum 1,25-(OH)D. In this study, advanced HIV stage was not associated with serum 25-(OH)-D.

Another explanation would have been irregulation of the tightly factors that determine 1,25-(OH)₂D hydroxylation. Under normal physiological condition, parathyroid hormone and calcium regulate 1,25(OH)₂D production, and low serum PTH, and calcium would have been expected to decrease the rate of 1-hydroxylation (108), however serum PTH and calcium among HIV patients in this study were within normal levels.

The rate of 1α- hydroxylation of serum 25-(OH)-D in the kidney normally determines the level of serum 1, 25-(OH)₂D concentration. But previously study have shown that advanced HIV disease was associated with chronic kidney diseases (109) which ultimately lead to kidney failure or reduced kidney functions. Therefore, a reasonable assumptions would be the inadequate 1α-hydroxylation might be the cause of low serum 1, 25-(OH)₂D in advanced stage of HIV infection. However, inadequate renal hydroxylation could not be the reason as patients with renal disorders were excluded in this study.

Studies done in vitro found cells in the body such as macrophages also express enzyme 1-α-hydroxylase (30). In the late phase of macrophage activation, macrophage- 1-α-hydroxylase produces 1,25-(OH)₂D under the influence of tumour necrotic factor alpha (TNF-α) (110) which apparently has a local rather than a systemic effect on immune cells (36). However, during HIV infection there is down regulation of TNF-α receptors and cells differentiation in advanced clinical disease (111;112) and a granulomas formation is not commonly seen in these patients following coinfection with tuberculosis. Although the macrophage-1-α-hydroxylase is identical to the renal 1-α-hydroxylase, its expression is not down-regulated by the parathyroid hormone (PTH) neither the active vitamin D and is mainly up-regulated by inflammatory cytokines such as interferon gamma (IFN-γ) and by lipopolysaccharides (30;36). We did not assess both interferon gamma and tumor necrosis alpha in this study however, Haug et al (49) found the combination of low 1,25(OH)₂D and high tumor necrosis factor may further impair the immune response in HIV infected patients both independently and in combination and may represent an important feature of HIV pathogenesis. Therefore a reasonable assumption of low serum 1,25-(OH)₂D in advanced stage of HIV would be the reduction of the activation of 25(OH)D to 1,25(OH)₂D in the macrophages.
4.2.3. Vitamin D and highly active antiretroviral therapy (HAART)

There was highly statistical significant difference between HIV patient receiving HAART and HIV naïve. Patients receiving HAART were greater than 4 fold more likely to demonstrate hypovitaminosis D. Our subjects were on first line treatment which comprised of two nucleoside reverse transcriptase inhibitors (NRTIs) stavudine (d4T) or zidovudine (ZDV), combined with lamivudine (3TC), and one non-nucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine (NVP) or efavirenz (EFV).

Based on Stavudine or Zidovudine regimen, we found a statistically significant association of Zidovudine with lower serum level of the potent metabolite of vitamin D (1,25-(OH)₂D). Vitamin D deficiency (serum 25-(OH)-D < 50nmol/l) was also associated with Zidovudine based regimen and this was also present following adjustment with sex, age, BMI and duration of HIV. These findings are consistent with a recent study conducted in Spain (113).

Our finding of low serum 1,25-(OH)₂D following use of HAART in HIV infected patients may have some explanation and implications; Use of HAART especially protease inhibitors (PI) and non nucleoside reverse transcriptase inhibitors (NNRTIs) have been found to inhibit the function of hepatic- 25-hydroxylase and macrophage-1,25-hydroxylase which are critical for active vitamin D synthesis. The net effect is a reduced production of 1, 25-(OH)₂D (61) that could influence immunity. Our patients were on combination of NRTIs and NNRTIs. The finding of low 1, 25-(OH)₂D based on zidovudine regimen might have the same explanation as above. This may also explain the elevated PTH levels that we found among HIV patients on antiretroviral therapy in this study.

Low 1, 25-(OH)₂D in HIV patients has been associated with immune reconstitution inflammatory syndrome (IRIS) (112;114) i.e. a temporary worsening of clinical presentation after starting HAART despite immunological improvement. About 30% of HIV infected persons living in resources limited settings and high burden of tuberculosis such as Sub-Saharan Africa are highly affected by this problem (115). And by far, Mycobacterium tuberculosis has been the most pathogenic organism involved (112).

The active form of vitamin D (1,25(OH)₂D) play a role on immunity through different mechanisms; first, based on Mycobacterium tuberculosis infection, the active form of vitamin D (1,25-(OH)₂D ) produced by macrophage-1,25 hydroxylase at the inflammation site, has many local actions leading to a negative feedback loop avoiding macrophage overstimulation.
Second, 1, 25-(OH)\(_2\)D acts also directly on macrophages by inducing intracellular *Mycobacterium tuberculosis* destruction via the cathelicidin-mediated system. If macrophages are over stimulated, high local level of 1,25-(OH)\(_2\)D could lead to systemic spill over and thus hypercalcemia, as has been described in *Mycobacterium tuberculosis*-IRIS (56) since no systemic negative feedback by the parathyroid axis exists on macrophage-1,25-hydroxylase (98).

We did not asses the presence or absent of IRIS in this study however its interrelation with ARV, low vitamin D and immunity appear to be importance in the clinical aspect of HIV patients’ management, warranting more studies to explore low vitamin D status on the pathogenesis of IRIS.

### 4.2.4. Season variation

Outdoor sun exposure and time spent outdoors are better predictors of serum 25-(OH)-D concentration than dietary vitamin D intake (64). Although well known at higher latitudes above 35\(^0\), the seasonal variation in serum 25-(OH)-D, with concentration raising from May to October, was unexpected in this study, given the fact that our study site lies between 3\(^0\) 40ˈ and 6\(^0\) 0ˈ latitudes.. Only brief daily sun light exposure converts cholecalciferol to inactive metabolites. Hence seasonal variation is not expected in countries around the equator, such as Puerto-Rico (116) and Guinea-Bissau(98). In Manyara region, Tanzania; the mean number of daily sunshine hours per day ranges from 6.9 to 10.0 h/d. It was lower in January with 6.9 h/d and higher in August with 10.0 h/d. The increased serum vitamin D (25-[OH]-D) in harvest through post-harvest (from May to peak in August, September and October) coincides with the peaks with sunshine hours. Although we didn’t enquire the time spent outdoor, but the increase may be explained by a combination of slightly more sunlight hours, more outdoors farming activities, and more sun exposure during the cooler days of June to July. Furthermore, although the contribution of food in vitamin D is about 10\%, the availability of food and money (from selling crops) following harvest may have contributed the slight increased serum vitamin D levels.

We found a lower albumin-corrected serum calcium concentration in the HIV/TB-coinfected patients than in the HIV-1 patients, which was also, present when serum calcium concentrations were not corrected for serum albumin. This finding was likely explained by the fact that hypovitaminosis was more frequent among HIV/TB patients than among HIV patients; Calcium absorption is known to be impaired when serum 25(OH)D concentrations are below 75 nmol/l (117).
HIV-1 infected patients were older compared to those who were HIV negative. Most of those who were HIV positive were in the age group 25-35 years. These results are consistent with the data from Tanzania National AIDS Control Programme of 2008 which shows that the age group 20-49 years remained the most affected by HIV for both sexes, an observation that has remained consistent for several years since the beginning of HIV epidemic (27).

Another limitation in this study was the cross-sectional design of it which hinders strong conclusions on the direction of association. A prospective study following individuals with hypovitaminosis for the development of tuberculosis, and optimal vitamin D HIV individuals on ARV for the development of hypovitaminosis, and changes of vitamin D status during the course of disease would be appropriate, however be difficult and costly.

4.2.5. Measurement precision
The high levels of hypovitaminosis D observed in our study cannot merely be the result of imprecise measurements. Serum vitamin D levels were determined in an international reference recognized laboratory (Hormone Laboratory, Department of Endocrinology, Oslo University Hospital), ensuring high precision and reliability. If small inaccuracies in the measurement of weight and height should have occurred, they would not materially change the observed range and distribution of BMI. Our study is not the only one to have identified very high levels of hypovitaminosis D. Several studies, for example in West Africa (98) and recent one among Tanzanian in urban center (68) found hypovitaminosis D profiles that were equally unfavourable. This further reduces the likelihood that measurement error or selection bias could largely explain our findings. By all accounts, the risk of hypovitaminosis D in this community is alarmingly high.
4.2.6. Conclusion and recommendation

This cross-sectional study showed that, hypovitaminosis D was highly prevalent among HIV, HIVTB and healthy individuals in rural Tanzania; advanced stage of HIV disease was associated with lower serum 1,25(OH)₂D concentrations, possibly due to reduced hydroxylation of 25-(OH)-D to 1,25(OH)₂D in the macrophages. Serum 25-(OH)-D concentration was higher in HIV monoinfected than HIV/TB coinfected patients, hence hypovitaminosis D was more common among HIV/TB coinfected compared to HIV-1 monoinfected but the difference was not statistically significant due to small sample size. Hypovitaminosis D was significantly higher in HIV patients on antiretroviral therapy compared with patients not on ART.

Advanced management of HIV have resulted in a dramatic decline of mortality, and increased long term complications such as fracture due to reduced mineral bone density (118). Our study found a high frequency of hypovitaminosis D among these patients which may be another added risk factor. Our findings suggest that periodic screening of serum vitamin D and supplementation should be considered in routine care of HIV infected patients, with emphasis given to patients with coinfection however, safety and cost effectiveness need to be evaluated.

Given the high frequency of hypovitaminosis D found in this study, there is a need to work more on health promotion activities to this community targeting the importance of physical activities, exposure to sunlight and use of diets rich in vitamin D.

Areas for future research

Prospective well designed (studies) intervention based trials are difficult and costly however, are needed for further evaluation on the relationship between adequate vitamin D repletion and treatment/prevention of bacterial infections, especially Mycobacterium tuberculosis. Emphasis should be put on effectiveness of repletion therapy, large sample size and factors that may confound the results, such as; exogenous intake of vitamin D irrespective of the groups assignment, independent effect of nutritional status improvement with TB therapy and seasonal variation with vitamin D status.

The role of vitamin D status in modulating host immune response to HIV infection appears complex. Evidence based information from both clinical and laboratory driven studies are clearly needed to help clarify the complex encircled by vitamin D status, vitamin D metabolism, ART and HIV infection in human host.
5. References


(51) Villamor E. A potential role for vitamin D on HIV infection? Nutr Rev %2006 May ;64 (5 Pt 1):226 -33(5 Pt 1:226-33).


(62) Mueller NJ FCLBELSPDTMLCAVAB-FH. High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients. AIDS Res Hum Retroviruses 2010;24(00).


(100) Chan TY. Differences in vitamin D status and calcium intake: possible explanations for the regional variations in the prevalence of hypercalcemia in tuberculosis. Calcif Tissue Int 1997 Jan;60(1):91-3.


(104) Villamor E. A potential role for vitamin D on HIV infection? Nutr Rev %2006 May ;64 (5 Pt 1):226 -33 2006;(5 Pt 1:226-33).


6. Appendices

6.1. Patient consent form

Studies on HIV, nutrition and co-infections:

You are invited to participate in a research study looking for vitamin D status and co-infections in people living with HIV/AIDS. HIV/TB co-infection is among the main causes of morbidity and mortality in Sub-Saharan Africa, but other infections such as hepatitis B and cytomegalovirus (CMV) are also frequent.

Purpose of the study: to establish the interaction between Vitamin D, HIV and HIV/TB co-infection, and to study other co-infections such as hepatitis B and CMV.

Information registered: The study is based on information routinely registered in the hospital files on demographic, clinical and laboratory data. Blood samples for additional evaluation of the vitamin D-metabolism will be obtained in connection with routine laboratory tests. In addition to routinely registered information and blood samples, face to face interview on food frequency recall will be conducted.

Storage of specimens: The collected blood samples will be analyzed as described and any surplus material will be stored (maximum 30 years) for possible future studies. If you consent to participate in the study you also consent to the use of remnant specimens for other HIV-relevant studies. Oslo University Hospital, Ullevål (Norway) is responsible for stored samples.

Risks and Benefits of Study Participation: All patients will receive the best treatment in accordance with national and WHO guidelines for HIV/AIDS and TB. Any pathology during examination will be referred to the appropriate clinic for further managements. The study will also pay for the additional lab tests such as vitamin D, calcium and phosphorus. In the long term, the findings from this study could be used to plan effective programmes to benefit management of HIV patients. Minimal risks include loss of time and the discomfort of an additional venipuncture.

Participation in the study is voluntary. Patients have the right to withdraw from the study at any time without being excluded from further care and treatment. They can claim access to their registered data at any point.
Any information given by the patient is confidential. Only the clinicians and the researchers will have access to patient information. Data in the study are kept anonymous and secure. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject.

Responsible for the study: Dr Sokoine Kivuyo, National Institute for Medical Research (Tanzania) master student at University of Oslo (Norway) will be responsible for the registration of data and the practical follow up of the study. Project responsible is professor Johan N. Bruun and dr Asgeir Johannessen, Oslo University Hospital, Ullevål (Norway).

**Statement of Consent:** I consent to participate in the study.

Signature of Subject__________________________________________________________

Date__________________________

Signature of Investigator_______________________________________________________

Date__________________________

### 6.2. Swahili Patient Consent form.

**Ridhaa ya kushiriki katika utafiti**

**Utafiti wa ukimwi, Lishe, na magonjwa nyemelezi.**

Unakaribishwa kushiriki katika utafiti huu kuhusu viwango vya vitamini D pamoja na magonjwa nyemelezi kwa watu wanaoishi na virusi vya Ukimwi. Ukimwi na Kifua kikuu kwa pamoja ni miongoni mwa magonjwa yanayosababisha matatiza na vifo vingi kwa nchi za Africa zilizo chini ya Jangwa la Sahara, pia hepatitis B na Cytomegalovirus ni miongoni.

Kazi hii ni utafiti wa kisayansi kufahamu zaidi kuhusu sababu za upungufu wa vitamini D kwa watu waishio na virusi vya ukimwi pamoja na kifua kikuu. Kamati ya maadili ya utafiti nchini Tanzania na ng’ambo (Chuo kikuu cha Oslo nchini Norway) wamehalalisha utafiti huu kufanyika.
Utafiti huu unafanyika kwa sababu Ukimwi na Kifua kikuu ni tatizo kubwa katika nchi yetu, na labda kushiriki kwako au mtoto wako huenda ukasaidia kupata njia mpya za kuingia au kutibu upungufu wa vitamini D. Kwa mtazamo huu moja ya faida ya kushiriki utafiti huu ni kuboresha afya yako au ya mtoto wako.

Tunaomba uwe mmojawapo katika utafiti huu kwa sababu ushiriki wako au wa mtoto wako unaweza kutusaidia sisi kujua sababu za upungufu wa vitamini D kwa watu wenye Ukimwi au Kifua Kikuu.

Ni mambo gani yatatokea na kutendeka katika ipindi cha utafiti?

Iwapo wewe au mtoto wako utakubali kushiriki katika utafiti huu tutafanya yafuatayo:

Tutachukua sampuli ndogo ya damu (kiasi cha kijiko cha chai) ili kuangalia kiwango cha vitamini D, kinga(CD4), wingi wa virusi pamoja na madini ya calcium. Tutakukupima wewe (au mtoto) uzito na urefu wako. Tutakusaili pia mambo yahusuito vyakula vya kuongeza vitamini D mwilini.


Faida za utafiti.

Vitu vizuri vinaweza pia kutokea kwa watu kwa sababu ya kushiriki kwao katika tafuli huu. Faida yako kushiriki katika utafiti huu ninaweza kuwa kwamba tutaweza kutisha sourcing kuwa sababu za matatizo ya ukosefu wa vitamini D kwako/ mtoto wako, na ikitokea tutukupata ruaha kwenda kupata matibabu yanayostahili. Pia utafiti huu utagaramia mpya baadhi ya vipimo kama kiasi cha kinga(CD4), virusi (viral loads), kiwango cha vitamini D ambavyo ni muhimu kwa maendeleo ya afya yako. Daktari au mtafiti atakuliza taarifa muhimu itokanayo na utafiti huu ambayo ni muhimu kwa afya yako pia.

Athari za utafiti huu?

Wakati mwingine vitu hutokea kwa watu katika tafuli ambazo vinaweza kuwa muhimu wao au kuwafanya wajisikie vibaya. Inaweze katiwa katika utafiti huu ukajisikia vibaya kiasi unapojibu maswali. Wakati wa kuchoma sindano kuchukua damu wewe au mtoto wako mnaeza kupata
maumivu kidogo kwa muda, na dalili ndogo au mchubuko kutokeza na kuchoma sindano. Kwa kuwa taarifa zinazokusanyika katika utafiti huu zinawezekutambua wewe na mtoto wako, tutakuwa makini sana kuzilinda taarifa zinazokuhusu wewe binafsi.

**Kushiriki ni lazima au la?**


Ukiamua kutokezishiriki, au baadaye ukijitaka utafitini wakati wowote hakuna mtu atakayekulaimisha. Hakuna atakayekuhudumia tofauti kwa sababu umekataa kushiriki katika utafiti huu. Huduma yako ya kawaida haitabadilika.

**Nitawasiliana na nani iwapo nitakuwa na maswali yanayohusiana na utafiti huu?**

Sokoine Lesikari Kivuyo
National Institute for Medical Research (NIMR)
2448, Ocean Road, Junction of Luthuli / Sokoine Drive
P.O.BOX 9653  Dar es salaam, Tanzania  Tel: +255-682887292

Professor Johan N. Bruun and Dr Asgeir Johannessen,  Tel: +4797983264
Oslo University Hospital, Ullevål (Norway).

**Saini**

Naomba uulize maswali yako kuhusu utafiti huu kabla ya kusaini.

<table>
<thead>
<tr>
<th>Jina la mshiriki</th>
<th>Jina</th>
<th>Tarehe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(saini)</td>
<td>(chapa)</td>
<td></td>
</tr>
</tbody>
</table>
6.3. Medical Record Abstraction Tool

Unique Abstraction Code: _______ ART Site Name: ________

Abstractor’s Initials: _______ Date of Abstraction__________

A. Record Identifiers

Facility Name: _________ Unique Study ID Number: ________

B. Demographic

Sex: Male-------- Female --------Missing-------

Marital Status:

Single --------Married----------Cohabitig---------Divorced/Separated---------Widow/Widowed -------
--------Missing-----------

Date of Birth: --------------- Missing----------------

Age: Years-------- Missing--------

Anthropometric measurements

    A. Body weight…………………Kg   B. Height…………………cm

    BMI…………………..Kg/m^2
C. Treatment Information

Prior ARV Exposure:

None ........................PMTCT ......................... Combination Therapyzell

Prior Therapy (transfer in without records) .......... Missing ............

PMTCT Monotherapy Transfer In, date: ___/___/___ or, 

date missing ____________

Prior regimen ___________ Date Confirmed HIV+ ___________ Missing ________

Date Enrolled in Care __________ Missing ____________________

Date Medically Eligible for ART: __________ Missing ________________

Date Eligible & Ready for ART: __________ Missing __________

Date Start ART: __________ Missing ________

Prior to anti-TB exposure:

Prior regimen ___________ Date confirmed TB positive __________ Missing ________

Date started anti-TB __________ Missing __________

Why Eligible for ART (circle as appropriate and record value)

Clinical Only--------CD4 Count %--------TLC--------

Status at Start ART:

WHO Clinical Stage........Missing....... ......Weight: ........ . .Kg    Height.......Missing.........

Functional Status: (W, A or B)........Missing.......... CD4: cells/dl.......... Missing............

Enrollment Visit
Enrollment Visit Date: .......... Missing...... Weight......... Kg  Missing ........

WHO Clinical Stage............ Missing............

Functional Status: Working ......Ambulatory..... Bed-ridden....... Missing......

**TB Status Code:**

1. NO  2. REFER  3. SP  4. CONFIRM  5. INH  6. TB/RX

7. Missing

**ART Status at Enrollment :( select one)**


7. Missing

If on ARV, which combination..................Hb  at ART Initiation: .......... ( g/dl) Missing.........

ALT at ART Initiation........Missing.......ALP at ART Initiation.........Missing.....

**H. Follow-up Visit (ideally at 6 months)**

ART Visit Date: .......... Missing...........Weight: ........ Kg  Missing.......... 

Height...........Missing..........................WHO Clinical Stage ...... Missing........

**Functional Status:**

Working ...... Ambulatory.....Bed-ridden....... Missing........

**TB Status Code:**

1. NO  2. REFER  3. SP  4. CONFIRM  5. INH  6. TB/RX

7. Missing
ARV Status:
1 - No ARV  2 - Start ARV  3 – Continue  4 - Change  5 - Stop
6 – Restart  7. Missing

ARV combination………………..Hb at 6months: ……. (g/dl) Missing………..

ALT at 6months…….Missing……..ALP at 6months …….Missing……..

Follow-up Visit (ideally at 12 months)

ART Visit Date: ………. Missing…….Weight: ………. Kg Missing………..

Height………….Missing……..WHO Clinical Stage…………. Missing……………..

Functional Status:

Working ………. Ambulatory………..Bed-ridden…….. Missing……………..

TB Status Code:
1. NO  2. REFER  3. SP  4. CONFIRM  5. INH  6. TB/RX

7. Missing g

ARV Status:
1 - No ARV  2 - Start ARV  3 – Continue  4 - Change  5 - Stop
6 – Restart  7. Missing

ARV combination………………..Hb at 12months: ….. (g/dl) Missing………..

ALT at 12months … ……….Missing……..ALP at 12months … …..Missing………..

Follow-up Visit (ideally at 18 months)
ART Visit Date: ……………… Missing………Weight: …………… Kg Missing………
Height……………Missing……………WHO Clinical Stage….. Missing……

**Functional Status:**

Working ….. Ambulatory…..Bed-ridden….. Missing……

**TB Status Code:**

1. NO  2. REFER  3. SP   4. CONFIRM  5. INH  6. TB/RX
7. Missing

**ARV Status:**

1 - No ARV  2 - Start ARV  3 – Continue  4 - Change  5 - Stop
6 – Restart  7. Missing

ARV combination……………….Hb at 18months: …… (g/dl) Missing………..
ALT at 18months … …Missing…………ALP at 18months … …Missing…………

**Follow-up Visit (ideally at 24 months)**

ART Visit Date ……………… Missing………Weight: …………… Kg Missing………
Height……………Missing……………WHO Clinical Stage………… Missing…………

**Functional Status:**

Working ……….. Ambulatory………..Bed-ridden….. Missing……

**TB Status Code:**
ARV Status:
1 - No ARV       2 - Start ARV       3 – Continue       4 - Change       5 - Stop
6 – Restart       7. Missing

Hb at 24months: ....... ( g/dl) Missing........ALT at 24months ...............Missing........

ALP at 24months ... ........Missing.........

Follow – Up Status (last visit/retention)

Date of most recent (last) follow-up visit.............

ARV combination..............

ART Status most recent: (select one)
1 - No ARV       2 - Start ARV       3 – Continue       4 - Change       5 - Stop
6 – Restart       7. Missing

Hb at last visit: ....... ( g/dl) Missing.........ALT at last visit ...............Missing.........

ALP at last visit ... ..............Missing.........Weight.........Kg    Height...........

Was CD4 count recorded at any time?

Yes       No       Missing

If yes, please list the dates and values?
Was viral load recorded at any time?

Yes........ No ....... Missing.......... 

If yes, please list the dates and values?

DD MM YY

DD MM YY

Chest examination: Normal........ Abnormal...........

Chest X-ray results: Normal........ Cavity......... Other abnormalities (specify) ............... 

Laboratory parameters:

Serum calcium--------------mmol/l   Serum álbúmin----------g/l
Serum Phosphorus----------mmol/l   Serum 25(OH)D----------nmol/l
Serum 1,25(OH)D---------pmol/l   Serum PTH----------pmol/l
ALAT...................... u/l   ASAT..................... u/l
Creatinine.................. umol/l