BONE MINERAL DENSITY ABNORMALITIES IN HIV INFECTED PATIENTS AND HIV NEGATIVE RESPONDENTS AT MBAGATHI HOSPITAL USING CALCANEAL QUANTITATIVE ULTRASOUND.

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DECLARATION

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

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ACRONYMS/ABBREVIATIONS

ALP – Alkaline Phosphatase

ART- Anti-Retroviral Therapy

BMD – Bone Mineral Density

BMI- Body Mass Index

BUA – Broadband Ultrasound Attenuation

CCC – Comprehensive Care Clinic

DEXA – Dual Energy X-ray Absorptiometry

DPA – Dual Photon Absorptiometry

DXA – Dual X-ray Absorptiometry

D4T - Stavudine

HAART – Highly Active Anti- Retroviral Therapy

HIV – Human Immunodeficiency Virus

PI – Protease Inhibitors

QUS – Quantitative Ultrasound

SoS – Speed of Sound

TDF – Tenofovir Disoproxil Fumarate

VCT- Voluntary Counseling and Testing

WHO – World Health Organization

ABSTRACT

Background

Osteoporosis is a systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and fracture.

Kenya has an estimated 1.6 million people living with HIV. Use of Highly Active Anti-Retroviral Therapy (HAART) has been associated with prolonged survival and consequently with an increase in the prevalence of decreased bone mineral density.

Quantitative Ultrasound (QUS) is gaining popularity as an appropriate tool for determination of bone mineral density profiles in resource- poor settings.

Objectives

To determine and compare the difference in the prevalence of Bone Mineral Density(BMD) abnormalities using quantitative calcaneal ultrasound between HIV infected patients on a TDF based first-line regime for at least one year, HAART-naive HIV positive patients in Mbagathi Comprehensive Care Clinic(CCC) and a HIV negative control group seen at the Mbagathi Voluntary Counselling and Testing Centre(VCT). To describe the occurrence of traditional risk factors associated with decreased BMD in the above populations (oral corticosteroid use, smoking, alcohol, previous bone fracture, body mass index and physical inactivity)

Methods

This is a cross-sectional comparative group descriptive study of HIV positive adult patients on TDF based first-line regime (exposed), HIV positive HAART- naive adult patients (unexposed) and HIV negative adult group (control) at Mbagathi hospital.

Random sampling was used to recruit 315 participants (105 in each arm). An interviewer administered questionnaire was used to document risk factors for low BMD. Quantitative ultrasound bone mineral density was done using a heel ultrasonic gel- coupled QUS system, the Sunlight Mini Omni (Beam Med Ltd, Israel)

Results

The prevalence of osteoporosis among HIV positive respondents on HAART was significantly higher (58.1%) compared to HIV positive respondents not on HAART (32.6%) (Z-test p-value = .001) and HIV negative respondents (9.3%) (Z-test p-value = .001). Older patients had lower levels of BMD (i.e. more negative BMD. p-value = .032)

HIV positive respondents on HAART had lower BMI than HAART naïve and HIV negative individuals(23.6%,24.8% and 26.1% respectively).There was a significant positive correlation between T-score and BMI(p-value .043). There was no significant correlation between T- score and the other traditional risk factors (oral corticosteroid use, smoking, alcohol use, history of bone fractures and physical activity)

Conclusions

Use of TDF based HAART regimes is associated with higher rates of osteoporosis compared to HAART naïve and HIV negative populations which may be partly mediated by lower Body Mass Index(BMI).

CHAPTER ONE: INTRODUCTION

Background

As the dynamics of a country continue changing, there is an improvement in lifestyle and by extension the life span of the population. There is also a shift from acute and infectious illnesses to more chronic ailments, including osteoporosis [1].

Human immune-deficiency virus (HIV) infection is one of the heaviest infectious disease burdens afflicting sub-Saharan countries. Kenya has the fourth-largest HIV epidemic in the world and in 2012, an estimated 1.6 million people were living with HIV, and roughly 57,000 people died from AIDS-related illnesses. Moreover there are now 1.1 million orphans to the epidemic in the country. [1, 2]

HIV prevalence in Kenya peaked at 10.5% in 1996 and by 2012; this had fallen to 5.6% mainly due to the rapid scaling up of anti-retroviral treatment (ART) [3]. Since 2008, the expansion of ART services throughout the national healthcare system had increased the number of adults on treatment from 64% to 80% in 2013. [3]

Use of Highly Active Anti-retroviral Therapy (HAART) has been associated with viral suppression and improved patient survival. With prolonged life, the prevalence of osteoporosis and osteopenia increases due to differential bone remodeling associated with aging. [4, 5].

HIV causes osteopenia through cytokine and inflammatory- mediated pathways [6, 7]. , Highly Active Anti-Retroviral Therapy (HAART) drugs have been associated with decreased Bone Mineral Density (BMD) especially tenofovir disoproxil fumarate (TDF) and protease inhibitor (PI) based regimens. This is probably through the effect of these medications on cellular DNA synthesis and gene expression involved in bone re-modelling. [8]

The International Society for Clinical Densitometry (ISCD) recommends testing for BMD in patients who suffer from conditions that could precipitate bone loss, are going to be prescribed or are on prescription drugs known to cause bone loss or are being treated for bone loss and require monitoring [9].

The WHO recommends the use of Dual Energy X- ray Absorptiometry (DXA, previously DEXA) method to determine BMD levels, and has provided guidance on classifying the levels into clinically relevant outcomes depending on the number of standard deviations (SDs) below the mean BMD for a healthy, young (25–35 years of age), sex- and ethnicity-matched reference population (T-score).

Other methods used to determine bone mineral density include Quantitative Computer Tomography (QCT) and Quantitative Ultrasound (QUS).Both DXA AND QCT involves utilization of specialized equipment, generate ionizing radiation, are expensive and require relative expertise.

Quantitative calcaneal ultrasonography offers several benefits. It is cheaper and more portable than DEXA, there is no exposure to ionizing radiation [10] and is as effective as DEXA at predicting femoral neck, hip, and spine osteoporotic fractures [4, 11]

With advancement in technology, the quantitative ultrasound (QUS) is gaining popularity as an appropriate tool for determining bone mineral density profiles in resource poor settings. [12, 13]

CHAPTER TWO: LITERATURE REVIEW

What is bone mineral density?

Bone mineral density (BMD) refers to the amount of mineral matter per square centimeter of bone. It is commonly used as an indicator of the risk of a fracture or development of osteoporosis. A low or decreased bone density indicates higher probability of the development of osteoporosis or a fracture [9].

In general, fracture risk approximately doubles for each standard deviation below the mean young adult (25-35 years) BMD (for each -1 decrease in T score) regardless of fracture type and BMD measurement site [9, 14, 15].

In addition to BMD, bone strength and susceptibility to a fracture depend on trabecular connectivity and arrangement, biochemical properties (such as elasticity, strain/stress response, and failure point) and other factors such as bone size, shape, turnover, and architecture. [16]

BMD levels can be determined through several methods, with the gold standard being the dual X-ray absorptiometry (DXA), though Quantitative Ultrasound (QUS) is gaining popularity as a good epidemiological and screening tool in resource poor settings. [17, 18].

Using the DXA, BMD levels, the World Health Organization (WHO) developed guidelines for classifying the levels into clinically relevant outcomes depending on the number of standard deviations (SDs) below the mean BMD for a healthy, young (25–35 years of age), sex- and ethnicity-matched reference population (T-score).

This classification was initially used on post-menopausal women, but has now being generalized to other adult populations [15]. A T-score less than or equal to −2.5 at the hip or spine is defined as osteoporosis, a T-score of between −1 and −2.49 is defined as osteopenia while a T-score of more than -1 is regarded as normal [14].

Risk factors of reduced bone mineral density

Risk factors for osteoporosis can be classified into two broad categories:

i) Non-modifiable risk factors.
ii) Modifiable risk factors.

Non- modifiable risk factors

- ***History of previous fracture***

Individuals who have suffered a previous fragility fracture (defined as a fracture occurring after a fall from a standing height or less) are at an increased risk of further fractures, independent of BMD. Women who develop a vertebral fracture have a 19.2% (95%CI 13.6-24.8%) risk of a further vertebral fracture within one year [19]. Men and women aged 65 years or older with a vertebral fracture have a five year risk of femur or hip fracture of 6.7% and 13.3% respectively [20]. In women, the presence of one or two vertebral fractures increases the risk of further fracture 7.4 fold [21]

- ***Age***

As BMD decreases, the risk of osteoporosis increases with age. A significant increase in the prevalence with each decade after age 60 has been demonstrated. [22]

- ***Sex***

Women are at greater risk of osteoporosis as they have smaller bones and hence lower total bone mass. Additionally, women lose bone more quickly following menopause, and typically live longer. Osteoporosis is less common in men but is still a significant problem. The rate of bone loss in men is less than that in women. In the Framingham Osteoporosis Study, annualized percent bone loss for women was 0.86-1.21% at different sites and 0.04- 0.90% for men at different sites. [23]

- ***Ethnicity***

African women have a higher BMD than white women at all ages due to a higher peak bone mass and a slower rate of loss. White women have a 2.5-fold greater risk of getting osteoporosis [23, 24].

- ***Reproductive factors***

A late menopause is associated with higher BMD. There is consistent evidence that low BMD is associated with early menopause [25]. Consequently, women with an early menopause should be considered at higher risk of osteoporosis than others at a similar age. [26]

There is no consistent evidence that tubal ligation, parity, number of previous miscarriages, or breastfeeding affect bone mineral density. [25]

Current use of estrogen replacement therapy is associated with a higher BMD. [23] Those individuals currently taking estrogen therapy should therefore be considered as being at a lower risk than others at a similar age.

- ***Family history of osteoporosis***

Lower BMD is found in women and men with a family history of osteoporosis (defined as a history of osteoporosis or brittle bones, or low trauma fracture after age 50 years as reported by the offspring).

Individual BMD decreases as the number of family members with osteoporosis increases. Overall family history is a more sensitive predictor of osteoporosis risk than maternal or paternal history alone. Prevalence of a positive history in sisters is similar to prevalence reported for mothers [22, 27]

Modifiable risk factors

- ***Weight***

Weight loss is a frequent symptom in HIV infection and is used as a diagnostic criterion in the classification of HIV disease by the Centre for Disease Control (CDC). HIV infected individuals experience weight loss due to the virus itself, due to wasting syndrome and malnutrition [28].

Low body mass index is an indicator of lower bone mineral density. Individuals in the lowest quartile of BMI have a two-fold greater bone loss than those in the highest quartile over a follow-up period of two years. Thus HIV infected individuals are at an increased risk of osteopenia and osteoporosis due to low body mass index.[29]

- ***Corticosteroid use***

There is up to a six fold increase in the risk of developing fractures due to osteoporosis in individuals on long term steroids [20]. Ingestion of as little as 5mg of prednisone daily over 3 months is associated with an increased risk of developing osteoporosis [30].

It is postulated that the long term use of corticosteroids may lead to osteoporosis through two mechanisms. Firstly, through the inhibition of osteoblasts and activation of osteoclasts leading to an imbalance of bone remodeling due to higher rates of bone resorption and secondly, through the interference with calcium absorption from the gut, leading to increased bone resorption[30].

- ***Alcohol***

Chronic consumption of alcohol affects bone structure mainly through two postulated mechanisms. It reduces the level of activated Vitamin D in the body thereby reducing calcium absorption from the diet, leading to increased bone resorption to restore normal calcium homeostasis.

Secondly, it reduces the production of parathyroid hormone which is centrally involved in calcium regulation. Alcohol has also been shown to have direct toxic effects on the osteoblasts in in-vitro studies [31].

- ***Smoking***

A meta-analysis of studies looking at the effect of smoking found that BMD in smokers was 2% lower with each increasing decade after menopause compared to non- smokers, with a 6% difference at 80 years [32].

Men who smoke show greater bone loss at the trochanter, while female smokers have been shown to be at greater risk of hip fracture than non-smokers, with the risk increasing in line with cigarette consumption [23]. The level of risk declines on giving up smoking, but is not significantly reduced until 10 years after cessation of cigarette smoking [33].

Proposed mechanisms for reduced BMD include alterations in calciotropic hormone metabolism and intestinal calcium absorption, dysregulation in sex hormone production and metabolism, alterations in adrenal cortical hormone metabolism and direct toxic effects on bone cells [34].

- ***Physical activity***

Generally balanced physical activity in childhood and adolescence has been shown to improve bone health through mechanical stimulation which enables skeletal tissue development.

Regular physical activity also increases muscle mass, reduces blood cholesterol and triglyceride levels, decreases fatigue, improves cardio-respiratory function and increases bone mineral density [26].

Decreased physical activity and sedentary lifestyles are more common in HIV infected populations due to malnutrition and wasting syndrome associated with HIV as well as other co-morbid illnesses and opportunistic infections linked to HIV.

Individuals with a sedentary adolescent lifestyle should be considered at higher risk of osteoporosis. Those adults who currently have a sedentary lifestyle are also at higher risk [26].

- ***Diet***

Past dietary intake of milk in adult pre-menopausal women has been positively associated with higher bone mineral density. Evidence of association between current calcium intake and low bone mineral density is inconsistent. No consistent association has been found between other dietary factors and BMD [35].

Table 1: Risk factors for osteoporosis (when there is no history of fracture)

STRONGEST RISK FACTORS	OTHER SIGNIFICANT RISK FACTORS
Female sex Age > 60 years Family history of osteoporosis	Caucasian origin Early menopause Low BMI Smoking Sedentary lifestyle Long term(≥3 months) corticosteroid use

It is difficult to offer evidence based advice about particular combinations of risk factors which justify further investigation since the evidence is lacking, but there seems to be an additive effect of risk factors(more risk factors means a greater risk of osteoporosis)[36].

Effect of HIV on bone mineral density

The etiology and pathogenesis of reduced bone mineral density in HIV infection has not been fully elucidated. Multiple factors have been proposed that may provide the likely mechanisms of the effect of HIV infection on bone mineral density. This includes:

- Direct cytopathic effects of HIV upon osteogenic cells characterized by increased serum markers of bone resorption(C-telopeptide) and markedly depressed osteocalcin levels associated with increased bone turnover [37-39]
- Persistent upregulation and activation of pro-inflammatory cytokines, especially tumour necrosis factor-alpha (TNF-α) whose levels are negatively correlated with osteocalcin levels and subsequent reduced BMD [40-42]
- Alterations in the metabolism of Vitamin D and its deratives due to cytokine dysregulation leading to low levels of 1,25- $(OH)_2D$ and subsequent decreased BMD [41, 43]
- Presence of opportunistic and/or chronic diseases associated with HIV infection which affects bone health via chronic inflammation [44]
- Mitochondrial abnormalities related to chronic inflammation and HIV infection leading to lactic acid acidosis which promotes bone mineral dissolution and may affect BMD in this population. [45]

Knobel et al [46] found both osteopenia and osteoporosis in patients on HAART treatment and in therapy naïve HIV infected patients. The HIV infected groups showed significant differences from the non- HIV, healthy control group with respect to BMD, with a similar percentage of osteoporosis and osteopenia in the HAART naïve and HAART experienced patients.

Bruera et al [47] concluded that BMD was significantly lower in HIV seropositive patients when compared with healthy HIV-negative individuals, with no significant differences among patient groups on different therapeutic regimes. This suggested a deleterious effect of HIV on bone health, independent of anti-retroviral therapy.

HIV treatment and its effect on BMD

Use of HAART has significantly decreased the morbidity and mortality associated with HIV infection. The long term survival of the treated patients has revealed several metabolic complications such as lipodystrophy, insulin resistance, diabetes mellitus, dyslipidaemia, and more recently, alterations in phosphocalcic metabolism affecting bone health. [47]

A variable pattern for the biochemical parameters controlling bone formation and resorption has been described. Teichmann et al [48] showed a decrease in bone formation markers and increases in bone resorption markers in those patients on HAART, regardless of variables such as the type and duration of treatment therapy. Most longitudinal studies involving HAART-naïve individuals showed bone density declined by 2-6% within 24-48 weeks after HAART initiation [49-52].

Several studies [51, 53, 54] have reported an increase in bone resorption markers relative to bone formation markers, suggesting an increase in bone turnover, with resultant decrease in BMD in patients on Protease Inhibitor based (PI) regimes. Other studies [55, 56] analyzing BMD profiles in HIV positive patients did not show any deleterious effect of PI based treatment on bone mineral density.

It is interesting to note a study carried out in male Caucasian participants in a Western Australian HIV cohort proposed that Indinavir (a Protease Inhibitor) therapy may be associated with an increase in bone mineral density over time.[57]

A population based study conducted at a large U.S health care system with 10733 patients concluded that fracture prevalence was increased in HIV infected patients on a TDF based regimen compared to non- HIV infected patients. [6] A prospective randomized double- blind multicenter study concluded that TDF compared to Stavudine (D4T) was associated with significant increase in levels of biochemical markers of bone metabolism (bone-specific ALP, serum osteocalcin, serum C-telopeptide and urinary N- telopeptide) suggesting increased bone turnover. Of note was that the proportion of patients who met a protocol defined value of BMD loss (5% decrease in spine or 7% decrease in hip) was higher in the TDF group compared to the D4T group [52].

In general, regimens with a Tenofovir base, have shown a more significant reduction of BMD, probably through renal toxicity leading to phosphate loss with a compensatory mechanism of bone resorption [4, 51].

Complications of reduced bone mineral density

A lower BMD will lead to a higher risk and rates of bone fractures. Common fractures include vertebral compression fractures, and fractures of the distal radius and proximal femur [58].
Osteoporotic fractures occurring at the spine and the forearm are associated with significant morbidity, but the most serious consequences arise in patients with hip fractures, which is associated with a significant increase in mortality (up to 30%)[59].

Patients with osteoporotic vertebral fractures experience reduced quality of life, loss of independence, difficulties with activities of daily living, depression or low self-esteem, impaired gait and poor balance. Such fractures, especially when multiple, can result in reduction in volume of the thoracic and abdominal cavities leading to reduced pulmonary function and early satiety, respectively [60].

In many Western countries the combined lifetime fracture risk in women is 30-40%. Thus, more than one-third of adult women will sustain one or more osteoporotic fractures in their lifetime. In comparison, risks for men are about one-third of those in women, and are even lower for forearm fractures, but still represent a considerable burden [59].
This estimate is conservative since it only takes into account those fractures which come to clinical attention, so that the true risk of fracture is higher. This indicates the widespread prevalence of osteoporosis, with its substantial and growing economic burden in the society.

How is bone mineral density measured?

BMD can be measured by several methods. This includes Dual-energy X-ray absorptiometry, Quantitative computed tomography, Quantitative ultrasound, single photon absorptiometry, dual photon absorptiometry and digital X-ray radiogrammetry. [18].

The gold standard method for measuring BMD is the dual-energy X-ray absorptiometry (DXA) formerly referred to as DEXA scan. A DXA scanner produces two X-ray beams; one beam is high energy while the other is low energy. The amount of X-rays that pass through the bone (dependent on bone thickness) is measured for each beam. The difference between the two

beams helps determine the bone density and is presented as the ratio of bone content to the scanned area [61]. It emits low radiation levels coupled with high precision and is non-invasive in nature.

Dual photon absorptiometry (DPA) uses a radioactive substance to measure bone density. It can measure BMD at the hip and spine. DPA also uses very low doses of radiation but has a slower scan time than the DXA.

Mechanism of action of Quantitative Ultrasound

Quantitative ultrasound for bone assessment typically involves placing ultrasound transducers on either side of the bone of interest: one acts as a wave transmitter, and the other acts as the receiver [62].

These devices assess three main parameters:

- Broadband ultrasound attenuation(BUA)
- Speed of sound or velocity of sound(SOS)
- Quantitative ultrasound index stiffness

Broadband ultrasound attenuation measures the frequency of dependence of attenuation of the ultrasound signal that occurs as energy is removed from the wave, primarily by absorption and scattering in the bone and soft tissue [62].

Speed of sound and velocity of sound measure the distance the ultrasound signal travels per unit of time [63].

Quantitative ultrasound index and stiffness are composite parameters derived from broadband ultrasound attenuation and speed of sound or velocity of sound [62, 63].

Ultrasound parameters are typically lower in osteopenic/osteoporotic bone than in healthy bone [63].

Utility and comparability of QUS to other diagnostic tools for BMD

Several large prospective studies have shown that calcaneal quantitative ultrasound can predict future fracture risk nearly as well as DXA [64-67].

A meta-analysis of 25 studies in 2006 concluded that QUS is non- inferior to DXA using the current WHO recommended cut-offs [68].

Quantitative ultrasound performed similarly to BMD measured at the spine and femur by DXA in evaluating glucocorticoid induced osteoporosis.[69, 70]

Indirect studies and studies in-vitro have suggested that QUS might give information not only about bone density, but also about bone architecture and elasticity [71, 72].

Other studies have shown that unlike DXA, quantitative ultrasound may be able to assess bone quality in addition to BMD [17, 65, 73].

Other studies have noted that QUS appears to have the ability to discriminate between normal and osteoporotic patients partly independent of BMD in some cases [65].

Other advantages of QUS over the other methods are that it is radiation free, relatively cheaper, easier to use, and more portable [10].

Both cross-sectional and prospective studies have demonstrated that QUS can be used to discriminate normal from osteoporotic subjects nearly as effectively as traditional bone densitometry approaches [59, 64].

Specifically in HIV it has been used in countries like Senegal, to determine the level of BMD in patients on anti-retroviral medications [74].

These benefits, combined with clinical results showing good diagnostic sensitivity for fracture discrimination, have encouraged increased utilisation in clinical settings.

Osteoporosis in Kenya

Kenya like many sub-Saharan countries has a dearth of information on osteoporosis not only in the people afflicted by HIV but even in the general population. Several studies done in Kenya have concentrated on osteoporosis in the post- menopausal and elderly female populations [75-77] and in epileptics (87).We came across a single study from Senegal determining bone status using Quantitative ultrasound in a HIV infected population(74) .To the best of our knowledge, no

study has been done in East and Central Africa to determine the burden of decreased bone density among the HIV/AIDS population.

Statement of the problem

Kenya is home to one of the world's harshest epidemics-HIV and AIDS. An estimated 1.6 million people are living with HIV [3], and in 2012 approximately 57,000 people died due to AIDS- related illnesses.

The prevalence of osteoporosis is estimated to be around three times higher in HIV infected individuals compared to uninfected controls. [4, 5]. Reduced bone mineral density has been reported with increasing frequency on patients receiving HAART. Despite growing concerns about this complication, the impact on bones of HIV and its treatment in third world countries is poorly documented.

Use of HAART has been implicated in reduced bone mineral density with both protease inhibitors and nucleoside analogues singled out [52, 54, 79]. Chronic inflammation caused by HIV infection itself has been associated with increased osteoclastic activity [80] and bone resorption [81].

Available screening methods to determine reduced bone mineral density include dual energy X-ray absorptiometry (DEXA), calcaneal quantitative ultrasonography (QUS) and clinical risk assessment tools.

For screening epidemiological purposes QUS provides an appropriate tool for comparing bone mineral density between different groups and identifying factors associated with variation in bone density especially in settings where DEXA is unavailable[12, 13]

To our knowledge, there are no studies carried out in East and Central Africa to determine the prevalence of bone mineral density in HIV patients using quantitative calcaneal ultrasound despite sub Saharan Africa's poor social economic status and heavy burden of HIV.

This may be due to low index of suspicion among clinicians, limited availability and prohibitive cost of DEXA for assessing bone mineral density.

Justification

Osteoporosis is the most common metabolic bone disease worldwide and can result in devastating physical, psycho-social and economic consequences. Affected individuals experience pain, disability and diminished quality of life. It is often overlooked and under treated. However, in large part it is often clinically silent before manifesting in the form of a fracture. Up to two-thirds of vertebral fractures are painless [82].

HIV infection and HAART use has unequivocally been associated with decreased bone mineral density with a more than three-fold and more than six-fold increase in the risk of osteopenia and osteoporosis respectively compared to HIV negative populations [4].

With increasing number of people on HAART in Kenya and the changing epidemiological landscape of HIV infection to a chronic disease, there is increased need to explore and identify factors that may compromise the quality of life of patients on these medications. Early identification and provision of preventive/supportive care to overcome the increased morbidity and to improve quality of life of this population is of paramount importance.

Following recent developments in densitometry technology that has provided alternative methods of determination of bone mineral density; quantitative ultrasound appears to be the most widely used, providing a cheap, efficient and low risk alternative for estimating the prevalence of osteoporosis in Kenya. DEXA scans are virtually unavailable with only one machine available in the country in the private sector whose cost is prohibitive to the majority of Kenyans.

Furthermore, Mbagathi hospital has a population of 4426 patients (as of 31st July 2014) attending the CCC and therefore provides an ideal setting for exploring the burden of osteoporosis and osteopenia in this patient population.

Finally, there is paucity of data on studies looking at bone mineral density in HIV populations within Eastern Africa and the African continent at large. We have not come across any studies carried out in East and Central Africa determining bone mineral density in HIV infected patients.

Research question

Is there a difference in the prevalence of Bone Mineral Density (BMD) abnormalities using calcaneal quantitative ultrasound between HIV-infected patients on a TDF based first-line regimen for at least one year, HAART-naive HIV positive patients in Mbagathi Comprehensive Care Clinic (CCC) and a HIV negative control group seen at the Mbagathi Voluntary Counselling and Testing Centre(VCT)?

Null hypothesis

There is no difference in BMD abnormalities using calcaneal quantitative ultrasound between HIV-infected patients on a TDF based first-line regimen for at least one year, HAART-naive HIV positive patients in Mbagathi Comprehensive Care Clinic (CCC) and a HIV negative control group seen at the Mbagathi Voluntary Counselling and Testing Centre (VCT).

Objectives of the study

Broad objective

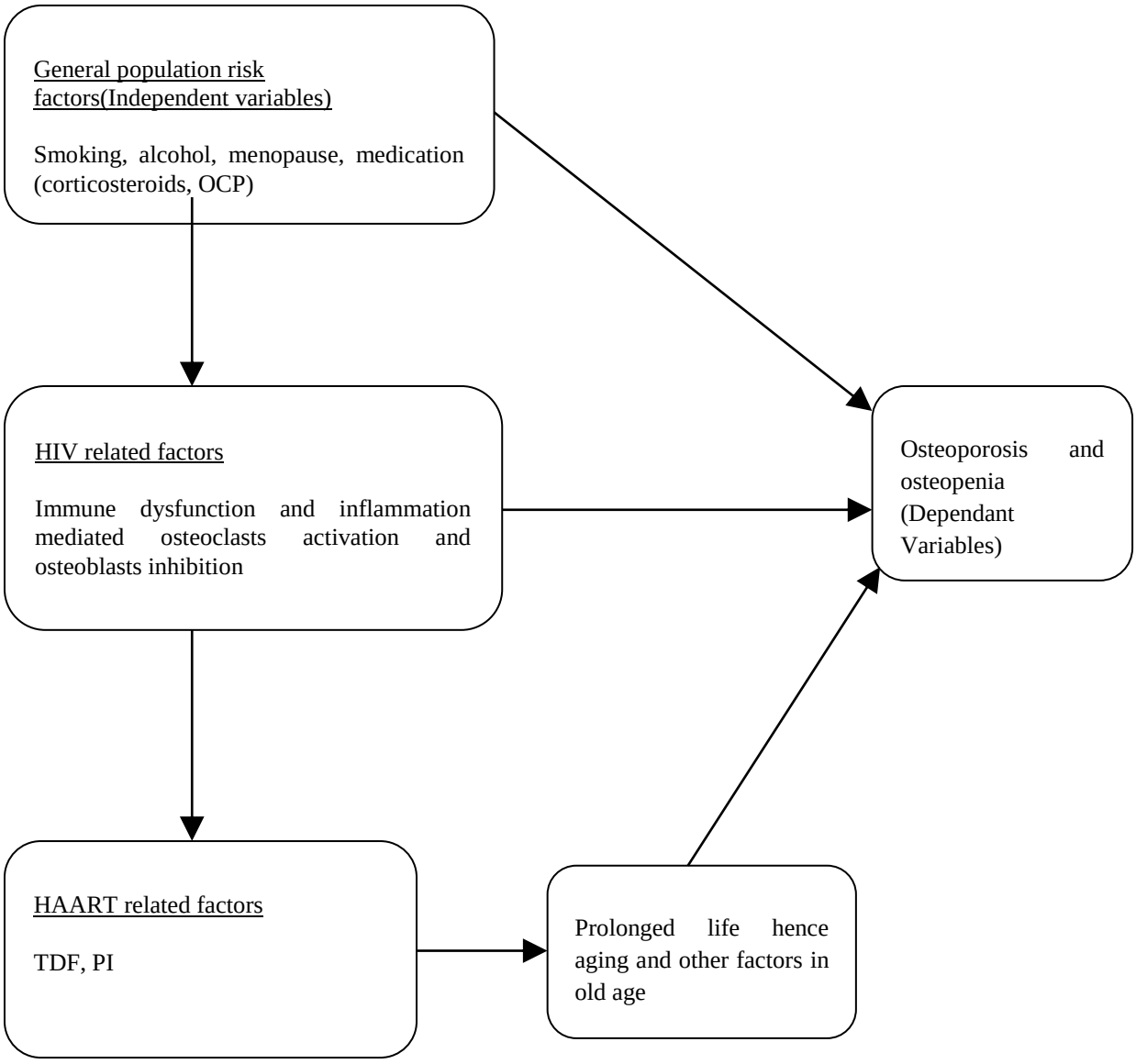
To determine and compare the difference in the prevalence of Bone Mineral Density (BMD) abnormalities using calcaneal ultrasound between HIV-infected patients on a TDF based first-line regimen for at least one year, HAART-naive HIV positive patients in Mbagathi Comprehensive Care Clinic (CCC) and a HIV negative control group seen at the Mbagathi Voluntary Counselling and Testing Centre(VCT).

Specific objectives

1. To determine the calcaneal BMD using calcaneal quantitative ultrasound in the HIV- infected patients on a TDF based first-line regimen for at least one year at Mbagathi Comprehensive Care Clinic.
2. To determine the calcaneal BMD using calcaneal quantitative ultrasound in HAART-naive HIV infected patients at Mbagathi Comprehensive Care Clinic.
3. To determine the calcaneal BMD using calcaneal quantitative ultrasound in HIV negative control group at the Mbagathi Voluntary Counselling and Testing Centre.
4. To compare the calcaneal BMD using calcaneal quantitative ultrasound among the HIV-infected patients on a TDF based first-line regimen for at least one year,HAART-naive HIV infected patients at Mbagathi Comprehensive Care Clinic and HIV negative control group at the Mbagathi Voluntary Counselling and Testing Centre.
5. To describe the occurrence of traditional risk factors associated with decreased BMD in the above populations, namely; previous fracture, oral corticosteroid use, oral contraception use, smoking, alcohol, and physical inactivity.

Figure 1: Conceptual framework

Conceptual framework



CHAPTER THREE: METHODOLOGY

Study design

This was a cross sectional comparative group descriptive study between HIV positive adult patients on TDF based first line regimen (exposed), HIV positive HAART-naïve adult patients (unexposed) and HIV negative adult group (control).

Study site and setting

Mbagathi level V hospital is a ministry of health facility located in Nairobi County, within Dagoretti constituency. The hospital was originally known as 'Infectious Disease Hospital' (IDH) under the then "King George VI Hospital" (currently Kenyatta National Hospital). It was built in the 1950's to offer health care services, mainly for infectious diseases which required isolation such as Tuberculosis, Measles, Meningitis and Leprosy. In 1995, IDH was curved from Kenyatta National Hospital and transformed into an autonomous District Hospital.

The hospital mainly serves people of low socio-economic status from the neighbouring Kibera slums but also the wider urban population. The hospital hosts one of the largest comprehensive care clinics that provides free health services to HIV patients and maintains a database of 4426 active adult patients who are on HAART and 1115 HAART naïve patients as at 31st July 2014.

On a typical day the Comprehensive Care Clinic (CCC) reviews an average of 100 patients. Presently the CCC has 1 consultant physician, 1 medical officer, 1 pharmacist, 7 clinical officers, 4 nurses and 2 VCT counsellors. The nurses also double up as counsellors. The CCC has a fully set up laboratory to support provision of care but is dependent on the hospitals radiology department for all imaging services.

The current standard first-line HAART regime used in Mbagathi is TDF/3TC/EFV or TDF/3TC/NVP which is consistent with the national guidelines.

Mbagathi Hospital also runs a weekday Voluntary Counselling and Testing (VCT) centre that attends to an average of 12 people daily. The HIV prevalence rate for the year 2014 was 7.2% which is above the national average.

Both the CCC and VCT centre operates every weekday from 8.00am to 5.00pm.

Study population

From a cohort of 4426 patients attending the Mbagathi CCC, HIV infected adult patients aged ≤40 years on a TDF based first line therapy for at least one year and HIV naïve adult patients aged ≤ 40 years were selected.

A HIV negative adult control group was selected from those attending the VCT centre in Mbagathi Hospital.

Inclusion criteria

Group 1

1. Those who are HIV positive and are on a TDF based first-line regimen for at least one year.
2. Adults between 18- 40 years of age.
3. Those who give informed consent.

Group 2

1. Those who are HIV positive and are HAART-naive
2. Adults between 18- 40 years of age.
3. Those who give informed consent.

Group 3

1. Those who are HIV negative.
2. Adults between 18-40 years of age.
3. Those who give informed consent.

Exclusion criteria

1. Those who are single/double lower limb amputees.
2. Those with bilateral calcaneal/foot wounds.

Sample size

In this study we set out to detect a 20% difference in the BMD between HAART-naïve and HAART exposed patients. We therefore used a prevalence of 52% for osteopenia based on a meta-analysis of 37 studies on BMD prevalence between 1996 and 2005 [4]

Using the sample size formula for comparison of proportions suggested by Smith and Morrows [83] the required sample size in each group was:

n=[(z1+z2)22p(1-p)]/(p1- p2)2

Where P_1 is proportion of osteopenia in ART naïve patients (32%)

P_2 is proportion of osteopenia in ART exposed patients (52%) i.e. 52-20% detectable difference

P is the average of p_1 and p_2 (42%) – (52+32)/2

Z_1 is the specified significance level of 5% (1.96)

Z_2 id the power of the study of 80% (0.84)

$n= [(1.96+0.84)^2 \times 2 \times 0.42 (1-0.42)]/ (0.52 – 0.32)^2$

$=[7.84x2x0.42x0.58]/0.04$

$=95$

Therefore 95 patients were required in each arm hence a total of 285 patients.

A 10% adjustment in sample size was made for non-response and the total sample size was 315 patients (105 participants in each arm).

Sampling Procedure

Study Procedure

Mbagathi CCC has an electronic system used to scheduled patients for their next clinic visit, with patients being scheduled in advance. The database has appointment bookings for the next 3 months, which is updated daily.

This database holds key demographic characteristics of age, gender and treatment status. The data was stratified into groups based on the treatment status (HAART exposed or naïve) and serially organized after excluding patients older than 40 years and less than 18 years.

A random number generation table was then used to randomly select 20 patients on each clinic visit by the principal investigator of which 10 patients were from the HAART exposed arm and 10 patients from the HAART-naïve arm. A random number generation table was also used to select 5 participants from the VCT center by the principal investigator (twice weekly).

Approval for consent from the selected participants was then sought by the principal investigator after which their clinical appointment was expedited. Those who met the exclusion criteria were informed (and excluded) and another random number(s) generated and the process repeated. Those selected to undergo the study then had the study procedure carried out (questionnaire and ultrasound procedure).

The questionnaire captured demographic data, duration of HIV/HAART use and occurrence of traditional risk factors among the respondents. The study procedure is laid out in a flowchart. (Appendix IV)

QUS bone mineral density was assessed using a heel ultrasonic gel-coupled QUS system, the Sunlight Mini Omni (Beam Med Ltd, Israel). The Mini Omni ultrasound device measures two parameters at mid-calcaneus: bone ultrasound attenuation (BUA) (in dB/MHz) and speed of sound (in m/s).

The participants were asked to remove their shoes and stand with one foot on the ultrasound machine. Three repeated measurements with repositioning was performed on the same foot for all participants. BUA was expressed as a T-score (standard deviations from the mean value in normal young individuals of the same sex) using the manufacturer's age- and sex-specific reference data.

A bone densitometry form was filled for each participant showing their bone mineral density. This form was handed over to their clinician for appropriate advice or treatment based on the T-score findings.

Data variables

Dependent variable

Reduced bone mineral density was categorized into either osteopenia (T-score between −1 and −2.49 versus normal) or osteoporosis (T score ≤ -2.5 versus normal).

Independent variables

Data documenting risk factors for low bone mineral density was collected during a face-to-face interview for both patients and controls. They include previous bone fracture, smoking status, alcohol consumption, oral corticosteroid use, oral contraception use and physical activity.

Physical activity was assessed by a short frequency questionnaire (the International Physical Activity Questionnaire- IPAQ). Weight and height was measured during the interview to calculate BMI (weight (kg)/height (m^2).

Data management and analysis

Data was collected by the Principal investigator, trained research assistants (by the Principal Investigator) and quantitative ultrasound technician. Data was entered into a password protected Microsoft Access database managed by the statistician. Once data entry was complete, entries in the database were compared to the hard copies to ensure accurateness. Inconsistencies were detected by use of simple frequencies and correlations and those identified were rectified before data analysis began. Data was analysed using SPSS software version 20 for windows.

Descriptive statistics (mean, mode, frequencies) was reported to describe the dependent and independent variables while inferential statistics to establish the association between bone mineral density and the various risk factors using a chi-square and mantel-Hansel analysis. A bi-variate logistic regression was used to analyze the independent predictors of decreased bone mineral density.

Factors that were significant at the bi-variate stage underwent a multivariate analysis to identify the predictors of decreased bone mineral density.

Ethical considerations

This was a non-invasive descriptive study undertaken only after approval by the Department of Clinical Medicine & Therapeutics, University of Nairobi and the KNH/UoN Scientific and Ethical Review Committee. Ref:KNH-ERC/A/192.

The objectives and purpose of the study was clearly explained to eligible participants in a language suitable to them prior to inclusion into the study. Only patients who gave informed consent were enrolled. Patients were free to withdraw during the study period without discrimination. Information gathered from the participants was kept confidential.

All information collected onto the data collection tool was stored in a locked cabinet accessible only to the principal investigator. The information collected was only used for the purposes of this study and will not be shared with any other persons.

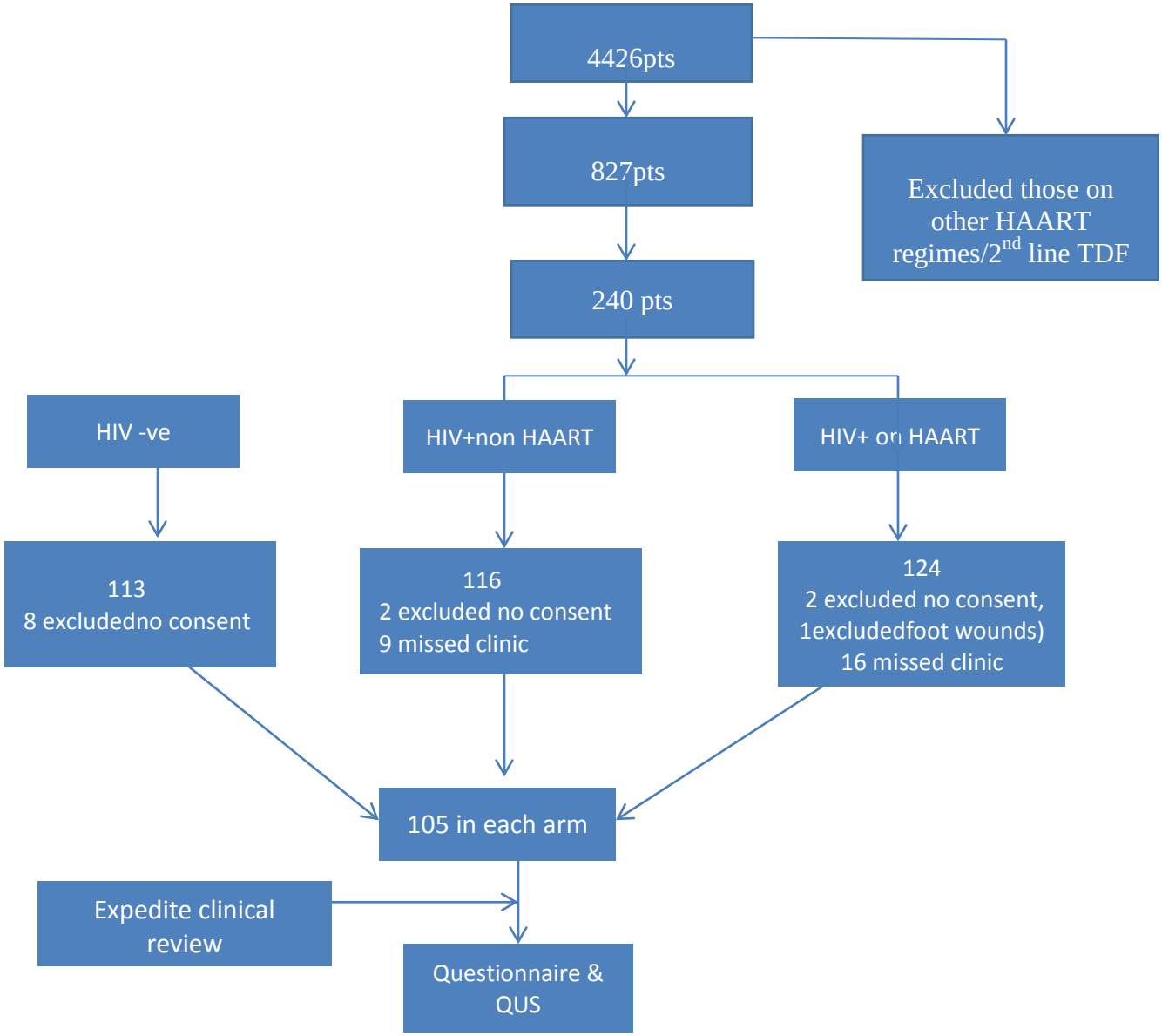
This will be stored for two years upon completion of the study in the event of any need for verification or clarification purposes. Upon two years, the stored data will then be destroyed.

CHAPTER FOUR: RESULTS

Introduction

Data was collected over a fifteen week period. Out of the 4426 adult patients attending Mbagathi CCC, 105 patients were recruited in each arm (HAART naïve and those on HAART). 105 individuals who were HIV negative were recruited from the Mbagathi VCT centre.

Figure 2: Recruitment process



Socio-demographic characteristics

56.5% of the total respondents were female while 43.5% were male. 50.5% were single, 94.3% lived in urban areas and 39.0% of them had attained secondary level of education. 37.8% of the respondents were self-employed while 34% of the respondents were earning below Kenya shillings 2500 per month. Gender (p-value = .066) of the respondents was similarly distributed across treatment arms while residence, marital status, highest level of education, occupation and income level were significantly different among the treatment arms (p-values < .001). This was partly attributed to the inability to match for age and sex among the comparative arms.

Table 2 : Socio-demographic characteristics

Characteristic	Categories	HIV negative (%)	HIV non-HAART (%)	HIVon HAART (%)	Total (%)	P-value
Age	18-22 23-28 29-34 35-40	15(14.1) 46(44.7)31(29.5) 12(11.6)	13(12.5) 22(21.1) 43(40.6) 27(26.8)	7(6.8) 15(14.2) 31(29.5) 52(49.5)	43.5% (29- 28yrs)	**<.001**
Gender	Male Female	48(45.7) 57(54.3)	46(43.3) 59(56.7)	44(41.4) 61(58.6)	137(43.5) 178(56.5)	.066
Marital status	Married Single Divorced Widowed	14(13.2) 83(52.2) 8(19.5) 0(.0)	32(30.2) 53(33.3) 18(43.9) 2(22.2)	60(56.6) 23(14.5) 15(36.6) 7(77.8)	106(33.7) 159(50.5) 41(13.0) 9(2.9)	**<.001**
Residence	Rural Urban	8(44.4) 97(32.7)	1(5.6) 104(35.0)	9(50.0) 96(32.3)	18(5.7) 297(94.3)	**<.001**
Highest education level	None Primary	0(.0) 21(20.6)	1(100.0) 46(45.1)	0 (.0) 35(34.3)	1(0.3) 102(32.4)	**<.001**

	Secondary	21(17.1)	50(40.7)	52(42.3)	123(39.0)	
	Tertiary	63(60.8)	8(9.0)	18(20.2)	89(28.3)	
Occupation	Unemployed	12(25.0)	23(47.9)	13(27.1)	48(15.2)	**<.001**
	Student	60(85.7)	8(11.4)	2(2.9)	70(22.2)	
	Self employed	21(17.6)	45(37.8)	53(44.5)	119(37.8)	
	Civil servant	3(25.0)	4(33.3)	5(41.7)	12(3.8)	
	Other	9(13.6)	25(37.9)	32(48.5)	66(21.0)	
Income level per month	Below 2500	55(51.4)	39(36.4)	13(12.1)	107(34.0)	**<.001**
	2500 - 5000	22(36.1)	25(41.0)	14(23.0)	61(19.4)	
	5000 - 10000	16(23.2)	22(31.9)	31(44.9)	69(21.9)	
	10000 - 30000	10(14.9)	18(26.9)	39(58.2)	67(21.3)	
	>30000	2(18.2)	1(9.1)	8(72.7)	11(3.5)	

The mean age of HIV negative patients was 28.02(±1.12) years with 29.5% aged between 21 and 24 years. The mean age of HIV positive patients not on HAART was 30.61(±1.15) years with 21.6% aged between 30 and 33 years while the mean age of HIV positive patients on HAART was 34.69(±1.27) years with 28.4% aged between 36 and 39 years. There was significant age difference between the comparative arms (Kruskal Wallis test p-value < .001) as shown in figure 3. This was attributed to the inability to match for age and sex among the comparative arms.

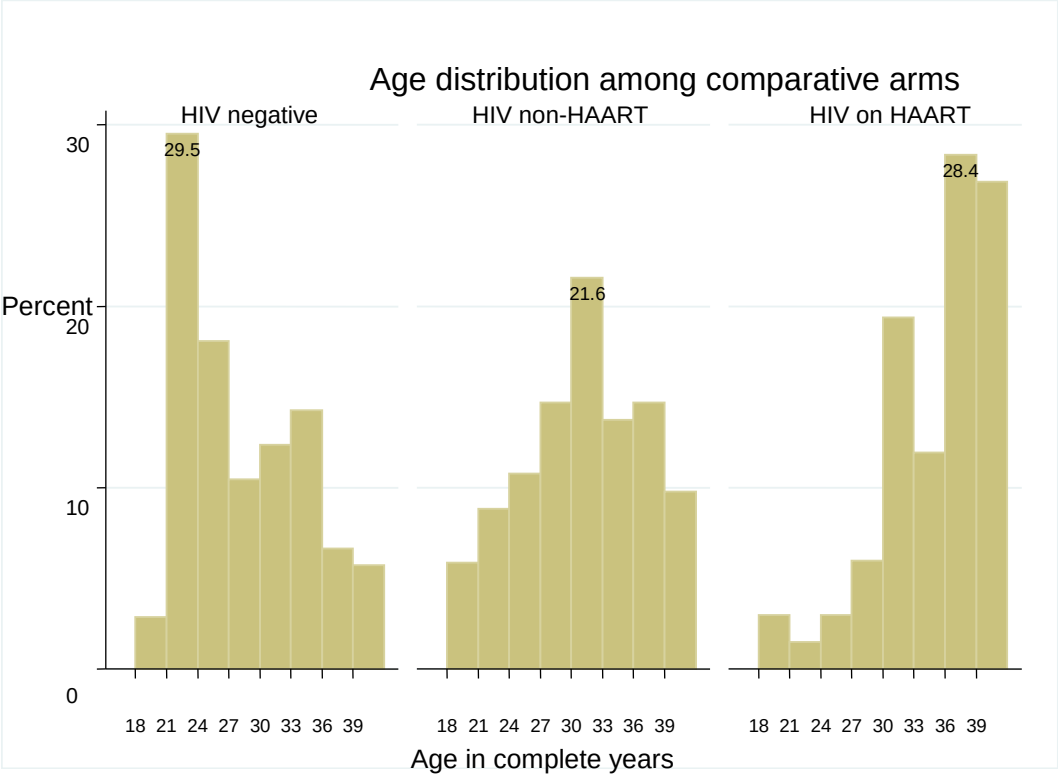


Figure 3: Age distribution among comparative arms

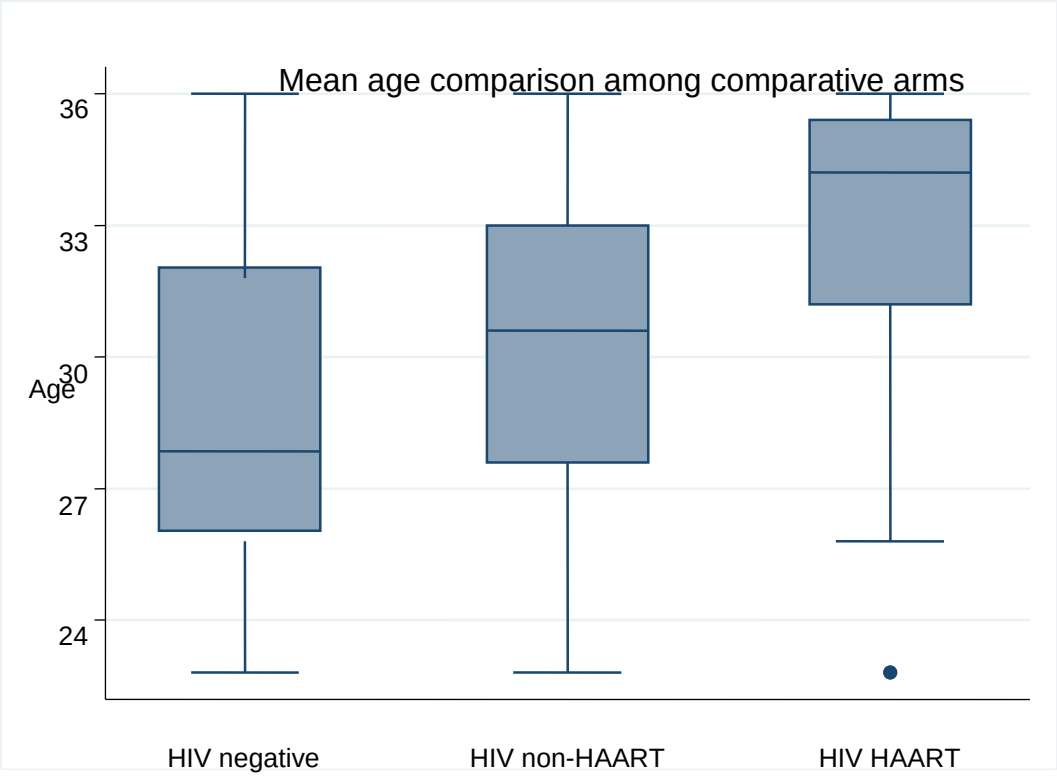


Figure 4: Mean age comparison among the comparative arms

T-score distribution

Majority 112(35.6%) of the total respondents had a T-score of between -2 and -1 with a mean of -1.416(±0.115).

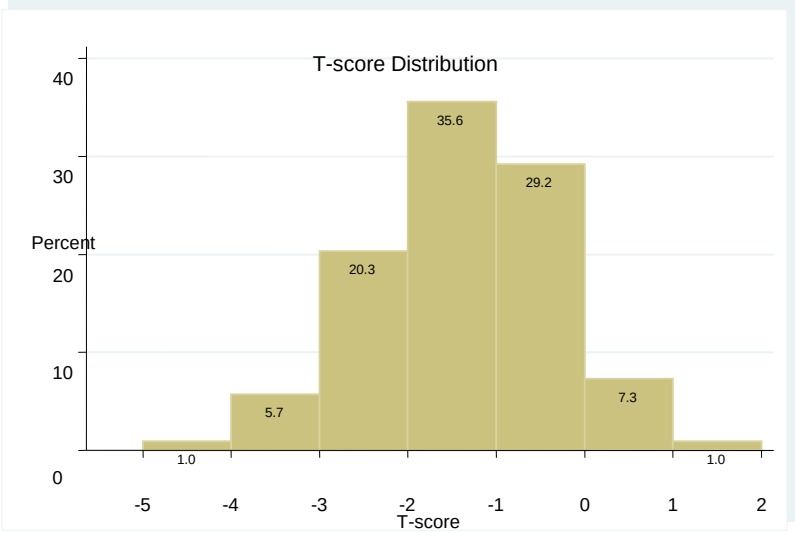


Figure 5: T-scores distribution

Of the total respondents, 118(37.5%) had normal BMD, 154(48.9%) had osteopenia and 43(13.7%) had osteoporosis.

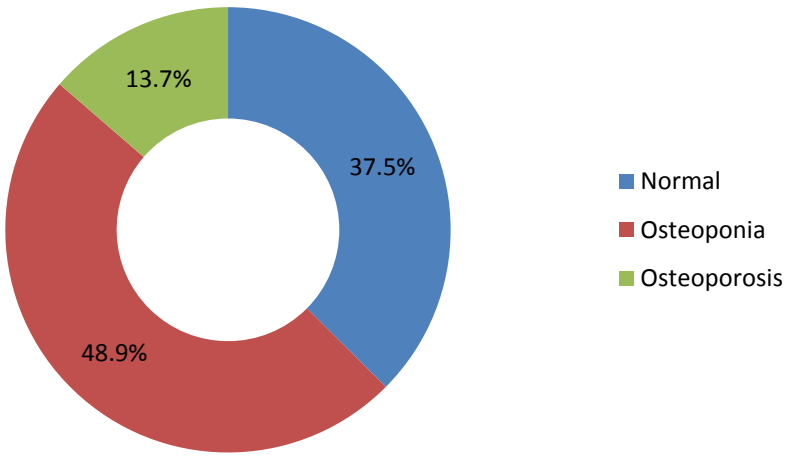


Figure 6: T-score distribution among the total respondents

Majority of respondents who had normal BMD 45(38.1%) and those who had osteopenia 56(36.4%) were HIV negative while most 25(58.1%) of respondents who had osteoporosis were HIV positive on HAART. The prevalence of osteoporosis among HIV positive respondents on HAART was significantly higher compared to HIV positive respondents not on HAART (Z-test p-value = .001) and HIV negative respondents (Z-test p-value = .001).

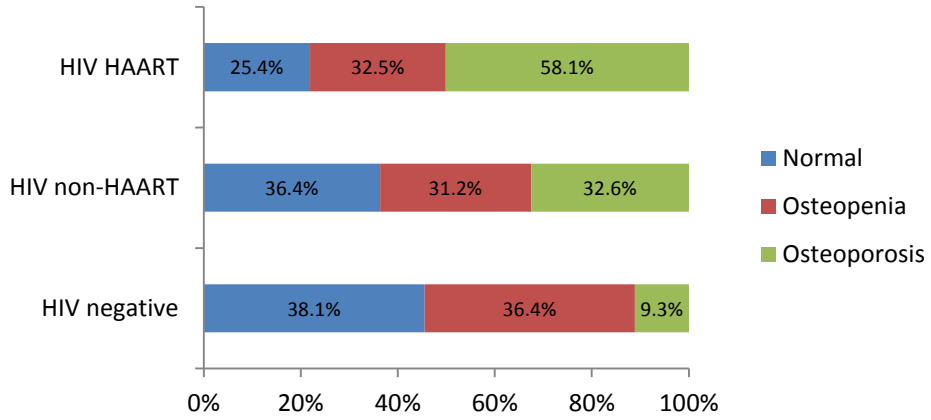


Figure 7: Prevalence of BMD abnormalities among comparative arms

The mean T-score of HIV negative respondents was -1.197(±0.168) compared to mean T-scores of -1.311(±0.184) and -1.740(±0.231) in the HIV positive HAART naïve and HIV positive on HAART respondents respectively. There was a significant difference in mean T-score between the comparative arms (ANOVA p-value < .001).

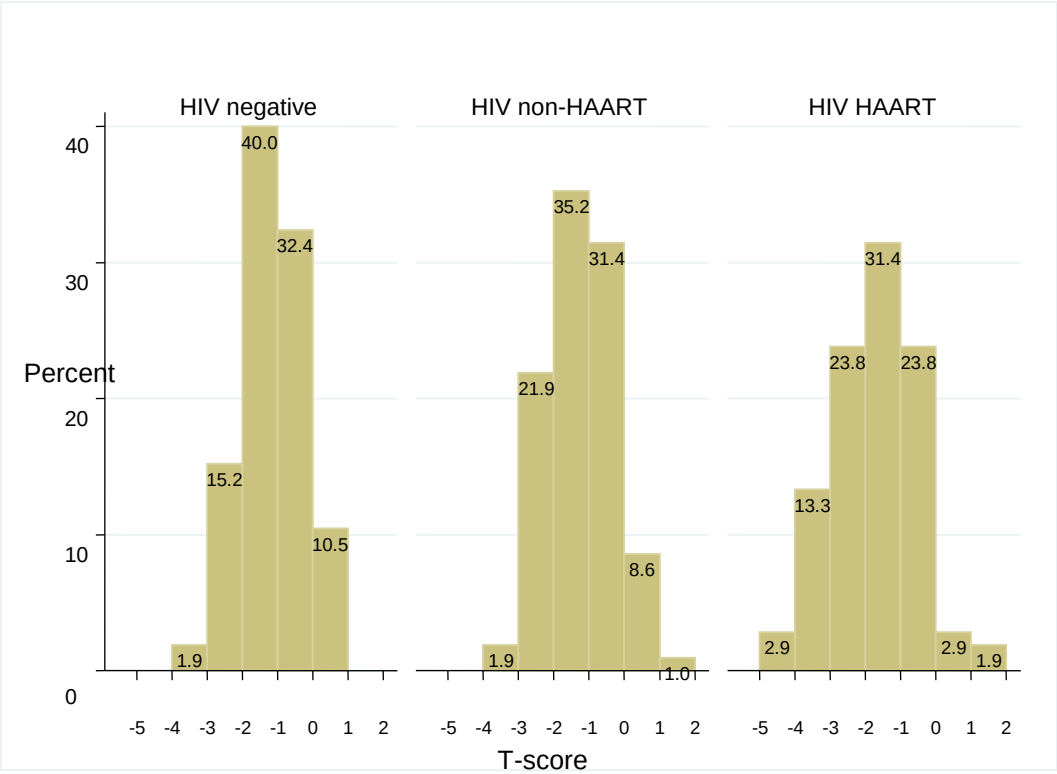


Figure 8: T-score distribution and comparative arms

Post-hoc Tamhane test pair wise comparison revealed that HIV positive respondents on HAART had significantly the least (most negative) T-score as compared to other treatment arms.

Table 3: T-scores mean comparison among comparative arms(Tamhane test)

(I) Arm	(J) Arm	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower	Upper
HIV negative	HIV non-HAART	.1143	.1252	.741	-.187	.416
	HIV HAART	.5424*	.1440	.001	.196	.889
HIV non-HAART	HIV negative	-.1143	.1252	.741	-.416	.187
	HIV HAART	.4281*	.1487	.013	.070	.786
HIV HAART	HIV negative	-.5424*	.1440	.001	-.889	-.196
	HIV non-HAART	-.4281*	.1487	.013	-.786	-.070
*. The mean difference is significant at the 0.05 level.						

Among respondents living with HIV, there was an insignificant correlation (Pearson r = - 0.085, p-value = .218) between T-score and length of living with HIV since diagnosis.

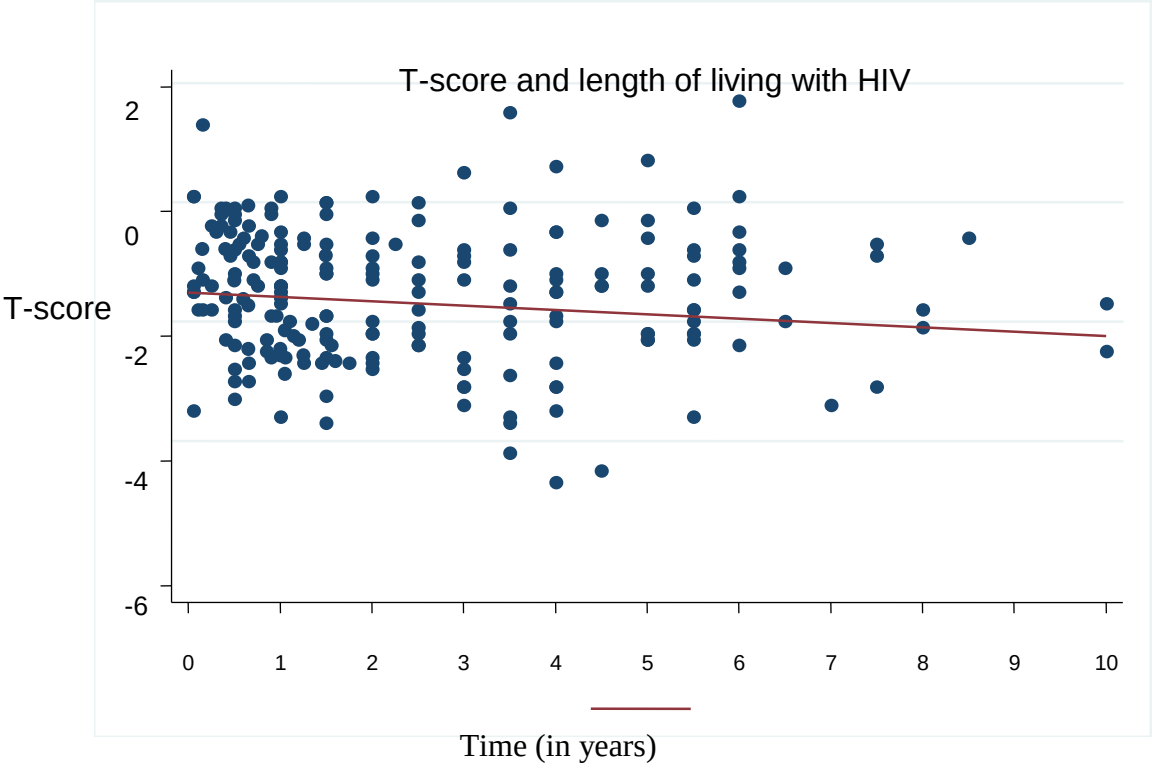


Figure 9: T-score and Length of living with HIV since diagnosis

Among respondents living with HIV and taking HAART, there was an insignificant correlation (Pearson r = .105, p-value = .288) between T-score and length of taking HAART.

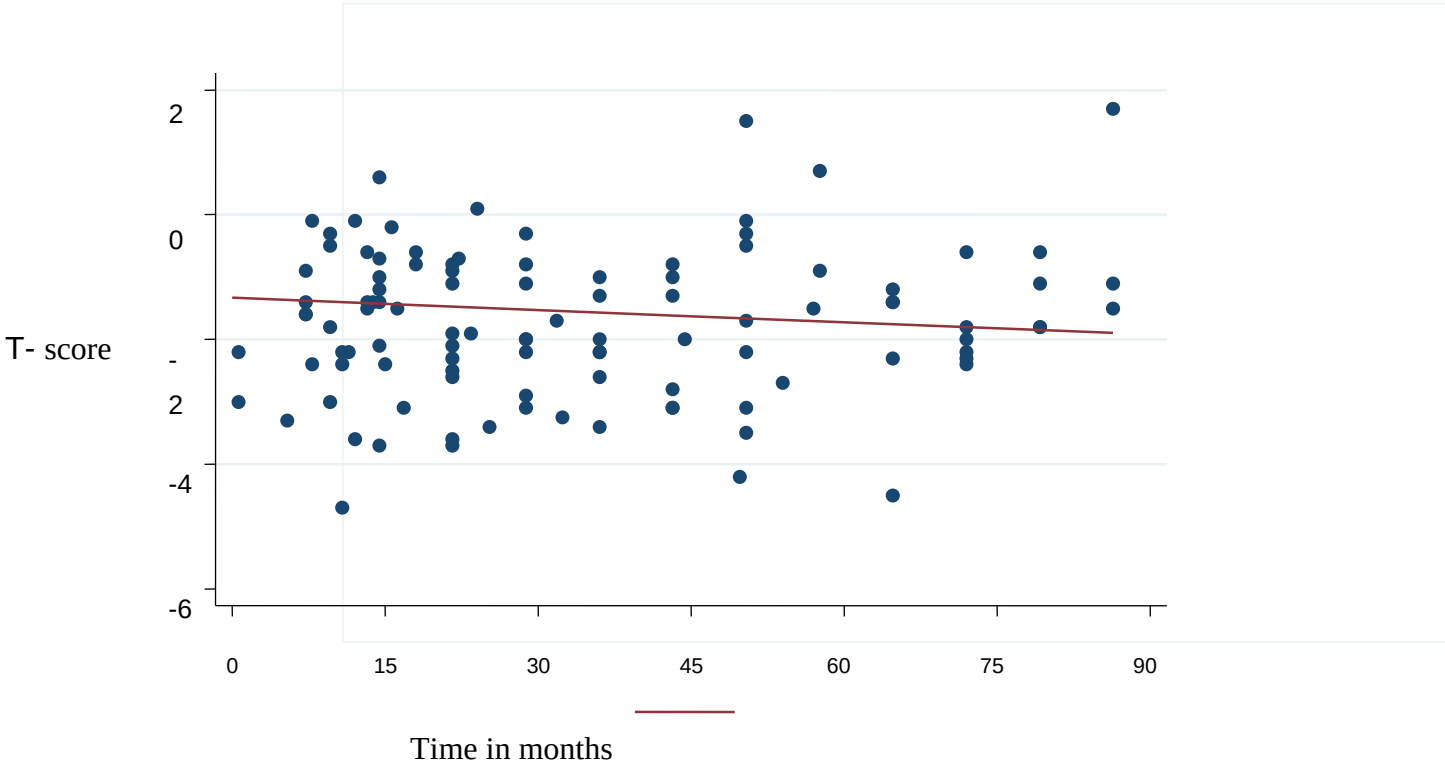


Figure 10: T-score and length of taking HAART

Socio-demographic characteristics and BMD

There was significant negative correlation between T-score and age of the respondents (Spearman r = -.121, p-value = .032). This implied that older patients were associated with lower levels of BMD (i.e. more negative BMD)

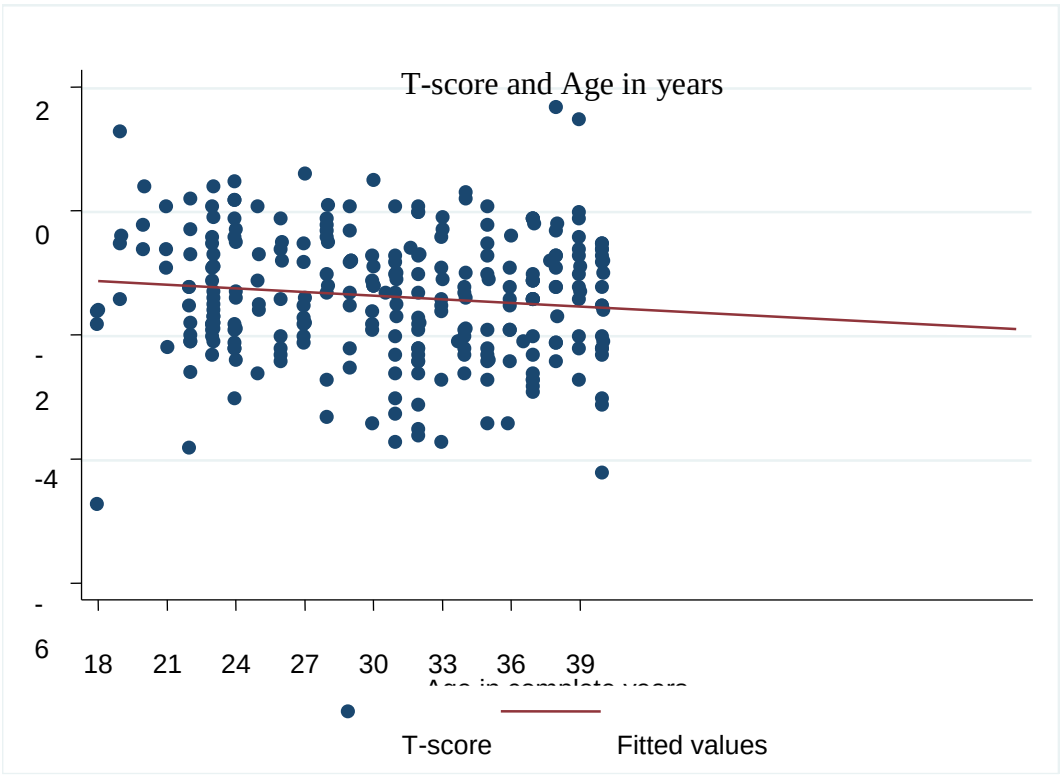


Figure 11: T-score and age of respondents

There was no significant association between low BMD and marital status (p-value = .086), gender (p-value = .257), residence (p-value = .195), highest education level (p-value = .756), occupation (p-value = .280) and income level per month (p-value = .798).

Table 4: Socio-demographic characteristics and BMD

Characteristics	Categories	Normal BMD	Osteopenia	Osteoporosis	P- value
Age	18-22	48.1	30.2	21.7	**.032**
	23-28	44.5	29.3	26.2	
	29-34	38.4	33.0	28.6	
	35-40	22.0	31.9	46.1	
Gender	Male	39.7%	50.9%	9.5%	
	Female	36.2%	47.7%	16.1%	.257
Marital status	Married	32.1%	46.2%	21.7%	
	Single	39.6%	52.8%	7.5%	
	Divorced	36.6%	46.3%	17.1%	.086
	Widowed	66.7%	22.2%	11.1%	
Residence	Rural	33.3%	38.9%	27.8%	.195
	Urban	37.7%	49.5%	12.8%	
Highest education level	None	100.0%	0.0%	0.0%	.756
	Primary	36.3%	47.1%	16.7%	
	Secondary	39.8%	48.0%	12.2%	
	Tertiary	34.8%	52.8%	12.4%	
Occupation	Unemployed	41.7%	45.8%	12.5%	

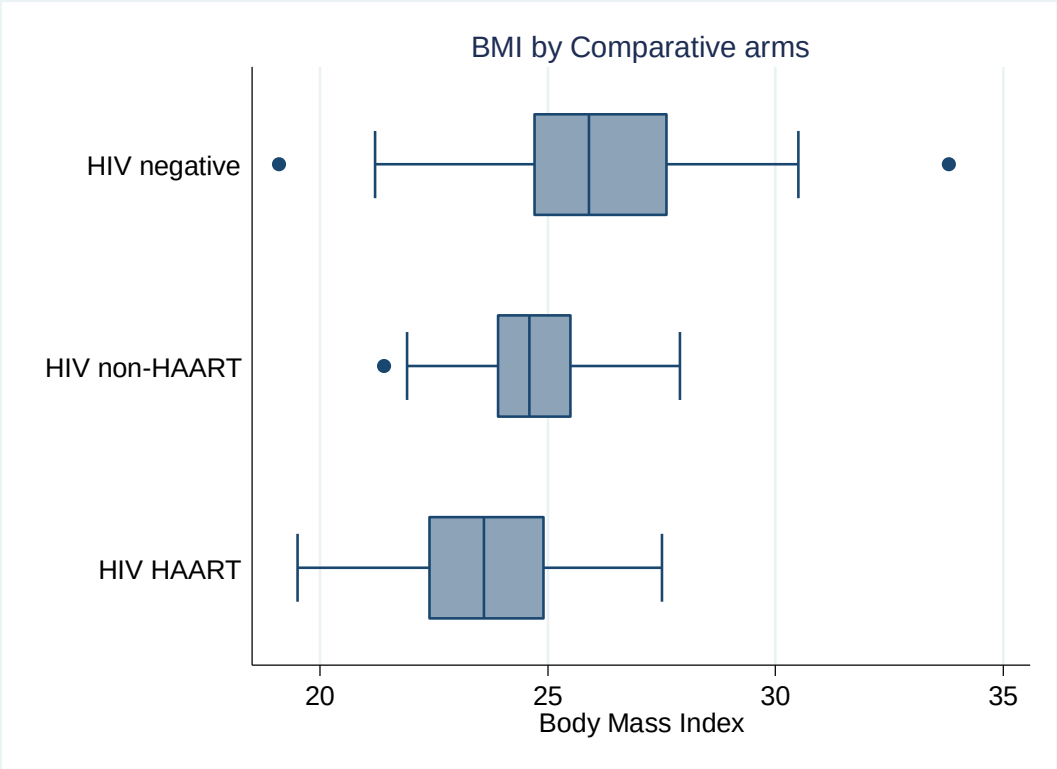
	Student	38.6%	55.7%	5.7%	
	Self employed	37.0%	46.2%	16.8%	.280
	Civil servant	58.3%	25.0%	16.7%	
	Other	30.3%	53.0%	16.7%	
Income level per month	Below 2500	40.2%	49.5%	10.3%	.798
	2500 – 5000	34.4%	47.5%	18.0%	
	5000 – 10000	39.1%	44.9%	15.9%	
	10000 – 30000	37.3%	50.7%	11.9%	
	>30000	18.2%	63.6%	18.2%	

Table 5: Traditional risk factor distribution among the comparative arms.

Risk factor	**HIV negative**	**HIV Non HAART**	**HIV On HAART**
Oral corticosteroid use	3.8%	1.9%	0.0%
Current smokers(n=11)	6.7%	1.9%	1.9%
Used to smoke(n=33)	8.6%	7.6%	15.2%
Alcohol intake(n=160)			
once monthly or less(122)	50.4%	45.7%	20.0%
weekly(35)	19.0%	8.6%	5.7%
daily(3)	1.9%	0.9%	0.0%
Sustained bone fracture	16.1%	20.0%	13.3%
Physical activity levels			
Vigorous	22.8%	13.3%	14.2%
Moderate	43.9%	35.3%	45.7%
minimal	33.3%	51.4%	40.0%
BMI(Kg/m^2)	26.1	24.8	23.6

Body Mass Index (BMI)

There was a significant difference in BMI among the comparative arms (ANOVA p-value < .001) with HIV negative patients having significantly the highest BMI on average followed by HIV non-HAART then HIV HAART patients.



There was significant positive correlation between T-score and BMI (Pearson R = .085, p-value = .043)

Corticosteroid use

Only 6 respondents of whom 4(3.8%) were HIV negative and 2(1.9%) were HIV positive HAART naive had used corticosteroids continuously for a period of more than 3 months. The mean T-score among respondents who used corticosteroids continuously for a period of more than 3 months was -1.92 while those who had not used was -1.41. There was no significant difference in T-scores with whether one had used oral corticosteroids continuously for a period of 3 months (t-test p-value = .260)

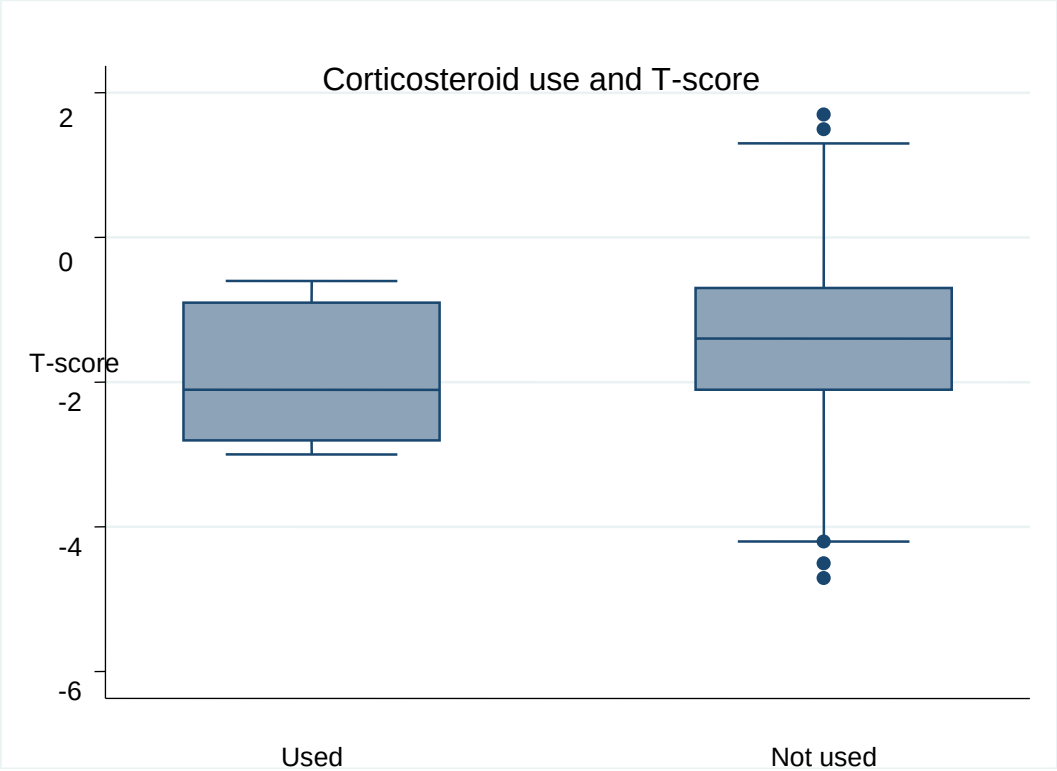


Figure 12: Corticosteroid use and T-score

Tobacco smoking

Of the total respondents, 11(7 HIV negative, 2 HIV HAART naïve and 2 HIV on TDF) were currently smoking while 33(9 HIV negative, 8 HIV HAART naïve and 16 HIV on TDF) used to smoke tobacco. The mean T-score among respondents who were current smokers was -1.84(±.48), the mean T-score among patients who used to smoke was -1.58(±.28) while among those who had not smoked was -1.42(±.13). There was no significant difference in T-scores with whether one had smoked tobacco (One Way ANOVA p-value = .117). There was also no significant correlation between pack years smoked and T-score (Spearman Rho = -.112, p-value = .547)

Alcohol intake

49.2% (155) of the total respondents had never taken alcohol. Of those who were taking alcohol, 75 %(122) were taking alcohol once monthly or less, 21.9% (35) were taking weekly while 1.9%(3) were taking daily. The majority of respondents who took alcohol monthly or less (50.4%), weekly (19.0%) and daily (0.9%) were HIV negative.

The mean T-score among respondents who had never taken alcohol was -1.47(±.15), the mean T-score among respondents who were taking monthly was -1.41(±.19), the mean T-score among respondents who were taking weekly was -1.13(±.39) while among those who were taking daily was -2.30(±1.24). There was no significant difference in T-scores with frequency of alcohol use. (ANOVA p-value = .148).

Bone Fractures

16.5 %(52) of the total respondents had sustained bone fractures. Of the respondents who had sustained bone fractures, 34.6% (18) were HIV negative, 38.4% (20) were HIV HAART naïve and 26.9% (14) were HIV on TDF based regimes. The mean T-score among respondents who had sustained bone fractures was -1.53(±.28) while those who had not sustained bone fracture was -1.39(±.13). There was no significant difference in T-scores between those who had sustained bone fractures and those who had not. (T-test p-value = .391).

Physical activity

Majority(43.8%) of the HIV negative respondents were involved in moderate intensity physical activities, majority(51.4%) of the HIV HAART naïve respondents were involved in minimal intensity physical activities while majority(45.7%) of the HIV positive on HAART respondents were involved in moderate intensity physical activities. There was an insignificant difference in intensity of physical activities undertaken by the respondents between the comparative arms (Chi-square p-value = .447).

Table 6 : Physical activity intensity among the comparative arms

Physical activity	HIV negative	HIV non-HAART	HIV HAART	Total
Minimal	35	54	42	131
Moderate	46	37	48	131
Vigorous	24	14	15	53

The mean T-score among those involved in vigorous activity was -1.32(±0.19), among those involved in moderate activity was -1.45(±0.17) and among those involved in minimal activity was - 1.56(±0.29). There was no significant difference in T-scores among intesity of physical activities (ANOVA p-value = .325).

Multivariate analysis

Factors that were significant at the bi-variate stage (age and BMI) underwent multivariate analysis to identify the predictors of decreased bone mineral density. BMI was the only risk factor identified as significant to predict occurrence of decreased bone mineral density at a p-value of 0.046.

Table 7: Multivariate analysis

Variable	Significance	Odds Ratio	95% C.I. for Odds Ratio	
			Lower	Upper
Age in years	.364	1.017	.981	1.053
BMI (Ref ≥ 25)	**.046**	1.962	1.607	2.225

CHAPTER FIVE: DISCUSSION

Our study populations were adults between 18-40 years old. We did note a female preponderance (56.5%) among our respondents. This was in keeping with our national HIV demographics. (1, 3).We did not match age and gender among the comparative arms due to resource limitations.

Though we did not match age and gender among the comparative arms (resource constraints), our study showed a significant negative correlation between T-score and age of the respondents. This implied that older respondents were associated with lower Bone Mineral Density levels. John et al (58) attributed this to age related changes in bone homeostasis and increased bone fragility

Our study showed that the prevalence of osteoporosis among HIV positive respondents on HAART was significantly higher compared to HAART naïve and to HIV negative respondents. Our study showed 58.1%, 32.6% and 9.3% of those on HAART, HIV positive HAART naïve and HIV negative respondents respectively were osteoporotic. This reflects a six-fold higher prevalence of osteoporosis between HIV infected individuals and the HIV negative controls. In other studies comparing HIV infected to uninfected populations the T-score difference between the two groups(HIV infected and uninfected) varied from 2.5-fold to 10-fold(47,53,55,56).

QUS and DEXA simply measures different bone characteristics (bone quality and bone quantity respectively). QUS can thus determine the strength of bone micro-architecture which may be associated with impaired bone structure with a higher risk of fractures and lower BMD (90). QUS parameters including Speed Of Sound (SOS), Bone Ultrasound Attenuation (BUA) and bone stiffness provide additional, specific and different information which may be useful in the integrative assessment of bone health. (91)

It is also important to note that QUS has been extensively researched in large prospective studies and meta-analyses and has demonstrated comparable utility and diagnostic accuracy to DEXA at hip and non-spinal bone sites (64-70)

In our study, the prevalence of osteopenia was 32.5%, 31.2% and 36.4% in those HAART, HIV positive HAART naïve and HIV negative respondents respectively. This was in keeping with a

meta-analysis of 37 studies by Brown et al(4) which showed significant heterogeneity between the studies for reduced BMD with osteopenia of between 4%- 56% in the HIV negative respondents and 13%-62% in the HIV positive respondents on HAART. Poor dietary intake of calcium rich foods especially in childhood and adolescence could explain the similar rates of osteopenia across the comparative arms (12).

Several studies (4, 51) have shown the association of Tenofovir disoproxil fumarate (TDF) with nephrotoxicity and hypophosphatemia due to renal tubular dysfunction leading to impaired Vitamin D metabolism which may determine low BMD in HIV patients.

HIV infection has been associated with decreased BMD mainly through cytokine dysregulation and impaired Vitamin D metabolism (40-43).Thus the longer duration of living with HIV may be associated with low BMD (46).We did not find significant association between T-score values and length of living with HIV which could be attributed to the relatively short duration of living with HIV among the respondents, with a mean duration of 4.8 years.

Gender, marital status, residence, education level, occupation and income levels were insignificantly associated with BMD abnormalities.

Body Mass Index (BMI) was 23.6%, 24.8% and 26.1% among HIV positive on HAART, HIV positive HAART naïve and HIV negative respondents respectively. We did find a significant negative correlation between T-score and BMI. Respondents with low BMI were likely to have lower BMD values. Indeed, bone mass is known to be positively correlated with body weight or BMI, as an indicator of muscular mass, and HIV infected individuals usually have lower body weight compared with HIV negative persons(88). This observation is in agreement with Amandine et al (74) and Bolland et al (85) who suggested that the relationship between HIV infection and low bone mineral density was mediated by low body weight.

The difference in Bone Mineral Density abnormalities was in part, related to the difference in Body Mass Index between those on HAART and HIV negative respondents. Bone mass is known to be positively correlated with BMI, as an indicator of muscular mass, and HIV infected individuals usually have lower body weight compared with uninfected persons(22,26). A meta-

analysis by Bolland et al(84) showed that, after adjustment for weight, residual between-group differences in bone mineral density were small(2.2-4.7%) and unlikely to be clinically significant.

Poor dietary intake of milk especially in childhood and adolescence has been associated with low bone mineral density (12, 35). This could partly explain low BMD in our study participants who are from a low socioeconomic catchment area.

Most longitudinal studies involving HAART-naïve individuals showed that bone mineral density declined by 2-6% within 24-48 weeks after initiation of HAART (49-52). Thereafter, bone mineral density values remained stable or even increased slightly (57). We did not find any association between QUS bone mineral density and duration of treatment with HAART. This could be attributed to the fact that majority of the respondents (68%) had received HAART for at least 45 months.

Persons who consume moderate amounts of alcohol have a lower risk of hip fractures compared to heavy drinkers (85).We did not find significant difference in bone mineral density in the respondents who consumed alcohol. This could be due to the fact that 50.8% of the total respondents in our study took alcohol of whom76.3% consumed alcohol once a month or less and only 0.9% of the study participants who consumed alcohol daily.

Karnis et al (86) in a multi-center prospective study concluded that the risk of fractures is greater for smokers and those with a history of smoking compared to non-smokers. We did not find significant difference in BMD between those who smoked, had prior history of smoking and non-smokers. There was also no significant correlation between pack years smoked and BMD. This could be attributed to the fact that only 3.5% of the total respondents smoked, with 2.7 average pack years and a study population of young adults.

We did not find any association between oral corticosteroid use with decreased BMD. This could be attributed to the low number of respondents on oral corticosteroids (5.7%), though duration of steroid use and preventive measures against steroid induced osteoporosis (vitamin D and calcium

supplementation use) was not assessed. Further studies are required to determine the relationship of duration of corticosteroid use with BMD in the HIV population.

43.9%, 45.3% and 45.7% of HIV negative, HAART naïve and those on TDF based regime respectively were involved in moderate physical activities. We did not find any difference in BMD values in the intensity levels of physical activity among the comparative arms. This could be attributed to the fact that the respondents were from a low socio-economic background (53.4% earned <kshs 5000/month) and could not afford public transport and would therefore walk to work.

We have shown, in an African setting, that HIV infected patients on a TDF based regime have reduced Quantitative Ultrasound bone mineral density in comparison to HAART naïve and HIV negative populations. However, the clinical significance of this result in terms of osteoporosis remains unknown, since we could not use the validated reference by WHO for mineral density Assessment.

Conclusions

Our study has shown that HIV infection is associated with decreased bone mineral density. Use of TDF based HAART regimes is associated with higher rates of osteoporosis compared to HAART naïve and HIV negative populations.

Recommendations

HIV positive populations on a TDF based regime should undergo Bone Mineral Density studies to determine the prevalence of BMD abnormalities in this population.

Further studies are required to assess the impact of Body Mass Index (BMI), muscular mass and adiposity on different bone sites (weight/non-weight bearing)in determining bone density in HIV infected populations.

Comparative studies are required to determine the prevalence of bone mineral density abnormalities in HIV patients on different HAART regimes.

Limitations

Our study had several limitations. Due to resource constraints we could not match our respondents' age and sex among the three comparative arms.

Our study was unable to assess dietary calcium intake as a traditional risk factor for low BMD. This would have been difficult since determination of calcium amounts in our staple food is variable and not accurately quantified.

Our study did not compare QUS BMD findings with the gold standard (DEXA). Comparative studies between DEXA and QUS in HIV infected populations are required to fully validate use of quantitative ultrasound in bone densitometry.

Timeline


Oct 2015
Results Presentation
Apr - Aug 2015
Data Collection & Analysis
Jan - Mar 2015
Ethics Approval
Sep - Dec 2014
Proposal Development & Presentation

Study Budget

Category	Remarks	Units	Unit Cost (KShs)	Total (KShs)
Proposal Development	Printing drafts	600 pages	10	6,000
	Proposal Copies	8 copies	600	4,800
Data Collection	Stationery Packs (Pens, Paper and Study Definitions)	10	100	1000
	Training research assistants	1 day	1000 X 2	2, 000
	Research assistants (2)	8 weeks	1500 X 2X8	24, 000
	Ultrasound technician	8 weeks	1500X 8	12,000
Data Analysis	Statistician	1		20,000
Thesis Write Up	Computer Services			8,000
	Printing drafts	1000 pages	10	10,000
	Printing Thesis	10 copies	1000	10,000
Contingency funds				20,000
Total				**117,800**

APPENDICES

Appendix IA: Consent Form

STUDY PARTICIPANT CONSENT FORM

Name: Dr. Abdullahi A.M.

Qualification: MBchB, MMed Internal Medicine

Institution: UoN/KNH

Department: Clinical Medicine and Therapeutics

Position: Resident

Emergency Telephone number:

Dr. Abdullahi A.M, University of Nairobi, Tel no: 0725841811

Investigator's statement

I am asking you to be in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether to be in the study. Please read this form carefully. You may ask questions about what you will be asked to do, the risks, the benefits and your rights as a volunteer, or anything about the research that is not clear in this form. When all your questions have been answered, you can decide if you want to be in this study or not. This process is called "informed consent".

Purpose and benefits

Those participating in this study are patients with HIV (those on Anti-Retroviral Treatment and those who have not yet started Anti-Retroviral Treatment)and those who are HIV negative at Mbagathi Hospital. We are doing a study to compare the bone mineral density between the 3 groups of patients and assess the severity of bone mineral density abnormalities in these 3 groups. We will then provide this information to your attending clinician to provide the necessary advice and treatment to you.

Procedures

A brief questionnaire will be administered to you after which you will undergo an ultrasound of your heel. This entails you removing your shoe and sock in one leg (without a wound) and stepping on the machine which will automatically provide us with your bone mineral density T-SCORE. The process takes approximately 1-2 minutes and is not painful or uncomfortable in any way. The results are available immediately and will be made available to your attending clinician.

Risks, stresses or discomfort

Some of the questions asked will be of a personal nature. However, you are encouraged to answer them all to aid in strengthening the study. The questions will be asked in a private environment and confidentiality will be assured at all times to ensure your comfort.

No pain or discomfort is expected during the ultrasound procedure. Participation in the study will require you to commit your time. Completing the process will take 10-15 minutes.

Cost

The cost of standard care of the participants while at the hospital will be free under the CCC and VCT centre. However, the cost of the ultrasound procedure will be incurred by the study investigator.

Confidentiality

Your confidentiality will be maintained at all times. The questionnaires will not have any names but will be assigned identifiers. Only the investigator, the University of Nairobi Ethics and Research committee will have access to the information about you. There shall be no mention of names or identifiers in the report or publications which may arise from the study. The information obtained will be used only for the purpose of the study.

You may withdraw from the study or refuse to answer any of the questions asked at any time without loss of benefit or penalty. Your participation in the study is voluntary and will be highly appreciated.

If you have any questions regarding the study, contact Dr Abdullahi on Tel no: 0725842811

In case of any ethical concerns, please contact:

The Chairman, KNH/UON- Ethics and Research Committee

Hospital Road along Ngong Road

P.O BOX 20723, Nairobi (CODE 00202

Telephone number (+254-020)2726300 ext 44355

Chairperson: Professor K.M. Bhatt Email uonknherc@uonbi.ac.ke

STATEMENT OF CONSENT BY PATIENT

The purpose of this study, procedure, study benefits and my rights have been fully explained to me. I ……. hereby give my written consent to participate in the study.

Signature …… …….
Date……

Thumbprint……**.....**

Witness……
Date…….

Signature…….

Thumbprint…….

INTERVIEWERS STATEMENT

I have explained the purpose and benefits of this study to the respondent to the best of my Knowledge and conviction, he/she has understood and has given consent.

Interviewer……

Date ……

Signature ……

Appendix II: Questionnaire

1. Socio-Demographic characteristics of the respondent:

Age (Years)..... (to the nearest year)

Marital status

1. ▢ Married
2. ▢ Single
3. ▢ Divorced
4. ▢ Widowed

Residence

1. ▢ Rural,
2. ▢ Urban

Highest level of education

1. ▢ None
2. ▢ Primary
3. ▢ Secondary
4. ▢ Tertiary

Occupation

1. ▢ Unemployed
2. ▢ Student
3. ▢ Self employed
4. ▢ Civil servant
5. ▢ Other (Specify)…….

What is your income level per month (Ksh)

1. ▢ Below 2,500
2. ▢ 2,500-5,000
3. ▢ 6,000 – 10,000
4. ▢ 10,000 -30,000
5. ▢>30,000

A. HIV related factors

1) How long have you lived with HIV? ……..... years

2) How long have you taken HIV medication (HAART) ……Months

B. Osteoporosis risk factors

3) Have you used oral corticosteroids continuously for a period equal to or exceeding three months?

1) ▢ Yes
2) ▢ No

4) Have you ever smoked cigarettes?

1) ▢ Currently smoke
2) ▢ Used to smoke. ……. (pack years)
3) ▢ Never smoked

5) How often do you take an alcoholic drink?

1. ▢ Never
2. ▢ Monthly or less
3. ▢ Weekly
4. ▢ Daily

6) Have you used or are you currently using oral contraception?

1. ▢ Yes
2. ▢ No

7) Have you ever sustained a bone fracture?

1. ▢Yes
2. ▢No

Physical activity

We are interested in finding out about the kinds of physical activities you do as part of your everyday life. The questions are about the time you spent being physically active in the last seven (7) days. They include questions about activities you do at work, as part of your house work, to get from place to place, and in your spare time as exercise or sport.
In answering the following questions;
- Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.
- Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat/ slightly harder than normal.
- Minimal/no activities refer to activities that fall below moderate physical activity

8) During the last seven days, have you had any vigorous physical activity like, heavy lifting, digging, aerobics, or fast cycling? (activities for at least 10 minutes)

1. ▢Yes
2. ▢No

If yes, how many days per week? ……..

If yes, how many hours cumulatively per day? ……

9) During the last seven days, have you had any moderate physical activity like carrying light loads, or riding a bike at a regular pace (activities for at least 10 minutes)

1. ▢Yes
2. ▢No

If yes, how many days per week? ……..

If yes, how many hours cumulatively per day? ……

10) During the last seven days, have you walked for at least 10 minutes?

1. ▢Yes
2. ▢No

If yes, how many days per week? ……..

If yes, how many hours cumulatively per day? ……

11) In the last seven days, approximately how much time did you spend sitting on a weekday?
……hours/day

HOJAJI

A. Wasifu wa kijamii na hulka ya anaye jibu maswali
Umri (Miaka) …….(inayokaribia mwaka mzima)

Hadhi ya kindoa:
1. ▢Ameoa/ameolewa
2. ▢Kapera(hajaowa/hajaolewa
3. ▢Ametalikiwa
4. ▢Mjane

Makaazi :
1. ▢Mashambani(mashinani)
2. ▢Mjini

Kiwango cha juu cha elimu
1. ▢Hukusoma
2. ▢Shuleyamsingi
3. ▢Shuleyaupili(sekondari)
4. ▢Elimuyajuu

Kazi
1. ▢Hujaajiriwa
2. ▢Mwanafunzi
3. ▢Umejiajiri
4. ▢Mtumishiwaumma
5. ▢Nyengine (taja/eleza).....

Unakuwa na mapato ya kiasi gani kila mwezi (KSH)?
1. ▢Chini ya 2,500
2. ▢ Kati ya 2,500 na 5,000
3. ▢ Kati ya 6,000 na 10,000
4. ▢ Kati ya 10,000 na 30,000
5. ▢Zaidi ya 30,000

B. Masuala yanayohusiana na Ukimwi (HIV)
1. Kwa muda gani umeishi na Ukimwi? Miezi/ Miaka .....

2. Kwa muda gani umetumia dawa za Ukimwi(HAART) Miezi .....

C. Mambo ambazo inaweza kuchangia kwa upungufu wa madini kwenye mifupa

3. Je umekuwa ukitumia steroidi mfululizo kwa muda wa miezi mitatu au zaidi?
1) ▢Ndiyo
2) ▢Hapana

4. Je umewahi kuvuta sigara?
1) ▢Ninavuta (sigara)
2) ▢Nilikuwa ninavuta sigara. _____pakiti kwa mwaka(pack years)
3) ▢Sijawahi kuvuta sigara

5. Je unakunywa pombe mara kwa mara?
1) ▢Sinywi kabisa
2) ▢Kila mwezi au chini ya mwezi
3) ▢Kila wiki
4) ▢Kila siku

6. Je, umewahi kutumia au unatumia tembe zenye madini ya kalsiamu?
1) ▢Ndiyo
2) ▢Hapana

7. Umeshawahi kutumia au unatumia kwa sasa vidonge vya kuzuia mimba?
1) ▢Ndiyo
2) ▢Hapana

8. Umeshawahi kuvunjika mfupa wowote wa mwili?
1) ▢Ndio
2) ▢Hapana

D. SHUGHULI YA MWILI AU MAZOEZI

Tungependa kujua aina ya shughuli za mwili (mazoezi ya mwili) unazofanya kama sehemu ya kila siku ya maisha yako. Maswali ni kuhusu muda unaotumia katika kuushughulisha mwili katika siku saba (7) zilizopita.

Maswali hayo yanajumuisha shughuli unazofanya kazini, kama sehemu ya kazi yako ya nyumbani, kuenda eneo moja hadi nyengine, na katika muda wako wakujipumzisha kama mazoezi au mchezo

Katika kujibu maswali yafuatayo;

Shughuli za kutumia nguvu sana ni zile shughuli zinazohitaji nguvu zaidi na hukufanya upumue haraka Kuliko kawaida

Shughuli za wastani ambazo ni zile zinazohitaji nguvu kiasi na ambazo hukufanya upumue kidogo haraka kuliko ilivyokawaida.
Shughuli chache sana au panapo hamna shughuli yoyote ni zile shughuli ambazo kadri yake ni chini ya shughuli zawastani.

9. Katika siku saba zilizopita, je umekuwa na shughuli ya kutumia nguvu nyingi kama kunyanyua uzani, kulima, mazoezi ya viungo, au uendeshaji wakasi wa baiskeli? (shughuli ambazo hudumu kwa muda wa zaidi ya dakika kumi)

1. ☐Ndiyo
2. ☐Hapana

Ikiwa ni ndiyo, je ni siku ngapi kwa wiki? .....
Ikiwa ni ndiyo, je ni muda wa jumla wa saa ngapi kwa siku? .....

10.Katika siku saba zilizopita, umewahi kuwa na shughuli za wastani kama kubeba mizigo mepesi, au kuendesha basikeli katika kasi ya kawaida(shughuli ambazo hudumu kwa zaidi ya dakika 10)

1) ☐Ndiyo
2) ☐Hapana

Ikiwa ni ndiyo,je, ni siku ngapi kwa wiki? .....
Ikiwa ni ndiyo, je, ni muda wa jumla wa saa ngapi kwa siku? .....

11.Katika siku saba zilizopita, je umetembea kwa dakika kumi au zaidi?

1. ☐Ndiyo
2. ☐Hapana

Ikiwa ni ndiyo, je ni siku ngapi kwa wiki?.....
Ikiwa ni ndiyo, je ni muda wa jumla wa saa ngap ikwa siku?.....

12. Katika muda wa siku saba zilizopita, unatumia takriban muda gani kukaakwa wiki/siku? Saa..... kwa siku.

Appendix III: QUS Approval Certificates

KEMA Quality

CERTIFICATE

Number: 93741CE01

CE

CE MARKING OF CONFORMITY
MEDICAL DEVICES

Issued to:
BeamMed Ltd.
8 Ha-Lapid Street
Petah Tikva 49170
Israel

For the product category:

Ultrasound Diagnostic Systems

KEMA Quality grants the right to use the EC Notified Body Identification Number illustrated below to accompany the CE Marking of Conformity on the products concerned conforming to the required Technical Documentation and meeting the provisions of the EC-Directive which apply to them:

0344

Documents, that form the basis of this certificate:

Certification Notice 87757CN, initially dated July 10, 1998
Addendum, initially dated July 1, 2001

KEMA Quality hereby declares that the above mentioned manufacturer fulfils the relevant provisions of 'Besluit Medische Hulpmiddelen', the Dutch transposition of the Directive 93/42/EEC of June 14, 1993 concerning Medical devices, including all subsequent amendments, and that for the above mentioned product category the Conformity Assessment Procedure Annex V in combination with Annex VII for class IIa products, is executed by the Manufacturer in accordance with the provisions of the Council Directive 93/42/EEC of June 14, 1993. The necessary information and the reference to the relevant documentation, of the products concerned and the assessments performed, are stated in the Certification Notice which forms an integrative part of this certificate.

This certificate is valid until: July 1, 2013
Issued for the first time: July 10, 1998
Reissued: June 16, 2010

KEMA Quality B.V.

drs. G.J. Zoetbrood
Managing Director

ing. A.A.M. Laan
Certification Manager

KEMA Quality B.V. Utrechtseweg 310, 6812 AR Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 26 356 2000 F +31 26 352 5800 www.kemaquality.com Company registration 09085396

a DEKRA company

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Room – WO66-G609
Silver Spring, MD 20993-0002

OCT 12 2011

Ms. Rita Koremblum
QA/RA Manager
Beam-Med LTD
8 Halapid Str., P.O. Box 7520
Petach Tikva, 49170
ISRAEL

Re: K110646
Trade/Device Name: Sunlight MiniOmni Ultrasound Bone Sonometer
Regulation Number: 21 CFR 892.1180
Regulation Name: Bone sonometer
Regulatory Class: II
Product Code: MUA
Dated: September 11, 2011
Received: September 13, 2011

Dear Mr. Koremblum:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely Yours,

Mary S Pastel

Mary S. Pastel, Sc.D.
Director
Division of Radiological Devices
Office of In Vitro Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

K110646
P. 1 of 1

Indications for Use Statement

510(k) Number (if known): Not known

Device Name: Sunlight MiniOmni Ultrasound Bone Sonometer

Indications for Use: The Sunlight MiniOmni Ultrasound Bone Sonometer is a non-invasive device that is designed for the quantitative measurement of the signal velocity of ultrasound waves ("Speed of Sound" or "SOS" in m/sec) propagating at multiple skeletal sites (i.e., the distal one-third of the radius, the proximal third phalanx and the fifth metatarsal). SOS provides an estimate of skeletal fragility.

The output is also expressed as a T-score and a Z-score, and can be used in conjunction with other clinical risk factors as an aid to the physician in the diagnosis of osteoporosis and other medical conditions leading to reduced bone strength and, ultimately, in the determination of fracture risk.

Multiple skeletal site testing provided clinicians with alternatives if one site is not accessible and with additional skeletal information (i.e., from bones with different combinations of cortical and cancellous material and from weight bearing and non-weight bearing sites) that assists in diagnosing osteoporosis and risk fracture.

The SOS measured by MiniOmni has a precision error low enough in comparison with the expected annual change in a patient's measurement to make it suitable for monitoring bone changes which occur in the early years following menopause (i.e., age range approximately 50-65 years).

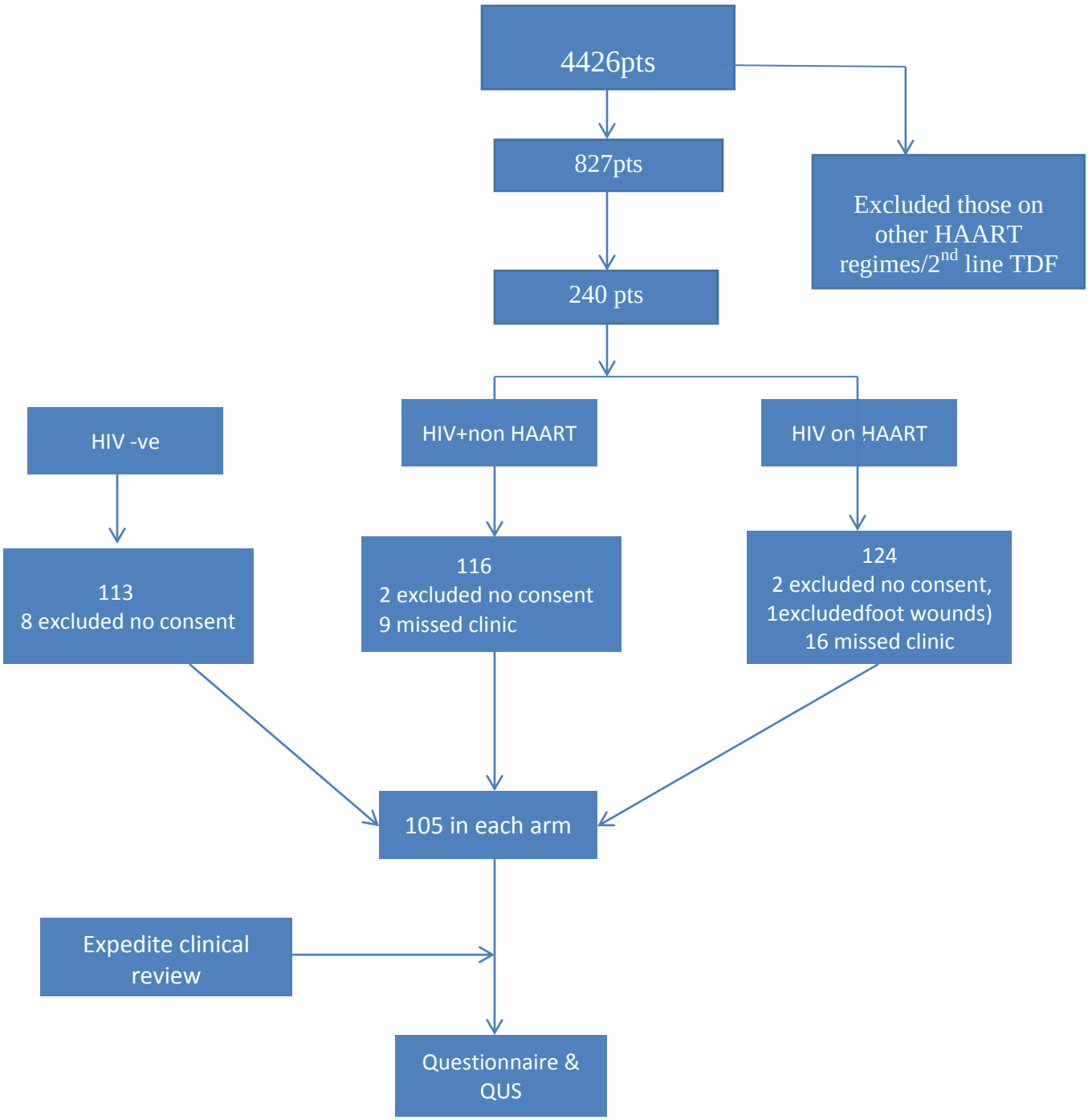
Prescription Use (Part 21 CFR 801 Subpart D)	☒	AND/OR	Over-The-Counter Use (21 CFR 801 Subpart C)	☒

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

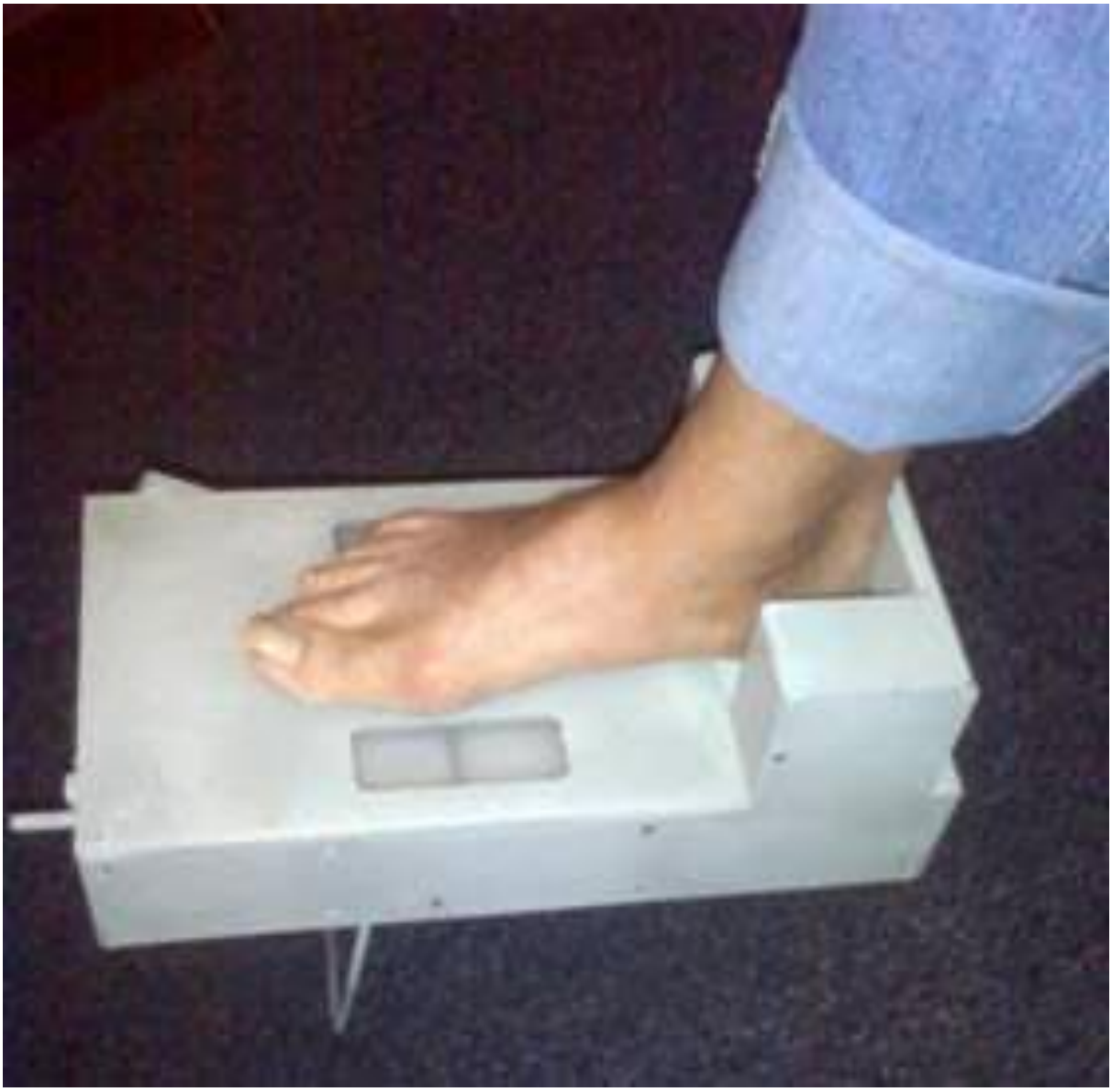
Concurrence of CDRH, Office of Device Evaluation (ODE)

(Division Sign-Off)
Division of Radiological Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
510K K110646

Appendix IV: Flowchart



Appendix V: Calcaneal Qus Pictogram



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Appendix VII: ETHICAL APPROVAL LETTER

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27th April, 2015

Dr. A.M. Abdullahi
Dept. of Clinical Medicine and Therapeutics
School of Medicine
University of Nairobi

Dear Dr. Abdullahi

Research Proposal : Bone Mineral density abnormalities in HIV infection at Mbagathi Hospital using calcaneal quantitative ultrasound (P60/02/2015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 27th April 2015 to 26th April 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

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