PREVALENCE AND RISK FACTORS OF REDUCED BONE MINERAL DENSITY IN HIV INFECTED INDIVIDUALS

SHERON GOH SIR LOON

FACULTY OF MEDICINE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2018

PREVALENCE AND RISK FACTORS OF REDUCED BONE MINERAL DENSITY IN HIV INFECTED INDIVIDUALS

SHERON GOH SIR LOON

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN MEDICAL SCIENCES

FACULTY OF MEDICINE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2018

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Sheron Goh Sir Loon

Matric No: MGN150006

Name of Degree: Masters in Medical Sciences

Title of Dissertation/Thesis ("this Work"): Prevalence and risk factors of reduced bone mineral density in HIV infected individuals

Field of Study: Osteoporosis and human immunodeficiency virus

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University ofMalaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

PREVALENCE AND RISK FACTORS OF REDUCED BONE MINERAL DENSITY IN HIV INFECTED INDIVIDUALS

ABSTRACT

The commencement of antiretroviral therapy (ART) in human immunodeficiency virus (HIV)-infected individuals has decreased HIV-related complications and improved survival. However, studies have shown that there is a higher prevalence of osteopenia/osteoporosis [reduced bone mineral density (BMD)] in HIV-infected individuals. The aim of our study was to determine the prevalence of osteopenia/osteoporosis (reduced BMD), vitamin D level, the 10-year probability of fracture risk, and its associated risk factors in HIV-infected and HIV-uninfected individuals in Malaysia. HIV-infected individuals aged ≥25 years and virologically suppressed on ART for at least 1 year, were recruited from September 2014-September 2016, at a tertiary hospital in Malaysia. HIV-uninfected individuals were recruited from the community. BMD was assessed using dual X-ray absorptiometry (DXA), whilst the 10-year probability of fracture risk was calculated using the fracture risk assessment tool (FRAX®). A total of 684 potential participants were approached; of which 640 participants agreed to participate (response rate=93.6%). Participants were then matched for gender and age, finally giving 206 participants in each group. The majority of participants were male (73.8%) and Chinese (64.1%) with the median age of 40 years old. A significantly higher number of HIV-infected individuals (73.8%) had reduced BMD when compared to HIV-uninfected individuals [(57.3%), p<0.001]. The prevalence of osteoporosis was found to be significantly higher in HIV-infected individuals (14.1%) when compared to HIV-uninfected individuals [(5.3%), p<0.001]. Similarly, vitamin D deficiency (<50nmol/L) was significantly higher in HIV-infected (65.0%) compared to uninfected individuals [(30.1%), p<0.001]. The 10-year probability of sustaining a hip

fracture in HIV-infected individuals (0.4%) was significantly higher than in HIV-uninfected individuals (0.2%, p=0.003), but not in major osteoporotic fracture [HIV-infected (1.7%); HIV-uninfected (1.3%)] (p=0.066). Lower body mass index (BMI), reduced physical activity and older age were risk factors that significantly associated with reduced BMD in HIV-infected individuals. In conclusion, the prevalence of reduced BMD, vitamin D deficiency and the 10-year probability of sustaining a hip fracture was higher in HIV-infected compared to HIV-uninfected individuals. Lower BMI, reduced physical activity and older age were found to be associated with reduced BMD in HIV-infected individuals.

Keywords: HIV, bone density, osteopenia, osteoporosis

KEKERAPAN DAN FAKTOR RISIKO PENGURANGAN KETUMPATAN TULANG DI KALANGAN INDIVIDU MENGHIDAPI HIV

ABSTRAK

Penggunaan terapi antiretroviral telah berjaya mengurangkan komplikasi HIV dan melanjutkan usia dalam kalangan pesakit HIV. Namun, kajian telah menunjukkan bahawa kekerapan *'osteopenia or osteoporosis'* (kekurangan ketumpatan mineral tulang) adalah tinggi dalam kalangan pesait HIV. Tujuan kajian ini adalah untuk mengenalpasti kekerapan *'osteopenia or osteoporosis'*(kekurangan ketumpatan mineral tulang), tahap vitamin D, kebarangkalian risiko tulang retak dalam masa10 tahun dan faktor risiko yang berkaitan dalam kalangan pesakit HIV dan individu yang tidak menghidapi HIV di Malaysia. Para peserta yang dikumpul terdiri daripadapesakit HIV yang berumur ≥25 tahun, berada dalam keadaan *'virologically suppressed'*dan telah dirawat dengan terapi antiretroviralselama setahun. Para pesertatelah dikumpul daripada September 2014-September 2016 dari sebuah hospital tertiari di Malaysia. Individu-individu yang tidak menghidapi HIV dikumpul daripada komuniti. Ketumpatan mineral tulang diukur menggunakan *'dual X-ray absorptiometry (DXA)'* manakala kebarangkalian risiko tulang retak dalam masa 10 tahun diukur menggunakan *'fracture risk assessment too'l (*FRAX®). Seramai 684 orang yang berpotensi untuk menjadi peserta dijemput menyertai kajian ini. Daripada itu, seramai 640 peserta bersetuju untuk mengambil bahagian (kadar sambutan=93.6%). Para peserta daripada setiap kumpulan kemudiannya dipadankanberdasarkan jantina dan umur, dan seramai 206 peserta berjaya dipadankan. Majoriti peserta adalah lelaki (73.8%), berbangsa Cina (64.1%) dengan median umur 40 tahun. Para peserta yang menghidapi HIV (73.8%) mempunyai kekerapan kekurangan ketumpatan mineral tulang *(osteopenia atau osteoporosis)*yang lebih tinggiberbanding individu-individu yang tidak menghidapi HIV [(57.3%), p<0.001].Kekerapan kerapuhan

tulang *(osteoporosis*) dalam kalangan peserta yang menghidapi HIV (14.1%) didapati adalah lebih tinggi berbanding individu-individu yang tidak menghidapi HIV [(5.3%), p<0.001]. Kekurangan vitamin D (<50nmol/L) dalam kalangan peserta yang dijangkiti HIV (65.0%) juga adalah lebih tinggi berbanding individu-individu yang tidak dijangkiti HIV [(30.1%, p<0.001]. Kebarangkalian tulang pinggul retak dalam masa 10 tahun dalam kalangan penghidap HIV (0.4%) adalah lebih tinggi berbanding individu-individu yang tidak menghidapi HIV (0.2%, p=0.003). Namum, tiada perbezaan dikenal pasti dalam kebarangkalian tulang retak *(major osteoporosis)*[HIV-infected (1.7%); HIV-uninfected (1.3%)] (p=0.066). Faktor risiko yang berkaitan dengan kekurangan ketumpatan mineral tulang dalam pesakit HIV adalah individu yang mempunyai indeks jisim badan yang lebih rendah, kurang aktiviti fizikal dan lebih tua. Kesimpulannya, kekerapan kekurangan ketumpatan mineral tulang, tahap vitamin D, kebarangkalian risiko tulang pinggul retak dalam masa 10 tahunadalah lebih tinggi dalam kalangan pesakit HIV berbanding dengan individu yang tidak dijangkiti HIV. Pesakit HIV yang mempunyai indeks jisim badan yang rendah, kurang aktiviti fizikal dan lebih tua antara faktor risiko yang berkaitan dengan kekurangan ketumpatan mineral tulang.

Kata kunci: HIV, ketumpatan mineral tulang, *osteopenia, osteoporosis*

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisors, Associate Professor Pauline Lai Siew Mei, Associate Professor Alexander Tan Tong Boon, and Associate Professor Dr. Sasheela A/p Sri La Sri Ponnampalavanar for their constant guidance and encouragement throughout this study. I would also take this opportunity to thank all the lecturers, staffs and colleagues from the Malaysian Elders Longitudinal Research (MELoR) - Health & Wellbeing: HIV & Aging (MHIVA) team, the Nuclear Medicine Department and the Infectious Disease Clinic for their guidance and co-operation throughout my study period. Additionally, I would like to thank all the participants in this study for their precious time spent in participating in this study. Without all of them this study would not be possible. A special thanks to Ms Ranita Bt. Hisham Shunmugam (medical librarian) for her assistance in designing a comprehensive search strategy in our meta-analysis. Last but not least, I would like to take this opportunity to express my profound gratitude to my beloved parents, family and friends for their patience and continuous moral support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

°C	:	Degree celsius
µg	:	Microgram
µL	:	microliter
ABONE	:	Age Bulk One or Never Estrogens
AIDS	:	Acquired immunodeficiency syndrome
ALP	:	Alkaline phosphatase
ALT	:	Amino alanine transferase
ART	:	Antiretroviral therapy
ARV	:	Antiretroviral
AST	:	Aspartate aminotransferase
BMD	:	Bone mineral density
BMI	:	Body mass index
BWC	:	Body weight criterion
CCR5	:	Chemokine co-receptor 5
CDC	:	United States Centers for Disease Control and Prevention
cells/µL	:	Cells per microliter
copies/mL	:	Copies per milliliter
DNA	:	Deoxyribonucleic acid
DXA	:	Dual X-ray absorptiometry
EDTA	:	Ethylenediaminetetraacetic acid
eGFR	:	Glomerular filtration rate
ELISA	:	Enzyme-linked immunosorbent assay
FDA	:	Food and Drug Administration
FRAX®	:	Fracture risk assessment tool

g	:	Gram
g/dL	:	Grams per deciliter
g/cm^2	:	Gram per square centimeter
GARVAN	:	Garvan fracture risk calculator
GGT	:	Gamma-glutamyl transferase
HAART	:	Highly active, antiretroviral therapy
HDL	:	High-density lipoprotein
HIV	:	Human immunodeficiency virus
HRT		Hormone replacement therapy
i.e	:	that is
IFA	:	Indirect immunofluorescence assay
IOF	:	International Osteoporosis Foundation
IOM	:	The Institute of Medicine
ISCD	:	International Society for Clinical Densitometry
IQR	:	Interquartile range
IU	:	International unit
IV	:	Intravenous
kg	:	Kilogram
kg/m^2	:	Kilogram per square meter
LDL	:	Low-density lipoprotein
m	:	Meter
MESH	:	Medical Subject Heading
MELoR	:	Malaysian Elders Longitudinal Research
mg	:	Milligram
MHIVA	:	Malaysian Elders Longitudinal Research (MELoR) - Health & Wellbeing: HIV & Aging

mIU/L	:	Milli-international units per liter
ml	:	Milliliter
mm	:	Millimeter
mmol/L	:	Milimole per liter
MOST	:	Malaysian Osteoporosis Screening Tool
N	:	Number
NA	:	Not applicable
ng/dl	:	Nanogram per desiliter,
nmol/L	:	Namomol per litre
NGOs	:	Non-government organizations
NHANES	:	National Health and Nutrition Examination Survey
nmol/L	:	Nanomole per liter
NNRTI	:	Non-nucleoside reverse transcriptase inhibitor
NR	:	Not reported
NRTI	:	Nucleoside reverse transcriptase inhibitors
NtRTI	:	Nucleotide reverse transcriptase inhibitor
OPG	:	Osteoprotegerin
OR	:	Odds ratio
ORAI	:	Osteoporosis Risk Assessment Instrument
OSIRIS	:	Osteoporosis Index of Risk
OSTA	:	Osteoporosis Self-Assessment Tool for Asians
PreP	:	Pre-exposure oral prophylaxis
PI	:	Protease inhibitor
PLWH	:	People living with HIV
PRISMA	:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PTH	:	Parathyroid hormone
PWID	:	People who inject drugs
QCT	:	Quantitative computed tomography
QUS	:	Quantitative Ultrasound
RANKL	:	Receptor of activated NF-κB ligand
RCT	:	Randomized control trial
RevMan	:	Review Manager
RNA	:	Ribonucleic acid
SC	:	Subcutaneous
SERMs	:	Serum estrogen receptor modulators
SCORE	:	Simple Calculated Osteoporosis Risk Estimation
SD	:	Standard deviation
SPSS	:	Statistical Package for the Social Sciences
TDF	:	Tenofovir disoproxil fumarate
TNF	:	Tumor necrosis factor
UMMC	:	University Malaya Medical Centre
UMRIC	:	University of Malaya Research Imaging Centre
umol/L	:	Micromole per liter
U/L	:	Units per liter
USA	:	United States of America
UVB	:	Ultraviolet B
WHO	:	World Health Organization

LIST OF APPENDIX

CHAPTER 1: INTRODUCTION

In 1981, the first cases of acquired immunodeficiency syndrome (AIDS) were reported in the United States (Centers for Disease Control and Prevention, 1982). AIDS is the advance stage of human immunodeficiency virus (HIV) infection. HIV-infected individuals are considered to have progressed to AIDS when their CD4+ cell counts fall to <200 cells/μL (Hidalgo, Macarthur, & Crane, 2000). In this stage, the immune system of a HIV-infected individual is badly damaged and becomes vulnerable to common opportunistic infections (Low et al., 2016).

The first anti-retroviral drug, zidovudine [a nucleoside reverse transcriptase inhibitor (NRTI)] was approved in the mid-1980s, and was given as monotherapy for the treatment of HIV (Young, 1988). Since then, other classes of antiretroviral agents (ARV) have been developed, such as non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, integrase inhibitors and chemokine co-receptor 5 (CCR5) antagonists(Arts & Hazuda, 2012). Effective treatment of HIV has evolved to a combination of different classes of ARV agents known as highly active antiretroviral therapy (HAART) (Arts & Hazuda, 2012).

The advent of HAART has significantly reduced the morbidity and mortality associated with HIV infection and AIDS (Palella et al., 1998). As a result, life expectancy of HIV-infected individuals has increased (Bolland, Grey, & Reid, 2015). Globally, in 2013, there were 4.2 million HIV-infected individuals aged 50 years old and older, which was a two-fold increase from 1995 (Mahy, Autenrieth, Stanecki, & Wynd, 2014). In the United States, people living with HIV (PLWH) ≥ 50 years old has increased from 40% in

2012 to 50% in 2015 (Wing, 2016). Similarly, in Malaysia, PLWH has increased from 5% in 2005 to 10% in 2015 (Ministry of Health, 2016b).

Studies have shown that HIV infection and the use of ART may accelerate the ageing process in HIV-infected individuals (Effros et al., 2008; Onen et al., 2010; Wing, 2016). As a result, HIV-infected individuals are more susceptible to age-related illnesses (Wing, 2016)such as osteoporosis, metabolic syndrome, ocular degeneration, mitochondrial toxicity, neurocognitive dysfunction and depression (Effros et al., 2008; Onen et al., 2010).

Osteoporosis is a "systemic skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture"(National Institutes of Health, 2001). However, the aetiology of bone loss in HIV-infected individuals is multifactorial (Qaqish & Sims, 2004). It is a complex interaction between HIV infection itself, traditional osteoporosis risk factors and ART-related factors (Saccomanno & Ammassari, 2011). According to a meta-analysis on the prevalence of osteopenia/osteoporosis conducted in 2006, it was found that HIV-infected individuals has an increased risk of 3.7 times of developing osteoporosis when compared to HIV-uninfected individuals (Brown & Qaqish, 2006).

If osteoporosis is left untreated, fragility fracture will occur. Osteoporosis fracture can cause pain, severe disability, loss of independence and reduced quality of life (Madureira, Ciconelli, & Pereira, 2012). Studies have shown that the mortality rate within the first year after sustaining a hip fracture is 20%, with 50% of these hip fracture survivor are permanently incapacitated without regaining their mobility after one year of sustaining

the hip fracture (Vochteloo et al., 2013). Therefore, it is important to screen HIV-infected individuals at risk for osteoporosis and initiate treatment accordingly

To date, there is a paucity of local data on osteopenia/osteoporosis (reduced BMD) in HIV-infected individuals in Malaysia. As HIV-infected individuals are now live longer, it is important to determine the local prevalence of reduced BMD and its associated risk factors so that clinicians are able to develop specific screening strategies and implement management algorithms targeted specifically towards PLWH in Malaysia. Therefore, the aim of our study was to determine the prevalence of osteopenia or osteoporosis in HIV-infected versus HIV-uninfected individuals in Malaysia; and its associated risk factors.

CHAPTER 2: LITERATURE REVIEW

2.1 Human immunodeficiency virus

AIDS was first reported in 1981 as a fatal disease affecting young homosexual men and intravenous drug users in the United States (Centers for Disease Control and Prevention, 1982). Patients with AIDS were found to have a marked impairment of cellular response and were susceptible to opportunistic infection such as candidiasis, cryptococcal meningitis, pneumocystis pneumonia, herpes, toxoplasmosis, tuberculosis, kaposi sarcoma and cytomegalovirus retinitis (Centers for Disease Control and Prevention, 1982; Low et al., 2016). As a consequence, the number of cases and deaths among individuals with AIDS were high during 1980s (Centers for Disease Control and Prevention, 1982). In 1984, a new human retrovirus called human immunodeficiency virus (HIV), was isolated and identified as the causative agent of AIDS by Barre-Sinoussi et al. from the Pasteur Institute (Barre-Sinoussi et al., 1983).

There are two main types of HIV: HIV-1 and HIV-2. Both HIV-1 and HIV-2 are similar in their basic gene arrangement, modes of transmission, intracellular replication pathway and clinical manifestations (Nyamweya et al., 2013). However, the differences between these two types of HIV includes geographic distribution, clinical progression of the disease, possible chances of HIV transmission via perinatal route and choice of ART used [Table 2.1] (Clavel et al., 1986; Ingole et al., 2013; Ndour et al., 2000; Poulsen et al., 1993).

Table 2:1: Key differences between human immunodeficiency virus-1 and human immunodeficiency virus-2

	HIV-1	HIV-2
Geographic distribution	Found commonly worldwide	Found commonly in western Africa, India, France and Portugal
Clinical progression towards AIDS	More infectious and progresses faster to AIDS (7-10 years)	Less infectious and progresses more slowly to AIDS (10-25 years)
Perinatal transmission	Relatively common (15-45%)	Relatively rare (<5%)
Choice of ART used	NNRTIs (such as such as efavirenz and nevirapine) are commonly used as first-line ART regimen	NNRTIs (such as efavirenz and nevirapine) ineffective

HIV=human immunodeficiency virus; AIDS=acquired immunodeficiency syndrome; ART=antiretroviral therapy; NNRTIs=non-nucleoside reverse transcriptase inhibitors

2.2 Modes of transmission of the human immunodeficiency virus

The HIV is primarily transmitted via sexual, parenteral or perinatal contact (Gershon, Vlahov, & Nelson, 1990).

2.2.1 Sexual contact

Sexual transmission of HIV can occur through unprotected anal, vaginal or oral sexual intercourse (Marks, Crepaz, & Janssen, 2006). The probability of HIV transmission via anal intercourse is 0.1-0.4% per sexual contact depending on whether it is receptive or insertive; and via vaginal intercourse is 0.1-1.5% per sexual contact (Jin et al., 2010). In Malaysia, in the year 2015 approximately 70% of the transmission of HIV infection occurred through sexual contact (Ministry of Health, 2016b).

2.2.2 Parenteral route

The use of contaminated needles or other injection paraphernalia by intravenous drug users is the main cause of parenteral transmission (Kaplan, 1989). According to the Malaysian Ministry of Health statistics, in 2016, 20% of HIV transmission occurred through intravenous drug use (Ministry of Health, 2016b).

Exposure to contaminated equipment during piercing and tattooing can also be a vehicle for HIV infection (Tweeten & Rickman, 1998). Health care workers have an occupational risk in acquiring HIV, through accidental injuries such as percutaneous needle prick injury or splash of body fluids on orifice or open wounds (Gershon et al., 1990). Direct exposure through contaminated blood, tissues or organs from HIV-infected individuals can also cause HIV infection (Gershon et al., 1990).

2.2.3 Perinatal route

Perinatal or vertical transmission (during ante, intra or post-partum) is the most common cause of HIV acquisition in children (Coutsoudis, Kwaan, & Thomson, 2010). In 2008, 90% of HIV transmission in children were via perinatal route(World Health Organisation, 2010). Without appropriate intervention, the risk of transmission via perinatal route ranged from 20% to 45%(World Health Organisation, 2010). Preventive treatment which involves ART treatment in pregnant mother and in new-born babies, elective caesarean section, and not breastfeeding can decrease the risk of perinatal transmission to about 1% (Coutsoudis et al., 2010).

2.3 Pathogenesis of the human immunodeficiency virus

The pathogenesis of HIV is an interrelationship between the HIV virus and the host immune system [Figure2.1] (Levy, 1993).

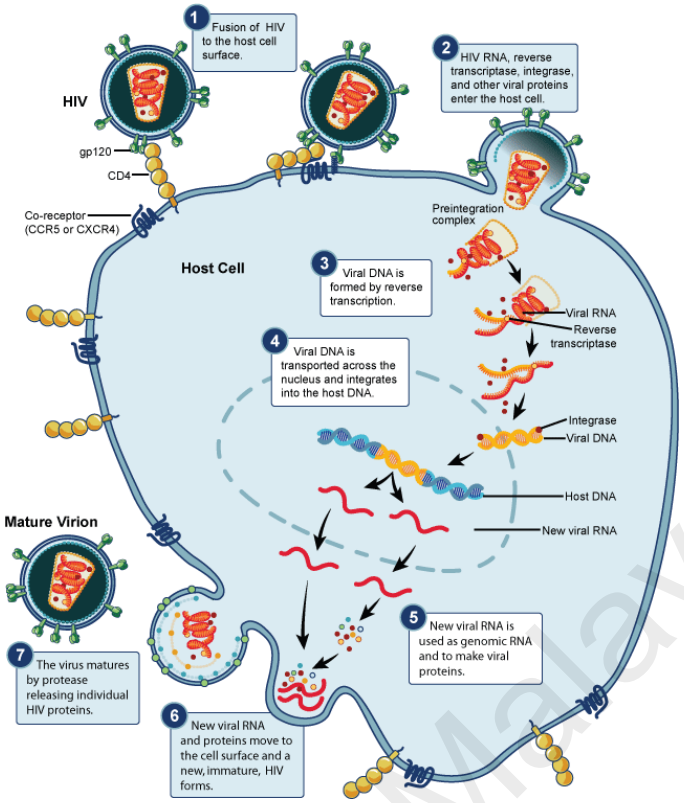


Figure 2:1: The pathogenesis of the human immunodeficiency virus
Source: Sierra, S., Kupfer, B., & Kaiser, R. (2005). Basics of the virology of HIV-1 and its replication. Journal of Clinical Virology, 34(4), 233-244. (Sierra, Kupfer, & Kaiser, 2005)

HIV is a virus that possesses the enzyme reverse transcriptase. It consists of a lipid bilayer membrane which surrounds the capsid (Levy, 1993). The surface of the HIV consists of a glycoprotein molecule, which has a strong affinity towards the CD4+ receptor protein (which is found predominantly on the host T-helper cell or inducer lymphocytes) (Levy, 1993).

The entry process of the HIV into the host cell is complex. The HIV also binds to the chemokine co-receptors (CCR5 and CXCR-4)when membrane fusion occurs (Levy, 1993). After penetration, the virus sheds its outer coat and releases its genetic material [HIV ribonucleic acid (RNA), reverse transcriptase, integrase and other viral proteins] into the host cell (Levy, 1993).

Viral deoxyribonucleic acid (DNA) is formed by using reverse transcriptase enzyme (Levy, 1993). Viral DNA is then transported across the nucleus and integrates into the host DNA (Levy, 1993). Viral DNA then undergoes transcription and translation in the host cell nucleus and enables the production of new viral proteins (Levy, 1993). The new viral RNA and proteins move to the host cell surface and a new immature HIV forms (Levy, 1993). Virus particles assemble and bud out of the host cell, to mature into infectious virions by protease enzyme (Levy, 1993). These new virions are produced at a rate of approximately 10^8 virions per day (Weber, 2001).

2.4 Human immunodeficiency virus staging and classification

Two major clinical classification system are currently used by physicians to screen and monitor the clinical progression of HIV in PLWH. These two systems are the United States Centers for Disease Control and Prevention (CDC) classification (United States Centers for Disease Control and Prevention, 1993), and the World Health Organization (WHO) clinical staging and disease classification for HIV/AIDS(World Health Organisation, 2006).

2.4.1 The United States Centers for Disease Control and Prevention (CDC) classification system

The CDC classification system which (was recently revised in 1993) helps physicians to assess the severity of HIV disease by assessing CD4+ cell counts and by determining the presence of specific HIV-related conditions (United States Centers for Disease Control and Prevention, 1993). According to the CDC, HIV infection is divided into nine categories (A1-A3, B1-B3 and C1-C3), based on CD4+ cell counts [Table 2.2] (United States Centers for Disease Control and Prevention, 1993). AIDS is defined as any HIV-

infected individuals with CD4 cell counts of <200 cells/μL, with or without AIDS-defining conditions (United States Centers for Disease Control and Prevention, 1993).

Table 2:2:The United States Centers for Disease Control and Prevention classification system for human immunodeficiency virus-infected adults

Absolute CD4+cell count /μL)	A: Asymptomatic or persistent generalized lymphadenopathy or acute seroconversion illness	B: HIV-related conditions, not A or C	C: AIDS-defining conditions
>500	A1	B1	C1
200-499	A2	B2	C2
<200	A3	B3	C3

AIDS-defining conditions

- Candidiasis of bronchi, trachea or lungs
- Candidiasis, oesophageal
- Cervical carcinoma, invasive
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (1-month duration)
- Cytomegalovirus (CMV) disease (other than liver, spleen or nodes)
- CMV retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex, chronic ulcers (1-month duration); or bronchitis, pneumonitis or oesophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis; chronic intestinal (1-month duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma (primary) of brain
- Mycobacterium avium-intracellulare complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent
- Progressive multifocal leucoencephalopathy
- Salmonella septicaemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome, due to HIV

HIV=human immunodeficiency virus; AIDS=acquired immunodeficiency disease syndrome; μL=microliter; Source: Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. (United States Centers for Disease Control and Prevention, 1993)

2.4.2 The World Health Organization (WHO) clinical staging and disease classification system for human immunodeficiency/acquired immunodeficiency deficiency syndrome

The WHO Clinical Staging and Disease classification can be used readily in resource limited facilities, as it does not require CD4+ cell count assessment (World Health Organisation, 2006). The WHO system (which was recently revised in 2007) classifies HIV disease based on patient's clinical manifestations (World Health Organisation, 2006). The clinical stages of HIV are classified as stage 1 to 4, progressing from primary HIV infection to AIDS [Table 2.3] (World Health Organisation, 2006).

Table 2:3: The World Health Organization clinical staging of human immunodeficiency/acquired immunodeficiency deficiency syndrome for adults

Clinical Stage	Clinical conditions or symptoms
Primary HIV infection	• Asymptomatic • Acute retroviral syndrome
Clinical stage 1	• Asymptomatic • Persistent generalized lymphadenopathy
Clinical stage 2	• Moderate unexplained weight loss (<10% of presumed or measured body weight) • Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis) • Herpes zoster • Angular cheilitis • Recurrent oral ulceration • Papular pruritic eruptions • Seborrheic dermatitis • Fungal nail infections
Clinical Stage 3	• Unexplained severe weight loss (>10% of presumed or measured body weight) • Unexplained chronic diarrhea for >1 month • Unexplained persistent fever for >1 month (>37.6°C, intermittent or constant) • Persistent oral candidiasis (thrush) • Oral hairy leukoplakia • Pulmonary tuberculosis (current) • Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia) • Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis • Unexplained anemia (hemoglobin <8 g/dL) • Neutropenia (neutrophils <500 cells/µL) • Chronic thrombocytopenia (platelets <50,000 cells/µL)

Table 2.3: The World Health Organization clinical staging of human immunodeficiency/acquired immunodeficiency deficiency syndrome for adults *(continued)*

Clinical stage 4	• HIV wasting syndrome • Pneumocystis pneumonia • Recurrent severe bacterial pneumonia • Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site) • Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs) • Extrapulmonary tuberculosis • Kaposi sarcoma • Cytomegalovirus infection (retinitis or infection of other organs) • Central nervous system toxoplasmosis • HIV encephalopathy • Cryptococcosis, extrapulmonary (including meningitis) • Disseminated nontuberculosis mycobacteria infection • Progressive multifocal leukoencephalopathy • Candida of the trachea, bronchi, or lungs • Chronic cryptosporidiosis (with diarrhea) • Chronic isosporiasis • Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis) • Recurrent nontyphoidal Salmonella bacteremia • Lymphoma (cerebral or B-cell non-Hodgkin) • Invasive cervical carcinoma • Atypical disseminated leishmaniasis • Symptomatic HIV-associated nephropathy • Symptomatic HIV-associated cardiomyopathy • Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

HIV=human immunodeficiency virus; °C=degree Celsius; g/dL=grams per deciliter; µL=microliter; Source: WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. (World Health Organisation, 2006)

2.5 Clinical progression of the human immunodeficiency virus infection

Progression of HIV infection can be divided into three phases: acute infection, clinical latency and AIDS [Figure 2.2] (Fauci, 2007; Hidalgo et al., 2000).

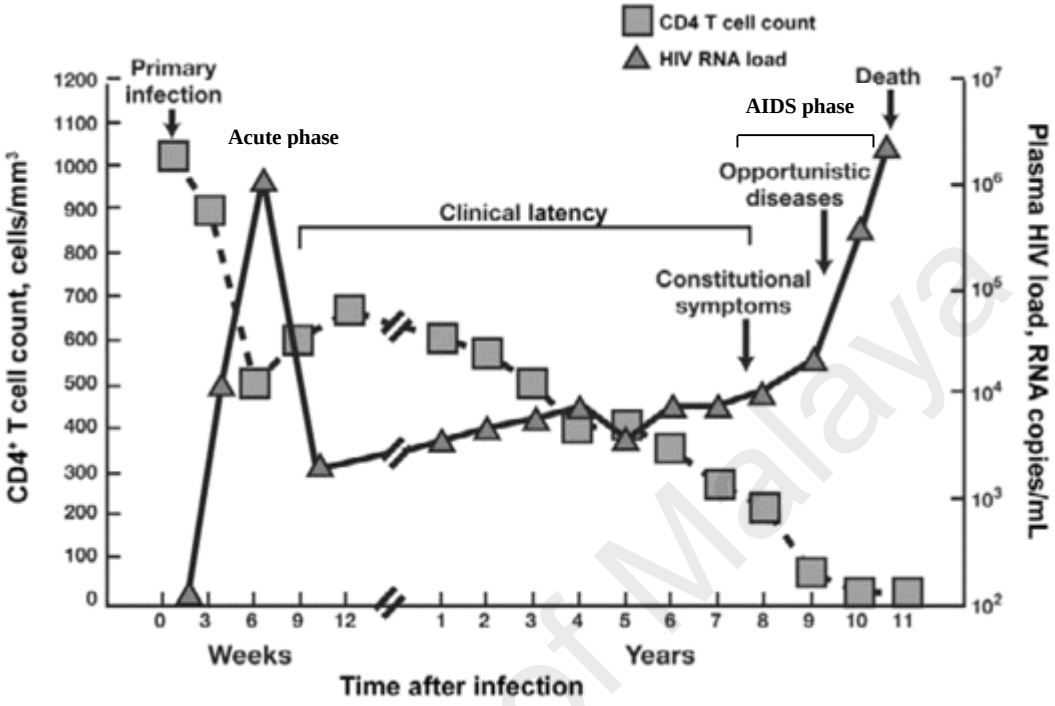


Figure 2:2:Progression of untreated human immunodeficiency virus infection
Source: Pathogenesis of HIV Disease: Opportunities for New Prevention Interventions. Clinical Infectious Diseases, 45 (Supplement 4) (Fauci, 2007)

2.5.1 Phase 1: Acute human immunodeficiency virus infection

The first phase of HIV infection is called the acute infection or primary infection. It occurs after the initial entry of the HIV into the host body. During this phase, there is a high rate of viral turnover, and a sharp decrease in CD4+ cell count (Fauci, 2007). At this phase, most individuals develop non-specific symptoms such as influenza-like syndrome or mononucleosis-like illness, pharyngitis, adenopathy, rash, myalgia, headache, diarrhoea, nausea, or vomiting; while others have no significant symptoms (Hidalgo et al., 2000). This occurs within 2-4 weeks post HIV exposure. Symptoms usually last for 2 weeks, are usually self-limiting, and individuals will recover completely. However, around 10% of individuals may also develop AIDS-defining illnesses (i.e. opportunistic

infections) (Fauci & Lane, 2012). During this phase, HIV antibody testing is usually negative.

2.5.2 Phase 2: Clinical latency

After the acute phase, HIV-infected individuals will enter the clinical latency phase. During this phase, viral turnover slows down until it reaches an equilibrium (Coffin & Swanstrom, 2013). The HIV infection may appear to be clinically latent, but the HIV continues to replicate and CD4+ cell counts steadily decreases (Hidalgo et al., 2000). The majority of HIV-infected individuals are asymptomatic for a substantial, but variable length of time (Coffin & Swanstrom, 2013). Without treatment, the clinical latency period can range from 3-20 years (with an estimated average of nine years) before progressing to AIDS (Hidalgo et al., 2000).

2.5.3 Phase 3: Acquired immunodeficiency syndrome

As HIV infection progresses, viral load increases and CD4+ cell counts reduces (Coffin & Swanstrom, 2013). During this phase, HIV-infected individuals develop clinical presentations associated with immunosuppression (Hidalgo et al., 2000). HIV-infected individuals reach this phase when their CD4+ cell counts fall below 200 cells/μL (United States Centers for Disease Control and Prevention, 1993). Severe symptoms and life-threatening complications due to AIDS-defining illnesses (as described in Section 2.4) are seen, as their immune system become vulnerable and badly damaged (Low et al., 2016).

2.6 Epidemiology of individuals infected by the human immunodeficiency virus

In 2015, there were approximately 36.7 million PLWH, globally. This was an increase of 10.2% from 2010 (33.3 million people) [Table 2.4] (UNAIDS, 2016b). The highest

prevalence of HIV-infected individuals was from Africa. In 2015, there were approximately 25.7 million PLWH; where the vast majority were from eastern and southern Africa (19 million people) (UNAIDS, 2016b). The second highest HIV rates were found in the Asia Pacific region (with approximately 5.1 million people) (UNAIDS, 2016b). In 2015, it was estimated that were 91000 PLWH in Malaysia (Ministry of Health, 2016b).

Table 2:4: Number of people living with the human immunodeficiency virus globally, in 2010 and 2015

Region	PLWH	
	2010	2015
Global	33.3 million	36.7 million
Eastern and southern Africa	17.2 million	19.0 million
Western and central Africa	6.3 million	6.5 million
Asia Pacific	4.7 million	5.1 million
Western and central Europe and North America	2.1 million	2.4 million
Latin America and the Caribbean	1.8 million	2.0 million
Eastern Europe and central Asia	1.0 million	1.5 million
Middle East and north Africa	190 000	230 000

PLWH=people living with human immunodeficiency virus; Source: Global AIDS Update. (UNAIDS, 2016b)

2.7 Epidemiology of new human immunodeficiency virus infections

New HIV infections were found to have decreased by 5% from 2010 to 2015, globally [Table 2.5] (UNAIDS, 2016b). In 2015, there were approximately 2.1 million new HIV infections globally, of which 150,000 of them were children (UNAIDS, 2016b).

Table 2:5: Number of new human immunodeficiency virus infections globally, in 2010 and 2015

Region	New HIV infections	
	2010	2015
Global	2.2 million	2.1 million
Eastern and southern Africa	1.1 million	960 000
Western and central Africa	450 000	410 000
Asia Pacific	310 000	300 000
Western and central Europe and North America	92 000	91 000
Latin America and the Caribbean	100 000	100 000
Eastern Europe and central Asia	120 000	190 000
Middle East and North Africa	20 000	21 000

HIV=human immunodeficiency virus; Source: Global AIDS Update. (UNAIDS, 2016b)

The largest reduction of new HIV infections occurred in eastern and southern Africa (UNAIDS, 2016b). This could be due to vigorous efforts by non-government organizations (NGOs) to reduce HIV transmission by introducing various national campaigns to encourage uptake of HIV testing and counselling, condoms distribution for sex workers (>3 million male condoms were distributed per year) and voluntary medical male circumcision (11.7 million men). In 2012, the approval of pre-exposure oral prophylaxis (PreP), emtricitabine and tenofovir disoproxil fumarate (Truvada®) for HIV/AIDS prevention in HIV-uninfected individuals may have also contributed to the decrease of new HIV infection (World Health Organization, 2012).

In contrast, eastern Europe and central Asia were the only regions where new HIV infection continues to rise rapidly (UNAIDS, 2016b). New HIV infection in this region increased from 2010 to 2015 by 58% (UNAIDS, 2016b). This could be due to low

coverage of harm reduction programmes in people who inject drugs (PWID) poor surveillance of HIV epidemic, and minimal coverage of ART used in HIV-infected individuals (only 20% HIV-infected individuals were treated with ART in 2015) (UNAIDS, 2016b).

In Malaysia, new HIV infections have decreased by 50% from 2000 to 2015(Ministry of Health, 2016b).The Malaysian Ministry of Health and NGOs have introduced several programmes such as harm reduction programmes, needle exchange programmes, national campaigns to encourage uptake of HIV testing and counselling, HIV screening for pregnant mothers and pre-marital couples to reduce HIV transmission (Ministry of Health, 2016b).

2.8 Diagnosis of the human immunodeficiency virus

Test used to diagnose HIV are enzyme immunoassays, HIV confirmatory tests and rapid HIV tests (Cornett & Kirn, 2013).

2.8.1 Rapid human immunodeficiency virus tests

The rapid HIV antibody test can be used to detect HIV antibody IgG and IgM in saliva and blood samples (Cornett & Kirn, 2013). The advantage of this test is that it is able to detect HIV within 30 minutes (Cornett & Kirn, 2013). However, the accuracy of this test for the diagnosis of HIV still remains a concern, as false-negative results may occur. If a positive result is obtained, a further confirmatory test needs to be performed.

2.8.2 Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay is the most common test used to screen for HIV (Murphy & Aitken, 2011). Fourth generation enzyme

immunoassays are highly sensitive and specific (Cornett & Kirn, 2013). It can detect both the p24 antigen and HIV-1/HIV-2 antibodies in a single assay (Cornett & Kirn, 2013). The ELISA test is usually performed 3-4 weeks from initial HIV exposure to avoid false negative results (Mylonakis, Paliou, Lally, Flanigan, & Rich, 2000). False positive results may occur if a patient has autoimmune/renal/liver disease, multiple pregnancies, blood transfusions, haemodialysis, hepatitis B, influenza or rabies (Mylonakis et al., 2000).

2.8.3 Human immunodeficiency virus confirmatory tests

After ELISA, a further confirmatory test needs to be performed before the final diagnosis of HIV can be made(Cornett & Kirn, 2013). Both the Western blot test and the indirect immunofluorescence assay (IFA) can be used as confirmatory tests for HIV, due to their higher specificity than ELISA (Cornett & Kirn, 2013).

Both tests results are determined based on its ability to detect antibodies that bind to HIV (Cornett & Kirn, 2013; Gastaldello, Gallego, Isa, Nates, & Medeot, 1999). However, the Western blot test may produce a false negative result, as it can only detect IgG antibodies, and may lag behind a reactive ELISA by 3 weeks (Branson & Mermin; Cornett & Kirn, 2013). The IFA is a cheaper and more affordable test, that can be used in developing countries (Gastaldello et al., 1999; Kiptoo, Mpoke, & Ng'ang'a, 2004).

2.9 Treatments for human immunodeficiency virus-infected individuals

The first anti-retroviral drug, zidovudine was approved by the Food and Drug Administration (FDA) in the mid-1980s,as monotherapy for the treatment of HIV(Young, 1988). Since then, other classes of ART have been developed. These include the nucleotide reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) in 1996,

fusion inhibitors in 2003, and integrase inhibitors and CCR5 antagonists in 2007 (Arts & Hazuda, 2012). Over the past years, HIV treatment has evolved to a combination of different classes of ARV agents known as highly active antiretroviral therapy (HAART) (Arts & Hazuda, 2012). The advent of ART in HIV-infected individuals has decreased HIV-related complications and improved survival (Matovu, Wattanachanya, Beksinska, Pettifor, & Ruxrungtham, 2016).

2.9.1 Goals of antiretroviral therapy

HAART is a combination of three or more ARVs used to treat HIV. To date, HIV infection cannot be fully eradicated. Therefore, the primary goal of initiating ART in HIV-infected individuals is to reduce the morbidity and mortality of HIV-infected individuals, to improve their quality of life and life expectancy; and to reduce complications associated with HIV/AIDS (Bolland et al., 2015; Günthard, Saag, Benson, & et al., 2016). ART also helps restore and preserve the immunological function, maximally suppress viral load, prevent transmission of HIV via sexual contact and perinatal transmission, and prevent the emergence of HIV drug resistance (Bolland et al., 2015; Günthard et al., 2016).

2.9.2 Classes of antiretroviral drugs available in Malaysia

In Malaysia, there are six classes of ARV agents which target different phases of the HIV life cycle [Table 2.6] (Ministry of Health, 2016a). These classes include the nucleoside reverse transcriptase inhibitors (NTRIs)/nucleotide reverse-transcriptase (NtRTI)**,** protease inhibitors (PIs), integrase inhibitors, chemokine co-receptor 5 (CCR5) antagonists and fusion inhibitors (Arts & Hazuda, 2012).

Table 2:6: Antiretroviral drugs that are currently available in Malaysia

ARV class	Generic name	Formulation	Standard adult dose	Side effects
NRTI/NtRTI	Abacavir	300mg tablet	300mg twice a day or 600mg once a day	Common: Nausea, vomiting, diarrhea, fever, headache, abdominal pain, tiredness, loss of appetite Rare: Hypersensitivity reaction, lactic acidosis
	Emtricitabine	200mg capsule	200mg once a day	Common: Nausea, diarrhea, headache, raised creatine kinase levels, skin darkening Rare: Lactic acidosis, liver damage
	Lamivudine	150mg and 300mg tablets	150mg twice a day or 300mg once a day	Common: Nausea, vomiting, diarrhea, headache, abdominal pain, hair loss, fever, insomnia (difficulty sleeping), rash, tiredness, joint pain Rare: Lactic acidosis, liver damage
	Zidovudine	100mg and 250mg capsules	250mg twice a day	Common: Nausea, vomiting, fatigue, headache, dizziness, weakness, muscle pain, loss of appetite, fever Rare: Blood disorders, lipoatrophy, lactic acidosis
	Tenofovir	245mg tablet	245mg once a day	Common: Nausea, vomiting, diarrhea, flatulence, dizziness, low blood phosphate levels, weakness, rash, headache, stomach pains, fatigue, bloating Rare: Kidney problems, bone thinning
	Abacavir+ Lamivudine	600mg abacavir+ 300mg lamivudine	One tablet once a day	Refer to abacavir and lamivudine
	Tenofovir+ Emtricitabine	245mg tenofovir + 200mg emtricitabine	One tablet once a day	Refer to tenofovir and emtricitabine
	Zidovudine+ Lamivudine	300mg zidovudine+ 150mg lamivudine	One tablet twice a	Refer to lamivudine and zidovudine

Table 2.6: Antiretroviral drugs that are currently available in Malaysia *(continued)*

ARV class	Generic name	Formulation	Standard adult dose	Side effects
NNRTI	Efavirenz	600mg tablet and 200mg capsule	600mg once a day	Common: Rash, dizziness, sleep disturbance, abnormal dreams, impaired concentration, nausea, vomiting, headache, tiredness, diarrhea, anxiety, depression, suicidal thoughts Rare: Psychosis, severe rash, liver problems
	Nevirapine	200mg tablet	200mg once a day for two weeks then 200mg twice a day	Common: Liver toxicity, allergic reaction, rash, nausea, headache, fatigue, stomach pain, diarrhea Rare: Severe rash (Stevens Johnson syndrome)
		400mg tablet (prolonged release tablet)	400mg once a day after introductory period on non-extended-release nevirapine	Common: Liver toxicity, allergic reaction, rash, nausea, headache, fatigue, stomach pain, diarrhea Rare: Severe rash (Stevens Johnson syndrome)
	Etravirine	100mg and 200mg	200mg twice daily	Common: Rash, peripheral neuropathy Rare: Severe rash (Stevens Johnson syndrome)
	Rilpivirin	25mg tablet	25mg once a day	Common: Insomnia (difficulty sleeping), headache, rash, raised liver enzymes, depression, dizziness, stomach pains, vomiting Rare: At doses above 25mg may cause a disturbance to the heart rhythm
CCR5 Antagonist	Maraviroc	150mg and 300mg tablets	300mg twice a day or 150mg twice a day with ritonavir-boosted PI except tipranavir and fosamprenavir or 600mg twice a day with efavirenz or etravirine without a ritonavir-boosted PI	Common: Nausea, diarrhoea, fatigue, headache Rare: Allergic reaction, liver problem

Table 2.6: Antiretroviral drugs that are currently available in Malaysia *(continued)*

ARV class	Generic name	Formulation	Standard adult dose	Side effects
PI	Atazanavir	150mg, 200mg and 300mg capsule	300mg with 100mg ritonavir once a day	Common: Nausea, diarrhea, rash, stomach ache, headache, insomnia (difficulty sleeping), vomiting, hyperbilirubinaemia, lipodystrophy, liver toxicity, diabetes Rare: Kidney stones, abnormal liver function, changes in heart rhythm
	Ritonavir	100mg tablet	Full dose: 600mg twice a day To 'boost' other PIs: 100–200mg once or twice a day	Common (at full dose): Raised lipid and liver enzymes, nausea, vomiting,diarrhea, abdominal pain, headache, weakness, numbness aroundthe mouth, bad taste in mouth, lipodystrophy, liver toxicity, diabetes Common (at low dose): Raised lipid levels Rare: Changes in heart rhythm
	Darunavir	600mg and 800mg tablet	800mg with 100mg ritonavir once a day or 600mg with 100mg ritonavir twice a day	Common: Diarrhea, nausea, rash, stomach pain, vomiting, headache, lipodystrophy, liver toxicity, diabetes, fever Rare: Abnormal liver function, changes in heart rhythm
	Lopinavir / ritonavir	200mg Lopinavir + 50mg ritonavir	Two tablets twice a day or four tablets once a day	Common: Lipodystrophy, raised liver enzymes, nausea, vomiting, diarrhea, abdominal pain, weakness, heartburn, headache, raised lipids, liver toxicity, diabetes Rare: Changes in heart rhythm
Integrase Inhibitors	Raltegravir	400mg tablet	400mg twice a day	Common: Headache, insomnia (difficulty sleeping) Rare: Severe rash, hypersensitivity reaction, extreme thirst
Fusion Inhibitor	Enfuvirtide	108/vial	90 mg (1 mL) subcutaneous twice daily	Common: Injection site reaction,decrease in appetite, diarrhea, nausea, insomnia, peripheral neuropathy, conjunctivitis, anxiety, fatigue Rare:Pancreatitis, renal failure, bacterial pneumonia

NRTI=nucleoside reverse transcriptase inhibitor; NtRTI=nucleotide reverse-transcriptase inhibitors; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitor; ARV=antiretroviral; HIV=human immunodeficiency virus; DNA=deoxyribonucleic acid; mg=milligram; ml=mililiter; Source: HIV-1 Antiretroviral Drug Therapy. *Cold Spring Harbor Perspectives in Medicine*, 2(4) (Arts & Hazuda, 2012), Global AIDS Response Progress Report 2016. (Ministry of Health, 2016b), Basics of the virology of HIV-1 and its replication. *Journal of Clinical Virology*, 34(4), 233-244. (Sierra et al., 2005)

2.9.2.1 Nucleoside reverse transcriptase inhibitors/nucleotide reverse-transcriptase inhibitor

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drug approved by the FDA for the treatment of HIV (Young, 1988). To date, there are four types of NRTIs (i.e. abacavir, emtricitabine, lamivudine and zidovudine) and one type of nucleotide reverse-transcriptase inhibitor (NtRTI) [i.e tenofovir] available in Malaysia (Ministry of Health, 2016a). The mechanism of action of both NRTIs and NtRTI are similar. Both NRTIs and NtRTI primarily block HIV-1 replication by competitively inhibiting reverse-transcriptase [a HIV enzyme used to convert viral RNA into DNA] (Arts & Hazuda, 2012). They are nucleosides or nucleotides analogues that lack the 3' hydroxyl group in the ribose and act as substrates for the reverse transcriptase (Arts & Hazuda, 2012). This action terminates the viral DNA chain synthesis and prevents HIV from replicating (Arts & Hazuda, 2012).

2.9.2.2 Non-nucleoside reverse transcriptase inhibitors

In 1996, nevirapine was the first non-nucleoside reverse transcriptase inhibitor (NNRTI) drug approved by the FDA for the treatment of HIV (de Béthune, 2010). Since then, four types of NNRTIs (i.e efavirenz, nevirapine, etravirine and rilpivirin) have been introduced in the treatment of HIV in Malaysia (Ministry of Health, 2016a). NNRTIs primarily block HIV-1 replication by directly binding to HIV reverse transcriptase enzyme. This prevents HIV reverse transcriptase from completing reverse transcription of single stranded viral RNA genome into DNA (Arts & Hazuda, 2012).

2.9.2.3 Protease inhibitors

Protease inhibitors (PIs) are a class of ART that blocks the protease enzyme (a protein cutting enzyme which is involved in the production of functional new HIV particles) and

prevent the cell from maturing into new viruses (Arts & Hazuda, 2012). Atazanavir, ritonavir, darunavir, lopinavir/ritonavir are PIs drugs that are currently used in Malaysia for the treatment of HIV (Ministry of Health, 2016a).

2.9.2.4 Integrase inhibitors

Integrase inhibitors are a class of ART that blocks the action of integrase, a viral enzyme that help to insert its viral DNA into the host cell DNA (Arts & Hazuda, 2012). Raltegravir is the only integrase inhibitor available in Malaysia for the treatment of HIV (Ministry of Health, 2016a).

2.9.2.5 Chemokine co-receptor 5antagonists

Chemokine co-receptor 5 (CCR5) antagonists are a class of ART that blocks the CCR5 co-receptor on the surface membrane of CD4+ cells and prevents the entry of the HIV into the cell (Arts & Hazuda, 2012). Maraviroc is the only CCR5 antagonist available in Malaysia (Ministry of Health, 2016a).

2.9.2.6 Fusion inhibitors

Fusion inhibitors are a class of ART that block the entry of HIV into the host CD4+ cell, by preventing the HIV envelope from merging with host cell membrane (fusion) (Arts & Hazuda, 2012). Enfuvirtide is the only fusion inhibitor available in Malaysia (Ministry of Health, 2016a)

2.9.3 Choice of antiretroviral therapy regimen

The combination of triple-drug ART regimens was first introduced in 1996 to suppress HIV replication and to improve treatment in HIV-infected individuals (Arts & Hazuda, 2012). Three ARV drugs (two NRTIs as backbone agents and one NNRTI, PI or integrase

inhibitor as core agents) are used to reduce the likelihood of the HIV virus developing drug resistance (Llibre, Walmsley, & Gatell, 2016). The choice of ART used is based on the patient's disease state, impact of the drug itself and patient's drug preference and convenience (Tseng et al., 2012).

2.9.3.1 Recommendations on when to start antiretroviral therapy in human immunodeficiency virus-infected individuals in Malaysia

In 2013, the World Health Organization (WHO) recommends that ART should be initiated in all HIV-infected individuals regardless of CD4+ cell count (World Health Organisation, 2013). Early initiation of ART in HIV-infected individuals was found to improve survival, decrease risk of disease progression and reduce perinatal transmission (Matovu et al., 2016; Pandhi & Ailawadi, 2014). The recommendation on when to start ART in HIV-infected adults and pregnant/breastfeeding mothers in Malaysia is summarized in Table 2.7 (Ministry of Health, 2016a; World Health Organisation, 2015).

Table 2:7: Recommendation on when to start antiretroviral therapy in human immunodeficiency virus-infected adults in Malaysia

Target population	Specific recommendation
Adults (≥18years)	ART is recommended for all HIV-infected individuals, regardless of CD4+ cell count
	As a priority, ART should be initiated in: • All adults with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) • Individuals with CD4+ cell count≤350 cells/μL • HIV-associated nephropathy • HIV/hepatitis B virus co-infection • HIV/hepatitis C virus co-infection
Pregnant and breastfeeding women	ART should be initiated in all pregnant and breastfeeding women living with HIV regardless of CD4+ cell count and continue lifelong

ART=antiretroviral therapy; HIV= human immunodeficiency virus; Source: Consensus Guidelines on Antiretroviral Therapy 2016 (Ministry of Health, 2016a), Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV (World Health Organisation, 2015).

2.9.3.2 First line antiretroviral therapy regimen in Malaysia

In Malaysia, two NRTIs and one NNRTI are recommended as first-line ART for HIV(Ministry of Health, 2016a). This is due to their cost, safety, effectiveness and tolerability (Cihlar & Fordyce, 2016). If a patient is found unable to tolerate the side effects of a NNRTI, an integrase inhibitor (e.g. raltegravir) or PI drug (e.g. atazinavir, ritonavir, lopinavir/ritonavir) is then used to replace it [Table 2.8] (Ministry of Health, 2016a).

Table 2:8: First-line antiretroviral therapy regimens in Malaysia

Preferred first-line ART	Alternative combinations of ART if the preferred treatment is not available in the clinical setting
Tenofovir+emtricitabine+ efavirenz (2NRTIs+1NNRTIs)	• Zidovudine+lamivudine+ efavirenz (or nevarapine) • Abacavir+lamivudine+efavirenz (or nevarapine) • Tenofovir+emtricitabine+nevarapine
**Tenofovir+ emtricitabine+raltegravir (2NRTIs+1intergrase inhibitor)	• Tenofovir/emtricitabine+ atazinavir/ritonavir • Tenofovir/emtricitabine+ lopinavir/ritonavir

** If patient is intolerant to NNRTI group drug; ART=antiretroviral therapy Source: Consensus Guidelines on Antiretroviral Therapy 2016 (Ministry of Health, 2016a)

2.9.3.3 Second-line antiretroviral regimen in Malaysia

When first line ART treatment failure is identified, HIV-infected individuals are switched to second-line ART (World Health Organisation, 2013). ART treatment failure is defined as "suboptimal response to current therapy leading to loss of virological control". According to the WHO, virological failure is defined as "persistent detectable plasma viral load >1000 copies/ml within three months interval or at least six months after ART initiation" (World Health Organisation, 2013).

In Malaysia, a "one boosted PI and two NRTI combination" is recommended as the preferred choice in second-line ART for adults, when NNRTIs-containing regimens are used as first-line ART [Table 2.9] (Ministry of Health, 2016a).

Table 2:9: Second-line antiretroviral therapy regimen in Malaysia

Target population	Preferred second-line regimen	
Adults	If starvudine and zidovudine were used as first line regimen[3]	Tenofovir[1] + lamivudine (or emtricitabine)+ atazanavir/rotinavir or lopinavir/ritonavir[2]
	If tenofovir was used in first-line therapy[3]	Zidovudine+lamivudine+ atazanavir/rotinavir or lopinavir/ritonavir[2]
HIV and HBV co-infection	Zidovudine+tenofovir[4]+lamivudine (or emtricitabine) + (atazanavir/rotinavir or lopinavir/ritonavir[2])	

[1] Abacavir may be used as potential back-up NRTI options in special circumstances (e.g. concomitant renal failure that precludes use of tenofovir or a past history of anemia precluding use of zidovudine).
[2]Atazanavir/rotinavir and lopinavir/ritonavir are the preferred PI options. Darunavir/ritonavir can also be used as an alternative choice but is currently not available as a fixed-dose combination.
[3]Lopinavir/ritonovir and raltegravir combination had been proven to be as efficacious as standard second-line regimen consisting two NRTIs and one PI.
[4] Tenofovir should not be discontinued in the second-line regimen in HIV patients co-infected with hepatitis B as this can lead to hepatitis B withdrawal flares in hepatitis.
Source: Consensus Guidelines on Antiretroviral Therapy 2016 (Ministry of Health, 2016a), Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV(World Health Organisation, 2015).

2.9.4 Epidemiology of antiretroviral treat individuals and AIDS-related deaths globally, by region in 2010 and 2015

Globally, the number of people treated with ART has increased by 127% from 7.5 million people in 2010 to 17 million people in 2015 [Figure 2.3]. This means that 46% of PLWH globally have access to ART (UNAIDS, 2016a). As a result, AIDS-related deaths have reduced by 27% from 1.5 million people in 2010 to 1.1 million people in 2015 [Table 2.10]. In 2015, 77% of pregnant women living with HIV had access to pre-exposure oral prophylaxis (PreP) to prevent transmission of HIV to their children, globally (UNAIDS, 2016c). These intensive efforts to eliminate perinatal transmission of HIV achieved a

decline in the annual number of children who were infected with HIV from 290,000 in 2010 to 150,000 worldwide (UNAIDS, 2016b).

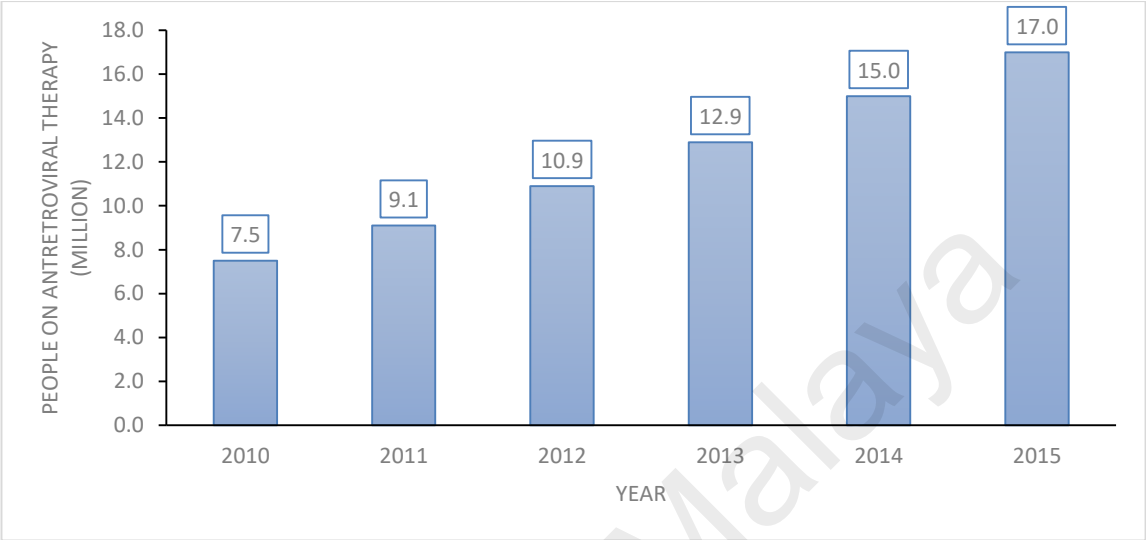


Figure 2:3:Number of human immunodeficiency virus-infected individuals treated with antiretroviral therapy, globally from 2010 to 2015
Source: Global AIDS Update.(UNAIDS, 2016b)

Table 2:10: Number of people living with human immunodeficiency virus that were on antiretroviral treatment, and AIDS-related deaths globally and by region in 2010 and 2015

Region	PLWH on ART		AIDS-related deaths	
	2010	2015	2010	2015
Global	7.5 million	17 million	1.5 million	1.1 million
Asia Pacific	907 600	2.1 million	240 000	180 000
Eastern and southern Africa	4.1 million	10 million	760 000	470 000
Eastern Europe and central Asia	112 100	321 800	38 000	47000
Latin America and the Caribbean	0.6 million	1.1 million	60 000	50 000
Middle East and north Africa	13 600	38 200	9 500	12 000
Western and central Africa	0.9 million	18 million	37 0000	330 000
Western and central Europe and north America	0.9 million	14 million	29 0000	22 000

PLWH=people living with human immunodeficiency virus; ART=antiretroviral therapy; AIDS=acquired immunodeficiency syndrome; Source: Global AIDS Update.(UNAIDS, 2016b)

Malaysia has made significant progress in expanding the availability and accessibility of ART since free medications to HIV-infected individuals was introduced in 2001 (Ministry of Health, 2016b). The number of PLWH on ART has increased by 16% from 21654 people in 2014 to 25700 people in 2015 (Ministry of Health, 2015, 2016b). This means that 30% of PLWH in Malaysia have access to ART in 2015 (Ministry of Health, 2016b). AIDS-related deaths in HIV-infected individuals from 2014 and 2015 showed no significant changes (approximately 17 000 people) (Ministry of Health, 2015, 2016b).

2.10 Epidemiology of people living with human immunodeficiency virus aged 50 years and older

The advent of HAART has significantly reduced the morbidity and mortality associated with HIV infection and AIDS (Palella et al., 1998). As a result, life expectancy of HIV-infected individuals has increased (Bolland et al., 2015). Increased coverage of ART allows HIV-infected individuals to live well past the age of 50 years (Mahy et al., 2014), and declines the rate of new HIV infection among younger adults (Negin & Cumming, 2010).

Globally, in 2013, there were 4.2 million HIV-infected individuals aged 50 years old and older, which was a two-fold increase from 1995 (Mahy et al., 2014). In the United States, the number of PLWH ≥50 years old has increased from 40% in 2012 to 50% in 2015 (Wing, 2016). Similarly, in Malaysia, the prevalence of PLWH over the age of 50 years has also gradually increased. It was estimated that PLWH that were ≥50 years old has doubled from 5% in 2005 to 10% in 2015 (Ministry of Health, 2016b).

2.11 Human immunodeficiency virus and ageing

Ageing is a complicated process which results in an increased vulnerability to multiple aged-related diseases (Pirrone et al., 2013). Recent studies have shown that HIV infection and the use of ART accelerates the ageing process in HIV-infected individuals (Effros et al., 2008; Onen et al., 2010; Wing, 2016). Premature ageing and development of certain age-related illnesses are demonstrated approximately 10-15 years younger in HIV-infected individuals when compared to the general population (Guaraldi et al., 2011; Pirrone et al., 2013). Data shows that death caused by aged-related illnesses in HIV-infected individuals has increased over the past years (Wing, 2016).

Age-related comorbidities in HIV-infected individuals include osteoporosis, metabolic syndrome, ocular degeneration, mitochondrial toxicity, neurocognitive dysfunction and depression (Effros et al., 2008; Onen et al., 2010). Older HIV-infected individuals were found to have a significant higher risk of developing age-related comorbidities when compared to younger HIV-infected individuals (Negin et al., 2012; Wu et al., 2014). In South Africa and Taiwan, HIV-infected individuals aged 50 years and older were found to have three times increased risk of developing aged-related comorbidities (>two chronic disease) [29.6%-30.6%] when compared to younger HIV-infected individuals [8.6-8.8%] (Negin et al., 2012; Wu et al., 2014).

In the United States, HIV-infected individuals ≥65 years old were found have 1.5-2.4 times as likely to have a chronic disease and 2.4–7 times as likely to have one to five co-morbid chronic conditions when compared to HIV-uninfected individuals (Friedman & Duffus, 2016). A study conducted in the Netherlands, of those HIV-infected individuals aged ≥45 years old (69.4%) with more than one aged-related comorbidities when compared to HIV-uninfected individuals (Schouten et al., 2014). Similarly in Malaysia, the proportion of HIV-infected individuals aged ≥25 years old (88%) who had one or more geriatric conditions was also higher than HIV-uninfected individuals (63%) (Rajasuriar et al., 2017).

2.12 Metabolic complications of human immunodeficiency virus and highly active antiretroviral therapy

The widespread use of effective ART regimens has been associated with metabolic complications such as lipodystrophy, dysregulation of glucose metabolism, mitochondrial abnormalities, and bone abnormalities (Sweet, 2005). These complications

can sometimes be severe and life threatening, which can profoundly affect the quality of life of HIV-infected individuals (Sweet, 2005).

2.12.1 Lipodystrophy

Lipodystrophy is a medical disorder that is characterized by fat loss and/or redistribution of body fat (Fiorenza, Chou, & Mantzoros, 2011). This disorder is frequently observed in HIV-infected individuals on long-term ART (Fiorenza et al., 2011). Studies found that body fat abnormalities occurred in 30%-50% of HIV-infected individuals receiving potent ART (Chow, Day, Souza, & Shikuma, 2006). Both PIs and NRTIs (such as starvudine) have been associated with the development of lipoathropic component in HIV-associated lipodystrophy syndrome (Chow et al., 2006).

2.12.2 Dysregulation of glucose metabolism

Insulin resistance, impaired glucose tolerance and flank diabetes mellitus are often associated with potent ART (Schambelan et al., 2002). Studies found that up to 40% of patients on a PI-containing regimen have impaired glucose tolerance due to significant insulin resistance (Hadigan et al., 2001); characterized by the reduced ability of insulin to inhibit hepatic gluconeogenesis and to increase muscle uptake of glucose (Schambelan et al., 2002). Hence, PIs may directly impair cellular glucose uptake (Murata, Hruz, & Mueckler, 2000) or indirectly affect the mechanisms related to body fat changes, including central obesity (Hadigan et al., 2001).

2.12.3 Mitochondrial abnormalities

NRTIs are known to significantly induce mitochondrial toxicity; which plays a major role in the development of the lipoatropic component of HIV-associated lipodystrophy syndrome (Chow et al., 2006). Studies found that NRTIs were associated with the

inhibitory effect on the principal enzyme responsible for mitochondrial DNA (mtDNA) replication (Chow et al., 2006), which led to the depletion of mitochondrial DNA or its qualitative changes (White, 2001). Additionally, studies found that PIs were associated with the inhibition of adipocyte differentiation and maturation of mitochondria (Dowell, Flexner, Kwiterovich, & Lane, 2000).

2.12.4 Bone abnormalities

Bone disorders like osteonecrosis and osteoporosis were found to be associated with HIV infection and ART (Ahmad, Ahmad, & Ahmad, 2017). Osteonecrosis (defined as bone tissue death as a result from circulatory insufficiency) (Fondi & Franchi, 2007)has been described as a complication of HIV infection since the late 1980s (Chow et al., 2006). Interruption of the vascular supply to bone results in a stepwise progression through ischemia, hyperaemia, an increase in intraosseous pressure, and eventually death of osteocytes (Chow et al., 2006). The areas most often affected are the femoral and humeral heads, femoral condyles, proximal tibia, and some of the small bones in the hand and wrist (Schambelan et al., 2002). A cross sectional study conducted in the United States found that 4.4% of HIV-infected individuals had osteonecrosis when compared to 0.02% to 0.14% of HIV-uninfected individuals (Miller et al., 2002)

2.13 Osteoporosis

2.13.1 Definition of osteoporosis

Osteoporosis is defined as a “systemic skeletal disease characterized by compromised bone strength predisposing a person to an increased risk of fracture” (National Institutes of Health, 2001). Bone strength consist of bone density and bone quality which are determined by multiple factors including peak bone mass, bone turnover rate, mineralization and micro-architecture (Leali et al., 2011).

2.14 Classification of osteoporosis

Osteoporosis can be classified as primary or secondary osteoporosis.

2.14.1 Primary osteoporosis

Primary osteoporosis occurs as a consequence of increasing age in both men and women (Clarke & Khosla, 2010; Ji & Yu, 2015). Primary osteoporosis mainly occurs in women after menopause and in men after their 70s [Figure 2.4] (Ji & Yu, 2015; Kruger & Nell, 2017).

Adults reach peak bone mass at 30 years old (Heaney et al., 2000; Kruger & Nell, 2017). Bone mass then remains relatively stable. Women then experience an accelerated bone loss during menopause (Clarke & Khosla, 2010; Kruger & Nell, 2017). On average, women will lose 35% to 55% of their bone mass in their lifetime whereas men will lose 20% to 30% of their bone mass (Riggs & Melton, 1986).

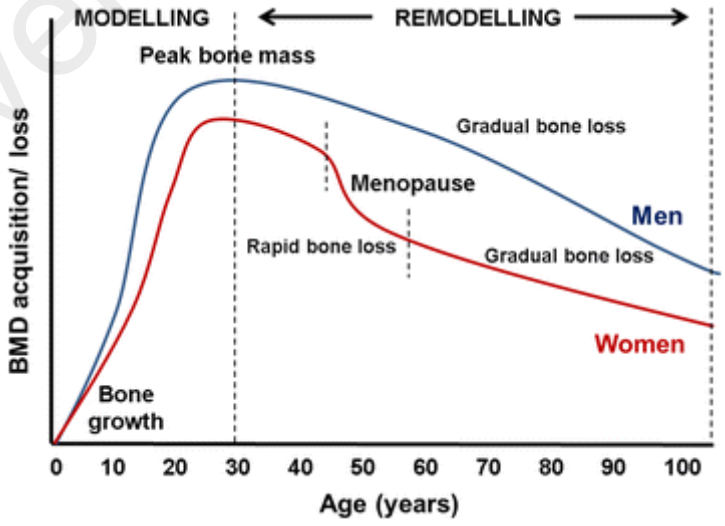


Figure 2:4:The general pattern of bone development and loss overtime
Source: Bone mineral density in people living with HIV: a narrative review of the literature. *AIDS Research and Therapy*, 14(1), 35. (Kruger & Nell, 2017)

2.14.2 Secondary osteoporosis

Secondary osteoporosis can caused by an underlying medical condition or medications that alter bone mass, which may subsequently lead to fragility fracture (Painter, Kleerekoper, & Camacho, 2006).The common causes of secondary osteoporosis are listed Table 2.11 (Hofbauer, Hamann, & Ebeling, 2010; Mazokopakis & Starakis, 2011). Although HIV infection is not listed as one of the common causes of secondary of osteoporosis, studies have found that HIV-infected individuals are more prevalent to have this disease when compared to the general population(Brown & Qaqish, 2006).

Table 2:11: Secondary causes of osteoporosis

Secondary causes of osteoporosis:
• Hyperparathyroidism (primary and secondary) • Cushing's syndrome • Hypogonadism • Thyrotoxicosis • Diabetes mellitus • Renal impairment • Chronic liver disease(e.g. liver cirrhosis) • Rheumatoid arthritis • Vitamin D deficiency • Calcium deficiency • Malabsorption • Nutritional deficiency (e.g. anorexia nervosa) • Malignancy (e.g. myeloma, bony metastasis) • Osteogenesis imperfect • Chronic lung disease • Medications (e.g. glucocorticoids, heparin, anticonvulsants, immunosuppressants, thiazolidinediones, oncology therapy)

Source: Approach to the patient with secondary osteoporosis. *Eur J Endocrinol*, 162(6), 1009-1020. (Hofbauer et al., 2010; Mazokopakis & Starakis, 2011)

2.15 Aetiology of osteopenia/osteoporosis in HIV-infected individuals

The pathophysiology of bone loss in HIV-infected individuals is complex and multifactorial (Qaqish & Sims, 2004). Bone loss may result from interactions between T-cells, osteoclast and osteoblasts (Saccomanno & Ammassari, 2011). In addition, bone loss can also be caused by the HIV infection itself and the use of ART (Saccomanno & Ammassari, 2011). Nutritional and hormonal changes in HIV-infected individual such as muscle wasting, malnutrition, malabsorption, hypogonadism, calcium and vitamin D deficiency may also contribute to bone loss (Saccomanno & Ammassari, 2011). In recent years, studies have found that changes in the immune system have drastically affected

skeletal metabolism (Ofotokun & Weitzmann, 2010). HIV infection is associated by chronic systemic inflammation (caused by an increase of pro-inflammatory cytokines) (Deeks, Tracy, & Douek, 2013). Pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, receptor of activated NF-κB ligand (RANKL) and osteoprotegerin (OPG) is associated with the inhibition of osteoblast function, an increase osteoclast formation and an induce bone resorption, which then accelerates bone loss (Azuma, Kaji, Katogi, Takeshita, & Kudo, 2000; Boyce & Xing, 2008).

2.16 Diagnosis of osteoporosis

2.16.1 Clinical evaluation

A thorough clinical evaluation which includes patient history, physical assessment, laboratory and radiological investigation is required to diagnose osteoporosis (Walker Harris & Brown, 2012). The diagnosis of osteoporosis is based on either low BMD on a dual X-ray absorptiometry (DXA) scan or a history of low trauma fracture (International Osteoporosis Foundation, 2015).

BMD results are presented as T-score and Z-score. The T-score is the standard deviation of an individual's BMD away from the mean BMD of healthy young adults of the same gender. Z-score is the standard deviation of an individual's BMD from the mean BMD of adults of the same age and gender (International Society for Clinical Densitometry, 2013; World Health Organisation, 1994).

When interpreting DXA scan results, the T-score is generally used in postmenopausal women and men age ≥ 50 years old, whilst the Z-score is used in premenopausal women, men ≤ 50 years old and children (International Society for Clinical Densitometry, 2013; World Health Organisation, 1994). The Z-score is used to identify possible secondary

causes of osteoporosis in younger adults (Sheu & Diamond, 2016). According to International Society for Clinical Densitometry (ISCD), Z-score of -2.0 or lower is defined as "low BMD for chronological age" and those above -2.0 being "within the expected range for age" (International Society for Clinical Densitometry, 2013).

According to the World Health Organisation, osteoporosis is diagnosed when T-score is ≤-2.5; and osteopenia is diagnosed when T-score is between -1 and -2.5 [Table 2.12] (World Health Organisation, 1994). DXA scan measures BMD of the lumbar spine and femoral neck as they are the most important sites for predicting fractures (Kanis, Melton, Christiansen, Johnston, & Khaltaev, 1994).

Table 2:12: Diagnosis of osteoporosis according to the World Health Organisation working group

Classification	T-score
Normal	≥-1.0
Osteopenia	-1.0 to -2.5
Osteoporosis	≤ -2.5
Severe osteoporosis	≤ -2.5 with existing fracture

Source: Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser, 843*, 1-129 (World Health Organisation, 1994)

2.16.2 Operational definition of reduced bone mineral density

For the purpose of this study, reduced BMD is defined as any individual who has been diagnosed with osteopenia or osteoporosis at any site.

2.16.3 Methods of measuring bone mineral density

Dual X-ray absorptiometry (DXA), quantitative computed tomography (QCT) and quantitative ultrasound (QUS) are non-invasive techniques that can be used to measure BMD; each of them having their own advantages and disadvantages [Table 2.13] (Faulkner et al., 1991; Stepan, 2002). QUS is the cheapest method to measure BMD, but cannot be used to diagnose of osteoporosis due to its lower precision when compared to QCT and DXA(Faulkner et al., 1991; Stepan, 2002). QCT is able to measure the true volumetric density of trabecular or cortical bone but emits higher radiation and require longer scanning time compared to DXA (Faulkner et al., 1991; Stepan, 2002).

According to the WHO, the "goal standard" to measure BMD in clinical practice by DXA (World Health Organisation, 1994), as it is precise and quick when compared to other techniques (Faulkner et al., 1991; Stepan, 2002). However, use of DXA scan alone as a screening and diagnostic tool for osteoporosis may not be feasible in some countries, as it is expensive, and is usually only available in tertiary hospitals (Chen et al., 2016).

Table 2:13: List of non-invasive methods measuring bone mineral density

Technique	Site of scan	Advantages	Disadvantages	Comments
DXA	Lumbar spine, proximal femur, total body, forearm	It yields a high precision (sensitivity=88.2%) and enables fast scanning.	It is expensive, radioactive, needs to shield radiographer from radiation, and is usually only available in tertiary hospitals	It is based on the use of photons emitted at two different energies, which allows measurements on the sites with the uneven soft tissue composition to be performed.
QCT	Lumbar spine	It is able to measure the true volumetric density of trabecular or cortical bone	It has a higher radiation dose and needs longer scanning time compared to DXA	An alternative technique to measure BMD in the axial skeleton
QUS	Calcaneus, tibia, radius, phalanges	It is less expensive than DXA or QCT, avoids ionising radiation, and is relatively portable	It is generally poorer precision than DXA, diversity of techniques, lack of standardization and comparable local normal ranges to conduct the test	It appears to be a good predictor of fracture risk but it is not recommended to be used for diagnosis of osteoporosis and treatment monitoring

DXA=dual X-ray absorptiometry, QCT=quantitative computed tomography, QUS= quantitative ultrasound; Noninvasive measurements of bone mass, structure, and strength: current methods and experimental techniques. *American Journal of Roentgenology*, *157*(6), 1229-1237(Faulkner et al., 1991). Stepan, J. J. (2002). Techniques for measuring bone mineral density. *International Congress Series*, *1229*, 63-68.(Stepan, 2002).

2.16.4 Self- assessment tools to evaluate osteoporosis and fracture risk

Several self-assessment tools have been developed to evaluate the risk of osteoporosis or fracture risk in patients (Ahmadzadeh, Emam, Rajaei, Moslemizadeh, & Jalessi, 2014). The advantages of these tools are that they are simple to use, and inexpensive and easily available when compared to DXA scan (Kling, Clarke, & Sandhu, 2014). However, these tools are unable to incorporate all clinical osteoporosis risk factors, and some tools may have low sensitivity to detect osteoporosis (Kling et al., 2014). Hence, these tools should are not be used as the "gold standard" to screen for osteoporosis; but rather as a tool to enhance patient's clinical assessment (Kling et al., 2014).

2.16.4.1 Self-assessment tools to evaluate osteoporosis risk

Several tools have been developed to assess osteoporosis risk [Table 2.14] (Chen et al., 2016; Lim, Ong, Suniza, & Adeeb, 2011). Both the Age Bulk One or Never Estrogens (ABONE) (Weinstein & Ullery, 2000)and Simple Calculated Osteoporosis Risk Estimation (SCORE) (Lydick et al., 1998)were developed in the United States, whilst the body weight criterion (BWC), the Osteoporosis Risk Assessment Instrument (ORAI), the Osteoporosis Index of Risk (OSIRIS) and the Malaysian Osteoporosis Screening Tool (MOST) was developed in Sweden (Michaelsson et al., 1996), Canada (Cadarette et al., 2000), Belgium (Sedrine et al., 2002), and Malaysia(Lim et al., 2011), respectively. The Osteoporosis Self-Assessment Tool for Asians(OSTA) was developed in eight countries [Singapore, China, Hong Kong, Korea, Philippines, Taiwan, Thailand and Japan] (Chen et al., 2016). Among these tools, only OSTA and MOST have been developed to assess osteoporosis risk in Asian population(Chen et al., 2016; Lim et al., 2011). The scoring of each of these tools are summarized in Table 2.14 (Chen et al., 2016; Lim et al., 2011). Among these tools, only the BWC and OSTA are able to assess osteoporosis risk in both

men and women whilst others tools can only be used in women (Chen et al., 2016; Lim et al., 2011).

Table 2:14: Characteristics of self-assessment tools used to evaluate osteoporosis risk

Screening tool	Developed in	Validated in	No. of questions	Cut-off point indicating increased risk of osteoporosis	Scoring based on
BWC	Sweden (Michaelsson et al., 1996)	• Singapore(Chan, S.-P. et al., 2006)	1	<70kg	Weight
ABONE	USA (Weinstein & Ullery, 2000)	• Singapore (Chan, S.-P. et al., 2006)	3	Score≥2	Age, weight and oral contraceptive or oestrogen used for ≥ 6 months
OSTA	Eight Asian countries	• USA (Geusens et al., 2002), • Canada (Cadarette et al., 2000), • Belgium (Gourlay et al., 2005; Richy et al., 2004), • Netherlands (Geusens et al., 2002), • Philippines (Li-Yu, Llamado, & Torralba, 2005), • Japan (Fujiwara, Masunari, Suzuki, & Ross, 2001), • Korea(Park, Park, Park, Paek, & Cho, 2003), • Thailand (Geater, Leelawattana, & Geater, 2004; Saetung, Ongphiphadhanakul, & Rajatanavin, 2008), • Taiwan(Li, Y. M., 2008), • Hong Kong(Kung, Ho, Sedrine, Reginster, & Ross, 2003), • Singapore (Chan, S.-P. et al., 2006)	2	High risk: Age (years): ≥65; weight (kg):40-69	Age, weight

Table 2.14: Characteristic of self-assessment tools used to evaluate osteoporosis risk *(continued)*

Screening tool	Developed in	Validated in	No. of questions	Cut-off point indicating increased risk of osteoporosis	Scoring based on
ORAI	Canada (Cadarette et al., 2000)	• USA (Geusens et al., 2002), • Canada (Cadarette et al., 2000), • Belgium (Gourlay et al., 2005; Richy et al., 2004), • Netherlands (Geusens et al., 2002), • Japan (Fujiwara et al., 2001), • Singapore (Chan, S.-P. et al., 2006)	3	Score≥9	Age, weight, oestrogen use
OSIRIS	Belgium (Sedrine et al., 2002)	• Belgium (Sedrine et al., 2002), • France (Reginster et al., 2004)	4	Score≤1	Age, weight, current oestrogen used, history of low impact fracture
SCORE	USA (Lydick et al., 1998)	• USA (Geusens et al., 2002; Lydick et al., 1998), • Belgium (Gourlay et al., 2005; Richy et al., 2004), • Netherlands (Geusens et al., 2002), • Japan (Fujiwara et al., 2001), • Singapore (Chan, S.-P. et al., 2006)	6	Score≥6	Age, weight, race, fracture history, rheumatoid arthritis history and oestrogen use
MOST	Malaysia (Lim et al., 2011)	• Malaysia(Lim et al., 2011)	4	Score≥4	Age, years post menopause, BMI, hip circumference

ABONE=Age Bulk One or Never Oestrogens, BWC=body weight criterion, BMI=body mass index; ORAI=Osteoporosis Risk Assessment Instrument, OSIRIS=Osteoporosis Index of Risk, OSTA=Osteoporosis Self-Assessment Tool for Asians, SCORE=Simple Calculated Osteoporosis Risk Estimation, MOST=Malaysian Osteoporosis Screening Tool; Source: Comparisons of Different Screening Tools for Identifying Fracture/Osteoporosis Risk Among Community-Dwelling Older People. *Medicine, 95*(20), e3415 (Chen et al., 2016), Developing a Malaysian Osteoporosis Screening Tool (MOST) for early osteoporosis detection in Malaysian women. *Sex Reprod Healthc, 2*(2), 77-82. (Lim et al., 2011)

2.16.4.2 Self-assessment tools to evaluate fracture risk

The Fracture risk assessment tool (FRAX) and the Garvan fracture risk calculator (GARVAN) were developed to assess the risk of fracture in both men and women [Table 2.15] (Chen et al., 2016). The FRAX was developed and validated in Europe, North America, Asia and Australia (Kanis et al., 2009); whilst the GARVAN was developed and validated only in Australia (Nguyen, Frost, Center, Eisman, & Nguyen, 2008). The FRAX calculates the 10-year probability of hip and major osteoporotic fracture whilst, the GARVAN calculates the 5- and 10-year risk of hip fracture and risk of any fragility fracture (van den Bergh, van Geel, Lems, & Geusens, 2010). Both tools can be used with or without BMD results (van den Bergh et al., 2010).

Table 2:15: Characteristics of self-assessment tools used to evaluate fracture risk

Screening tool	Gender /age availability	Developed in	Validated in	No of questions	Cut-off point indicating increased risk of fracture	Scoring based on
FRAX	Male &female ≥40 years old	Europe, North America, Asia and Australia(Kanis et al., 2009)	Europe, North America, Asia and Australia(Kanis et al., 2009)	13	≥20% probability of major osteoporotic fracture ≥3% probability of hip fracture	Age, gender, ethnicity, weight, height, history of prior fractures, parental history of hip fracture, current smoking, glucocorticoid use, rheumatoid arthritis, secondary osteoporosis, alcohol use (≥3 units per day) and with/without femoral neck BMD
GARVAN	Male &female ≥50 years old	Australia (Nguyen et al., 2008).	Australia(Nguyen et al., 2008).	6	≥20% probability of any osteoporotic fracture	Age, gender, body weight, history of prior fractures aged ≥ 50 years, history of falls in the past year and with/without femoral neck BMD

FRAX=Fracture Risk Assessment Tool, GARVAN=Garvan fracture risk calculator, BMD=bone mineral density; BMI=body mass index. Source: Chen, S. J., Chen, Y. J., Cheng, C. H., Hwang, H. F., Chen, C. Y., & Lin, M. R. (2016). Comparisons of Different Screening Tools for Identifying Fracture/Osteoporosis Risk Among Community-Dwelling Older People. *Medicine*, *95*(20), e3415 (Chen et al., 2016), Lim, P. S., Ong, F. B., Suniza, S. S., & Adeeb, N. (2011). Developing a Malaysian Osteoporosis Screening Tool (MOST) for early osteoporosis detection in Malaysian women. *Sex Reprod Healthc*, *2*(2), 77-82 (Lim et al., 2011)

2.17 Osteoporosis risk factors in human immunodeficiency virus-infected individuals

The risk factors to develop osteoporosis for a HIV-infected individual can be divided into traditional osteoporosis risk factors or HIV-related risk factors

2.17.1 Traditional osteoporosis risk factors

Traditional risk factors for osteoporosis can be further categorized as non-modifiable or modifiable risk factors [Table 2.16] (International Osteoporosis Foundation, 2015).

Table 2:16: Traditional risk factors of osteoporosis

Non-modifiable	Modifiable
• Age • Ethnicity • Gender • Premature menopause • Family history of osteoporotic hip fracture in first degree relative • Personal history of fracture as an adult	• Sedentary lifestyle • Smoking • Alcohol intake • Excessive caffeine intake • Low body weight • Estrogen deficiency • Frequent falls • Eating disorders • Calcium intake • Vitamin D intake

Source: (International Osteoporosis Foundation, 2015)

2.17.1.1 Non-modifiable risk factors

(a) ***Age***

Older age has been associated with increased risk of developing osteopenia or osteoporosis (Clarke & Khosla, 2010; Ji & Yu, 2015). This has been further discussed in Section 2.13.1.

(b) ***Ethnicity***

Osteoporosis is more common in Caucasians and Asians when compared to Blacks (International Osteoporosis Foundation, 2015). Blacks have higher bone density and greater bone strength throughout their lives when compared to Caucasians (Gilsanz , Roe , Mora , Costin , & Goodman 1991). Besides, Caucasians and Asians tend to have lower average bone mass and smaller bones which may lead to an increased risk of developing osteoporosis (Cong & Walker, 2014). Additionally, Asians consume less dairy products, and more prone to lactose intolerance (Tung, 2012). In Malaysia, the prevalence of osteopenia or osteoporosis at the hip were higher in Chinese (62%), followed by Malay (26%) and Indian(10%) (Chan et al., 2014).

(c) ***Gender***

Women are more susceptible to osteoporosis compared to men especially after menopause as described in section 2.13.1 (Manolagas, O'Brien, & Almeida, 2013). Oestrogen regulates bone formation (Manolagas et al., 2013). In addition, women have smaller body frames which leads to greater risk of developing osteoporosis as they have less bone mass to lose when compared to men (Seeman, 2001).

(d) ***Premature menopause or hysterectomy***

Early menopause or hysterectomy with oophorectomy (removal of uterus and both ovaries) in women before the age of 45 years old may increase their risk of developing osteoporosis (Melton et al., 2007; Tuppurainen, Kroger, Saarikoski, Honkanen, & Alhava, 1995). Decrease oestrogen levels at an earlier age speeds up bone loss in a similar way which occurs in postmenopausal women as described in section 2.13.1 (Melton et al., 2007; Tuppurainen et al., 1995)

(e) ***Family history of osteoporotic hip fracture in a first degree relative***

Family history of an osteoporotic hip fracture in a first degree relative is associated with an increased risk of osteoporosis (Kanis et al., 2004; Keen, Hart, Arden, Doyle, & Spector, 1999). Osteoporosis is a genetic disease, and evidence suggests that osteoporosis can be hereditary (Fox, Cummings, Powell-Threets, & Stone, 1998).

(f) ***Personal history of fracture as an adult***

History of a previous fracture in adult (aged ≥45 years old) is associated with an increased risk of osteoporosis, as individuals may have lower BMD when compared to individuals without a history of fracture in adulthood (Gehlbach et al., 2012).

2.17.1.2 Modifiable risk factors

(a) ***Sedentary lifestyle***

Sedentary lifestyle is associated with an increased risk of developing osteoporosis. Physical inactivity causes reduced gravitational loading and muscle contraction forces on the skeleton, which leads to increased bone loss (Booth, Roberts, & Laye, 2012).

(b) ***Smoking***

Cigarette smoking is associated with an increased risk of developing osteoporosis (Kanis, Johnell, et al., 2005). This may be due to the induced alteration of bone metabolism caused by cigarette smoking (Yoon, Maalouf, & Sakhaee, 2012).

(c) ***Alcohol intake***

Excessive alcohol intake (more than 3 units daily) is associated with an increased risk of developing osteoporosis. It can lead to a transient parathyroid hormone (PTH) deficiency and increased urinary calcium excretion, which results in calcium loss from the body that causes bone loss (Epstein, 1997; Kanis, Johansson, et al., 2005).

(d) ***Excessive caffeine intake***

Excessive caffeine intake (more than 3 cups per day) can lead to a slight decrease of intestinal calcium absorption and an increase in urinary calcium excretion which could lead to bone loss (Li, X. L. & Xu, 2013).

(e) ***Low body weight***

Low body weight is associated with low BMD (Pruzansky, Turano, Luckey, & Senie, 1989), as individuals with body mass index (BMI) ($\leq$19 kg/m^2) tend to have smaller bones and lower bone mass (US Department of Health Human Services, 2004).

(f) ***Oestrogen deficiency***

Oestrogen deficiency in younger women is associated with accelerated bone loss and increased risk of osteoporosis (Weitzmann & Pacifici, 2006). The mechanism of bone loss in these women are similar and has been described in section 2.16.1.1 (d)

(g) ***Frequent falls***

Frequent falls is associated with increased risk of osteoporotic fracture (Ministry of Health, 2012). Ninety percent of hip fracture in elderly are resulted from falls (Woolf & Akesson, 2003).

(h) ***Eating disorders***

Individuals with eating disorders (such as anorexia nervosa and bulimia) have been associated with an increased risk of developing osteoporosis. Bone loss in these individuals are often due to hormone imbalance in response to low body weight, malnutrition and reduced muscle mass (International Osteoporosis Foundation, 2015).

(i) ***Calcium intake***

Adequate calcium intake is important to maintain bone health (Levenson & Bockman, 1994). The recommended daily calcium intake for adults is 800mg to 1000mg, which can be achieved through consumption of food high in calcium (such as dairy products) and supplements [Table 2.17] (Ministry of Health, 2012; National Coordinating Commitee on Food and Nutrition, 2005; Sunyecz, 2008). Examples of food rich in calcium are shown in Table 2.18 (National Coordinating Commitee on Food and Nutrition, 2005).

Table 2:17: Recommended daily calcium intake

	Age	Recommended intake
Men	19-49	800mg
	≥ 50 years	1000mg
Women	19-49	800mg
	≥ 50 years	1000mg

Source: *Recommended Nutrient Intakes for Malaysia: A Report of the Technical Working Group on Nutritional Guidelines*. Putrajaya, Malaysia (National Coordinating Commitee on Food and Nutrition, 2005)

Table 2:18: Calcium content of food high in calcium

Food	Calcium content (mg)
1 glass of high calcium milk (200 ml)	500
1 glass of skimmed milk (200 ml)	250
1 glass of full cream milk (200 ml)	220
1 cup of yoghurt (150 g)	200
1 piece of tofu (150 g)	200
1/2 cup of yellow dhal (100 g)	170
1 cup of spinach (56 g)	160
1 cup of ice-cream (156 g)	150
1 cup of watercress (sai-yong choy) (50 g)	100
1 piece of cheddar cheese (20 g)	100
1 cup of mussels (160 g)	100
1/2 cup of ikan bilis (dried without head & entrails) (20 g)	100
1 piece of canned sardine (40 g)	100
1 cup of baked beans (240 g)	100
1 cup of mustard green (sawi), cekur manis, kai lan or pucuk ubi kayu (50 - 80 g)	100
1 piece of tempeh (70 g)	50
1 cup of soyabean milk (200 ml)	40
1 cup of broccoli (95 g)	40
10 almonds (15 g)	30

* 1 cup = 200 ml; ml=millilitre; g=gram; Source: *Recommended Nutrient Intakes for Malaysia: A Report of the Technical Working Group on Nutritional Guidelines*. Putrajaya, Malaysia (National Coordinating Commitee on Food and Nutrition, 2005)

However, if dietary calcium intake is insufficient, calcium supplementation may be needed. The absorption of calcium supplements is variable ranging from 20-40% depending on formulation [Table 2.19] (Levenson & Bockman, 1994).

Table 2:19: Calcium content in different salts preparations

Type	Elemental calcium (%)
Calcium carbonate	40
Calcium citrate	21
Calcium lactate	13
Calcium gluconate	9
Milk (non calcium enriched)	33

Source: A review of calcium preparations. *Nutr Rev, 52*(7), 221-232. (Levenson & Bockman, 1994)

(j) Vitamin D level

Sufficient vitamin D intake is vital to sustain bone health, improve muscle strength, balance and reduce the risk of falling (Bischoff-Ferrari et al., 2004; Sunyecz, 2008). Vitamin D is important for the regulation of calcium and phosphorus balance (Eisman & Bouillon, 2014), and facilitates calcium absorption in the intestine (Christakos, Dhawan, Porta, Mady, & Seth, 2011).

A) Definitions of vitamin D deficiency

To date, there is no consensus on the "goal standard" to define vitamin D deficiency and insufficiency. Therefore, there are several definitions of vitamin D deficiency and insufficiency which are categorized based on the Institute of Medicine (IOM) and other experts like the Endocrine Society and the International Osteoporosis Foundation (IOF) cut-off points [Table 2.20] (Holick et al., 2011; Institute of Medicine, 2011; International Osteoporosis Foundation, 2015).

Table 2:20: Definitions of vitamin D deficiency and insufficiency

Classification of vitamin D level	IOM	Endocrine Society/IOF
Normal	>50nmol/L	>75nmol/L
Insufficiency	30-50nmol/L	50-75nmol/L
Deficiency	<30nmol/L	<50nnmol/L

IOM=Institute of Medicine; IOF=International Osteoporosis Foundation; nmol/L=nanomole per liter:
Source: Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab, 96*(7), 1911-1930. (Holick et al., 2011), The National Academies Collection: Reports funded by National Institutes of Health. In Ross, A. C., Taylor, C. L., Yaktine, A. L., & Del Valle, H. B. (Eds.), *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US) (Institute of Medicine, 2011), (International Osteoporosis Foundation, 2015)

According to IOM, vitamin D level > 50 nmol/L covers the requirement of at least 97.5% of the population (Institute of Medicine, 2011). Individuals who have vitamin D level <50nmol/L were associated with suppression of parathyroid hormone which can lead to secondary osteoporosis (Fahrleitner et al., 2002; Kuchuk et al., 2009). However, according to IOF, vitamin D level >75nmol/L is necessary to minimize the risk of falls and fracture in elderly (Dawson-Hughes et al., 2010).

B) Risk factors of vitamin D deficiency

Risk factors of vitamin D deficiency in HIV-infected individual can be classified into traditional osteoporosis risk factors or HIV-related risk factors.

1) Traditional risk factors of vitamin D deficiency

The traditional risk factors of vitamin D deficiency can be further categorized as non-modifiable or modifiable risk factors (Lake & Adams, 2011).

a. Non-modifiable traditional risk factors

Non-modifiable traditional risk factors of vitamin D deficiency include age, higher latitudes, winter season, darker skin pigmentation, malabsorption, medication use, liver and renal disease.

i. Age

Older individuals are more likely to develop vitamin D deficiency due to decrease dietary intake, ageing (skin of an older person is less efficient in converting sunlight to vitamin D3), impaired intestinal absorption and impaired hydroxylation in the liver and kidneys (Gloth & Tobin, 1995; Janssen, Samson, & Verhaar, 2002).

ii. Higher latitudes

Individuals living in higher latitudes have a higher risk of developing vitamin D deficiency, due to insufficient sunlight exposure that help stimulates the production of vitamin D in skin (Tangpricha, 2007; Wacker & Holick, 2013).

iii. Winter season

Individuals have higher risk of developing vitamin D deficiency during winter season when compared summer season due to lack of sunlight (Nair, R. & Maseeh, 2012; Wacker & Holick, 2013).

iv. Darker skin pigmentation

Individuals who have darker skin pigmentation to experience slower vitamin D synthesis due to a higher melanin content in their skin (Nair, R. & Maseeh, 2012).

v. Malabsorption

Individuals who have gastrointestinal absorption disorders are more likely to have vitamin D deficiency as they are unable to absorb sufficient fat-soluble vitamin D from food in the intestine (Nair, R. & Maseeh, 2012).

vi. Liver and renal disease

Individuals who have liver and renal disease have a higher risk of developing vitamin D deficiency due to impaired vitamin D hydroxylation (Nair, S., 2010).

vii. Medication use

Patients who use antiepileptic drugs (e.g. phenobarbital, phenytoin, carbamazepine) are associated with vitamin D deficiency. Antiepileptic drugs induce vitamin D catabolism through hepatic induction of the cytochrome P450 enzyme (Nair, S., 2010).

b. Modifiable traditional risk factors of vitamin D deficiency

Modifiable traditional risk factors of vitamin D deficiency include obesity, lack of sunlight exposure and lack of dietary intake.

i. Obesity

Individuals who had greater BMI (>30 kg/m^2) are more prone to developing vitamin D deficiency. This may be because obese individuals have a greater amount of subcutaneous fat that may trap vitamin D and interrupt its release into circulation (Nair, R. & Maseeh, 2012).

ii. Lack of sunlight exposure

Individuals who avoid sunlight exposure for cultural of religious reasons are more likely to have vitamin D deficiency. Examples of cultural perception are preference for fairer skin as a sign of beauty (Jang et al., 2013; Li, E. P. H., Min, & Belk, 2008), having clothing styles that covered most parts of their body, using umbrellas and use sunblock and limit outdoor activities (Nimitphong & Holick, 2013)., Female Muslims are encourage by their religion to wear garments that cover the head, arms, body and legs (Shafinaz & Moy, 2016) and this decreases skin exposure to sunlight.

iii. Lack of dietary intake

Individuals who drink less milk and not taking vitamin D supplements have higher risk of getting vitamin D deficiency (Nair, R. & Maseeh, 2012).

2) HIV-related risk factors of vitamin D deficiency

HIV-related risk factors of vitamin D deficiency include HIV infection and ART use.

a) Human immunodeficiency virus infection

Chronic inflammation due to HIV infection may cause renal 1α-hydroxylase impairment which subsequently reduces the parathyroid hormone that helps to stimulate the production of vitamin D (Mansueto et al., 2015).

b) Antiretroviral therapy use

Both NNRTIs and PIs are associated with vitamin D deficiency in HIV-infected individuals by accelerating the hydroxylation of vitamin D (Cozzolino et al., 2003; Ellfolk, Norlin, Gyllensten, & Wikvall, 2009).

NNRTIs especially efavirenz have been found to increase vitamin D catabolism and production of inactive metabolites through interaction with cytochrome P450 enzymes (Brown & McComsey, 2009; Hariparsad et al., 2004).

NtRTI like tenofovir was found to promote renal phosphate wasting and reduce phosphate intestinal absorption, elevate parathyroid hormone, decrease function of 1α-hydroxylase related to renal toxicity (Gutierrez & Masia, 2011).

PIs such as darunavir and ritonavir may inhibit the 24-hydroxylases, which reduces the conversion of vitamin D to its active metabolite (Cozzolino et al., 2003). PIs such as ritonavir, indinavir and nelfinavir may inhibit 25-hydroxylase and 1α- hydroxylase enzymes (Gutierrez & Masia, 2011).

C) Vitamin D level and supplementation therapy

Individuals can obtain sufficient amount of vitamin D from exposure of skin to sunlight [ultraviolet B (UVB) rays] for 10-15 minutes per day, or from diet (International Osteoporosis Foundation, 2015; Nair, R. & Maseeh, 2012). The recommended daily intake for vitamin D is at least 800IU to 1000IU per day (Dawson-Hughes et al., 2005).

Vitamin D supplementation is recommended for individuals with vitamin D deficiency (<50nmol/L) [Table 2.21] (Ross et al., 2011). The optimal vitamin D level should 75 nmol/L to achieve optimal musculoskeletal health (Brown, Hoy, et al., 2015).

Table 2:21: Vitamin D level and supplementation therapy

Vitamin D level	Classification	Supplementation therapy
>75 nmol/L	Normal	1000IU/day vitamin D3 (cholecalciferol)
50-75 nmol/L	Insufficiency	2000 IU/day vitamin D3
<50 nmol/L	Deficiency	Vitamin D2 (ergocalciferol) or D3 50000 IU/week × 8 weeks (or equivalent of 6000 IU/day vitamin D3) Maintenance: vitamin D3 2000 IU/day

nmol/L=nanomole per liter; IU=international unit; Source: Bone Loss in the HIV-Infected Patient: Evidence, Clinical Implications, and Treatment Strategies. *The Journal of Infectious Diseases, 205*(Suppl 3), S391-S398 (Walker Harris & Brown, 2012)

Vitamin D requires hydroxylation by kidneys to its active form (Del Valle, Yaktine, Taylor, & Ross, 2011). Therefore, activated Vitamin D such as calcitriol (0.25 µg twice a day) or alfacalcidol (1µg daily) should be given to individuals with severe renal impairment who required vitamin D therapy (Fong & Khan, 2012).

2.17.2 Human immunodeficiency virus-related osteoporosis risk factors

HIV-related osteoporosis risk factors include the HIV infection itself, duration of HIV infection, use of ART including types and duration, CD4+ cell count and HIV viral load, hepatitis C co-infection and lipodystrophy (Cortés, Yin, & Reame, 2015; Mallon, 2010).

2.17.2.1 Human immunodeficiency virus infection

The pathogenesis of bone loss in HIV-infected individual is a complex and multifactorial process (Qaqish & Sims, 2004; Saccomanno & Ammassari, 2011). A detailed explanation on how HIV infection affects bone loss has been described in Section 2.14.

2.17.2.2 Duration of human immunodeficiency virus infection

The relationship of bone loss and duration of HIV infection remains unclear. A longer duration of HIV infection (>7 years) was associated with osteopenia/osteoporosis (Arnsten et al., 2007; Carr, Miller, Eisman, & Cooper, 2001; Mondy et al., 2003) and a higher risk of sustaining a fracture (Arnsten et al., 2007).

2.17.2.3 Use of antiretroviral therapy

The use of ART has been associated with reduced BMD among HIV infected individuals. Previous studies reported prevalence rates of reduced BMD which ranged from 18% to 83.7% (Aydın, Karaosmanoglu, Karahasanoglu, Tahmaz, & Nazlıcan, 2013; Carr et al., 2001; Garcia Aparicio et al., 2006; Libois et al., 2010; Madeddu et al., 2004; Tomazic et al., 2007). A meta-analysis conducted in 2006 reported that the odds of developing reduced BMD in ART-treated individuals was 2.5 times higher than in non ART-treated individuals (Brown & Qaqish, 2006).

(a) ***Types and duration of antiretroviral therapy used***

The mechanism of action of ART-related to bone loss remains controversial (Brown & Qaqish, 2006; Carr et al., 2001; Rivas et al., 2008). The possible mechanisms on the effects of ART associated with bone loss in HIV-infected individuals includes bone remodeling due to the direct action of drugs, an increase in phosphate levels due to renal loss, changes in vitamin D and parathyroid hormone (Gutierrez & Masia, 2011)

ART has been reported to affect osteoclast and osteoblast activity in vitro (Taylor & Rogers) and in animals (Pan et al., 2004). However, this finding could not be replicated in humans (Ofotokun & Weitzmann, 2011).

i. Types antiretroviral therapy used

NRTIs (like abacavir and zidovudine) have been reported to suppress osteoblast activity, (Taylor & Rogers) and promote osteoclastogenesis/osteoclast activity in animal studies (Pan et al., 2004). Tenofovir, a NtRTI has been reported to cause proximal renal tubular dysfunction that could result in excessive renal phosphate loss, which then impairs bone mineralization and increased bone turnover leading to bone loss (Fux, Christen, Zgraggen, Mohaupt, & Furrer, 2007; Walker Harris & Brown, 2012).

PIs (like ritonavir) have been reported to suppress osteoclastogenesis/osteoclast function in vitro and in vivo studies (Wang et al., 2004), whilst indinavir and nelfinavir have been reported to have no effects on osteoclastogenesis (Wang et al., 2004) and inhibits osteoblast function (Jain & Lenhard, 2002), respectively. Current evidence postulates that the cause of osteopenia/osteoporosis may be drug-specific, instead of class-specific (Libois et al., 2010; Mondy et al., 2003).

ii. Duration of antiretroviral therapy used

Some studies reported that the duration of tenofovir and PI used or HIV treatment was significantly associated with lower BMD (Jones et al., 2008) whilst other studies found that the duration of ART was not associated with lower BMD (Bolland et al., 2006; Libois et al., 2010). The difference in results could be due to different classes of ART used.

2.17.2.4 CD4+ cell count and HIV viral load

Low CD4+ cell count and high HIV viral load were associated with lower BMD (Dolan et al., 2004; Hansen, Obel, Nielsen, Pedersen, & Gerstoft, 2011; Libois et al., 2010). The underlying aetiology of low CD4+ cell count, HIV viral load and bone loss remains unclear. However, it has been suggested that the immune system may play a

potential role in skeletal maintenance (Grant et al., 2013).Evidence shows HIV-infected individuals with low baseline CD4+ cell count (<50 cells/μL) have approximately 3% greater bone loss than HIV-infected individuals with >500 cells/μL(Grant et al., 2013).

2.17.2.5 Hepatitis C co-infection

Hepatitis co-infection in HIV-infected individuals is associated with increased risk of developing osteopenia or osteoporosis (Dong, Cortes, Shiau, & Yin, 2014). Hepatitis C infection can lead to chronic liver dysfunction which mediates systematic immune activation (Carey, Balan, Kremers, & Hay, 2003; Neumann-Haefelin, Blum, Chisari, & Thimme, 2005; Pacifici, 2010). This activation has postulated to deteriorate bone health (Carey et al., 2003; Neumann-Haefelin et al., 2005; Pacifici, 2010).

2.17.2.6 Lipodystrophy

Lipodystrophy is associated with lower BMD (Bolland et al., 2006; Carr et al., 2001), fat mass is a significant predictor of BMD (Shapses & Sukumar, 2012).Changes of body composition from HIV infection and complications of ART mimic the normal ageing process of older individuals (Erlandson et al., 2013).

2.18 Meta-analysis on the prevalence of osteopenia/osteoporosis human immunodeficiency virus-infected individuals and its associating risk factors

A meta-analysis on the prevalence of osteopenia/osteoporosis (reduced BMD) in HIV-infected individuals was conducted in 2006 (Brown & Qaqish, 2006). Since then, an additional 13 cross-sectional and 6 longitudinal studies have been published from 2005 till 2015. This led us to perform a meta-analysis, to update findings from the previous meta-analysis (Brown & Qaqish, 2006).

2.18.1 Aim of the meta-analysis

This meta-analysis was conducted to combine data from published studies to quantify the prevalence of osteopenia or osteoporosis, percent change of BMD and its associating factors in HIV-infected, ART-treated, PI-treated and tenofovir-treated individuals

2.18.2 Study selection and search strategy

This meta-analysis was registered with PROSPERO (CRD: 42016047294) and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009) (Appendix A: PRISMA Check list). A search was conducted using six databases: MEDLINE, CINAHL, EMBASE, Science Direct, Cochrane, and Web of Science with all published studies from 1989 till May 2015. A combination of Medical Subject Heading (MESH) and free text terms were used to define the search ("HIV"[MeSH Terms] OR "HIV"[Text Word] OR "Human Immunodeficiency Virus"[Text Word]) AND ("Antiretroviral Therapy, Highly Active"[MeSH Terms] OR "HAART therapy"[Text Word] OR "HIV Protease Inhibitors"[Pharmacological Action] OR 'tenofovir') AND ("Osteoporosis"[MeSH Terms] OR "Osteoporosis"[Text Word] OR "Bone Diseases, Metabolic"[MeSH Terms] OR "Bone Density"[MeSH Terms] OR "Bone Density"[Text Word] OR "osteopenia*"[Text Word]) AND (epidemiologic studies"[MeSH Terms] OR "epidemiology"[MeSH Terms] OR "epidemiology"[Text Word]) OR "prevalence"[MeSH Terms] OR "prevalence"[Text Word] OR "incidence"[MeSH Terms] OR "incidence"[Text Word]). The results of the above search strategies were combined to yield a pool of preliminary studies. Reference mining and related citations of potential references were also examined. Duplicated studies with the same title and author were excluded.

2.18.3 Inclusion and exclusion criteria

Cross sectional or longitudinal studies published in English, original research articles that used dual-energy X-ray absorptiometry (DXA) to measure BMD on the lumbar spine, femoral neck or total hip, and compared at least two groups (e.g. HIV-infected versus HIV-uninfected, ART-treated versus non ART-treated, PI-treated versus non PI-treated, tenofovir-treated versus non tenofovir-treated individuals), aged ≥18 years old, and used a validated conversion equation if BMD were measured using different DXA machines, were included. Longitudinal studies were only included if change in BMD were reported >12 months from baseline as change in BMD has to be more than the least significant change of the DXA machine this can only occur 1-2 years from the previous DXA scan)(Foundation, 2017). Studies were excluded if the outcomes of interest (BMD or T-score) were not reported, and if the study was only published as editorials, commentaries, brief reports, expert opinions, case studies, theses, conference proceedings, newspapers, fact sheets, websites or policy documents. Articles that studied HIV and chronic viral hepatitis (Hepatitis B or Hepatitis C co-infection) were also excluded, as a review was recently published in 2014 (Dong et al., 2014).

2.18.4 Quality assessment and data extraction

The quality of each study was assessed independently by two teams of researchers using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies 2014, developed by the National Institutes of Health, United States [Appendix B] (National Institutes of Health, 2014). Data were extracted, and recorded in a standardized extraction form (Appendix C). When disagreements occurred between the two teams; each team consisted of a pair of researchers, both teams met and referred back to the original text to clarify the issue that was raised. Once the evidence was highlighted, the two teams then reached a consensus via discussion.

If authors only reported osteopenia/osteoporosis as continuous variables, they were contacted and asked to provide information on the proportion of participants with osteopenia/osteoporosis. Studies were excluded if authors did not provide the required data. The prevalence of osteopenia/osteoporosis included information from cross sectional and longitudinal studies at baseline. In longitudinal studies, the percent change of BMD from baseline to follow-up were examined.

2.18.5 Outcome and analysis

A fixed effects model was implemented to examine the heterogeneity between studies. Funnel plots [analysed using Review Manager v5 (Copenhagen, Denmark)], Begg's and Egger's test [analysed using Comprehensive Meta-Analysis v3 (New Jersey, United States of America)] were used to investigate for potential publication bias. Funnel plots that were found to be symmetrical or Begg's/Egger's test that had a p-value>0.05 indicated that publication bias was unlikely. However, Begg's and Egger test were found to be not so sensitive to detect bias if there were <25 studies (Sterne, Gavaghan, & Egger, 2000).

The quality of each study was assessed using the United States Preventative Services Task Force (USPSTF) guideline. A "good" study meets all criteria for that study design; a "fair" study does not meet all criteria but is judged to have no fatal flaw that invalidates its results; and a "poor" study contains a fatal flaw (Harris et al., 2001). Strength of evidence regarding the association between risk factors and BMD was analysed according to Table 2.22.

Table 2:22: Grading the association between risk factors and bone mineral density

Grade	Definition
Good	There is good evidence for or against an association between the risk factor and BMD *Determined by*: consistent results across studies; > three studies; at least one study graded as 'good' quality
Fair	There is fair evidence for or against an association between the risk factor and BMD *Determined by:* consistent results across studies but limited by quantity (≤ three studies) or quality (no studies graded as 'good')
Inconsistent	There is inconsistent evidence for or against an association between the risk factor and BMD *Determined by*: studies had conflicting results
Insufficient	There is insufficient evidence for or against an association between the risk factor and BMD *Determined by*: inadequate number of studies evaluating the risk factor (< three studies)

Source: Bone mineral density changes in protease inhibitor-sparing vs. nucleoside reverse transcriptase inhibitor-sparing highly active antiretroviral therapy: Data from a randomized trial. *HIV Medicine, 12*(3), 157-165 (Harris et al., 2001)

2.18.6 Findings

A total of 21 cross sectional and 8 longitudinal studies met our inclusion criteria (Figure 2.5). Four authors from the cross sectional studies were contacted for additional information regarding the proportion of reduced BMD. Unfortunately, none of the authors responded. Hence, these studies were excluded. No publication bias was observed during analysis (Appendix D).

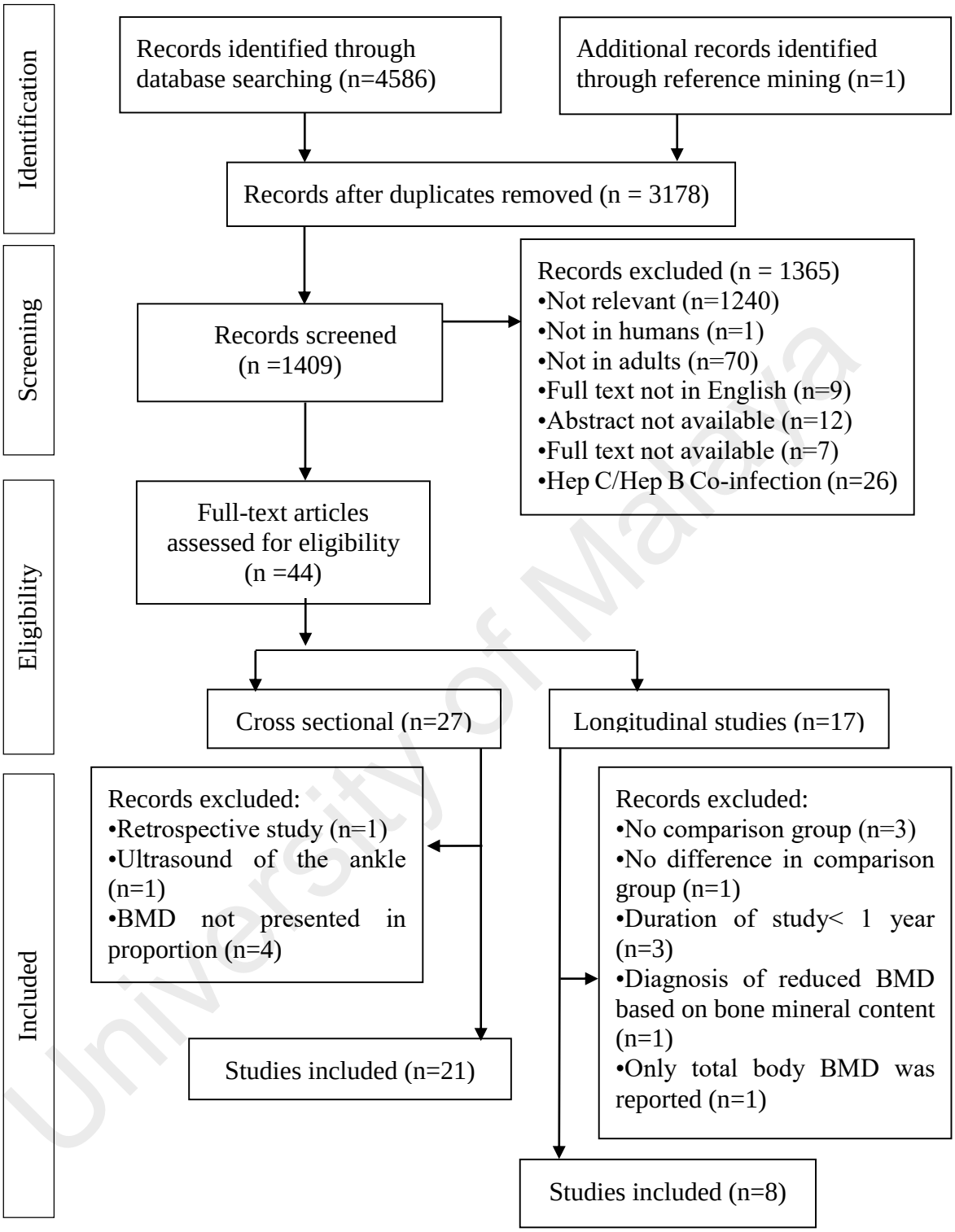


Figure 2:5: Flow chart of studies included in meta-analysis

2.18.6.1 Human immunodeficiency virus-infected versus uninfected individuals

Seventeen studies [cross sectional (n=14); longitudinal (n=3)] compared HIV-infected versus HIV-uninfected individuals [Tables 2.23 and 2.24] (Amiel et al., 2004; Arnsten et al., 2007; Arnsten et al., 2006; Bolland et al., 2012; Bolland et al., 2006; Brown et al., 2004; Bruera, Luna, David, Bergoglio, & Zamudio, 2003; Dolan et al., 2004; Dolan, Kanter, & Grinspoon, 2006; Grijsen et al., 2013; Jones et al., 2008; Loiseau-Pérès et al., 2002; Madeddu et al., 2004; Negredo et al., 2014; Tebas et al., 2000; Teichmann et al., 2009; Teichmann et al., 2003). Seven studies only included men (Amiel et al., 2004; Arnsten et al., 2007; Bolland et al., 2012; Bolland et al., 2006; Grijsen et al., 2013; Tebas et al., 2000; Teichmann et al., 2009), four studies only included women (Arnsten et al., 2006; Dolan et al., 2004; Dolan et al., 2006; Teichmann et al., 2003), whilst six studies included both men and women (Brown et al., 2004; Bruera et al., 2003; Jones et al., 2008; Loiseau-Pérès et al., 2002; Madeddu et al., 2004; Negredo et al., 2014). The majority of participants were male (range: 30%-86%) (Brown et al., 2004; Jones et al., 2008). All studies were matched for gender except for three studies (Jones et al., 2008; Madeddu et al., 2004; Negredo et al., 2014). Age and BMI were well matched between HIV-infected versus HIV-uninfected individuals. Three studies recruited only Caucasians (Amiel et al., 2004; Bolland et al., 2006; Loiseau-Pérès et al., 2002), nine studies recruited participants of mixed ethnicity (Arnsten et al., 2007; Arnsten et al., 2006; Bolland et al., 2012; Brown et al., 2004; Dolan et al., 2004; Dolan et al., 2006; Grijsen et al., 2013; Jones et al., 2008; Negredo et al., 2014), whilst five studies did not specify the ethnicity group (Bruera et al., 2003; Madeddu et al., 2004; Tebas et al., 2000; Teichmann et al., 2009; Teichmann et al., 2003).

Table 2:23: Baseline characteristics of cross sectional studies (n=14) and longitudinal study (n=1) and proportion of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD, n (%)
In HIV-infected individuals and HIV-uninfected individuals													
(Amiel et al., 2004)	Single site, France / cross sectional, prospective	Lunar DPX-L	•Lumbar Spine (L1-L4) •Femoral neck	HIV+	148	100	40±8	Caucasian(100)	23±3	NR	NR	Osteopenia: 98(66%) Osteoporosis 24(16%)	122(82)
				HIV-	81	100	39±10	Caucasian(100)	24±3	NA	NA	Osteopenia: 26(32%) Osteoporosis: 3(4%)	29(36)
(Arnsten et al., 2006)	Multiple sites, USA /cross sectional, prospective	Lunar Prodigy	•Lumbar Spine (L2-L4) •Femoral neck	HIV+	263	0	44±5	Black (59) Caucasian (6) Hispanic (34) Others (1)	NR	NR	NR	Osteopenia + osteoporosis: 71(27%)	71(27)
				HIV-	232	0	45±5	Black (44) Caucasian (12) Hispanic (42) Others (3)	NR	NA	NA	Osteopenia + osteoporosis: 44(19%)	44(19)
(Arnsten et al., 2007)	Single site, USA/ cross sectional, prospective	Lunar Prodigy	•Lumbar Spine •Femoral neck	HIV+	328	100	54.7±5	Black (61) Caucasian (12) Hispanic (23)	NR	NR	NR	Osteopenia + osteoporosis: 180(55%)	180(55)
				HIV-	231	100	55.8±5	Black (50) Caucasian (19) Hispanic (28)	NR	NA	NA	Osteopenia + osteoporosis: 118(51%)	118(51)

Table 2.23: Baseline characteristics of cross sectional studies (n=14) and longitudinal study (n=1) and proportion of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD, n (%)
In HIV-infected individuals and HIV-uninfected individuals *(continued)*													
(Bolland et al., 2006)	Single site, New Zealand/ cross sectional, prospective	Lunar Expert, Lunar Prodigy	•Lumbar Spine •Femoral neck •Total body	HIV+	59	100	50.1±8.3	Caucasian (100)	24.7±3.0	8.5±5.2	67.8±40.6	Osteopenia: 17(28.8%) Osteoporosis: 2(3.4%)	19(32.2)
				HIV-	118	100	49.8±8.7	Caucasian (100)	26.4±3.5	NA	NA	Osteopenia: 25(21.2%) Osteoporosis: 1(0.8%)	26(22)
(Brown et al., 2004)	Single site, USA/ cross sectional, prospective	Hologic-4500	•Lumbar Spine •Femoral neck •Total hip •Total forearm	HIV+	51	86	40.1±6.6	Caucasian (86) Others (14)	25.2±2.7	NR	NR	Osteopenia: 28(55%) Osteoporosis: 4(8%)	32(63)
				HIV-	22	82	39.2±6.5	Caucasian (82) Others (18)	25.7±4.0	NA	NA	Osteopenia: 7(32%) Osteoporosis: 0	7(32)
(Bruera et al., 2003)	Single site, Argentina / cross sectional, prospective	Hologic-4500w	•Lumbar Spine (L1-L4) •Femoral neck •Total body	HIV+	111	80	NR	NR	NR	NR	NR	<u>Lumbar spine data:</u> Osteopenia: 52(46.8%) Osteoporosis 8(7.2%)	<u>Lumbar spine data:</u> 60(54)
												<u>Hip data:</u> Osteopenia: 65(58.6) Osteoporosis: 15(13.5)	<u>Hip data:</u> 80(72.1)
				HIV-	31	77	31.4±6.2	NR	25.4±3.0	NA	NA	<u>Lumbar spine data:</u> Osteopenia: 11(36.52%) Osteoporosis: 2(5.22%)	<u>Lumbar spine data</u>: 13(42)
												<u>Hip data:</u> Osteopenia: 5(15.3%) Osteoporosis: 0	<u>Hip data:</u> 5(15.3)

Table 2.23: Baseline characteristics of cross sectional studies (n=14) and longitudinal study (n=1) and proportion of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD, n (%)
In HIV-infected individuals and HIV-uninfected individuals *(continued)*													
(Dolan et al., 2004)	Multiple site, USA/ cross sectional, prospective	Hologic 4500	•Lumbar Spine •Femoral neck •Total body •Total hip	HIV+	84	0	41±1	Asian(0) Black (36) Caucasian(40) Hispanic(14) Native American(2) Others(8)	26.0±0.6	Mean:8	NR	Osteopenia: 45(54%) Osteoporosis: 8(10%)	53(64)
				HIV-	63	0	41±1	Asian(5) Black (27) Caucasian(49) Hispanic(14) Native American(0) Others(5)	27.0±0.5	NA	NA	Osteopenia: 19(30%) Osteoporosis:3(5%)	22(35)
(Grijsen et al., 2013)	Single site, Netherlands /cross sectional, prospective	Hologic QDR 4500W	•Lumbar Spine (L1–L4) •Femoral neck • Total hip	HIV+	147	100	NR	Caucasian(80.8) Others (19.1)	NR	NR	NR	Osteopenia+ osteoporosis: 31(21.2%)	31(21.2)
				HIV-	30	100	38±6	Caucasian (80) Others (20)	24.4±4.8	NA	NA	Osteopenia+ osteoporosis: 4(13%)	4(13)

Table 2.23: Baseline characteristics of cross sectional studies (n=14) and longitudinal study (n=1) and proportion of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD, n (%)
In HIV-infected individuals and HIV-uninfected individuals *(continued)*													
(Jones et al., 2008)	Multiple site, USA / cross sectional, prospective	Hologic 4500	•Lumbar Spine (L1-L4) •Total hip	HIV+	57	60	61±5	Black (54) Caucasian(11) Hispanic(35)	26±5	Months median= 132 (IQR:72-156)	•tenofovir: Median= 15 (range: 0.5–42) •PI: Median= 22 (range: 1–84)	Lumbar spine data: Osteopenia: 22(39%) Osteoporosis: 16(28%)	Lumbar spine data: 38(67)
												Hip data: Osteopenia: 31(54%) Osteoporosis: 3(5%)	Hip data:33(59)
				HIV-	47	30	62±6	Black (34) Caucasian(30) Hispanic(36)	29±6	NA	NA	Lumbar spine data: Osteopenia: 12(26%) Osteoporosis: 6(13%)	Lumbar spine data: 18(39)
												Hip data: Osteopenia: 12(26%) Osteoporosis: 0	Hip data: 12(26)
(Loiseau-Pérès et al., 2002)	Single site, France/ cross sectional, prospective	Hologic QDR 4500 W	•Lumbar Spine •Total hip	HIV+	47	66	Males: 43.0± 11.2 Females: 38.8± 10.5	Caucasian (100)	NR	NR	NR	Osteopenia: 28(59.6%) Osteoporosis: 4(8.5%)	32(68.1)
				HIV-	47	66	NR	Caucasian (100)	NR	NA	NA	Osteopenia: 15(31.9%) Osteoporosis: 1(2.1%)	16(34)

Table 2.23: Baseline characteristics of cross sectional studies (n=14) and longitudinal study (n=1) and proportion of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In HIV-infected individuals and HIV-uninfected individuals *(continued)*													
(Madeddu et al., 2004)	Single site, Italy/ longitudinal, prospective	Hologic QDR 4500A	•Lumbar Spine (L1-L4) •Femoral neck	HIV+	172	NR	NR	NR	NR	NR	NR	Osteopenia: 63(36.6%) Osteoporosis: 25(14.5%)	88(51.2)
				HIV-	64	60.9	NR	NR	NR	N/A	N/A	Osteopenia: 5(7.8%)	5(7.8)
(Negredo et al., 2014)	Multiple site, Spain / cross sectional, prospective	Lunar DPX PRO, Lunar Prodigy, Hologic QDR-4500A*	•Lumbar Spine (L1-L4) •Total femur	HIV+	232	78.9	Median: 28 (IQR:26–29)	Black(1.3) Caucasian(90.5) Hispanic (8.2)	NR	Median:2 (IQR:0–4)	Years Median:2 (IQR:0–5)	Osteopenia: 121 (56.5%) Osteoporosis: 23 (10.7%)	144(67.3)
				HIV-	75	73	Median: 26 (IQR:24–29)	NR	NR	NA	NA	Osteopenia: 38 (50.7%) Osteoporosis: 3(4%)	41(54.7)
(Tebas et al., 2000)	Single site, USA/ cross sectional, prospective	Hologic QDR-2000	•Lumbar Spine •Femoral neck •Total body	HIV+	95	100	NR	NR	NR	NR	NR	Osteopenia+ osteoporosis: 38(40%)	38(40)
				HIV-	17	100	33±9	NR	23±4	NA	NA	Osteopenia+ osteoporosis: 5(29%)	5(29)
(Teichmann et al., 2003)	Single site, Germany / cross sectional, prospective	Lunar Radiation	•Lumbar Spine (L1 to L4)	HIV+	50	0	37.4±7.1	NR	25.2±3.9	NR	NR	Osteopenia: 31(62%) Osteoporosis: 7(14%)	39(76)
				HIV-	50	0	35.1±3.6	NR	26.9±2.4	NA	NA	Osteopenia: 2(4%) Osteoporosis: 0	2(4)

Table 2.23: Baseline characteristics of cross sectional studies (n=14) and longitudinal study (n=1) and proportion of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In HIV-infected individuals and HIV-uninfected individuals *(continued)*													
(Teichmann et al., 2009)	Single site, Germany / cross sectional, prospective	Lunar Radiation	•Lumbar Spine (L1 to L4)	HIV+	80	100	NR	NR	NR	NR	NR	Osteopenia: 28(35%) Osteoporosis: 0	28(35)
				HIV-	20	100	35.4±4.1	NR	26.1±5.5	NA	NA	Osteopenia:0 Osteoporosis:0	0

*This study used three different DXA machines, but an equation was used to standardized values of all BMD results; HIV=human immunodeficiency virus; NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; IQR=interquartile range; USA=United States of America

Table 2:24: Baseline characteristics of longitudinal studies (n=2) and percent change in bone density from baseline to follow-up in human immunodeficiency virus-infected versus uninfected individuals

Reference	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age ±SD	Ethnicity (%)	Duration of study (months)	Loss of follow up (%)	Mean BMI±SD (kg/m^2)	Baseline (mean BMD±SD (g/cm^2)	Percent change in BMD (%)
In HIV-infected individuals and HIV-uninfected individuals													
(Bolland et al., 2012)	Single site, New Zealand/ longitudinal, prospective	GE Lunar Expert	•Lumbar Spine • Total hip •Total body	HIV+	44	100	48.7± 9.1	European (82) Others (18)	72 Interval of follow-up: 0, 24, 72	27	24.6 ±3.3	Lumbar spine: 1.2± 0.2 Total hip:1.1± 0.1	**At lumbar spine** Baseline→72 months HIV+ve=↑5.3% vs HIV-ve=↑ 0.3% **At total hip:** Baseline→72 months HIV+ve= ↓0.6% vs HIV-ve=↓1.0%
				HIV-	37	100	46.0± 10.5	European (88) Others (12)		11	25.5 ±3.5	Lumbar spine:1.3± 0.2 Total hip: 1.1± 0.1	
(Dolan et al., 2006)	Multiple sites, USA/ longitudinal, prospective	Hologic 4500	•Lumbar Spine •Femoral neck •Total hip	HIV+	100	0	41±1	Black (36) Hispanic (14) Asian(1) Native American (2) Other(7)	24 Interval of follow-up 0, 6, 12, 18, 24	75	26.1 ±0.5	Mean: Lumbar spine:-0.68 Total hip:-0.25	**At lumbar spine:** Baseline→6months HIV+ve=↑0.7% vs HIV-ve= ↓0.1% Baseline→12 months HIV+ve= ↑1.5% vs HIV-ve= ↑1.1% Baseline→ 18 months HIV+ve= N/A HIV-ve= ↓0.4 **At total hip:** Baseline→6months HIV+ve =↑0.5% vs HIV-ve= ↑0.2% Baseline→12 months HIV+ve= ↓0.1% vs HIV-ve= ↑1.2% Baseline→ 18 months HIV+ve= N/A vs HIV-ve= ↑1.0%
				HIV-	100	0	41±1	Black(45) Hispanic (13) Asian(4) Native American (0) Other(3)		75	27.2±0.4	Mean: Lumbar spine: -0.20 Total hip:-0.01	

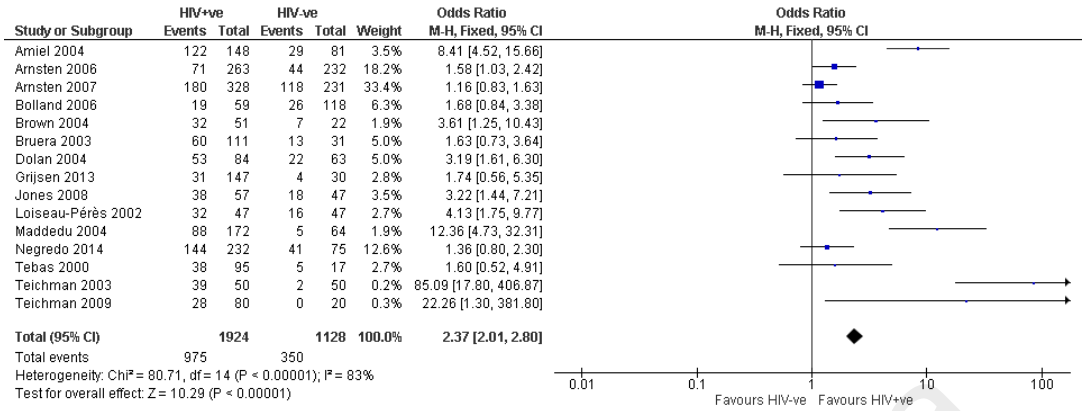
HIV=human immunodeficiency virus; NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; IQR=interquartile range

(a) ***Odds ratio of reduced bone mineral density in human immunodeficiency virus-infected versus uninfected individuals***

Fourteen cross sectional studies and one observational longitudinal study were included in this analysis [Table 2.23] (Amiel et al., 2004; Arnsten et al., 2007; Arnsten et al., 2006; Bolland et al., 2006; Brown et al., 2004; Bruera et al., 2003; Dolan et al., 2004; Grijsen et al., 2013; Jones et al., 2008; Loiseau-Pérès et al., 2002; Madeddu et al., 2004; Negredo et al., 2014; Tebas et al., 2000; Teichmann et al., 2009; Teichmann et al., 2003). Two longitudinal studies were excluded as the proportion of osteopenia/osteoporosis at baseline were not reported (Bolland et al., 2012; Dolan et al., 2006). The odds of developing osteopenia/osteoporosis at the lumbar spine and hip was OR=2.4 (95%Cl 2.0, 2.8), p<0.001 and OR=2.6 (95%Cl 2.2, 3.0), p<0.001, respectively (Figure 2.6). The overall assessment of heterogeneity between studies for osteopenia/osteoporosis at lumbar spine and hip was I^2=83% (Q=80.7, p<0.001), and I^2=85% (Q=92.7, p<0.001), respectively.

Our study found that the odds of developing osteopenia/osteoporosis in HIV-infected individuals was two times lower than a previous meta-analysis (Brown & Qaqish, 2006). This difference could be due to the additional seven cross sectional studies (Arnsten et al., 2007; Arnsten et al., 2006; Bolland et al., 2006; Grijsen et al., 2013; Jones et al., 2008; Negredo et al., 2014; Teichmann et al., 2009). Additionally, we excluded three studies as two studies were brief reports (Huang, Mulkern, & Grinspoon, 2002; Knobel, Guelar, Vallecillo, Nogues, & Diez, 2001), and the other included participants with hepatitis C co-infection (Yin, M. et al., 2005).

(a)



(b)

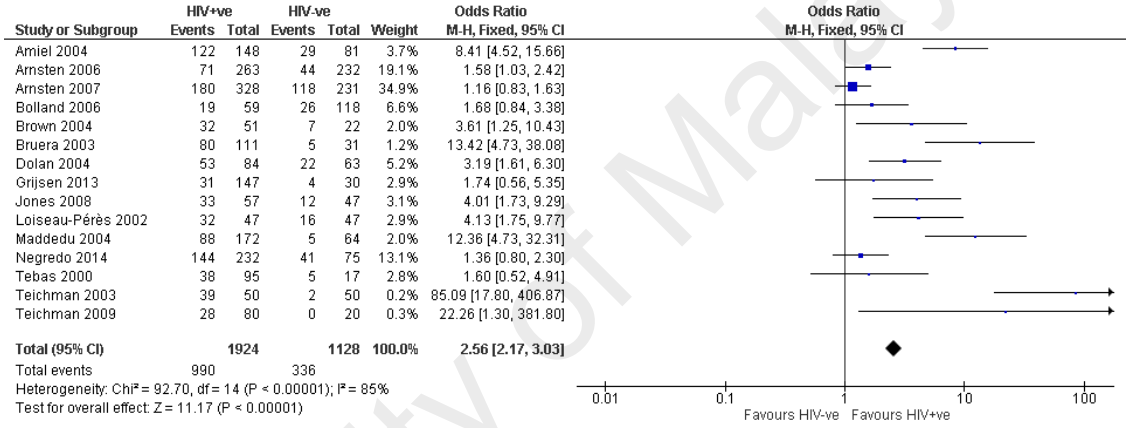


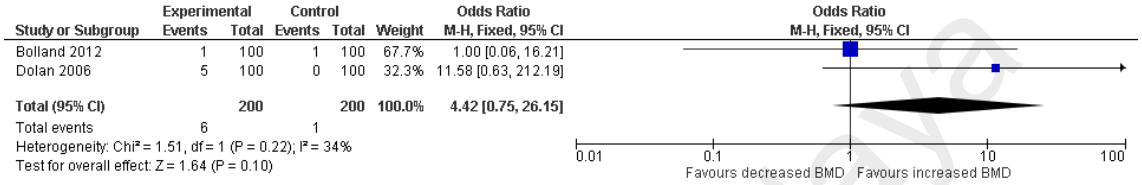
Figure 2:6: Odds ratio of reduced bone mineral density inhuman immunodeficiency virus-infected versus uninfected individuals at:(a) lumbar spine; (b) hip

(b) Percent change in bone mineral density from baseline to follow-up inhuman immunodeficiency virus-infected versus uninfected individuals

Two observational longitudinal studies were included in this analysis [Table 2.24] (Bolland et al., 2012; Dolan et al., 2006). The duration of follow-up ranged from 24-72 months (Bolland et al., 2012; Dolan et al., 2006). Duration of follow-up ranged from 24-72 months (Bolland et al., 2012; Dolan et al., 2006). Bone loss only occurred at the total hip from baseline to 12 months (Dolan et al., 2006) and 72 months (Bolland et al., 2012). However, when a meta-analysis was performed from baseline to 24-72 months, no significant difference was seen at the lumbar spine [OR=4.4 (95%Cl 0.8, 26.2), p=0.22]

and total hip [OR=0.6 (95%Cl 0.1,4.6), p=0.61] in HIV-infected and HIV-uninfected individuals (Figure2.7). The overall assessment of heterogeneity between studies for percent change in BMD analysis at lumbar spine and hip was I^2=34% (Q=1.5, P=0.16,) and I^2=0% (Q=0.3, P=0.62,), respectively.

(a)



(b)

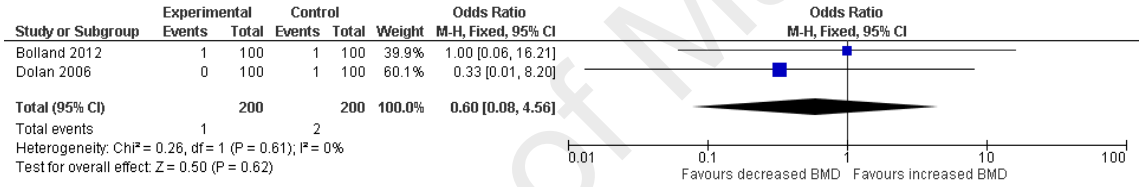


Figure 2:7: Percent change in bone mineral density from baseline in human immunodeficiency virus-infected versus uninfected individuals at: (a) lumbar spine; (b) total hip

2.18.6.2 Antiretroviral-treated versus non antiretroviral-treated individuals

Nine studies [cross sectional (n=8); longitudinal (n=1)] compared ART-treated versus non ART-treated individuals [Table 2.25] (Amiel et al., 2004; Aydın et al., 2013; Bruera et al., 2003; Carr et al., 2001; de Menezes Barbosa et al., 2013; Garcia Aparicio et al., 2006; Libois et al., 2010; Madeddu et al., 2004; Tomazic et al., 2007). Five studies only included men (Amiel et al., 2004; Carr et al., 2001; de Menezes Barbosa et al., 2013; Garcia Aparicio et al., 2006; Tomazic et al., 2007), one study only included women (Libois et al., 2010), whilst two studies included both men and women (Bruera et al., 2003; Madeddu et al., 2004). The majority of participants were male (range: 70%-91%). Two studies were not matched for gender (Bruera et al., 2003; Madeddu et al., 2004). One

study recruited only Caucasians (Amiel et al., 2004), whilst another study recruited both Caucasians and Blacks (Libois et al., 2010).

Table 2:25: Baseline characteristics of cross sectional studies (n=8) and longitudinal study (n=1) and proportion of reduced bone mineral density in antiretroviral-treated and non antiretroviral-treated individuals

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In antiretroviral-treated and antiretroviral-naive individuals													
(Amiel et al., 2004)	Single site, France / cross sectional, prospective	Lunar DPX-L	•Lumbar Spine (L1-L4) •Femoral neck	ART+	100	100	NR	Caucasian(100)	NR	NR	NR	Osteoporosis:18(18%)	18(18)
				ART-	48	100	36±7	Caucasian(100)	23±3	5±5	NA	Osteoporosis: 4(8.3%)	4(8.3)
(Aydın et al., 2013)	Single site, Turkey/ cross sectional, prospective	Norland	•Lumbar Spine •Femoral neck •Total body	ART+	80	NR	NR	NR	NR	NR	NR	Osteopenia+ osteoporosis: 67 (83.7%)	67 (83.7)
				ART-	46	NR	NR	NR	NR	NR	NA	Osteopenia+ osteoporosis: 31 (67.3%)	31(67.3)
(Bruera et al., 2003)	Single site, Argentina / cross sectional, prospective	Hologic-4500w	•Lumbar Spine (L1-L4) •Femoral neck •Total body	ART+	78	75.6	NR	NR	NR	NR	NR	<u>Lumbar spine data:</u> Osteopenia: 32(41%) Osteoporosis: 6(7.7%)	<u>Lumbar spine data:</u> 38(48.7)
												<u>Hip data:</u> Osteopenia:43(55.1%) Osteoporosis:14(17.9%)	<u>Hip data:</u> 57(73.0)
				ART-	33	91	31.1± 6	NR	23.8±3	4.1±3.4	NA	<u>Lumbar spine data:</u> Osteopenia: 20(60.87%) Osteoporosis: 2(4.35%)	<u>Lumbar spine data:</u> 22(66.7)
												<u>Hip data:</u> Osteopenia: 22(66.7%) Osteoporosis: 1(3.0%)	<u>Hip data:</u> 23(69.8)

Table 2.25: Baseline characteristics of cross sectional studies (n=8) and longitudinal study (n=1) and proportion of reduced bone mineral density in antiretroviral-treated and non antiretroviral naïve-treated individuals *(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In antiretroviral-treated and antiretroviral-naive individuals *(continued)*													
(Carr et al., 2001)	Single site, Australia/ cross sectional, prospective	Lunar DPXL	•Lumbar Spine •Total body	ART+	189	100	NR	NR	NR	NR	NR	Osteopenia+ osteoporosis: 47(24.9%)	47(24.9)
				ART-	32	100	NR	NR	NR	NR	NA	Osteopenia+ osteoporosis: 2(6%)	2(6)
(de Menezes Barbosa et al., 2013)	Multiple site, Brazil / cross sectional, prospective	Hologic QDR 4500A	•Lumbar Spine •Femoral neck •Total body	ART+	40	100	NR	NR	NR	NR	NR	Lumbar spine data: Osteoporosis: 1(2.5%) Osteopenia 20(50%)	Lumbar spine data: 21(52.5)
												Hip data: Osteopenia: 13(32.5%) Osteoporosis:1(2.5%)	Hip data: 14(35)
				ART-	10	100	30.8±9.4	NR	20.4±6.7	Months 34.8±20.7	NA	Lumbar spine data: Osteoporosis: 1(10%) Osteopenia: 3(30%)	Lumbar spine data: 4(40)
												Hip data: Osteopenia: 2(20) Osteoporosis:0	Hip data: 2(20)
(Garcia Aparicio et al., 2006)	Single site, Spain/cross sectional, prospective	Lunar	•Lumbar Spine •Femoral neck •Total hip	ART+	17	100	41±8.6	NR	22.7 ±2	8.15±4.0	NR	Osteopenia: 9(53%) Osteoporosis: 2(11.8%)	11(64.8)
				ART-	13	100	35±4.4	NR	24.1 ±3.1	9.3±4.6	NA	Osteopenia: 8(61.5%) Osteoporosis: 1(7.7%)	9(69.2)

Table 2.25: Baseline characteristics of cross sectional studies (n=8) and longitudinal study (n=1) and proportion of reduced bone mineral density in antiretroviral-treated and non antiretroviral-treated individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In antiretroviral-treated and antiretroviral-naive individuals *(continued)*													
(Libois et al., 2010)	Single site, Belgium/ cross sectional, prospective	Hologic QDR 4000	•Lumbar Spine •Femoral neck •Total hip	ART+	52	0	NR	Black (75.1) Caucasian(24.9)	NR	NR	Years: Median:3.5	Osteopenia+ osteoporosis: 19(36.5%)	19(36.5)
				ART-	37	0	Median: 36.5	Black (94.5) Caucasian(5.5)	Median: 25.7	Median: 2.04	NA	Osteopenia+ osteoporosis: 9(24.3%)	9(24.3)
(Madeddu et al., 2004)	Single site, Italy/ longitudinal, prospective	Hologic QDR 4500A	•Lumbar Spine (L1-L4) •Femoral neck	ART+	152	NR	NR	NR	NR	NR	NR	Osteopenia: 57(37.5%) Osteoporosis: 25(16.4%)	82(53.9)
				ART-	20	70	38±7	NR	21.7±3.2	5±6	N/A	Osteopenia: 6(30%)	6(30)
(Tomazic et al., 2007)	Single site, Slovenia/ cross sectional, prospective	Hologic Discovery -W SIN 70659	•Lumbar Spine (L1-L4) •Femoral neck •Total hip	ART+	72	100	NR	NR	NR	NR	NR	Osteopenia: 32(44.4%) Osteoporosis: 9(12.5%)	41(56.9)
				ART-	24	100	39.6± 10.3	NR	24.3±2.6	3.7±4.7	NA	Osteopenia: 13(54%) Osteoporosis: 3(12%)	16(66)

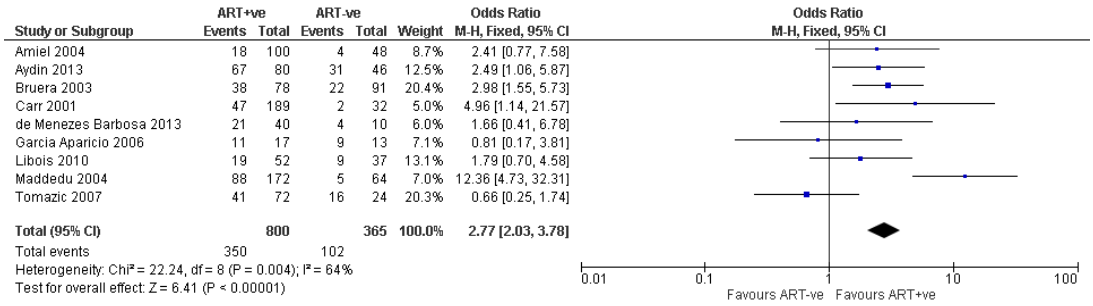
HIV=human immunodeficiency virus; NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; ART=antiretroviral therapy; IQR=interquartile range

(a) ***Odds ratio of reduced bone mineral in antiretroviral-treated versus non antiretroviral-treated individuals***

Eight cross-sectional studies and one observational longitudinal study were included in this analysis [Table 2.25] (Amiel et al., 2004; Aydın et al., 2013; Bruera et al., 2003; Carr et al., 2001; de Menezes Barbosa et al., 2013; Garcia Aparicio et al., 2006; Libois et al., 2010; Madeddu et al., 2004; Tomazic et al., 2007). The odds of developing osteopenia/osteoporosis at the lumbar spine and hip was OR=2.8 (95%Cl 2.0, 3.8), p=0.004and OR=3.4 (95%Cl 2.5, 4.7), p=0.0002, respectively (Figure 2.8),which was similar to previous findings (Brown & Qaqish, 2006).The overall assessment of heterogeneity between studies for osteopenia/osteoporosis at lumbar spine and hip was I^2=64% (Q=22.2, p<0.001) and I^2=74% (Q=30.4, p<0.001), respectively.

A meta-analysis on the percent change in BMD from baseline to follow-up for ART-treated individuals could not be calculated as there was only one observational longitudinal study (Madeddu et al., 2004).

(a)



(b)

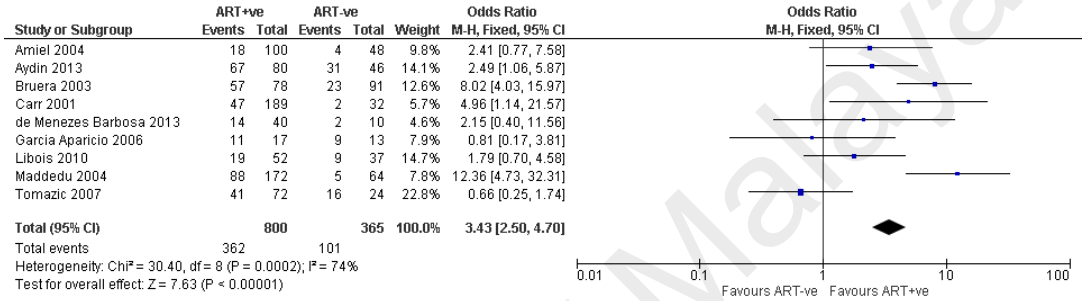


Figure 2:8:Odds ratio of reduced bone mineral density in antiretroviral-treated and non antiretroviral-treated individuals at: (a) lumbar spine; (b) hip

2.18.6.3 Protease inhibitor-treated versus non protease inhibitor-treated individuals

Eleven studies [cross-sectional (n=7); longitudinal (n=4)] compared PI-treated versus non PI-treated individuals [Tables 2.26 and 2.27] (Bonnet et al., 2013; Bruera et al., 2003; Calmy et al., 2009; Carr et al., 2001; de Menezes Barbosa et al., 2013; Hansen et al., 2011; Libois et al., 2010; Madeddu et al., 2004; Mondy et al., 2003; Tebas et al., 2000; Tomazic et al., 2007). Of the four longitudinal studies, only one was a randomized controlled trial (RCT) (Hansen et al., 2011). Four studies only included men (Carr et al., 2001; de Menezes Barbosa et al., 2013; Tebas et al., 2000; Tomazic et al., 2007), one study only included women (Libois et al., 2010), whilst six studies included both men and women (Bonnet et al., 2013; Bruera et al., 2003; Calmy et al., 2009; Hansen et al., 2011; Madeddu et al., 2004; Mondy et al., 2003). The majority of participants were male (range: 63%-96%) (Calmy et al., 2009; Madeddu et al., 2004). All studies were matched for

gender except for five studies (Bonnet et al., 2013; Bruera et al., 2003; Calmy et al., 2009; Hansen et al., 2011; Madeddu et al., 2004). Age and BMI were well matched between PI-treated and non-PI-treated individuals.

Table 2:26: Baseline characteristics of cross sectional studies (n=7) and longitudinal study (n=1) and proportion of reduced bone mineral density in protease inhibitor-treated and non protease inhibitor-treated individuals

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In protease inhibitor-treated and non protease inhibitor-treated individuals													
(Bruera et al., 2003)	Single site, Argentina/ cross sectional, prospective	Hologic-4500w	•Lumbar Spine (L1-L4) •Femoral neck •Total body	PI+	42	81	36.3 ±6.1	NR	23.9±3.1	7.4±3.5	NR	Lumbar spine data: Osteopenia:16(38.1 %) Osteoporosis: 3(7.1%)	Lumbar spine data: 19(45)
												Hip data: Osteopenia: 22(52.4%) Osteoporosis: 6(14.3%)	Hip data: 28(66.7)
				PI-	36	69	34.8 ±6.4	NR	23.8±2.8	6.2±3.1	NR	Lumbar spine data: Osteopenia: 16(44.4%) Osteoporosis:3(7.41 %)	Lumbar spine data: 19(52)
												Hip data: Osteopenia: 21(59.3%) Osteoporosis:8(22.2 %)	Hip data: 29(80.5)
(Calmy et al., 2009)	Single site, Australia/ cross sectional, prospective	Lunar Prodigy	•Lumbar Spine •Femoral neck	PI+	81	96.3	Median: 47 (IQR: 43–54)	NR	Median: 24.8 (IQR: (22.7–27.4)	Median: 15 (IQR:9–20)	Median: 56 (IQR:36.5–80.5)	Osteopenia: 39(53.4%)	39(53.4)
				PI-	72	100	Median: 49.5 (IQR: 42–57)	NR	Median: 24.3 (IQR: 22.5–26.6)	Median: 10 (IQR:5.3–16.0)	NR	Osteopenia: 26(39.4%)	26 (39.4)

Table 2.26: Baseline characteristics of cross sectional studies (n=7) and longitudinal study (n=1) and proportion of reduced bone mineral density in protease inhibitor-treated and non protease inhibitor-treated individuals*(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In protease inhibitor-treated and non protease inhibitor-treated individuals *(continued)*													
(Carr et al., 2001)	Single site, Australia/ cross sectional, prospective	Lunar DPXL	•Lumbar Spine •Total body	PI+	147	100	NR	NR	NR	NR	NR	Osteopenia+ osteoporosis: 36 (25%)	36 (25)
				PI-	42	100	NR	NR	NR	NR	NR	Osteopenia+ osteoporosis: 11 (26%)	11 (26)
(de Menezes Barbosa et al., 2013)	Multiple site, Brazil / cross sectional, prospective	Hologic QDR 4500A	•Lumbar Spine •Femoral neck •Total body	PI+	20	100	NR	NR	NR	NR	NR	Lumbar spine data: Osteopenia: 8(40%) Osteoporosis: 1(5%)	Lumbar spine data: 9(45)
												Hip data: Osteopenia: 6(30%) Osteoporosis: 0	Hip data: 6(30)
				PI-	20	100	NR	NR	NR	NR	NR	Lumbar spine data: Osteopenia: 12(60%) Osteoporosis: 0	Lumbar spine data: 12(60)
												Hip data: Osteopenia: 7(35%) Osteoporosis:1(5%)	Hip data: 8(40)
(Libois et al., 2010)	Single site, Belgium/ cross sectional, prospective	Hologic QDR 4000	•Lumbar Spine •Femoral neck •Total hip	PI+	25	0	Median: 37	Black(72) Caucasian(28)	Median: 24.8	Median: 5.3	Years: Median:2.3	Osteopenia+ osteoporosis: 11(40.74%)	11(40.7)
				PI-	27	0	Median: 37	Black (78) Caucasian(22)	Median: 24.3	Median: 7.5	Years: Median:4.6	Osteopenia+ osteoporosis: 8 (32%)	8 (32)

Table 2.26: Baseline characteristics of cross sectional studies (n=7) and longitudinal study (n=1) and proportion of reduced bone mineral density in protease inhibitor-treated and non protease inhibitor-treated individuals *(continued)*

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In protease inhibitor-treated and non protease inhibitor-treated individuals *(continued)*													
(Madeddu et al., 2004)	Single site, Italy/ longitudinal, prospective	Hologic QDR 4500A	•Lumbar Spine (L1-L4) •Femoral neck	PI+	92	65.2	39±6	NR	22.46 ±3.02	10±4	37.7±12.7	Osteopenia: 34(37%) Osteoporosis: 20(21.7%)	54(58.7)
				PI-	60	63.3	38±6	NR	22.64 ±3.1	9±4	34.6±14.1	Osteopenia: 23(38.3%) Osteoporosis: 5(8.3%)	28(46.6)
(Tebas et al., 2000)	Single site, USA/ cross sectional, prospective	Hologic QDR-2000	•Lumbar Spine •Femoral neck •Total body	PI+	60	100	41±8	NR	24±4	NR	Weeks: Median:104 (range: 16–363)	Osteopenia+ osteoporosis: 30(50%)	30(50)
				PI-	35	100	37±7	NR	22±6	NR	NR	Osteopenia+ osteoporosis: 8(23%)	8(23)
(Tomazic et al., 2007)	Single site, Slovenia/ cross sectional, prospective	Hologic Discovery -W SIN 70659	•Lumbar Spine (L1-L4) •Femoral neck •Total hip	PI+	35	100	45.5 ±10.0	NR	25.9±3.6	11.0±4.8 (range: 1.4–21.5)	Years: 5.5±2.5 (range: 1.3–9.3)	Osteopenia: 17 (46%) Osteoporosis 3(9%)	20(54)
				PI-	37	100	43.8 ±11.4	NR	24.9±2.4	11.5±6.0 (range: 1.5–25.5)	Years: 5.1±3.2 (range: 1.0–10.1)	Osteopenia: 15 (41%) Osteoporosis: 6(16%)	21(57)

NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; PI=protease inhibitor; IQR=interquartile range

Table 2:27: Baseline characteristics of longitudinal studies (n=4) and percent change from baseline to follow-up in in protease inhibitor-treated and non protease inhibitor individuals

Reference	Setting	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD	Ethnicity (%)	Duration of study (months)	Loss of follow up (%)	Mean BMI±SD (kg/m^2)	Baseline (mean BMD±SD (g/cm^2)	Percent change (%)
In protease inhibitor-treated and non protease inhibitor-treated individuals													
(Bonnet et al., 2013)	Single site, France , longitudinal, prospective	Lunar Corporation®	•Lumbar spine (L2-L4) • Total hip •Total body	PI+	16	62.5	42.3± 14.5	European (75) Black(25)	21 Interval of follow-up: 0,9,21	54	23.8 ±3.6	Mean: Lumbar spine: 1.233	**Lumbar spine:** Baseline→9 months PI+ve=↓4.2% vs PI-ve=↓1.4% 9months→21months PI+ve=↓0.3% vs PI-ve=↓0.2%
				PI-	19	63.2	39.7± 11.9	European (73.7) Black (26.3)		46	24.0 ±3.6	Mean Lumbar spine:1.249	
(Hansen et al., 2011)	Multiple site, Denmark/ longitudinal, prospective, RCT	Hologic, Norland XR 36	•Lumbar Spine •Femoral neck	PI+	30	90	Median: 43.6 (IQR: 39.5–54.6)	Caucasian (90) Other (10)	33 Interval of follow-up: 0, 6, 11 22, 33	17	Median: 21.6 (IQR:20 .4–22.9)	Median: Lumbar spine: 1.04 (IQR: 0.90–1.18) Femur: 0.91 (0.79–0.98)	**Lumbar spine:** Baseline→ 6 months PI+ve =↓3.2%vs PI-ve=↓2.7% Baseline→33 months PI+ve=↓1.9% PI-ve=↓2.5% **Femur:** Baseline→11 months PI+ve=↓6.1% vs PI-ve=↓5.1% Baseline→33 months PI+ve=↓5.0% vs PI-ve=↓4.5%
				PI-	29	89.7	Median :41.1 (IQR 37.5–50.9)	Caucasian (96.6) Other(3.4)		36	Median: 22.7 (IQR: 20.3–25.9)	Median: Lumbar spine: 1.11 (IQR: 1.0–1.2) Femur: 0.90 (IQR: 0.8–1.0)	
(Madeddu et al., 2004)	Single site, Italy/ longitudinal, prospective	Hologic QDR 4500A	•Lumbar Spine (L1-L4) •Femoral neck	PI+	92	65.2	39±6	NR	14 Interval of follow-up: 0, 14	81.5	22.5 ±3.0	Lumbar spine: 0.9± 0.1 Femur:0.8±0.1	**Lumbar spine:** Baseline→ 14 months PI+ve= ↑0.9% vs PI-ve=↓0.2% **Femur:** Baseline→ 14 months PI+ve= ↓0.9% vs PI-ve= ↓0.2%
				PI-	60	63.3	38±6	NR		83.3	22.6 ±3.1	Lumbar spine: 1.0± 0.1 Femur: 0.9±0.1	

Table2.27: Baseline characteristics of longitudinal studies (n=4) and percent change from baseline to follow-up in in protease inhibitor-treated and non protease inhibitor individuals *(continued)*

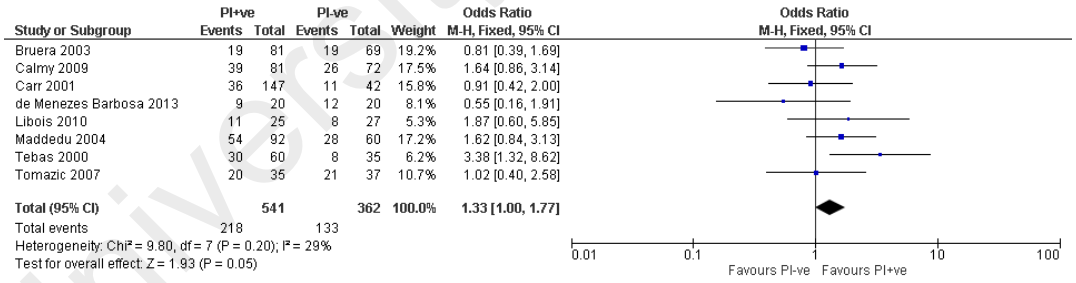
Reference	Setting	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD	Ethnicity (%)	Duration of study (months)	Loss of follow up (%)	Mean BMI±SD (kg/m^2)	Baseline (mean BMD±SD (g/cm^2)	Percent change (%)
In protease inhibitor-treated and non protease inhibitor-treated individuals *(continued)*													
(Mondy et al., 2003)	Multiple sites, USA/ longitudinal, prospective	Hologic QDR-2000)	•Lumbar Spine (L1-L4) •Femoral neck •Total body	PI+	61	86.4	NR	Black(16) Caucasian(84)	17	NR	NR	NR	**Lumbar spine** Baseline→17months PI+ve= ↑2.5% vs PI-ve=↑3.8%
				PI-	19	NR	NR	NR	Interval of follow-up: 0,17	NR	NR	NR	

NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; PI=protease inhibitor; IQR=interquartile range, RCT=randomized control trial

(a) ***Odds ratio of reduced bone mineral density in protease inhibitor-treated versus non protease inhibitor-treated individuals***

Seven cross-sectional studies and one observational longitudinal study were included in this analysis[Table 2.26] (Bruera et al., 2003; Calmy et al., 2009; Carr et al., 2001; de Menezes Barbosa et al., 2013; Libois et al., 2010; Madeddu et al., 2004; Tebas et al., 2000; Tomazic et al., 2007). Three longitudinal studies were excluded as the proportion of osteopenia/osteoporosis at baseline were not reported (Bonnet et al., 2013; Hansen et al., 2011; Mondy et al., 2003). The odds of developing osteopenia/osteoporosis at the lumbar spine and hip was OR=1.3 (95%Cl 1.0, 1.8), p=0.20and OR=1.3 (95%Cl 1.0, 1.7), p=0.17, respectively (Figure 2.9). However, the result did not reach statistical significance, which was similar to previous findings (Brown & Qaqish, 2006). The overall assessment of heterogeneity between studies for osteopenia/osteoporosis at lumbar spine and hip was I^2=29% (Q=9.8, p=0.05) and I^2=33% (Q=10.4, p=0.07), respectively.

(a)



(b)

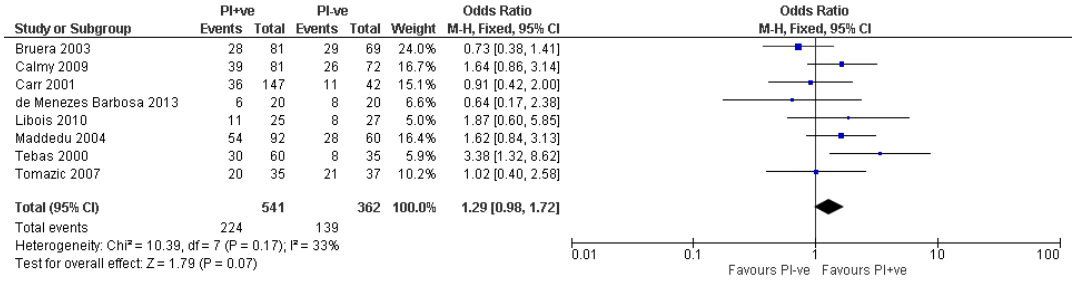
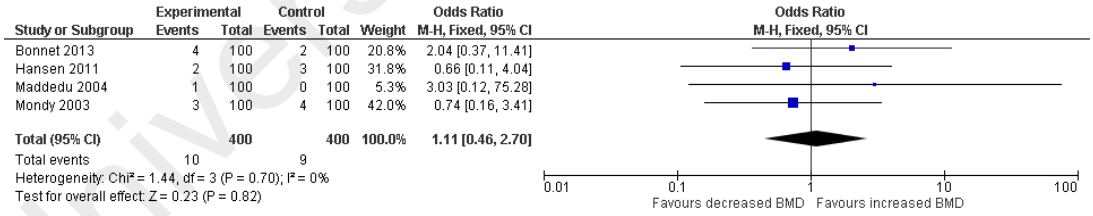


Figure 2:9: Odds ratio of reduced bone mineral density in protease inhibitor-treated and non protease inhibitor-treated individuals at: (a) lumbar spine; (b) hip

(b) ***Percent change in BMD from baseline to follow-up in protease inhibitor-treated versus non protease inhibitor-treated individuals***

Four longitudinal studies [observational (n=3); RCT (n=1)] were included in this analysis (Bonnet et al., 2013; Hansen et al., 2011; Madeddu et al., 2004; Mondy et al., 2003) [Table 2.27]. The duration of follow-up ranged from 14-33 months (Hansen et al., 2011; Madeddu et al., 2004). Bone loss occurred at both lumbar spine from baseline to 6 (Hansen et al., 2011), 9, 21 (Bonnet et al., 2013) and 33 months (Hansen et al., 2011); and at the femur from baseline to 11 (Hansen et al., 2011), 14 (Madeddu et al., 2004), and 33 months (Hansen et al., 2011). However, there was no significant difference between the percent change in BMD at the lumbar spine [OR=1.1 (95% Cl 0.5, 2.7)], p=0.70 and femoral neck [OR=1.2 (95% Cl 0.4, 3.8)], p=0.53 from baseline to 14-33 months in PI-treated versus non PI-treated individuals (Figure 2.10). The overall assessment of heterogeneity between studies for percent change in BMD analysis at lumbar spine and hip was I^2=0% (Q=1.5, p=0.82) and I^2=0% (Q=0.4, p=0.77), respectively.

(i)



(ii)

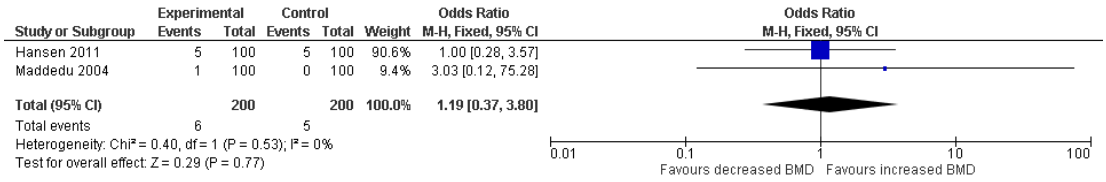


Figure 2:10: Percent change in bone mineral density from baseline to follow-up in protease inhibitor-treated versus non protease inhibitor-treated individuals at: (a) lumbar spine; (b)femur

2.18.6.4 Tenofovir-treated versus non tenofovir-treated individuals

Three studies [cross-sectional (n=1); longitudinal (n=2] compared tenofovir-treated versus non tenofovir-treated individuals [Tables 2.28 and 2.29] (Calmy et al., 2009; Gallant et al., 2004; Haskelberg et al., 2012). Only one longitudinal study was a RCT (Haskelberg et al., 2012). All three studies included both men and women (Calmy et al., 2009; Gallant et al., 2004; Haskelberg et al., 2012). The majority of the participants were male (ranged 74%-99%) (Calmy et al., 2009; Gallant et al., 2004), and all studies were not matched for gender (Calmy et al., 2009; Gallant et al., 2004; Haskelberg et al., 2012).

Table 2:28: Baseline characteristics of cross sectional studies (n=1) and proportion of reduced bone mineral density in tenofovir-treated and non tenofovir-treated individuals

References	Setting/ study design	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD (years)	Ethnicity (%)	Mean BMI±SD (kg/m^2)	Mean HIV duration± SD (years)	Mean Treatment duration± SD (months)	Prevalence of osteopenia/ osteoporosis (BMD results)	Overall prevalence of reduced BMD,n (%)
In tenofovir-treated and non tenofovir-treated individuals													
(Calmy et al., 2009)	Single site, Australia/ cross sectional	Lunar Prodigy	•Lumbar Spine •Femoral neck	TDF+	67	98.5	Median: 47 (IQR: 42–55)	NR	Median: 24.8 (IQR:22.9–27.2)	Median: 14 (IQR:7–20)	Median: 33 (IQR: 16–53)	Osteopenia: 30 (52.6%)	30(52.6)
				TDF-	86	97.7	Median: 49 (IQR:43–55)	NR	Median: 24.3 (IQR: 22.5–26.6)	Median: 12 (IQR: 6.8–17.3)	NR	Osteopenia: 35(42.7%)	35(42.7)

NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; TDF=tenofovir disoproxil fumarate; IQR=interquartile range

Table 2:29: Baseline characteristics of longitudinal studies (n=2) and percent change from baseline to follow-up in tenofovir-treated and non tenofovir-treated individuals

Reference	Setting	DXA machine	Site of DXA scan	Group	N	Male (%)	Mean age±SD	Ethnicity (%)	Duration of study (months)	Loss of follow up (%)	Mean BMI±SD (kg/m^2)	Baseline (mean BMD±SD (g/cm^2)	Percent change (%)
In tenofovir-treated and non tenofovir-treated individuals													
(Gallant et al., 2004)	Multiple sites, USA, South America, and Europe/ longitudinal, prospective	Not specified	•Lumbar Spine •Total hip	TDF+	299	74	Mean:36 (Absolute range: 19-61)	Caucasian (64) Black(21) Hispanic(7) Other(8)	33 Interval of follow-up: 0, 6, 11, 17, 28, 33	38	NR	NR	**Lumbar spine** Baseline→33 months TDF+ve= ↓2.2% vs TDF-ve= ↓1.0% **Total hip** Baseline→33 months TDF+ve=↓2.8% vs TDF-ve=↓2.4%
				TDF-	301	75	Mean: 36 (Absolute range:18-64)	Caucasian (64) Black(18) Hispanic(8) Other(11)		40	NR	NR	
*(Haskelberg et al., 2012)	Multiple site, Australia/ longitudinal, prospective, ,RCT	Lunar (72% sites), Other brands (27% sites)	•Lumbar Spine •Total hip (right)	TDF+	154	97	44.7 ±8.3	Caucasian (86) Others(14)	22 Interval of follow-up: 0, 11, 22	0	24.8 ±3.6	Lumbar spine: 1.2±0.2 Total hip: 1.0±0.1	**Lumbar spine** Baseline→11 months TDF+ve= ↓1.2% vs TDF-ve= ↑0.5% Baseline→22 months TDF+ve=↓0.3 vs TDF-ve=↑0.8 **Total hip** Baseline→11 months TDF+ve↓1.2% vs TDF-ve=↓0.6% Baseline→22 months TDF+ve=↓0.3 vs TDF-ve=↑0.8%
				TDF-	147	99	45.8 ±8.7	Caucasian (84) Others(16)		0	24.7 ±3.5	Lumbar spine: 1.2±0.2 Total hip: 1.0±0.1	

*All BMD scan was performed using standardized protocol but BMD scans were not centrally analyzed; HIV=human immunodeficiency virus; NR=not reported; NA=not applicable; N=number; SD=standard deviation; BMI=body mass index; BMD=body mineral density; TDF=tenofovir disoproxil fumarate; IQR=interquartile range; RTC=randomized control trial

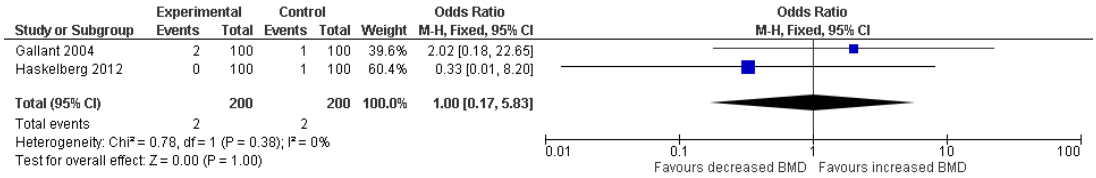
(a) ***Proportion of reduced bone mineral density in tenofovir-treated versus non tenofovir-treated individuals***

Only one cross sectional study was included in this analysis [Table 2.28] (Calmy et al., 2009). Hence, it was not possible to calculate the OR. A higher proportion of tenofovir-treated individuals 30(52.6%) had osteopenia/osteoporosis compared to non tenofovir-treated individuals 35(42.7%), but this result was not statistically significant (p=0.248).

(b) ***Percent change in bone mineral density from baseline to follow-up in tenofovir-treated versus non tenofovir-treated individuals***

Two longitudinal studies [observational (n=1); RCT (n=1)] were included in this analysis [Table 2.29](Gallant et al., 2004; Haskelberg et al., 2012). The duration of follow-up ranged from 22-33 months (Gallant et al., 2004; Haskelberg et al., 2012). Bone loss occurred at the lumbar spine and total hip from baseline to 11, 22 (Haskelberg et al., 2012) and 33 months (Gallant et al., 2004). However, when a meta-analysis was performed, no significant difference was found between the percent change in BMD at the lumbar spine [OR=1.0 (95% Cl, 0.2, 5.8)], p=0.38 and total hip [OR=1.8 (95% Cl , 0.4, 8.7)], p=0.71 from baseline to follow-up at 22-33 months in tenofovir-treated versus non tenofovir-treated individuals (Figure 2.11). The overall assessment of heterogeneity between studies for percent change in BMD analysis at lumbar spine and hip was I^2=0% (Q=0.8, p=1.00) and I^2=0% (Q=0.14, p=0.4), respectively.

(a)



(b)

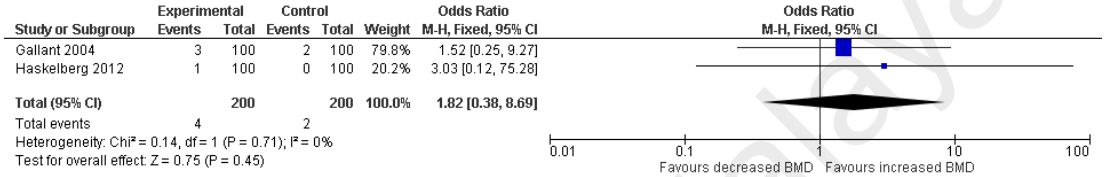


Figure 2:11: Percent change in bone mineral density from baseline in tenofovir-treated versus non tenofovir-treated individuals at: (a) lumbar spine; (b) total hip

2.18.6.5 Percent change in bone mineral density for longitudinal studies

Our study found that there was no significant difference in the percent change of BMD at the lumbar spine, femoral neck or total hip from baseline to follow-up at 14-33 months; between HIV-infected, PI-treated, tenofovir-treated versus their controls, respectively. Our meta-analysis suggests that short-term accelerated bone loss may occur within the first year of HIV infection or ART initiation. Our results were similar to previous studies which reported that 2%-6% of accelerated bone loss occurred over the first 2 years of ART initiation (McComsey et al., 2010).However, evidence concerning short term bone loss may not be as strong as the range of bone loss reported was small (0.1-5.0%) as our findings were based on a small number of studies (Davey, Turner, Clarke, & Higgins, 2011). This shows that the pattern of bone loss in HIV-infected individuals may be acute and accelerated, whilst in HIV-uninfected older people, their bone loss occurs gradually over time as they age (Clarke & Khosla, 2010).

Ideally, results from RCT and observational studies should be analysed separately as observational studies which recruited ART-treated individuals are more likely to have active disease or complications that may impact on outcome, whereas RCTs match for baseline conditions for both treated individuals and controls. However, it was not possible for us to perform any sub-group analysis based on study design as there were only two RCTs included.

2.18.6.6 Risk factors for low bone mineral density

Fifteen studies assessed the risk factors associated with low BMD [Table 2.30](Amiel et al., 2004; Arnsten et al., 2007; Arnsten et al., 2006; Aydın et al., 2013; Bolland et al., 2006; Calmy et al., 2009; Carr et al., 2001; de Menezes Barbosa et al., 2013; Dolan et al., 2004; Dolan et al., 2006; Garcia Aparicio et al., 2006; Hansen et al., 2011; Jones et al., 2008; Libois et al., 2010; Loiseau-Pérès et al., 2002). Thirteen studies were rated as fair quality as they did not perform a sample size calculation (Amiel et al., 2004; Arnsten et al., 2007; Arnsten et al., 2006; Aydın et al., 2013; Bolland et al., 2006; Calmy et al., 2009; Carr et al., 2001; de Menezes Barbosa et al., 2013; Dolan et al., 2004; Dolan et al., 2006; Hansen et al., 2011; Jones et al., 2008; Libois et al., 2010). Two studies were rated as poor quality as they did not perform any multivariate analysis (Garcia Aparicio et al., 2006; Loiseau-Pérès et al., 2002). Ten risk factors (age, history of bone fracture, BMI, body weight, ethnicity, testosterone level, smoking, lipodystrophy, CD4+ cell count, fat mass and lean body mass) had fair evidence of an association with low BMD, two risk factors (HIV viral load and lactic acid level) had insufficient evidence, whilst three risk factors (steroid use, opiate use and vitamin D level) had inconsistent evidence.

Table 2:30: Risk factors of low bone mineral density in human immune deficiency virus-infected individuals

Risk factor	References	Quality of study	Measurement/categorization of factor	Summary of results
(A) Traditional osteoporosis risk factors				
Strength of evidence: Fair evidence for an association between older age and low BMD				
Chronological age	(Arnsten et al., 2006)	Fair	years	Older age was associated with low BMD
	(Arnsten et al., 2007)	Fair		
	(Carr et al., 2001)	Fair		
	(de Menezes Barbosa et al., 2013)	Fair		
	(Hansen et al., 2011)	Fair		
	(Aydın et al., 2013)	Fair		No association was found between age and BMD (p=0.166)
Strength of evidence: Fair evidence for an association between history of bone fracture and low BMD				
History of bone fracture	(Arnsten et al., 2006)	Fair	Self-reported fracture	History of bone fracture was associated with low BMD
	(Arnsten et al., 2007)	Fair		
	(Amiel et al., 2004)	Fair		No association was found between history of bone fracture and BMD
Strength of evidence: Fair evidence for an association between low BMI and low BMD				
BMI	(Amiel et al., 2004)	Fair	kg/m^2	Low BMI was associated with low BMD
	(de Menezes Barbosa et al., 2013)	Fair		
	(Calmy et al., 2009)	Fair		
	(Dolan et al., 2004)	Fair		
	(Dolan et al., 2006)	Fair		
	(Hansen et al., 2011)	Fair		
	(Jones et al., 2008)	Fair		
	(Libois et al., 2010)	Fair		
	(Aydın et al., 2013)	Fair		No association was found between BMI and BMD (p=0.780).
Strength of evidence: Fair evidence for an association between low body weight is associated and low BMD				
Body weight	(Arnsten et al., 2006)	Fair	Kg	Low body weight was associated with low BMD
	(Arnsten et al., 2007)	Fair		
	(Carr et al., 2001)	Fair		
	(Dolan et al., 2004)	Fair		
	(Dolan et al., 2006)	Fair		
Strength of evidence: Fair evidence for an association between ethnicity is associated and low BMD				
Ethnicity	(Arnsten et al., 2006)	Fair	Black, Caucasian, Hispanic, Others	Hispanic and Caucasian were associated with low BMD
	(Arnsten et al., 2007)	Fair		
	(Libois et al., 2010)	Fair	Black, Caucasian	

Table 2.30: Risk factors of low bone mineral density in human immune deficiency virus-infected individuals *(continued)*

Risk factor	References	Quality of study	Measurement/ categorization of factor	Summary of results
(A) Traditional osteoporosis risk factors *(continued)*				
Strength of evidence: Fair evidence for an association between low testosterone level and low BMD				
Testosterone level	(Arnsten et al., 2007)	Fair	ng/dl	Low testosterone level was associated with lower BMD
	(Calmy et al., 2009)	Fair	nmol/L	
	(Garcia Aparicio et al., 2006)	Poor	NR	
Strength of evidence: Fair evidence for an association between smoking and low BMD				
Smoking	(Arnsten et al., 2007)	Fair	Current and ex-smoker: packs-years	Smoking was associated with low BMD
	(Hansen et al., 2011)	Fair	Current smoker	
	(Dolan et al., 2006)	Fair	pack-years	No association was found betweensmoking and low BMD
Strength of evidence: Insufficient evidence for an association between steroid use and low BMD				
Steroid use	(Arnsten et al., 2006)	Fair	Past prednisolone used: Duration used unknown	Steroid use was associated with low BMD
	(Arnsten et al., 2007)	Fair		
Strength of evidence: Insufficient evidence for an association between opiate use and low BMD				
Opiate use	(Arnsten et al., 2006)	Fair	•Current methadone treatment •Heroin, cocaine or crack cocaine used in past 5 years	Opiate use was associated with low BMD
	(Arnsten et al., 2007)	Fair		
Strength of evidence: Insufficient evidence for an association between vitamin D level and low BMD				
Vitamin D level	(Dolan et al., 2006)	Fair	nmol/L	No association was found between vitamin D level and low BMD
(B) HIV-related osteoporosis risk factors				
Strength of evidence: Fair evidence for an association between lipodystrophy and low BMD				
Lipodystropy	(Bolland et al., 2006)	Fair	Percentage	Lipodystrophy was associated with low BMD
	(Carr et al., 2001)	Fair	Peripheral lipoatrophy: any site	Lipoatrophy was associate with low BMD
	(Loiseau-Pérès et al., 2002)	Poor	NR	No association was found between lipodystrophy and BMD
Strength of evidence: Fair evidence for an association between low fat mass/low lean body mass and low BMD				
Fat mass/ lean body mass	(Carr et al., 2001)	Fair	Kg	Low fat mass and low lean body mass was associated with low BMD
	(Dolan et al., 2004)	Fair		
	(de Menezes Barbosa et al., 2013)	Fair	Percentage	

Table 2.30: Risk factors of low bone mineral density in human immune deficiency virus-infected individuals *(continued)*.

Risk factor	References	Quality of study	Measurement/ categorization of factor	Summary of results
(B) HIV-related osteoporosis risk factors *(continued)*				
Strength of evidence: Fair evidence for an association between CD4+ cells count and low BMD				
CD4+ cell count	(Dolan et al., 2006)	Fair	cells/µL	Low CD4+ cell count was associated with low BMD
	(Hansen et al., 2011)	Fair		
	(Libois et al., 2010)	Fair		
	(Arnsten et al., 2006)	Fair		No association was found between CD4+ cell count and BMD
	(Jones et al., 2008)	Fair		
Strength of evidence: Inconsistent evidence for an association between HIV viral load and low BMD				
HIV viral load	(Aydın et al., 2013)	Fair	copies/mL	Higher HIV viral load was associated with low BMD
	(Carr et al., 2001)	Fair		
	(Jones et al., 2008)	Fair		No association was found between HIV viral load and low BMD
	(Dolan et al., 2006)	Fair		
Strength of evidence: Inconsistent evidence for an association between lactate acid level and low BMD				
Lactic acid level	(Carr et al., 2001)	Fair	mmol/l	Higher lactic acid levels was associated with lower BMD
	(Dolan et al., 2004)	Fair		No association was found between lactic acid level and low BMD

Kg=kilogram; m=meter; BMD=bone mineral density,BMI=body mass index; NR=not reported, mm=millimeter, mmol/L=milimole per liter, µL=microliter, ng/dl= nanogram per desiliter, nmol/L=nanomole per liter

2.18.7 Strengths and limitations of the meta-analysis

One of the limitations of our meta-analysis was that we were not able to determine the effect of the individual types of antiretroviral medications on osteopenia/osteoporosis. Secondly, we excluded grey literature that were published as editorials, commentaries, brief reports, expert opinions, case studies, theses, conference proceedings, newspapers, fact sheets, websites or policy documents as they were not sufficiently detailed enough for us to assess the quality and to perform data extraction. However, we agree that omitting grey literature could potentially lose relevant work and cause publication bias as literature that has been published usually have a larger sample size and positive outcomes compared to grey literature. Lastly, we were not able to utilize z-score to diagnose osteoporosis in individuals <50 years, as the majority of the studies included did not present their results as z-score.

One of the strength of our study was that we performed our search on six databases. In addition, secondary research such as this meta-analysis provides higher level of evidence as compared to primary studies because it increases in power and precision; enables large enough number of patients so that the power of statistical tests ceases to be a limiting factor and estimates of association become precise enough to be more useful. Hence, it reduces bias that usually occurs in a single study. Finally, the use of fixed effects model in analysing the data is also another strength of this meta-analysis; funnel plots that show no publication bias or reporting bias in this meta-analysis.

2.19 Consequences of untreated osteoporosis in human immunodeficiency virus-individuals

If osteoporosis is left untreated, fragility fracture will occur.Previous studies found that HIV-infected individuals had 1.4 increased odds of developing fragility fracture when

compared to HIV-uninfected individuals (Shiau, Broun, Arpadi, & Yin, 2013). In Malaysia, a study conducted in 1997 shows that the incidence of hip fracture among individual ≥50 years was 90 per 100000 population with 63% of patients were Chinese, followed by Malays (20%) and Indians (13%) (Lee & Khir, 2007). These hip fractures were most likely due to osteoporosis (Lee & Khir, 2007).

Osteoporotic fractures have a profound impact on the daily life of an individual as it can cause pain, severe disability, loss of independence and reduced quality of life (Madureira et al., 2012). The most common sites for a fragility fractures are wrist, vertebrate and hip (Bianchi et al., 2005).

Studies have shown that the mortality rate within the first year after sustaining a hip fracture is 20% whilst in individuals that sustained vertebral fractures, 46 % will die after 3 years, 69 % die after 5 years and 90% die after 7 years (Lau, Ong, Kurtz, Schmier, & Edidin, 2008; Leibson, Tosteson, Gabriel, Ransom, & Melton, 2002). Besides that, for those who have survived, around 20% of them will require long term nursing care within their first year after a hip fracture (Tajeu et al., 2014). In addition, 50% of hip fractures survivors are permanently incapacitated without regaining their mobility after 1 year of sustaining the hip fracture (Vochteloo et al., 2013).

2.20 Treatment of osteoporosis in human immunodeficiency virus-individuals

The treatment of osteoporosis in HIV-infected individuals can be divided into pharmacological and non-pharmacological therapy (Walker Harris & Brown, 2012).

2.20.1 Pharmacological treatment for osteoporosis in human immunodeficiency virus-infected individuals

Pharmacological treatment of osteoporosis can be divided into first and second line therapy. Agents used in the treatment of osteoporosis include anti-resorptive drugs (bisphosphonates, hormone replacement therapy, serum oestrogen receptor modulators and denosumab, a human monoclonal antibody) , anabolic agent (teriparatide) and strontium [Table2.31] (Cotter & Mallon, 2012).

Table 2:31: Pharmacological treatment of osteoporosis treatment in HIV-infected individuals

Drugs	Route of administration	Dose/ frequency	Side effects
Bisphosphonate			
Alendronate	Oral	70 mg once weekly	Gastrointestinal effects (difficulty swallowing, esophageal inflammation, dyspepsia, and gastric ulcer), atypical femoral shaft fracture, osteonecrosis of the jaw and acute phase reactions (arthralgias, myalgias, headache, fever, and bone pain) in IV drugs
Ibandronate	Oral	150 mg once monthly	
	IV	3m once every 3 months	
Risedronate	Oral	35 mg once weekly	
Zoledronic acid	IV	5 mg once yearly	
Hormone replacement therapy (HRT)			
Conjugated Estrogen	Oral	0.3 or 0.625 mg daily	Increased risk of breast cancer and cardiovascular disease
Estradiol Valerate	Oral	1.0 or 2.0 mg mg daily	
Transdermal estradiol	Transdermal	25 -100 ug twice weekly	
Micronised estradiol	Oral	0.5 or 1.0 mg daily	
Tibolone	Oral	2.5 mg daily	
Serum estrogen receptor modulators (SERMs)			
Raloxifene	Oral	60 mg daily	Hot flushes, leg cramp, venous thromboembolism
Human monoclonal antibody (IgG_2)			
Denosumab	SC	60 mg twice yearly	Osteonecrosis of the jaw, serious skin infections, dermatitis, rashes, eczema, cellulitis
Recombinant parathyroid hormone (PTH)			
Teriparatide	SC	20 µg daily	Dizziness, leg cramps
Strontium ranelate			
Strontium ranelate	Oral	2 g daily	Headache, diarrhea, Drug Rash with Eosinophilia Systemic Symptoms (DRESS)

IV=intravenous; SC=subcutaneous; µg=microgram, mg=milligram Source: Therapeutic options for low bone mineral density in HIV-infected subjects. *Curr HIV/AIDS Rep, 9*(2), 148-159 (Cotter & Mallon, 2012)

2.20.1.1 Bisphosphonates

Bisphosphonates such as alendronate and zoledronic acid (Brown, Hoy, et al., 2015)are used as first-line therapy to treat osteoporosis in HIV-infected individuals(Walker Harris & Brown, 2012) as these bisphosphonate have been found to significantly increase BMD at the lumbar spine and total hip (Pinzone, Moreno, Cacopardo, & Nunnari, 2014). If HIV-infected individuals with osteoporosis are unable to tolerate alendronate, zoledronic acid is given as second line therapy. To date, evidence concerning the use of ibandronate and risedronate in HIV-infected individuals is limited (Brown, Hoy, et al., 2015).

Short-term side effects of bisphosphonate like fever, myalgias and arthralgias may occur within 24 to 72 hours in patients who received IV bisphosphonate therapy (i.e. Ibandronate and Zoledronic acid)(Kennel & Drake, 2009).

Individuals treated with bisphosphonates should be evaluated for efficacy of therapy after 3 yearsafter initiation of therapy and every 2 years thereafter (Sharma & Stevermer, 2009). After 5 years of therapy, clinicians will need to evaluate fracture risk and consider a ‘drug holiday’ (a period of time when treatment is stopped after continuous treatment) (Diab & Watts, 2013). This is to avoid side effects (such asatypical femoral shaft fractures or osteonecrosis of the jaw)which have been associated with long term use of bisphosphonate (>5 years) (Cosman et al., 2014).

2.20.1.2 Hormone replacement therapy

In the past, HRT was used for the treatment for osteoporosis in postmenopausal women (de Villiers et al., 2013). However, recent data reported that HRT can increase the risk of breast cancer and cardiovascular events (Rossouw et al., 2002). Since then, initiating HRT

in postmenopausal women for the sole purpose of preventing osteoporotic fractures is no longer recommended (Rossouw et al., 2002). Short-term use of HRTare indicated only to relieve postmenopausal symptoms in women(Moyer, 2013).

2.20.1.3 Serum estrogen receptor modulators (SERMs)

SERMs (e.g. raloxifene) is used to treat osteoporosis in HIV-uninfected individuals. To date, there is no data on the efficacy of raloxifene in HIV-infected individuals. SERMs were developed to act as an oestrogen antagonist that mimics the positive effects of estrogen on bones in postmenopausal women (Cotter & Mallon, 2012; Cummings, Eckert, Krueger, & et al., 1999). Currently, raloxifene is only approved by FDA for post-menopausal women and data on its efficacy in the treatment of osteoporosis in men was limited (Khosla, 2010). One study found that raloxifene increases BMD at the lumbar spine by 2.6% and femoral neck by 2.1% after 4 years in postmenopausal women (Delmas et al., 2002).

The side effects of raloxifene are hot flushes, leg cramp and venous thromboembolism (Kung, A. W. C. et al., 2003).

2.20.1.4 Human monoclonal antibody (IgG_2)

Denosumab is a human monoclonal antibody specific for receptor of activated NF-κB ligand (RANKL) that reduce bone resorption by inhibiting the formation, function and survival of osteoclast (Narayanan, 2013). However, to date, there is no study found to use denosumab in HIV-infected individuals for treatment of osteoporosis. A study found that denosumab given subcutaneously twice yearly for 36 months was associated with the increased of BMD, reduced risk of veterbral, nonvertebral and hip fractures in HIV-

uninfected women(Cummings et al., 2009). Denosumab increases the BMD of lumbar spine by 9.2% and total hip by 6.0% when compared to placebo (Cummings et al., 2009).

Side effects of denosumab are cellulitis, hypocalcemia, atypical femoral fractures and osteonecrosis of the jaw (Aspenberg, 2014; Boquete-Castro, Gómez-Moreno, Calvo-Guirado, Aguilar-Salvatierra, & Delgado-Ruiz, 2016; Iqbal, Sun, & Zaidi, 2010; Laskowski et al., 2016).

2.20.1.5 Recombinant parathyroid hormone

Teriparatide is a recombinant parathyroid hormone (PTH)which stimulates osteoblast activity and increases new bone formation (Dubois, Rissmann, & Cohen, 2011). Teriparatide may also be considered, but evidence concerning its use in in HIV-infected populations is limited.In 2015, A case study in HIV-infected men found that after two years of teriparatide use, the BMD at the lumbar spine, total hip, and femoral neck hasincreased to 35.4%, 3.5%, and 12.5%, respectively(Wheeler, Tien, Grunfeld, & Schafer, 2015).

Teriparatide, a biosynthetic peptide fragment of the biologically active region of the human parathyroid hormone, is a regulator of bone metabolism. By preferentially stimulating the osteoblastic activity over osteoclastic activity, it stimulates new bone formation on trabecular and cortical bone surfaces. Anabolic effects of teriparatide are seen in an increase in skeletal mass, bone strength and bone formation and resorption markers.

The common side effects of teriparatide include hypercalcemia and headache. There is an increased risk of osteosarcoma seen in rats treated with high dose teriparatide (Vahle

et al., 2002). Hence, to avoid potential harm in humans, it is recommended that teriparatide should only use for a maximum of 24 months (Vahle et al., 2002). To date, potential teriparatide–induced osteosarcoma in human are rare (Subbiah, Madsen, Raymond, Benjamin, & Ludwig, 2010).

2.20.1.6 Strontium ranelate

Strontium reduces bone resorption and promotes bone formation(Meunier et al., 2004). Evidence concerning the use of strontium in HIV-infected individuals is limited. After 3 years, strontium was found to have increased the BMD of lumbar spine, femoral neck and total hip by 12.7%, 7.2% and 8.6%, respectively in HIV-uninfected postmenopausal women(Meunier et al., 2004).

Side effects include diarrhoea, increased risk of cardiac events, venous thromboembolism and drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome (Jonville-Béra, Crickx, Aaron, Hartingh, & Autret-Leca, 2009; Osborne, Layton, Perrio, Wilton, & Shakir, 2010; Reginster, 2014).

2.20.2 Non-pharmacological treatment of osteoporosis in human immunodeficiency virus-infected individuals

Calcium, vitamin D and lifestyle changes areimportant adjunct therapy to increase the its effectiveness of the pharmacological treatment of osteoporosis(International Osteoporosis Foundation, 2015).

2.20.2.1 Adequate calcium and vitamin D intake

Calcium and vitamin D supplements are often use together as an adjunct therapy in the treatment of osteoporosis(Morgan, 2001). Calcium and vitamin D supplementation

alongside with antiresoptive drugs are important to reduce fracture rates in osteoporotic patients as they help to improve and maintain bone strength(Morgan, 2001). The recommended daily intake of calcium and vitamin D has been described in Section 2.16.1.2 (i-j).

2.20.2.2 Lifestyle changes

(a) ***Regular physical exercise***

Regular weight bearing exercise (e.g. brisk walking, jogging, dancing) and muscle strength exercise (e.g. weight training) is essential to maximize peak bone mass, decrease bone loss, maintain muscle strength and balance (Vuori, 2001). Regular weight bearing exercise is recommended for 30 minutes at least 3 days in a week (Howe et al., 2011).

(b) ***Smoking cessation***

Smoking cessation should be encouraged for individuals to reduce the risk of developing osteoporosis (Yoon et al., 2012).

(c) ***Moderate alcohol intake***

Individuals are advice to stop or to decrease alcohol consumption (<3 units per day) in order to reduce the risk of developing osteoporosis (Brown, Hoy, et al., 2015; Kanis, Johansson, et al., 2005).

2.21 Algorithm for the screening, assessment, management and monitoring of bone disease in human immunodeficiency virus-infected individuals

Clinical guidelines published in 2015 recommended that all HIV-infected men and women aged ≥40 years should have their 10-year probability of a fracture risk assessed using FRAX (Brown, Hoy, et al., 2015). DXA scan is recommended in HIV-infected:

individuals aged ≥40 years old who have a FRAX score ≥10%; men aged ≥50 years, post-menopausal women; individuals with a history of fragility fracture, individuals receiving chronic glucocorticoid treatment and individuals who are at higher risk of falls as previous recommended [Figure 2.12] (Brown, Hoy, et al., 2015).

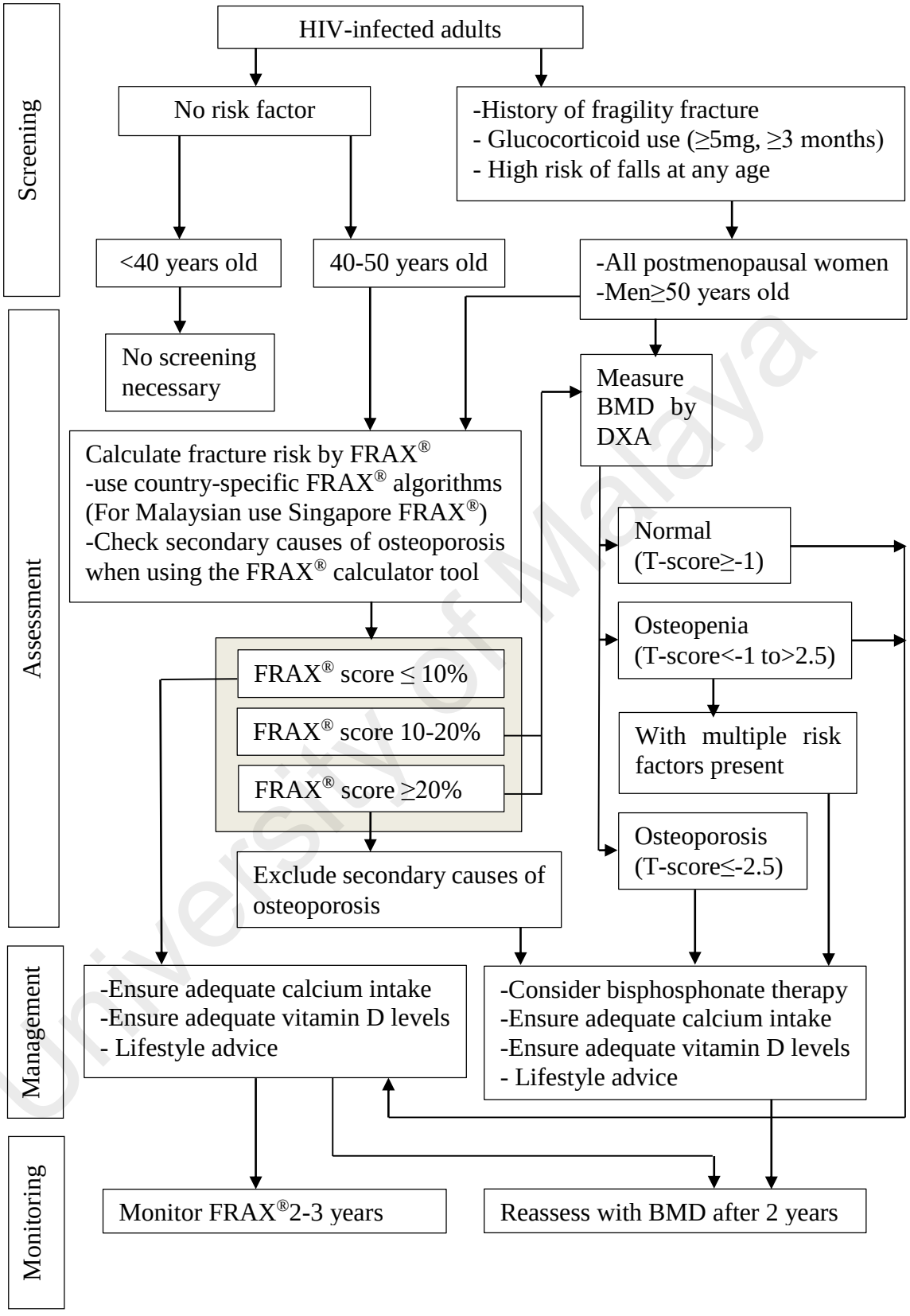


Figure 2:12: Algorithm for the screening, assessment, management and monitoring of bone disease in human immunodeficiency virus-infected individuals (Brown, Hoy, et al., 2015)

2.22 Managing antiretroviral therapy in human immunodeficiency virus-infected individuals with osteoporosis

Anti-osteoporosis treatment should be initiated in HIV-infected individuals who have osteoporosis. In the treatment of HIV-infected individuals diagnosed with osteoporosis, ARTs (i.e. tenofovir and PIs) are associated with an increased risk of developing osteoporosis should be avoided [Figure 2.13] (Brown, Hoy, et al., 2015). These ARTs should be replaced with other ARTs (such as abacavir and raltegravir) which have less effects on bone loss (Brown, Moser, et al., 2015; Stellbrink et al., 2010).

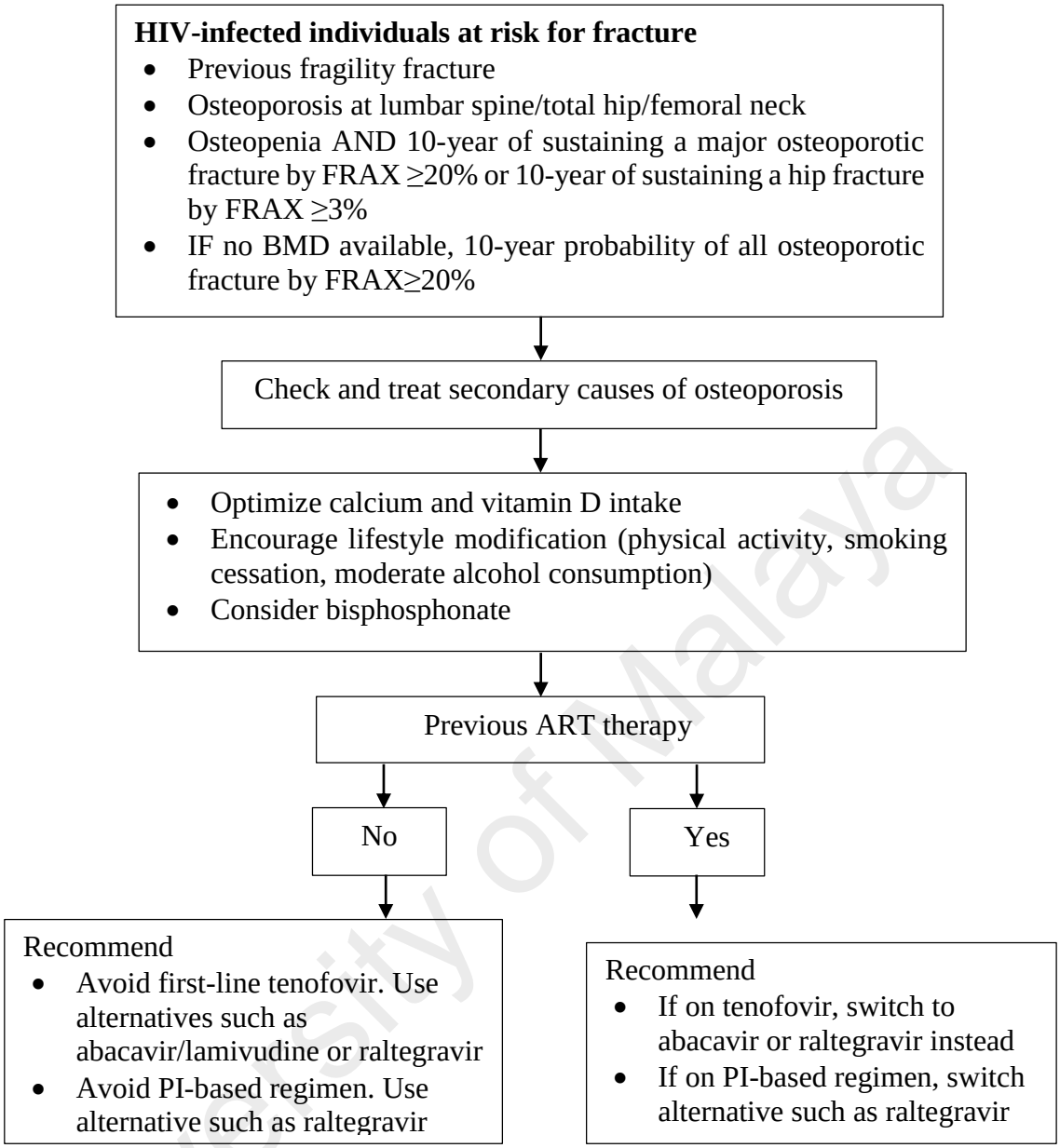


Figure 2:13: Algorithm for the management of antiretroviral therapy in HIV-infected patients at risk of bone disease (Brown, Hoy, et al., 2015)

2.23 Justification

To date, there is a paucity of local data on the prevalence of osteopenia/osteoporosis (reduced BMD) amongst HIV-infected individuals in Malaysia. Therefore, it is important to determine the prevalence of reduced BMD among HIV individual in Malaysia as patients are able to live more longer with the help of ART. Data from our study will help physicians identify the risk factors associated with osteoporosis in HIV-infected individuals, and to assess the 10-year probability of a fracture risk in HIV-infected individuals. Findings from this study will assist the development of a screening strategy for osteoporosis among HIV-infected individuals in Malaysia.

CHAPTER 3: AIMS AND OBJECTIVES

3.1 Aim

To determine the prevalence of osteopenia/osteoporosis (reduced BMD) in HIV-infected individuals versus HIV-uninfected individuals in Malaysia; and its associated risk factors.

3.2 Specific objectives

1) To determine the prevalence of osteopenia/osteoporosis (reduced BMD) in HIV-infected individuals versus HIV-uninfected individuals in Malaysia
2) To assess the vitamin D level in HIV-infected individuals versus HIV-uninfected individuals in Malaysia
3) To compare the 10-year probability of a fracture risk in HIV-infected individuals versus HIV-uninfected individuals in Malaysia
4) To determine the risk factors associated with osteopenia/osteoporosis (reduced BMD) in HIV-infected individuals

CHAPTER 4: METHODOLOGY

4.1 Study design and period

This cross sectional study was conducted from September 2014-September 2016.

4.2 Study setting

HIV-infected individuals were recruited from the Infectious Disease Clinic, University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia; whilst HIV-uninfected individuals of the same gender and age were recruited from the community. Our study was a subset of the MeLOR study. Hence, HIV-uninfected individuals aged ≥55 years were recruited via the Malaysian Elders Longitudinal Research (MELoR) study, which was to assess and investigate the needs, issues and challenges of the Malaysian growing older population. Whereas, we had to recruit HIV-uninfected participants between age 24-54 years old from the community to match the HIV-infected individuals recruited.

4.2.1 Participants

4.2.1.1 Human immunodeficiency virus--infected individuals

All participants recruited were adults aged ≥25 years old, reported to be virologically suppressed (HIV RNA <50 copies/mL), on ART for at least one year, not having no acute illness at the point of recruitment, and of Malaysian citizenship (defined as by having a blue identification card). We only recruited Malaysians because the aim of our study was to Malaysian population data on the prevalence of osteopenia or osteoporosis among HIV-infected individuals. Excluded were any participant reported or suspected to be pregnant, or having implants in the femoral neck or/and lumbar spine.

4.2.1.2 Human immunodeficiency virus-uninfected individuals

All participants recruited were adults aged ≥25 years old, who were not infected with HIV at the point of recruitment and of Malaysian citizenship. All HIV-uninfected individuals were consented to a rapid HIV screening test prior to enrolment to exclude HIV infected individuals. Excluded were any participant who were reported or suspected to be pregnant, or having implants in the femoral neck or/and lumbar spine.

4.2.2 Sample size calculation

Sample size was calculated using the Open Source Epidemiologic Statistics for Public Health (OpenEpi version 3.01) ("Open Source Epidemiologic Statistics for Public Health," 2013). A search of published literature found that the proportion of HIV-infected individuals with osteoporosis was 21.2% (Knobel et al., 2001). Hence, the total number of participants required for our study was 158 participants (i.e. 79 participants in the HIV-infected and HIV-uninfected individuals, respectively),with a confidence level of 95%, and 80% power.

4.2.3 Instruments used

Six instruments were used in our study: the rapid HIV screening test, the baseline structured questionnaire, the digital medical scale, the DXA scan, the vitamin D machine and the FRAX.

4.2.3.1 Rapid human immunodeficiency virus screening test

A rapid HIV screening test (Alere HIV Combo, Chiba, Japan) was used to screen for HIV in uninfected participants prior to enrolment.

4.2.3.2 Baseline structured questionnaire

A baseline structured questionnaire was used to collect participant's socio-demographic data, family & social history, medical history, past and current medication use.It took about 45 minutes to complete this questionnaire (Appendix E).

4.2.3.3 Digital medical scale

A digital medical scale (SECA, Hamburg, Germany) was used to measure the height and weight of participants.

4.2.3.4 Dual X-ray absorptiometry scan

A DXA machine (GE Lunar Prodigy Advance, Diegem, Belgium) was used to measure the BMD of the femoral neck and lumbar spine. Each DXA scan was performed by a trained radiographer from the Nuclear Medicine Department, UMMC. Each DXA scan took approximately 15 minutes to complete.

4.2.3.5 Vitamin D machine

Vitamin D levels sent to Gribbles Pathology (Malaysia) Sdn Bhd, in Petaling Jaya, Selangor, Malaysia. We were not able to obtain the make and model of the machine that measured vitamin D levels, as Gribbles Pathology's policy was that they could not release this information.

4.2.3.6 Fracture risk assessment tool (FRAX®)

FRAX is an online tool used to calculate the 10-year probability of a fracture risk. However, the Malaysian FRAX tool was not available during our study period. Hence, we used the FRAX that was validated in Singaporean Malays, Chinese and Indians in our participants aged between 40 to 90 years old (Kanis et al., 2009). This tool was selected

as the Malays, Chinese and Indians in Singapore and Malaysia are similar [Appendix F](Ministry of Health, 2012).

4.2.4 Outcomes measures

4.2.4.1 Primary outcomes

The primary outcome of our study was to compare the prevalence of osteoporosis or osteopenia (reduced BMD) in both HIV-infected versus HIV-uninfected individuals.

4.2.4.2 Secondary outcomes

The secondary outcomes were: to compare the vitamin D levels, the 10-year probability of a fracture risk in HIV-infected individuals versus HIV-uninfected individuals. Factors associated with reduced BMD in HIV-infected individuals were also studied.

(a) ***Comparison of vitamin D level in HIV-infected individuals versus HIV-uninfected individuals***

Vitamin D levels were measured using serum concentration of 25-hydroxy vitamin D obtained from blood. Fasting blood samples were collected in the morning from participants between 8amuntil 11 am.

Our study adopted the vitamin D level definition from the International Osteoporosis Foundation (IOF), which the cut-off values are of 50-75 nmol/L for vitamin D deficiency and <75nmol/L for vitamin D insufficiency. This is due to vitamin D level >75nmol/L is necessary to minimize the risk of falls and fracture in elderly (Dawson-Hughes et al., 2010). While, individuals who have vitamin D <50nmol/L were associated with

parathyroid hormone suppression which can lead to secondary osteoporosis (Fahrleitner et al., 2002; Kuchuk et al., 2009) as described in section 2.16.1.2 (j).

(b) ***Comparison of the 10-year probability of a fracture risk in HIV-infected individuals versus HIV-uninfected individuals***

The 10-year probability of a fracture risk in both HIV-infected versus HIV-uninfected individuals aged 40 years old and above was calculated using the FRAX.

(c) ***Risk factors associated with reduced bone mineral density in HIV-infected individuals***

Traditional risk factors for reduced BMD that were assessed in this study were: age, gender, ethnicity, family history of osteoporosis, personal history of a fracture, social history (physical activity, smoking, alcohol intake, caffeine intake), BMI, incidence of fall and menopause status. HIV-related risk factors that were assessed were; CD4+ cell count, HIV viral load, duration of HIV and types of ART use.

4.2.5 Pilot study

A pilot study was conducted in October 2013 to assess the feasibility of the study. A research assistant approached 39 potential HIV-infected individuals. Of the 39 potential participants that were approached, 14 refused to participate, and 17 defaulted clinical assessment and blood taking on the appointed date. Therefore, only eightparticipants were recruited for the pilot study (response rate=20.5%).

The low response rate of this pilot study could be due to several reasons: too time consuming to come for another clinic assessment/blood taking appointment, lack of compensation and a lengthy baseline questionnaire were too lengthy.

Therefore, several modifications were made. Firstly, doctors in the Infectious Disease clinic approached potential participants, and explained the purpose of the study, instead of research assistant. If the patient agreed to participate, written informed consent was obtained by the doctor. The patient was then referred to the research assistant, who provided a mutually agreed date for the patient to come back for clinic assessment and blood taking.

Secondly, participants were explained on benefits of participating in this study. Clinical assessments and blood taking charges (worth RM 950) would be waived.

Thirdly, the baseline structured questionnaire interview took more than 30 minutes to complete. To solve this issue, participants were informed upfront that this interview would last approximately 45 minutes. Initially, the interview was only conducted in English, but many participants were not fluent in English. Hence, the Malay and Chinese baseline structured questionnaire were created to overcome this problem.

4.2.6 Study protocol

The flow on how participants were recruited is shown in Figure 4.1.

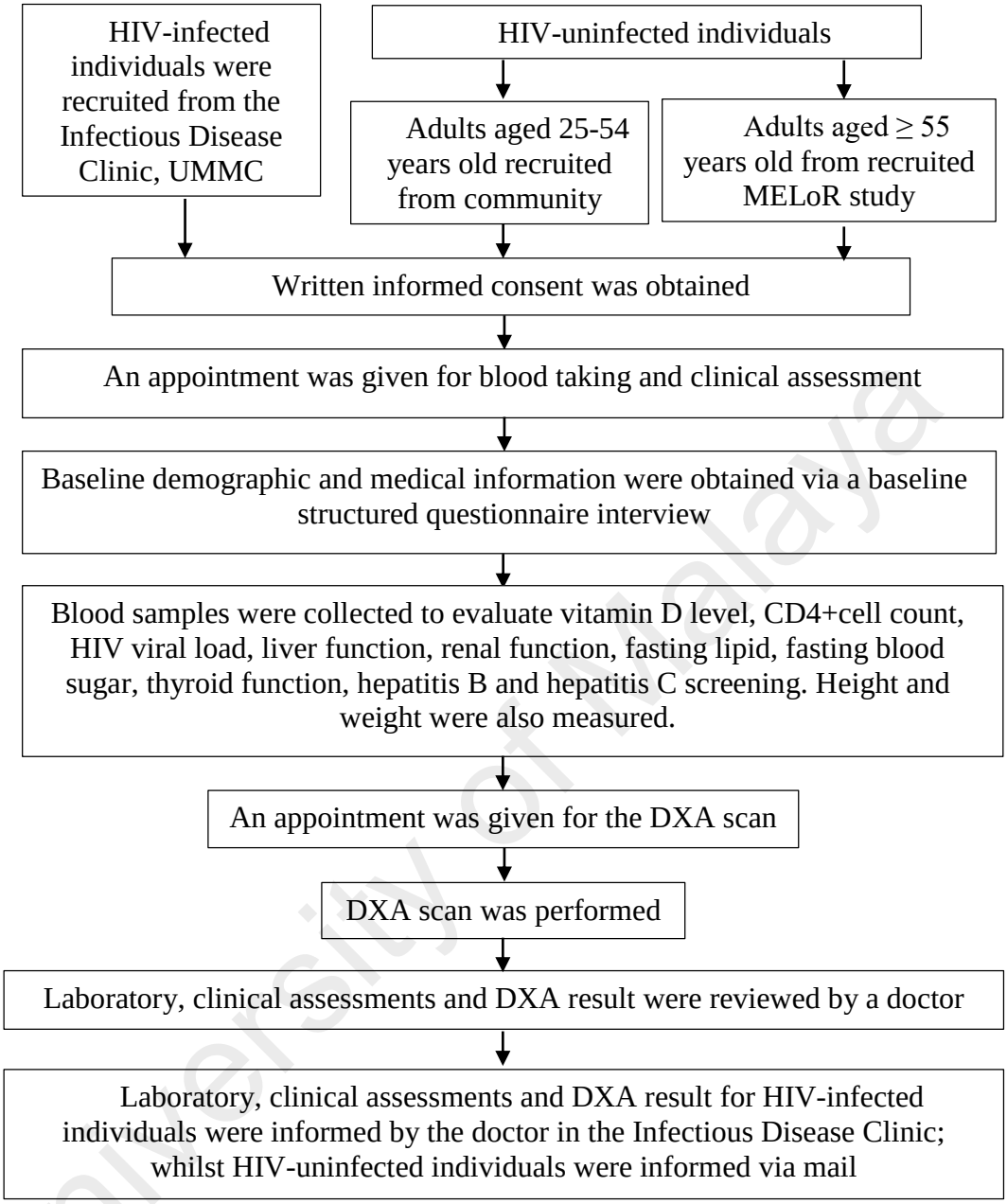


Figure 4:1: Flowchart on how participants were recruited

4.2.6.1 Sampling

Participants were recruited via convenient sampling. Ideally, random sampling is the best method in selecting participants. This is because sampling bias can be eliminated as each individual in the target population will have the equal chance of being selected to participate in the study. In random sampling, the entire target population list is needed before the randomization of potential participants can be performed. However, it is not possible for us to conduct this sampling method as it is too labour intensive and costly. Infectious disease clinic is a high patient flow clinic and patients generally do not come into the clinic in a particular order. Hence, we use convenient sampling because of its lower cost and easier accessibility to participants.

4.2.6.2 Study procedure during participants' recruitment

(a) ***HIV-infected individuals***

Potential participants who came to the Infectious Disease clinic, and who fulfilled the inclusion criteria were identified by researchers. The medical folder of potential participants were tagged so that the doctor would know which participants were eligible. The doctor explained the purpose of the study to each potential participant by using the patient information sheet (Appendix G). For those who agreed to participate, a written informed consent (Appendix I) was obtained by the doctor. Participants were then referred to the research assistant, who provide a mutually agreed appointment date for the participant to come in for their blood taking and clinic assessment at the Gynaecology clinic, Women and Children Health Complex, UMMC. Participants were instructed to fast overnight before coming for their appointment.

(b) ***HIV-uninfected individuals***

Participants aged ≥55 years were recruited via the MELOR study, whilst those aged 25-54 years old were recruited from the community. Potential participants who fulfilled the inclusion criteria were identified and approached by a research assistant. The research assistant explained the purpose of the study by using the patient information sheet (Appendix H). For those who agreed to participate, a written informed consent (Appendix I) was obtained. The research assistant then provides a mutually agreed appointment date for the participant to come for their blood taking and clinic assessment at the Gynecology clinic, Women and Children Health Complex, UMMC. Participants were instructed to fast overnight before coming for their appointment.

4.2.6.3 Study procedure during blood taking and clinical assessment

On the appointed date and time, participants were registered at the registration counter. Fasting blood samples were collected from the participant using a serum tube without clot activator gel to evaluate vitamin D level and viral load (only applicable to HIV-infected individuals); serum tube with clot activator gel, to evaluate liver function, renal function, fasting lipid, thyroid function, hepatitis B and hepatitis C screening; ethylenediaminetetraacetic acid (EDTA) tubes to evaluate HbA1CandCD4+cell count (only applicable to HIV-infected individuals);sodium fluoride tube to evaluate fasting glucose.

The height and weight of each participant was measured. Participants were interviewed by research assistant using a baseline structured questionnaire. An appointment for DXA scan was then given to participants. Participants were advised to stop any calcium and multivitamin supplements intake three days before the DXA scan.

On the appointed date, participants had to register at the Nuclear Medicine Department, UMMC, using the Request for Nuclear Medicine Study (BK-MIS-181-E02) (Appendix J) form and the University of Malaya Research Imaging Centre (UMRIC) Investigation Approval form (Appendix K). Each DXA scan was performed by a trained radiographer. Participants were instructed to lie still on the machine for approximately 15 minutes until the test was completed. All laboratory, clinical assessments and DXA results were informed by doctors to HIV-infected participant on their next clinic visit. While in HIV-uninfected individuals, all results were reviewed by doctors and then informed to participants via mail.

4.2.7 Ethics approval

Ethics approval was obtained from the University Malaya Medical Centre, Medical Ethic Committee (approval number: 943.6) prior to the commencement of the study (Appendix L).

4.2.8 Data analysis

Data was analysed using Statistical Package for the Social Sciences (SPSS) version 20 (New York, United States). Both HIV-infected and HIV-uninfected participants were first matched for gender, then age (that were within the same 10-year age range). This is because women are known to be more susceptible to osteoporosis compared to men. After menopause, women will experience a drastic decreased of oestrogen hormone which helps regulates bone formation and this leads to significant bone loss (Manolagas et al., 2013). In addition, women have smaller body frames which lead to greater risk of developing osteoporosis as they have less bone mass to lose when compared to men (Seeman, 2001). Besides, older age has been associated with increased risk of developing

osteopenia/ osteoporosis as individuals losses bone mass over time (Clarke & Khosla, 2010; Ji & Yu, 2015).

Normality was assessed using the Kolmogorov–Smirnov test. As our data were not normally distributed, continuous variables were expressed in median and interquartile range, whilst categorical data were expressed in frequency and percentage.

Univariate analysis between continuous and categorical data in HIV-infected individuals was performed using the Mann-Whitney U test. The chi-square test was used to determine if there was any association between categorical variables. Any association between variables that had a p value of <0.25 were then included in the multiple logistic regression.

Multiple logistic regression was then performed to determine the association between traditional and HIV-related risk factors with reduced BMD in HIV-infected individuals. A p value of <0.05 was regarded as statistically significant.

CHAPTER 5: RESULTS

A total of 684 potential participants were approached; of which 640 participants agreed to participate (response rate=93.6%). Participants were then matched for gender and age, finally giving 206 participants in each group [Figure 5.1].

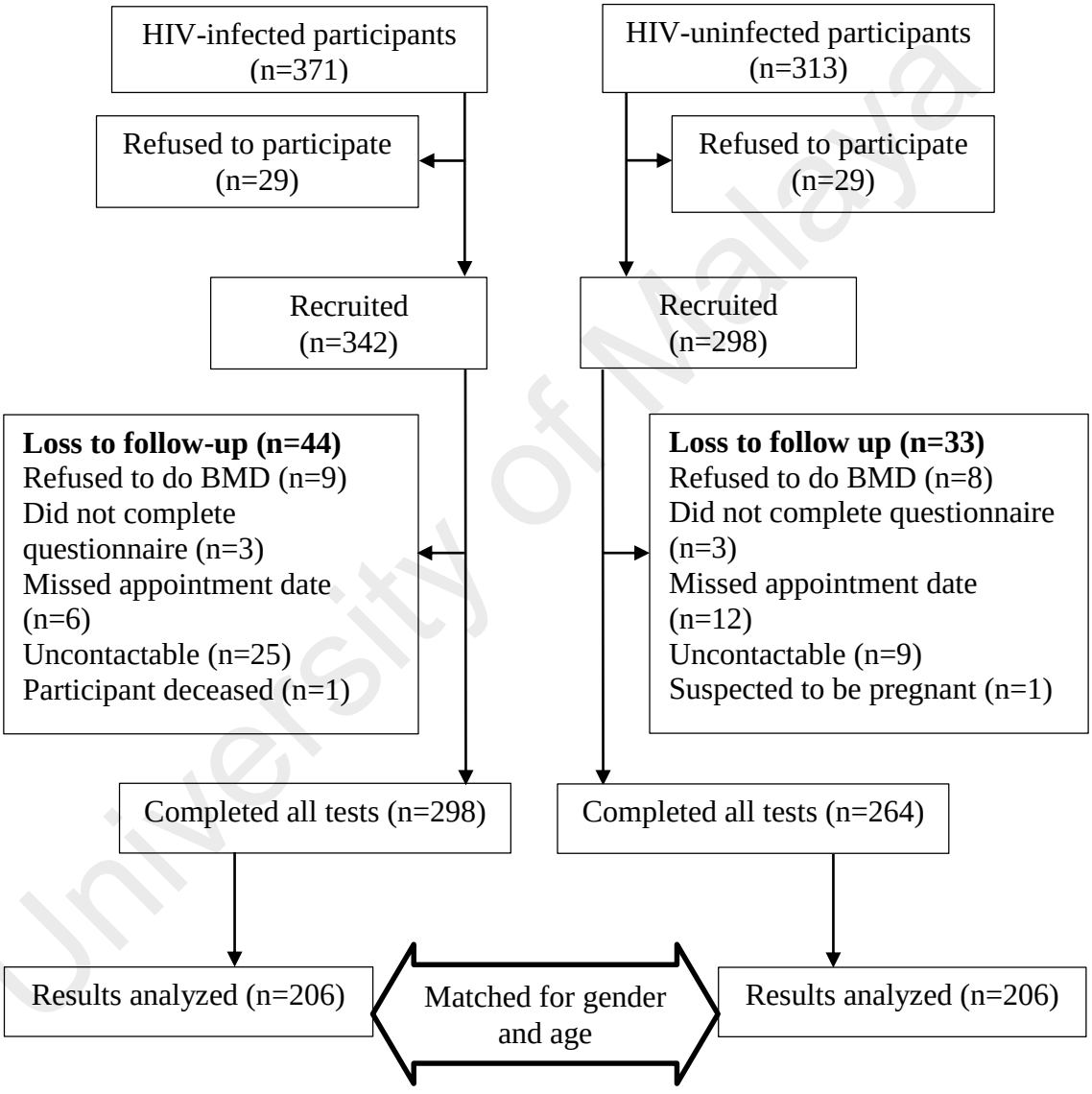


Figure 5:1: Flow chart of response rate and recruitment process

5.1 Demographic characteristics of participants

The majority of participants were male (73.8%) and Chinese (64.1%) with the median age of 40 years old. HIV-infected individuals were found to have significantly lower BMI, and have lost ≥4.5kg within the past 12 months. In addition, significantly more HIV-infected individuals were found to be current smokers [Table 5.1].

Table 5:1: Demographic and clinical characteristics of participants

	HIV-infected (n=206) n (%)	HIV-uninfected (n=206) n (%)	p-value
Gender			
Male	152 (73.8)	152 (73.8)	1.000
Female	54 (26.2)	54 (26.2)	
Median age (years) [IQR]	40.0 [35.0-51.0]	39.5 [31.8-51.0]	0.202
Ethnicity			
Malay	36(17.5)	59 (28.7)	0.027*
Chinese	142(68.9)	122 (59.2)	
Indian	28(13.6)	25 (12.1)	
Median BMI (kg/m^2)[IQR]	22.8[20.1-25.8]	24.8 [22.3-28.2]	<0.001*
Underweight (<18.5)	20(9.8)	9 (4.5)	0.001*
Normal weight (18.5 – 24.9)	121(59.3)	95 (47.3)	
Overweight (25.0 – 29.9)	52(25.5)	68 (33.8)	
Obese (≥30.0)	11(5.4)	29 (14.4)	
Family history of osteoporosis	8 (4.0)	12 (6.2)	0.312
No. of women who were post-menopausal	14 (28.0)	13 (24.5)	0.689
Previous fracture	30 (14.6)	24 (11.7)	0.381
Site of previous fracture			
Hip fracture	4(13.3)	1(4.0)	0.465
Wrist fracture	7(23.3)	7(28.0)	
Backbone fracture	1(3.3)	0	
Other sites (e.g leg, arm,ankle)	18(60.0)	17(68.0)	
No. of participants that fell within the past 12 months [median, IQR]	26 (12.8) [1.0,1.0-2.0]	29 (14.1) [1.0, 1.0-2.0]	0.707 0.786
No. of participants that lost ≥4.5kg within the past 12 months	33 (16.3)	19 (9.2)	0.033*
Currently consuming alcohol	86 (42.8)	82 (39.8)	0.541
>6 units/day	2 (2.3)	2(2.4)	0.369
3-6 units /day	15 (17.2)	9(11.0)	
≤2 units /day	25 (28.7)	18 (22.0)	
None	45 (51.7)	53 (64.6)	
Current smoker [median, IQR]	47 (23.5) [10, 3.5-17.5]	28 (13.7) [5.0, 3.0-15.0]	<0.001* 0.421
Exercise regularly (≥3 times/week)	94 (46.8)	101 (52.3)	0.269
No. of participants that consumed caffeinated drinks	134 (66.7)	137 (71.0)	0.355
No. of participants that consumed calcium	8 (4.0)	17 (12.3)	0.051

BMI=Body mass index, IQR=interquartile range, kg=kilogram, m=metre, No.=numberHIV=human immunodeficiency virus

* Statistically significant=p-value<0.05

HIV infection were mainly transmitted through heterosexual (48.3%) or homosexual contact (49.3%). The median duration of HIV infection and ART use were 6.0 years [IQR:3.0-10.0] and 60 months [IQR: 34.5-111.0], respectively. The majority of HIV-infected individuals were treated with 2NRTIs+1NNRTI (NNRTI-based) regimens (87.8%); with types of ART used as listed in Table 5.2. The median baseline and current CD4+ cell count in HIV-infected individuals was 147.5 [IQR: 44.5-265.0] cells/μL and 573.5 [IQR: 413.8-761.8] cells/μL, respectively. It was found that 97.6% HIV-infected individuals that had undetectable HIV viral load (≤50copies/mL).

Table 5:2: Types of antiretroviral therapy used

ART-contained in the regimen	HIV-infected (n=206); n (%)
Efavirenz	158(77.1)
Tenofovir	135(65.9)
Emtricitabine	135(65.9)
Lamivudine	69(33.7)
Zidovudine	62(30.2)
Nevirapine	18(8.8)
Ritonavir	20(9.8)
Lopinavir	17(8.3)
Abacavir	7(3.4)
Raltegravir	4(2.0)
Atazanavir	3(1.5)
Darunavir	2(1.0)
Indinavir	1(0.5)

ART=Antiretroviral therapy

5.2 Laboratory parameters of participants

Significantly more HIV-infected individuals had abnormal liver function (elevated ALP and GGT level) and lipid profile (elevated triglyceride and LDL cholesterol level) when compared to HIV-uninfected individuals ($p<0.005$). There was no significant difference in renal function, thyroid function and HbA1c level. [Table 5.3]

HIV-infected individuals co-infected with hepatitis B (5.3%) were significantly more when compared with HIV-uninfected individuals (1.5%) [$p=0.032$]. However, there were no significant difference between HIV-infected individuals co-infected with hepatitis C (1.0%) when compared with HIV-uninfected individuals (0.5%) [$p=0.571$].

Table 5:3: Laboratory parameters of participants

	HIV-infected (n=206); n (%)	HIV-uninfected (n=206); n (%)	p-value
Abnormal renal function (eGFR <60ml/min/1.73m^2)	6 (2.9)	1 (0.5)	0.122
Abnormal liver function			
ALP (>147 U/L)	13 (6.3)	2 (1.0)	0.004*
GGT (>51 U/L)	101 (49.0)	34 (16.5)	<0.001*
AST (>123 U/L)	1 (0.5)	0	0.100
ALT (>153 U/L)	1 (0.5)	2 (1.0)	0.100
Total bilirubin (>21(umol/L)	6 (2.9)	14 (6.8)	0.067
Abnormal thyroid function			
Thyroid stimulating hormone (mIU/L) Low level (<0.2 mIU/L) High level (>4.7 mIU/L)	 4 (1.9) 4 (1.9)	 6 (2.9) 6 (2.9)	1.000
Abnormal fasting blood sugar profile			
HbA1c (>6.1%)	25 (12.1)	17 (8.3)	0.254
Abnormal fasting lipid profile			
Triglyceride (>1.68mmol/L)	87 (42.2)	62 (30.1)	0.010*
HDL cholesterol (<1.03mmol/L)	29 (14.1)	26 (12.6)	0.664
LDL cholesterol (>2.58mmol/L)	142 (68.9)	178 (86.4)	<0.001*

IQR=interquartile range, eGFR=estimated glomerular filtration rate, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase, AST=aspartate aminotransferase, ALT=amino alanine transferase, HDL=high-density lipoprotein, LDL= low-density lipoprotein, U/L=units per liter, umol/L=micromole per liter, mmol/L=milimol per liter; mIU/L=milli-international units per litre

* Statistically significant=p-value<0.05

5.3 Prevalence of reduced bone mineral density in human immunodeficiency virus-infected individuals versus human immunodeficiency virus-uninfected individuals in Malaysia

A significantly higher number of HIV-infected individuals [152 (73.8%)] had osteopenia/osteoporosis (reduced BMD) when compared to HIV-uninfected individuals [118 (57.3%), p<0.001] [Table 5.4]. The prevalence of osteoporosis was found to be significantly higher in HIV-infected individuals [29(14.1%)] when compared to HIV-uninfected individuals [11(5.3%), p<0.001]. The percentage difference of BMD between

HIV-infected and HIV-uninfected individuals at the lumbar spine (5.1%) and femur neck (5.3%), respectively.

Table 5:4: Prevalence of reduced bone mineral density in HIV-infected versus HIV-uninfected individuals

	HIV-infected (n=206); n (%)	HIV-uninfected (n=206); n (%)	p-value	%BMD difference
Bone mineral density				
Normal Reduced BMD	54 (26.2) 152 (73.8)	88 (42.7) 118 (57.3)	<0.001*	
Normal Osteopenia Osteoporosis	54 (26.2) 123 (59.7) 29 (14.1)	88 (42.7) 107 (51.9) 11 (5.3)	<0.001*	
Lumbar spine [median, IQR]				5.1
BMD (g/cm^2)	1.11 [1.03-1.21]	1.17 [1.09-1.28]	<0.001*	
T-score	-0.90 [-1.60- -0.18]	-0.30 [-1.00-0.53]	<0.001*	
Z-score	-0.80 [-1.40-0]	-0.10 [-0.93-0.70]	<0.001*	
Femoral neck [median, IQR]				5.3
BMD (g/cm^2)	0.88 [0.78-0.95]	0.93 [0.86-1.03]	<0.001*	
T-score	-1.40 [-2.00- -0.80]	-1.00 [-1.60- -0.20]	<0.001*	
Z-score	-1.00 [-1.50- -0.30]	-0.60 [-1.20-0.13]	<0.001*	

BMD=bone mineral density, HIV=human immunodeficiency virus; IQR=interquartile range, g/cm^2=gram per square centimeter
* Statistically significant=p-value<0.05

We conducted a sub-analysis on the prevalence of reduced BMD in PI-treated and tenofovir-treated HIV-infected individuals. Significantly, more PI-treated HIV-infected individuals had reduced BMD, but our results did not reach statistical significance (p=0.066). Reduced BMD was not significantly different between tenofovir treated and non-tenofovir treated individuals [Table 5.5].

Table 5:5: Prevalence of reduced bone mineral density in protease inhibitor-treated and tenofovir-treated HIV-infected individuals

HIV-infected individuals	Normal (n=75)	Reduced BMD (n=222)	p-value
PI-treated individuals	5 (6.7)	33 (14.9)	0.066
Tenofovir-treated individuals	53 (70.7)	147 (66.2)	0.477

BMD=bone mineral density; HIV=human immunodeficiency virus; PI=protease inhibitor

5.4 Vitamin D level in human immunodeficiency virus-infected individuals versus human immunodeficiency virus -uninfected individuals in Malaysia

Similarly, vitamin D deficiency (<50nmol/L) was significantly higher in HIV-infected [134(65.0%)] when compared to HIV-uninfected individuals [62(30.1%)] (p<0.001). [Table 5.6]. Median vitamin D levels were significantly lower in HIV-infected (42.4, IQR=32.7-59.8) than HIV-uninfected individuals (57.0, IQR=47.5-67.0).

Table 5:6: Vitamin D level of individuals

	HIV-infected (n=206); n (%)	HIV-uninfected (n=206); n (%)	p-value
Vitamin D level			
Median vitamin D level[IQR]	42.4 [32.7-59.8]	57.0 [47.5-67.0]	<0.001*
Adequate (>75nmol/L)	17 (8.3)	25 (12.1)	<0.001*
Insufficient (50-75nmol/L)	55 (26.7)	119 (57.8)	
Deficient(<50nmol/L)	134 (65.0)	62 (30.1)	

HIV=human immunodeficiency virus; IQR=interquartile range
* Statistically significant=p-value<0.05

5.5 The 10-year probability of a fracture risk in human immunodeficiency virus -infected individuals versus human immunodeficiency virus-uninfected individuals in Malaysia

In this sub-analysis, we only managed to calculate the 10-year probability of a fracture risk in 109 HIV-infected individuals versus 103 HIV-uninfected individuals respectively as FRAX was developed and primarily validated in individuals that were over 40 years

of age (Kanis et al., 2009). To date, the modified-FRAX algorithm in HIV-infected individuals used to calculate the 10-year probability of a hip fracture or major osteoporotic fracture has not been fully developed and validated.

The 10-year probability of sustaining a hip fracture in HIV-infected individuals was 0.4% compared to 0.2% in HIV-uninfected individuals (p=0.003). There was no significant difference in the probability of major osteoporotic fracture between the two groups [HIV-infected (1.7%); HIV-uninfected (1.3%)] (p=0.066).

5.6 Risk factors associated with reduced bone mineral density in human immunodeficiency virus-infected individuals

When univariate analysis was performed in HIV-infected individuals, traditional reduced BMD risk factors [older age, Malay or Chinese ethnicity, lower BMI, family history of osteoporosis, history of previous hip and wrist fracture, reduced physical exercise, post-menopausal women, history of alcohol consumption (≥6 units/ day), consumed caffeine drinks and calcium supplement] were found to be significantly associated with reduced BMD [Table 5.7]. More HIV-infected individuals with reduced BMD had a significantly higher percentage of 10-year probability of sustaining a hip fracture and major osteoporotic fracture. HIV-related reduced BMD risk factors that were found associated with reduced BMD were mode of transmission of HIV (heterosexual and homosexual), NNRTI-based ART, PI-based ART, nevirapine used, lopinavir used, and ritonavir used. Significantly, more HIV-infected individuals with reduced BMD had abnormal liver function (elevated GGT level) and fasting lipid profile (lower HDL cholesterol level) [p<0.25].

Table 5:7: Univariate analysis of the risk factors associated with reduced bone mineral density

Risk factors	Univariate analysis		
	HIV-infected (n=298); n (%)		
	Normal (n=76)	Reduced BMD (n=222)	p-value
Gender			
Male	65 (85.5)	179 (80.6)	0.339
Female	11 (14.5)	43 (19.4)	0.616
Median age (years) [IQR]	42.0 [36.0-48.8]	44.0 [38.0-52.0]	0.082*
Ethnicity			
Malay	17 (22.4)	35 (15.8)	0.191*
Chinese	49 (64.5)	163 (73.4)	0.137*
Indian	10 (13.2)	24 (10.8)	0.579
Median BMI (kg/m^2) [IQR]	24.5 [21.5-27.5]	22.4 [20.1-25.1]	<0.001*
Underweight (<18.5)	3(4)	24(10.8)	0.111*
Normal weight (18.5 – 24.9)	35(46.1)	140 (63.1)	0.007*
Overweight (25.0 – 29.9)	25(32.9)	51(23.0)	0.061*
Obese (≥30.0)	12(15.8)	7(3.2)	<0.001*
Mode of transmission of HIV			
Homosexual	38 (50.7)	91 (41.2)	0.152*
Heterosexual	34 (45.3)	122 (55.2)	0.139*
Other mode of transmission (e.g blood transfusion, intravenous drug use)	3 (4.0)	8 (3.6)	0.880
Median duration of HIV diagnosis (years)[IQR]	6.0 [3.0-11.0]	7 [4.0-11.0]	0.552
Current ART regimen			
2NRTIs+1NNRTI (NNRTI-based)	69 (92.0)	183 (82.4)	0.046*
2NRTIs+PIs(PI-based)	5 (6.7)	33 (14.9)	0.066*
**Other combinations (e.g. integrase inhibitor-based)	1 (1.3)	6 (2.7)	0.499
ART-contained in the regimen			
Efavirenz	57(76.0)	165(74.3)	0.773
Tenofovir	53(70.7)	147(66.2)	0.477
Emtricitabine	53(70.7)	147(66.2)	0.477
Lamivudine	21(28.0)	74(33.3)	0.392
Zidovudine	20(26.7)	65(29.3)	0.665
Nevirapine	12(16.0)	18(8.1)	0.050*
Ritonavir	5(6.7)	32(14.4)	0.079*
Lopinavir	4(5.3)	26(11.7)	0.113*
Abacavir	1(1.3)	9(4.1)	0.259
Raltegravir	1(1.3)	6(2.7)	0.499
Atazanavir	1(1.3)	5(2.3)	0.625
Darunavir	1(1.3)	1(0.5)	0.419
Indinavir	0	1(0.5)	0.560
Median duration of ART (months)[IQR]	68 [39.0-111.0]	76.5[42.8-124.5]	0.489

Table 5.7: Univariate analysis of the risk factors associated with reduced bone mineral density *(continued)*

Risk factors *(continued)*	Univariate analysis		
	HIV-infected (n=298); n (%)		
	Normal (n=76)	Reduced BMD (n=222)	p-value
Median baseline CD4+cell count (cells/µL) [IQR]	127.0 [44.0-254.0]	123.0 [35.8-255.3]	0.830
Median current CD4+cell count (cells/µL) [IQR]	579.0 [412.5-733.5]	547.5 [393.3-764.0]	0.712
Viral Load (copies /mL)			
Detected (>50 copies/mL)	3 (3.9)	10 (4.5)	0.837
Not detected (≤50copies/mL)	73 (96.1)	212 (95.5)	0.837
Abnormal renal function (eGFR <60ml/min/1.73m^2)	2 (2.6)	7 (3.2)	0.819
Abnormal Liver function			
ALP (>147 U/L)	3(3.9)	16(7.2)	0.308
GGT (>51 U/L)	46(60.5)	109(49.1)	0.085*
AST (>123 U/L)	0	2(0.9)	0.406
ALT (>153 U/L)	0	3(1.4)	0.573
Total bilirubin (>21(umol/L)	3(3.9)	9(4.1)	0.967
Abnormal thyroid function			
Thyroid stimulating hormone (mIU/L)			
Low level (<0.2 mIU/L)	1(1.3)	1(0.5)	0.425
High level (>4.7 mIU/L)	3(3.9)	5(2.3)	0.430
Abnormal fasting blood sugar level			
HbA1c (>7.5%)	2(2.6)	10(4.5)	0.437
Abnormal fasting lipid profile			
Triglyceride (>1.68mmol/L)	31(40.8)	104(46.8)	0.360
HDL cholesterol (<1.03mmol/L)	18(23.7)	32(14.4)	0.062*
LDL cholesterol (>2.58mmol/L)	47 (63.5)	144 (67.9)	0.488
Hepatitis Bs Antigen [detected]	4 (5.3)	10 (4.5)	0.787
Hepatitis C Antibody [detected]	1 (1.3)	7 (3.2)	0.392
Vitamin D level			
Adequate (>75nmol/L)	5 (6.6)	23 (10.4)	0.325
Insufficient (50-75nmol/L)	23 (30.3)	65 (29.4)	0.888
Deficient(<50nmol/L)	48 (63.2)	133 (60.2)	0.646
Family history of osteoporosis	1 (1.4)	11 (5.0)	0.179*
No. of women who were post-menopausal	0	14 (35.9)	0.019*
Previous fracture	8 (10.5)	32 (14.4)	0.391
Hip fracture	2 (28.6)	2 (6.2)	0.078*
Wrist fracture	0	7 (21.9)	0.172*
Backbone fracture	0	2 (6.2)	0.497
Other fracture	5 (71.4)	21 (65.6)	0.768

Table 5.7: Univariate analysis of the risk factors associated with reduced bone mineral density *(continued)*

Risk factors *(continued)*	Univariate analysis		
	HIV-infected (n=298); n (%)		
	Normal (n=76)	Reduced BMD (n=222)	p-value
No. of participants that fell within the past 12 months	6 (7.9)	27 (12.4)	0.286
Median no. of times that the participants fell in the past 12 months [median, IQR]	1.0 [1.0-2.8]	1.0 [1.0-1.0]	0.512
No. of participants that lost ≥4.5kg within the past 12 months	58 (76.3)	175 (80.3)	0.464
History of alcohol consumption	32 (42.7)	97 (44.5)	0.783
>6 units/day	3 (8.6)	3 (2.7)	0.121*
3-6 units /day	7 (20.0)	17 (15.0)	0.487
≤2 units /day	8 (22.9)	36 (31.9)	0.309
None	17 (48.6)	57 (50.4)	0.847
Current smoker [median , IQR]	19 (25.3)	64 (29.5)	0.491
Median number of cigarettes smoked/ day IQR]	10.0 [3.0-20.0]	10.0 [5.0-20.0]	0.988
No. of participants that consumed caffeinated drinks	45 (62.5)	156 (71.2)	0.164*
No. of participants that consumed calcium	0	8 (3.7)	0.100*
FRAX score [IQR]			
Median major osteoporotic fracture	0.9 [0.8-1.3]	1.8 [1.2-3.0]	<0.001*
Median hip fracture	0.1 [0-0.1]	0.4 [0.2-1.1]	<0.001*
Regular exercise (≥3 times/week)	42 (58.3)	90 (41.1)	0.011*

IQR=interquartile range, HIV=human immunodeficiency virus, BMI=body mass index, NRTI=nucleoside reverse transcriptase inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, PI=protease inhibitor, ART=antiretroviral therapy, kg=kilogram, m=meter, mmol/L=milimol per liter, g=gram, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase, AST=aspartate aminotransferase, ALT=amino alanine transferase, BMD=bone mineral density

*Any association between variables that had a p value of <0.25 consider as statistically significant and were then included in the multiple logistic regression.

When a multiple logistic regression was performed, HIV-infected individuals with older age, lower BMI and reduced physical exercise were found to be associated with reduced BMD (Table 5.8).

Table 5:8: Multivariate analysis of the risk factors associated with reduced bone mineral density

Risk factors	OR (95%,Cl)	p-value
Age	1.04 (1.01, 1.07)	0.018*
BMI	0.84 (0.78, 0.91)	<0.001*
Physical activity	2.23 (1.26, 3.97)	0.06*

BMI=body mass index, OR=odds ratio

The odds ratio of developing osteoporosis was significantly higher in HIV-infected individuals aged ≥50 years and those who were underweight (<18.5 kg/m^2). In addition, the odds ratio of developing reduced BMD were significantly higher in HIV-infected individuals who did not exercise regularly (Table 5.9).

Table 5:9: Odds ratio of developing reduced BMD or osteoporosis in human immunodeficiency virus (HIV)-infected individuals

		Reduced BMD	Osteoporosis
Variables		OR (95 %,Cl)	OR (95%,Cl)
Age	< 40 years	Reference	Reference
	≥40 years	1.509 (0.878, 2.594)	1.442 (0.710, 2.927)
	< 50 years	Reference	Reference
	≥50 years	1.837 (0.990,3.410)	2.531 (1.331, 4.812)
BMI	Not underweight	Reference	Reference
	Underweight (<18.5 kg/m^2)	2.182 (0.732, 6.504)	2.983 (1.255, 7.089)
	Not overweight	Reference	Reference
	Overweight (≥25 kg/m^2)	0.373 (0.217, 0.640)	0.719 (0.354,1.460)
Physical activity	Regular exercise (≥ 3 times/ week)	Reference	Reference
	No regular exercise	2.007(1.169, 3445)	1.686 (0.874, 3.251)

BMI=body mass index, OR=odds ratio

CHAPTER 6: DISCUSSION

Our study found that the prevalence of osteopenia/osteoporosis (reduced BMD) was significantly higher in HIV-infected compared to HIV-uninfected individuals. Among the HIV-infected individuals, there was no difference in the prevalence of osteopenia/osteoporosis between those treated with PI or tenofovir compared to those treated with other agents. Vitamin D deficiency was significantly higher in HIV-infected compared to uninfected individuals. The FRAX derived probability of sustaining a hip fracture over the next 10 years was significantly increased in HIV-infected individuals but not in the probability of major osteoporotic fracture. Older age, lower BMI and reduced physical exercise were found to be associated with reduced BMD.

6.1 Prevalence of osteopenia/osteoporosis in human immunodeficiency virus-infected individuals versus human immunodeficiency virus-uninfected individuals

The prevalence of osteopenia/osteoporosis (reduced BMD) was significantly higher in HIV-infected individuals (73.8%) when compared to HIV-uninfected individuals (57.3%). A meta-analysis conducted in 2006 reported that the odds of developing reduced BMD in HIV-infected individuals was 6.4 times higher than HIV-uninfected individuals (Brown & Qaqish, 2006). Similarly, a recent meta-analysis by Goh et al., which included 13 cross-sectional and 6 longitudinal studies found that the odds of developing reduced BMD at the lumbar spine and hip in HIV-infected individuals was 2.4 and 2.6 times higher than HIV-uninfected individuals, respectively (Goh, Lai, Tan, & Ponnampalavanar, 2018).The difference in rates between the two meta-analysis could be due to the addition of seven cross sectional studies (Arnsten et al., 2007; Arnsten et al., 2006; Bolland et al., 2006; Grijsen et al., 2013; Jones et al., 2008; Negredo et al., 2014;

Teichmann et al., 2009), and a larger sample size. The reason for a higher prevalence of lower BMD among HIV-infected individuals when compared to uninfected individuals is likely to be due to a complex interaction of the HIV infection itself, traditional osteoporosis risk factors and/or antiretroviral-related factors (McComsey et al., 2010). A detailed explanation on how HIV infection affects on how HIV infection has been described in section 2.14.

In our study, we found that the prevalence of reduced BMD in HIV-infected individuals was 74%. Previous studies reported rates that ranged from 21% to 82% (Amiel et al., 2004; Arnsten et al., 2007; Arnsten et al., 2006; Bolland et al., 2012; Bolland et al., 2006; Brown et al., 2004; Bruera et al., 2003; Dolan et al., 2004; Dolan et al., 2006; Grijsen et al., 2013; Jones et al., 2008; Loiseau-Pérès et al., 2002; Madeddu et al., 2004; Negredo et al., 2014; Tebas et al., 2000; Teichmann et al., 2009; Teichmann et al., 2003). The difference in the prevalence rates could be attributed to the type of participants recruited. Studies conducted in France (82%) and Germany (76%) reported a higher prevalence of reduced BMD as they only recruited Caucasians (Amiel et al., 2004)and women (Teichmann et al., 2003), whilst studies that reported rates of 27% (Arnsten et al., 2006) and 21.2% (Grijsen et al., 2013) recruited more Blacks (59%) (Arnsten et al., 2006), individuals who were younger (average mean age=37) (Grijsen et al., 2013), and had a higher BMI (62%)(Arnsten et al., 2006). Caucasian, female, older age and lower BMI to be associated with lower BMD as they are known traditional osteoporosis risk factors (International Osteoporosis Foundation, 2015).

The prevalence of osteoporosis in our study was significantly higher in HIV-infected individuals (14.1%) when compared to HIV-uninfected individuals (5.3%). HIV-infected individuals had nearly three times increased risk of developing osteoporosis when

compared to HIV-uninfected individuals, which was similar to previous meta-analysis conducted in 2006 (Brown & Qaqish, 2006).

6.2 Prevalence of osteopenia/osteoporosis in antiretroviral therapy-treated verses non antiretroviral therapy-treated individuals

All HIV-infected individuals in our study were treated with ART. Hence, we were unable to compare the effects of bone loss in ART-treated versus non ART-treated individuals.

6.2.1 Prevalence of osteopenia/osteoporosis in protease inhibitor-treated versus non protease inhibitor-treated individuals

The prevalence of osteopenia/osteoporosis (reduced BMD) in PI-treated individuals in our study was 14.9%. Previous studies reported rates which ranged from 25% to 59% (Bruera et al., 2003; Calmy et al., 2009; Carr et al., 2001; de Menezes Barbosa et al., 2013; Libois et al., 2010; Madeddu et al., 2004; Tebas et al., 2000; Tomazic et al., 2007). The difference in the prevalence rates could be because only 13% of our participants were treated with PIs. In Malaysia, PIs are generally used as second-line treatment (Ministry of Health, 2016a). First-line ART which consists of NNRTI-based drugs are replaced by PIs when patients are unable to tolerate first-line drugs or when viral load suppression is not achieved (Ministry of Health, 2016a; World Health Organisation, 2013). Previous meta-analysis reported that the odds of developing reduced BMD in PI-treated individuals was approximately 1.5 times higher when compared to non PI-treated individuals (Goh et al., 2018).However, this result was not statistically significant. It is postulated that the cause of reduced BMD in PI-treated individuals may be based on specific drug instead of specific drug class. PI like ritonavir has been reported to suppress osteoclastogenesis and osteoclast function whilst indinavir had no effect on osteoclastogenesis (Wang et al.,

2004). A detailed explanation on how PIs affect bone loss has been described in Section 2.16.2.3(a).

6.2.2 Prevalence of osteopenia/osteoporosis in tenofovir-treated versus non tenofovir-treated individuals

The prevalence of osteopenia/osteoporosis (reduced BMD) in tenofovir-treated individuals in our study was 66.2%, which was slightly higher than a previous study (52.6%) (Calmy et al., 2009). Tenofovir is mainly used as the first-line ART in Malaysia (Ministry of Health, 2016a) and 67% of our participants were on tenofovir. This could have accounted for the slightly higher prevalence rate. Tenofovir-treated individuals were found to have proximal renal tubular dysfunction(Walker Harris & Brown, 2012). This could result in excessive renal phosphate loss, which can then impair bone mineralization leading to BMD loss (Walker Harris & Brown, 2012).

6.3 Vitamin D level in human immunodeficiency virus-infected individuals versus human immunodeficiency virus -uninfected individuals in Malaysia

The proportion of HIV-infected individuals with vitamin D deficiency (<50nmol/L) was twice higher (65.0%) when compared to HIV-uninfected individuals (30.1%). Our finding was different from previous studies which reported no significant difference in prevalence of vitamin D deficiency between HIV-infected individuals and HIV-uninfected individuals (Adeyemi et al., 2011; Chotalia et al., 2012). The difference in the prevalence rate could be due to the fact that almost 80% of our HIV-infected individuals were prescribed with efavirenz (a NNRTI), which was nearly twice higher than the study conducted by Adeyemi el al (44%) (Adeyemi et al., 2011). Efavirenz has been associated with low vitamin D levels (Dao et al., 2011; Gyllensten, Josephson, Lidman, & Saaf,

2006; Welz et al., 2010) as it can cause induced vitamin D catabolism (Brown & McComsey, 2009; Hariparsad et al., 2004).

6.4 Vitamin D level among human immunodeficiency virus-infected individuals

The prevalence of vitamin D deficiency (<50nmol/L) in our study was 65.0%. Other studies reported that the prevalence of vitamin D deficiency ranged from 27% to 70% (Adeyemi et al., 2011; Avihingsanon et al., 2016; Cervero et al., 2012; Chotalia et al., 2012; Crutchley et al., 2012; French et al., 2011; Kwan, Eckhardt, Baghdadi, & Aberg, 2012; Lerma et al., 2012; Rodriguez, Daniels, Gunawardene, & Robbins, 2009; Rwebembera et al., 2013; Wiboonchutikul et al., 2012). The difference in the prevalence rate could be due the variations in the populations studied.

Our study found that the prevalence of vitamin D deficiency was higher when compared to most studies conducted in United States [range:30%-59%] (Adeyemi et al., 2011; Crutchley et al., 2012; French et al., 2011; Kwan et al., 2012; Rodriguez et al., 2009; Wasserman & Rubin, 2010) and European countries [range:39%-44%] (Cervero et al., 2012; Lerma et al., 2012). This could be due to the cultural perception among Asians which has a preference for fairer skin as it is a sign of beauty when compared to their Western counterparts which preferred tanned skin (Jang et al., 2013; Li, E. P. H. et al., 2008). Hence, it may influence Asians to avoid sunlight exposure by wearing clothing styles that covered most parts of their body, using umbrellas and using sunblock or limit outdoor activities which can prevent vitamin D synthesis (Nimitphong & Holick, 2013).

One study reported lower prevalence of vitamin D deficiency when compared to previous study as they recruited mainly Blacks (94.4%) and older persons (mean age=49 years) (Chotalia et al., 2012). Being Black and older are known traditional risk factors for

vitamin D deficiency (Gloth & Tobin, 1995; Janssen et al., 2002; Nair, R. & Maseeh, 2012)

The prevalence of vitamin D deficiency in our study (65.0%) was twice higher than in Thailand [range: 26.8%-29.9%] (Avihingsanon et al., 2016; Wiboonchutikul et al., 2012). Both Thailand and Malaysia are located in South-East Asia which have abundant sunshine. The difference in results could be due to cultural and religious belief. Approximately 60% of Malaysians are Muslims (Department of Statistics Malaysia, 2010). However, only 5% of Thais are Muslims (National Statistical Office, 2011). According to the Quran, female Muslims are encouraged to wear garments that cover the head, arms, body and legs (Shafinaz & Moy, 2016). This decreases skin exposure to sunlight.

We recommend that HIV-infected individuals be exposed to sunlight approximately 10-15 minutes every day (International Osteoporosis Foundation, 2015; Nair, R. & Maseeh, 2012). In addition, vitamin D3 supplements should be prescribed at 6000 IU/day for the first 8 weeks, followed by 2000 IU/day as maintenance dose.

6.5 The 10-year probability of fracture risk in human immunodeficiency virus -infected versus human immunodeficiency virus-uninfected individuals

FRAX was used to evaluate the 10-year probability of fracture risk in our participants aged ≥40 years. Our study found that the 10-year probability of sustaining a major osteoporosis fracture in HIV-infected individuals was higher (1.7%) when compared to HIV-uninfected individuals (1.3%), but our result was not statistically significant. Our finding (1.7%) was slightly higher than a previous study conducted in the United States (1.5%) (Stephens et al., 2016). When we compared the 10-year probability of sustaining

a hip fracture, HIV-infected individuals (0.4%) was significantly higher than HIV-uninfected individuals (0.2%). Our finding was higher (0.4%) than a previous study conducted in the United States (0.1%) (Stephens et al., 2016). These differences could be due to the fact that Stephens et al recruited 90.1% Blacks which are known to have low risk of osteoporosis (International Osteoporosis Foundation, 2015), whereas we recruited 59.2% Chinese which are known to be at high risk for osteoporosis (Chan et al., 2014).

To date, there is no self-assessment tool that has been validated to evaluate osteoporosis and fracture risk in HIV-infected individuals. Hence, the DXA scan is still used as a screening and diagnostic tool for osteoporosis in HIV-infected individuals (Brown, Hoy, et al., 2015). However, in some settings, DXA scans may not be feasible as it is expensive, and only available in tertiary hospitals (Chen et al., 2016). Medications to treat osteoporosis (e.g. bisphosphonates) should be initiated if the 10-year risk of major osteoporotic fracture is ≥20% and/or the risk of sustaining a hip fracture is ≥3% (Brown, Hoy, et al., 2015).

FRAX has its own shortcomings if used in HIV-infected individuals. Firstly, FRAX underestimates the overall fracture risk in HIV-infected individuals as it only considers traditional osteoporosis risk factors and not HIV-related osteoporosis risk factors (Calmy et al., 2009; Gazzola et al., 2010). Secondly, according to published literature, FRAX had a sensitivity of only 22% to screen for reduced BMD (Short, Shaw, Fisher, Gilleece, & Walker-Bone, 2014), and a sensitivity of 31% to screen for osteoporosis in HIV-infected individuals (Gazzola et al., 2010; Short et al., 2014); when compared to DXA scan(Yin, M.T. et al., 2016). A low sensitivity means that if a person has osteoporosis, FRAX may not show a positive result. Thirdly, FRAX cannot be used in individuals aged <40 years as it was constructed from real data population-based cohorts around the world that have

a limited age range from 40 to 90 years old (Kanis et al., 2009; Zhang, Ou, Sheng, & Liao, 2014). If HIV-infected individuals aged <40 years are suspected to have osteoporosis, a DXA scan needs to be performed (National Clinical Guideline Centre, 2012).Lastly, during the period of our study, FRAX has not been validated in Malaysia. Hence, we used FRAX that was validated in Singapore as the Malays, Chinese and Indians in Singapore and Malaysia are ethnically similar (Ministry of Health, 2012).

Therefore, FRAX is not recommended to replace DXA scan as a gold standard to screen for osteoporosis. It should be used initially to assess the possible risk of fracture in HIV-infected individuals. Further studies are needed to develop a more comprehensive risk assessment tool to screen HIV-infected individuals who have osteoporosis.

6.6 Risk factors associated with osteopenia/osteoporosis in human immunodeficiency virus-infected individuals

In this section, we analysed the risk factors associated with osteopenia/osteoporosis (reduced BMD) in HIV-infected individuals only. The risk factors associated with reduced BMD in HIV-infected individuals can be divided into traditional and HIV-related osteoporosis risk factors.

6.6.1 Traditional osteoporosis risk factors

Lower BMI, reduced physical activity and older age were significantly associated with reduced BMD, and our findings were similar to previous studies (Amiel et al., 2004; Bonjoch et al., 2010; Carr et al., 2001; Cazanave et al., 2008). However, we did not find any association between gender, ethnicity, family history of osteoporosis, personal history of fracture, smoking history, history of alcohol consumption and caffeinated drinks, with reduced BMD. This may be because the majority of our participants were

male (82%), and males are known to be less susceptible to osteoporosis than females (Manolagas et al., 2013). In our study, ethnicity did not play a significant role in bone loss. This was different from a previous study conducted in postmenopausal women in a tertiary hospital in Malaysia, as the prevalence of reduced BMD at the hip were higher in Chinese (62%), followed by Malay (26%) and Indian(10%) (Chan et al., 2014).

Only a small proportion of HIV-infected individual with reduced BMD had family history of osteoporosis (5%), personal history of fracture (14.4%) and smoking history (29.5%) as this may be the reason that we did find any association with reduced BMD.

Our data also shows that history of alcohol consumption (44.5%) and caffeinated drinks (71.2%) in HIV-infected individuals did not play a significant role in bone loss. This may be because the majority of our participants were younger (median age=44 years old) when compared to other previous studies (Guerri-Fernandez et al., 2013; Yin, M. T. et al., 2010).

6.6.2 HIV-related osteoporosis risk factors

We did not find any association between the duration of HIV diagnosis and duration of ART with reduced BMD in our study. According to our meta-analysis, the accelerated bone loss primarily occurs during the first year of HIV infection and ART initiation and stabilized thereafter (Goh et al., 2018). The median duration of HIV diagnosis and ART were 7 years and 6.4 years, respectively. This shows that majority of our participants were no more in their first year of HIV infection and ART initiation were accelerated bone loss occurs.

6.7 Limitations

This was a cross sectional study. Hence, we were unable to evaluate the causal relationships between BMD and HIV infection. Secondly, we only recruited participants from one site. The results may not be generalizable to all HIV-infected patients in Malaysia. Thirdly, we were unable to perform the sub-analysis of ART-treated versus non ART-treated individuals because all HIV-infected individuals in this study were treated with ART. Besides, FRAX can only be performed in individuals aged 40 years old and above. Hence, we were unable to assess the 10-year probability of a fracture risk in individuals that were younger than 40 years old. Finally, we did not assess oestrogen levels, testosterone levels, bone remodeling biochemical markers (i.e. osteocalcin), the total amount of sunlight exposure, food intake (i.e. fruit and vegetable intake as proxy measure) and supplements used in our cohort due to lack of funds.

6.8 Strengths

Firstly, the number of participants recruited in our study was bigger than the required calculated sample size. This is because we also analysed the association between risk factors and reduced BMD in HIV-infected individuals. Secondly, the number of participants recruited in our study was the larger when compared to previous studies. Thirdly, our participants were matched for gender and age in both HIV-infected and HIV-uninfected individuals. In addition, our study overall response rate was high (93.6%). Finally, our participants were also a representative of the three biggest Malaysian ethnic groups (i.e. the Malays, Chinese and Indians).

6.9 Clinical implications and recommendations

We recommend that all HIV-infected and ART–treated individuals should be screened for reduced BMD during the first year of HIV-infection or ART initiation, regardless of

age or gender. DXA scan should be performed at baseline (before the commencement of ART) and one year later, to screen for reduced BMD. Risk factors for osteoporosis should be identified and treated before the commencement of ART. If reduced BMD is identified in ART-naïve HIV-infected individuals, abacavir and raltegravir should be used instead of tenofovir or PIs. If reduced BMD is identified in HIV-infected individuals treated with tenofovir or PIs, switching to abacavir or raltegravir is recommended (Brown, Hoy, et al., 2015).

Our study found a high number of HIV-infected individuals (65%) had vitamin D deficiency. Hence, we recommend that routine supplementation of vitamin D should be prescribed to all HIV-infected individuals regardless of gender or age. Vitamin D supplement should be given to HIV-infected individuals before ART initiation even though vitamin D level result is not available.

CHAPTER 7: CONCLUSION

Reduced BMD is a serious problem that need to be addressed accordingly to prevent unwanted osteoporotic fracture sin HIV-infected individuals. The prevalence of reduced BMD was significantly higher in HIV-infected versus HIV-uninfected individuals. Similarly, vitamin D deficiency was significantly higher in HIV-infected versus HIV-uninfected individuals. The 10-year probability of sustaining a hip fracture derived from the FRAX was significantly increased in HIV-infected individuals but not in the probability of major osteoporotic fracture. Older age, lower BMI and reduced physical exercise were found to be associated with reduced BMD.

Short-term accelerated BMD loss may occur within the first year of HIV infection and ART initiation but stabilized thereafter. Therefore, we recommend that all HIV-infected and ART–treated individuals should be screened for reduced BMD during the first year of HIV-infection or ART initiation, regardless of age or gender. A high number of HIV-infected individuals with vitamin D deficiency were found in our study. Hence, we recommend routine vitamin D supplement to be prescribed to all HIV-infected individuals regardless of their age or gender.

REFERENCES

Adeyemi, O. M., Agniel, D., French, A. L., Tien, P. C., Weber, K., Glesby, M. J., . . . Cohen, M. (2011). Vitamin D deficiency in HIV-infected and HIV-uninfected women in the United States. *J Acquir Immune Defic Syndr, 57*(3), 197-204. doi:10.1097/QAI.0b013e31821ae418

Ahmad, A. N., Ahmad, S. N., & Ahmad, N. (2017). HIV Infection and Bone Abnormalities. *The Open Orthopaedics Journal, 11*, 777-784. doi:10.2174/1874325001711010777

Ahmadzadeh, A., Emam, M., Rajaei, A., Moslemizadeh, M., & Jalessi, M. (2014). Comparison of three different osteoporosis risk assessment tools: ORAI (osteoporosis risk assessment instrument), SCORE (simple calculated osteoporosis risk estimation) and OST (osteoporosis self-assessment tool). *Medical Journal of the Islamic Republic of Iran, 28*, 94-94.

Amiel, C., Ostertag, A., Slama, L., Baudoin, C., N'Guyen, T., Lajeunie, E., . . . De Vernejoul, M. C. (2004). BMD is reduced in HIV-infected men irrespective of treatment. *Journal of Bone and Mineral Research, 19*(3), 402-409.

Arnsten, J. H., Freeman, R., Howard, A. A., Floris-Moore, M., Lo, Y., & Klein, R. S. (2007). Decreased bone mineral density and increased fracture risk in aging men with or at risk for HIV infection. *Aids, 21*(5), 617-623.

Arnsten, J. H., Freeman, R., Howard, A. A., Floris-Moore, M., Santoro, N., & Schoenbaum, E. E. (2006). HIV infection and bone mineral density in middle-aged women. *Clinical Infectious Diseases, 42*(7), 1014-1020. doi:10.1086/501015

Arts, E. J., & Hazuda, D. J. (2012). HIV-1 Antiretroviral Drug Therapy. *Cold Spring Harbor Perspectives in Medicine, 2*(4), a007161. doi:10.1101/cshperspect.a007161

Aspenberg, P. (2014). Denosumab and atypical femoral fractures. *Acta Orthopaedica, 85*(1), 1-1. doi:10.3109/17453674.2013.859423

Avihingsanon, A., Kerr, S. J., Ramautarsing, R. A., Praditpornsilpa, K., Sophonphan, J., Ubolyam, S., . . . Ruxrungtham, K. (2016). The Association of Gender, Age, Efavirenz Use, and Hypovitaminosis D Among HIV-Infected Adults Living in the Tropics. *AIDS Res Hum Retroviruses, 32*(4), 317-324. doi:10.1089/aid.2015.0069

Aydın, O. A., Karaosmanoglu, H. K., Karahasanoglu, R., Tahmaz, M., & Nazlıcan, O. (2013). Prevalence and risk factors of osteopenia/osteoporosis in Turkish HIV/AIDS patients. *The Brazilian Journal of Infectious Diseases, 17*(6), 707-711. doi:http://dx.doi.org/10.1016/j.bjid.2013.05.009

Azuma, Y., Kaji, K., Katogi, R., Takeshita, S., & Kudo, A. (2000). Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. *J Biol Chem, 275*(7), 4858-4864.

Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., . . . Montagnier, L. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science, 220*(4599), 868-871.

Bianchi, M. L., Orsini, M. R., Saraifoger, S., Ortolani, S., Radaelli, G., & Betti, S. (2005). Quality of life in post-menopausal osteoporosis. *Health and Quality of Life Outcomes, 3*, 78-78. doi:10.1186/1477-7525-3-78

Bischoff-Ferrari, H. A., Dawson-Hughes, B., Willett, W. C., Staehelin, H. B., Bazemore, M. G., Zee, R. Y., & Wong, J. B. (2004). Effect of Vitamin D on falls: a meta-analysis. *JAMA, 291*(16), 1999-2006. doi:10.1001/jama.291.16.1999

Bolland, M. J., Grey, A., Horne, A. M., Briggs, S. E., Thomas, M. G., Ellis-Pegler, R. B., . . . Reid, I. R. (2012). Stable bone mineral density over 6 years in HIV-infected men treated with highly active antiretroviral therapy (HAART). *Clinical Endocrinology, 76*(5), 643-648. doi:10.1111/j.1365-2265.2011.04274.x

Bolland, M. J., Grey, A., & Reid, I. R. (2015). Skeletal health in adults with HIV infection. *The Lancet Diabetes and Endocrinology, 3*(1), 63-74.

Bolland, M. J., Grey, A. B., Horne, A. M., Briggs, S. E., Thomas, M. G., Ellis-Pegler, R. B., . . . Reid, I. R. (2006). Bone mineral density is not reduced in HIV-infected Caucasian men treated with highly active antiretroviral therapy. *Clinical Endocrinology, 65*(2), 191-197. doi:10.1111/j.1365-2265.2006.02572.x

Bonjoch, A., Figueras, M., Estany, C., Perez-Alvarez, N., Rosales, J., del Rio, L., . . . Negredo, E. (2010). High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study. *Aids, 24*(18), 2827-2833. doi:10.1097/QAD.0b013e328340a28d

Bonnet, E., Ruidavets, J. B., Genoux, A., Mabile, L., Busato, F., Obadia, M., . . . Perret, B. (2013). Early loss of bone mineral density is correlated with a gain of fat mass in patients starting a protease inhibitor containing regimen: The prospective Lipotrip study. *BMC Infectious Diseases, 13*(293), 1-10.

Booth, F. W., Roberts, C. K., & Laye, M. J. (2012). Lack of exercise is a major cause of chronic diseases. *Comprehensive Physiology, 2*(2), 1143-1211. doi:10.1002/cphy.c110025

Boquete-Castro, A., Gómez-Moreno, G., Calvo-Guirado, J. L., Aguilar-Salvatierra, A., & Delgado-Ruiz, R. A. (2016). Denosumab and osteonecrosis of the jaw. A systematic analysis of events reported in clinical trials. *Clinical Oral Implants Research, 27*(3), 367-375. doi:10.1111/clr.12556

Boyce, B. F., & Xing, L. (2008). Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Archives of Biochemistry and Biophysics, 473*(2), 139-146. doi:10.1016/j.abb.2008.03.018

Branson, B. M., & Mermin, J. Establishing the diagnosis of HIV infection: New tests and a new algorithm for the United States. *Journal of Clinical Virology, 52*, S3-S4. doi:10.1016/j.jcv.2011.09.024

Brown, T. T., Hoy, J., Borderi, M., Guaraldi, G., Renjifo, B., Vescini, F., . . . Powderly, W. G. (2015). Recommendations for evaluation and management of bone disease in HIV. *Clin Infect Dis, 60*(8), 1242-1251. doi:10.1093/cid/civ010

Brown, T. T., & McComsey, G. A. (2009). *Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D.* Paper presented at the Antivir Ther.

Brown, T. T., Moser, C., Currier, J. S., Ribaudo, H. J., Rothenberg, J., Kelesidis, T., . . . McComsey, G. A. (2015). Changes in Bone Mineral Density After Initiation of Antiretroviral Treatment With Tenofovir Disoproxil Fumarate/Emtricitabine Plus Atazanavir/Ritonavir, Darunavir/Ritonavir, or Raltegravir. *The Journal of Infectious Diseases, 212*(8), 1241-1249. doi:10.1093/infdis/jiv194

Brown, T. T., & Qaqish, R. B. (2006). Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *Aids, 20*(17), 2165-2174. doi:10.1097/QAD.0b013e32801022eb

Brown, T. T., Ruppe, M. D., Kassner, R., Kumar, P., Kehoe, T., Dobs, A. S., & Timpone, J. (2004). Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. *Journal of Clinical Endocrinology & Metabolism, 89*(3), 1200-1206. doi:10.1210/jc.2003-031506

Bruera, D., Luna, N., David, D. O., Bergoglio, L. A., & Zamudio, J. (2003). Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *Aids, 17*(13), 1917-1923. doi:10.1097/01.aids.0000076322.42412.6f

Cadarette, S. M., Jaglal, S. B., Kreiger, N., McIsaac, W. J., Darlington, G. A., & Tu, J. V. (2000). Development and validation of the Osteoporosis Risk Assessment Instrument to facilitate selection of women for bone densitometry. *CMAJ: Canadian Medical Association Journal, 162*(9), 1289-1294.

Calmy, A., Fux, C. A., Norris, R., Vallier, N., Delhumeau, C., Samaras, K., . . . Carr, A. (2009). Low Bone Mineral Density, Renal Dysfunction, and Fracture Risk in HIV Infection: A Cross-Sectional Study. *Journal of Infectious Diseases, 200*(11), 1746-1754. doi:10.1086/644785

Carey, E. J., Balan, V., Kremers, W. K., & Hay, J. E. (2003). Osteopenia and osteoporosis in patients with end-stage liver disease caused by hepatitis C and alcoholic liver disease: Not just a cholestatic problem. *Liver Transplantation, 9*(11), 1166-1173. doi:10.1053/jlts.2003.50242

Carr, A., Miller, J., Eisman, J. A., & Cooper, D. A. (2001). Osteopenia in HIV-infected men: association with asymptomatic lactic acidemia and lower weight pre-antiretroviral therapy. *Aids, 15*(6), 703-709. doi:10.1097/00002030-200104130-00005

Cazanave, C., Dupon, M., Lavignolle-Aurillac, V., Barthe, N., Lawson-Ayayi, S., Mehsen, N., . . . Dabis, F. (2008). Reduced bone mineral density in HIV-infected patients: prevalence and associated factors. *Aids, 22*(3), 395-402. doi:10.1097/QAD.0b013e3282f423dd

Centers for Disease Control and Prevention. (1982). A cluster of Kaposi's sarcoma and Pneumocystis carinii pneumonia among homosexual male residents of Los Angeles and Orange Counties, California. *MMWR Morb Mortal Wkly Rep, 31*(23), 305-307.

Cervero, M., Agud, J. L., Garcia-Lacalle, C., Alcazar, V., Torres, R., Jusdado, J. J., & Moreno Guillen, S. (2012). Prevalence of vitamin D deficiency and its related risk factor in a Spanish cohort of adult HIV-infected patients: effects of antiretroviral therapy. *AIDS Res Hum Retroviruses, 28*(9), 963-971. doi:10.1089/aid.2011.0244

Chan, Nurul, Z. Z., Chuah, J. S., Nabil, M. M. A., Isa, N. M., Sabarul, A. M., & Nazrun, A. S. (2014). Association between Risk Factors of Osteoporosis and Bone Mineral Density in Women of Different Ethnic Groups in a Malaysian Hospital. *International Journal of Osteoporosis and Metabolic Disorders*(7), 1-11.

Chan, S.-P., Teo, C. C., Ng, S. A., Goh, N., Tan, C., & Deurenberg-Yap, M. (2006). Validation of various osteoporosis risk indices in elderly Chinese females in Singapore. *Osteoporosis International, 17*(8), 1182-1188. doi:10.1007/s00198-005-0051-4

Chen, S. J., Chen, Y. J., Cheng, C. H., Hwang, H. F., Chen, C. Y., & Lin, M. R. (2016). Comparisons of Different Screening Tools for Identifying Fracture/Osteoporosis Risk Among Community-Dwelling Older People. *Medicine, 95*(20), e3415. doi:10.1097/MD.0000000000003415

Chotalia, J., Frontini, M., Tatini, P., Nsuami, M. J., Martin, D. H., & Clark, R. A. (2012). Vitamin D Deficiency in HIV-Infected and -Uninfected Women in the United States. *Journal of acquired immune deficiency syndromes (1999), 59*(4), e77-e77. doi:10.1097/QAI.0b013e31824a0d1d

Chow, D., Day, L., Souza, S., & Shikuma, C. (2006). Metabolic complications of HIV therapy. *HIV InSite Knowledge Base Chapter*, 1-27.

Christakos, S., Dhawan, P., Porta, A., Mady, L. J., & Seth, T. (2011). Vitamin D and Intestinal Calcium Absorption. *Molecular and Cellular Endocrinology, 347*(1-2), 25-29. doi:10.1016/j.mce.2011.05.038

Cihlar, T., & Fordyce, M. (2016). Current status and prospects of HIV treatment. *Current Opinion in Virology, 18*, 50-56. doi:http://dx.doi.org/10.1016/j.coviro.2016.03.004

Clarke, B. L., & Khosla, S. (2010). Physiology of Bone Loss. *Radiologic clinics of North America, 48*(3), 483-495. doi:10.1016/j.rcl.2010.02.014

Clavel, F., Guetard, D., Brun-Vezinet, F., Chamaret, S., Rey, M. A., Santos-Ferreira, M. O., . . . et al. (1986). Isolation of a new human retrovirus from West African patients with AIDS. *Science, 233*(4761), 343-346.

Coffin, J., & Swanstrom, R. (2013). HIV pathogenesis: dynamics and genetics of viral populations and infected cells. *Cold Spring Harb Perspect Med, 3*(1), a012526. doi:10.1101/cshperspect.a012526

Cong, E., & Walker, M. D. (2014). The Chinese skeleton: insights into microstructure that help to explain the epidemiology of fracture. *Bone research, 2*, 14009.

Cornett, J. K., & Kirn, T. J. (2013). Laboratory Diagnosis of HIV in Adults: A Review of Current Methods. *Clinical Infectious Diseases, 57*(5), 712-718. doi:10.1093/cid/cit281

Cortés, Y. I., Yin, M. T., & Reame, N. K. (2015). Bone density and fractures in HIV-infected postmenopausal women: A systematic review. *Journal of the Association of Nurses in AIDS Care*(0). doi:http://dx.doi.org/10.1016/j.jana.2015.03.005

Cosman, F., de Beur, S. J., LeBoff, M. S., Lewiecki, E. M., Tanner, B., Randall, S., & Lindsay, R. (2014). Clinician's Guide to Prevention and Treatment of Osteoporosis. *Osteoporosis International, 25*(10), 2359-2381. doi:10.1007/s00198-014-2794-2

Cotter, A. G., & Mallon, P. W. (2012). Therapeutic options for low bone mineral density in HIV-infected subjects. *Curr HIV/AIDS Rep, 9*(2), 148-159. doi:10.1007/s11904-012-0117-9

Coutsoudis, A., Kwaan, L., & Thomson, M. (2010). Prevention of vertical transmission of HIV-1 in resource-limited settings. *Expert Rev Anti Infect Ther, 8*(10), 1163-1175. doi:10.1586/eri.10.94

Cozzolino, M., Vidal, M., Arcidiacono, M. V., Tebas, P., Yarasheski, K. E., & Dusso, A. S. (2003). HIV-protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. *Aids, 17*(4), 513-520. doi:10.1097/01.aids.0000050817.06065.f8

Crutchley, R. D., Gathe, J., Jr., Mayberry, C., Trieu, A., Abughosh, S., & Garey, K. W. (2012). Risk factors for vitamin D deficiency in HIV-infected patients in the south central United States. *AIDS Res Hum Retroviruses, 28*(5), 454-459. doi:10.1089/aid.2011.0025

Cummings, S. R., Eckert, S., Krueger, K. A., & et al. (1999). The effect of raloxifene on risk of breast cancer in postmenopausal women: Results from the more randomized trial. *JAMA, 281*(23), 2189-2197. doi:10.1001/jama.281.23.2189

Cummings, S. R., San Martin, J., McClung, M. R., Siris, E. S., Eastell, R., Reid, I. R., . . . Christiansen, C. (2009). Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med, 361*(8), 756-765. doi:10.1056/NEJMoa0809493

Dao, C. N., Patel, P., Overton, E. T., Rhame, F., Pals, S. L., Johnson, C., . . . Brooks, J. T. (2011). Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis, 52*(3), 396-405. doi:10.1093/cid/ciq158

Davey, J., Turner, R. M., Clarke, M. J., & Higgins, J. P. (2011). Characteristics of meta-analyses and their component studies in the Cochrane Database of Systematic

Reviews: a cross-sectional, descriptive analysis. *BMC Medical Research Methodology, 11*(1), 160. doi:10.1186/1471-2288-11-160

Dawson-Hughes, B., Heaney, R. P., Holick, M. F., Lips, P., Meunier, P. J., & Vieth, R. (2005). Estimates of optimal vitamin D status. *Osteoporos Int, 16*(7), 713-716. doi:10.1007/s00198-005-1867-7

Dawson-Hughes, B., Mithal, A., Bonjour, J. P., Boonen, S., Burckhardt, P., Fuleihan, G. E., . . . Yoshimura, N. (2010). IOF position statement: vitamin D recommendations for older adults. *Osteoporos Int, 21*(7), 1151-1154. doi:10.1007/s00198-010-1285-3

de Béthune, M.-P. (2010). Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: A review of the last 20 years (1989–2009). *Antiviral Research, 85*(1), 75-90. doi:http://dx.doi.org/10.1016/j.antiviral.2009.09.008

de Menezes Barbosa, E. G., de Paula, F. J., Machado, A. A., de Assis Pereira, F., Barbosa Junior, F., & Navarro, A. M. (2013). Impact of antiretroviral therapy on bone metabolism markers in HIV-seropositive patients. *Bone, 57*(1), 62-67.

de Villiers, T. J., Pines, A., Panay, N., Gambacciani, M., Archer, D. F., Baber, R. J., . . . Sturdee, D. W. (2013). Updated 2013 International Menopause Society recommendations on menopausal hormone therapy and preventive strategies for midlife health. *Climacteric, 16*(3), 316-337. doi:10.3109/13697137.2013.795683

Deeks, S. G., Tracy, R., & Douek, D. C. (2013). Systemic Effects of Inflammation on Health during Chronic HIV Infection. *Immunity, 39*(4), 633-645. doi:10.1016/j.immuni.2013.10.001

Del Valle, H. B., Yaktine, A. L., Taylor, C. L., & Ross, A. C. (2011). *Dietary reference intakes for calcium and vitamin D*: National Academies Press.

Delmas, P. D., Ensrud, K. E., Adachi, J. D., Harper, K. D., Sarkar, S., Gennari, C., . . . Eastell, R. (2002). Efficacy of Raloxifene on Vertebral Fracture Risk Reduction in Postmenopausal Women with Osteoporosis: Four-Year Results from a Randomized Clinical Trial. *The Journal of Clinical Endocrinology & Metabolism, 87*(8), 3609-3617. doi:10.1210/jcem.87.8.8750

Department of Statistics Malaysia. (2010, 7 May 2015). Population Distribution and Basic Demographic Characteristic Report 2010 (Updated: 05/08/2011). Retrieved from https://www.dosm.gov.my/v1/index.php?r=column/ctheme&menu_id=L0pheU43NWJwRWVSZklWdzQ4TlhUUT09&bul_id=MDMxdHZjWTk1SjFzTzNkRXYzcVZjdz09

Diab, D. L., & Watts, N. B. (2013). Bisphosphonate drug holiday: who, when and how long. *Therapeutic Advances in Musculoskeletal Disease, 5*(3), 107-111. doi:10.1177/1759720X13477714

Dolan, S. E., Huang, J. S., Killilea, K. M., Sullivan, M. P., Aliabadi, N., & Grinspoon, S. (2004). Reduced bone density in HIV-infected women. *Aids, 18*(3), 475-483. doi:10.1097/00002030-200402200-00014

Dolan, S. E., Kanter, J. R., & Grinspoon, S. (2006). Longitudinal analysis of bone density in human immunodeficiency virus-infected women. *Journal of Clinical Endocrinology and Metabolism, 91*(8), 2938-2945.

Dong, H. V., Cortes, Y. I., Shiau, S., & Yin, M. T. (2014). Osteoporosis and fractures in HIV/hepatitis C virus coinfection: a systematic review and meta-analysis. *Aids, 28*(14), 2119-2131. doi:10.1097/qad.0000000000000363

Dowell, P., Flexner, C., Kwiterovich, P. O., & Lane, M. D. (2000). Suppression of preadipocyte differentiation and promotion of adipocyte death by HIV protease inhibitors. *Journal of Biological Chemistry, 275*(52), 41325-41332.

Dubois, E. A., Rissmann, R., & Cohen, A. F. (2011). Denosumab. *British Journal of Clinical Pharmacology, 71*(6), 804-806. doi:10.1111/j.1365-2125.2011.03969.x

Effros, R. B., Fletcher, C. V., Gebo, K., Halter, J. B., Hazzard, W. R., Horne, F. M., . . . High, K. P. (2008). Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin Infect Dis, 47*(4), 542-553. doi:10.1086/590150

Eisman, J. A., & Bouillon, R. (2014). Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. *BoneKEy Rep, 3*, 499. doi:10.1038/bonekey.2013.233

Ellfolk, M., Norlin, M., Gyllensten, K., & Wikvall, K. (2009). Regulation of human vitamin D(3) 25-hydroxylases in dermal fibroblasts and prostate cancer LNCaP cells. *Mol Pharmacol, 75*(6), 1392-1399. doi:10.1124/mol.108.053660

Epstein, M. (1997). Alcohol's impact on kidney function. *Alcohol Health Res World, 21*(1), 84-92.

Erlandson, K. M., Allshouse, A. A., Jankowski, C. M., MaWhinney, S., Kohrt, W. M., & Campbell, T. B. (2013). Functional Impairment is Associated with Low Bone and Muscle Mass among Persons Aging with HIV-Infection. *Journal of acquired immune deficiency syndromes (1999), 63*(2), 209-215. doi:10.1097/QAI.0b013e318289bb7e

Fahrleitner, A., Dobnig, H., Obernosterer, A., Pilger, E., Leb, G., Weber, K., . . . Obermayer-Pietsch, B. M. (2002). Vitamin D Deficiency and Secondary Hyperparathyroidism Are Common Complications in Patients with Peripheral Arterial Disease. *Journal of General Internal Medicine, 17*(9), 663-669. doi:10.1046/j.1525-1497.2002.11033.x

Fauci, A. S. (2007). Pathogenesis of HIV Disease: Opportunities for New Prevention Interventions. *Clinical Infectious Diseases, 45*(Supplement_4), S206-S212. doi:10.1086/522540

Fauci, A. S., & Lane, H. C. (2012). *Human immunodeficiency virus disease: AIDS and related disorders*. New York: McGraw-Hill.

Faulkner, K. G., Glüer, C. C., Majumdar, S., Lang, P., Engelke, K., & Genant, H. K. (1991). Noninvasive measurements of bone mass, structure, and strength: current methods and experimental techniques. *American Journal of Roentgenology, 157*(6), 1229-1237. doi:10.2214/ajr.157.6.1950872

Fiorenza, C. G., Chou, S. H., & Mantzoros, C. S. (2011). Lipodystrophy: Pathophysiology and Advances in Treatment. *Nature reviews. Endocrinology, 7*(3), 137-150. doi:10.1038/nrendo.2010.199

Fondi, C., & Franchi, A. (2007). Definition of bone necrosis by the pathologist. *Clinical Cases in Mineral and Bone Metabolism, 4*(1), 21-26.

Fong, J., & Khan, A. (2012). Hypocalcemia: Updates in diagnosis and management for primary care. *Canadian Family Physician, 58*(2), 158-162.

Foundation, N. O. (2017). Bone Density Exam or Testing. Retrieved from https://www.nof.org/patients/diagnosis-information/bone-density-examtesting/

Fox, K. M., Cummings, S. R., Powell-Threets, K., & Stone, K. (1998). Family history and risk of osteoporotic fracture. Study of Osteoporotic Fractures Research Group. *Osteoporos Int, 8*(6), 557-562.

French, A. L., Adeyemi, O. M., Agniel, D. M., Evans, C. T., Yin, M. T., Anastos, K., & Cohen, M. H. (2011). The association of HIV status with bacterial vaginosis and vitamin D in the United States. *J Womens Health (Larchmt), 20*(10), 1497-1503. doi:10.1089/jwh.2010.2685

Friedman, E. E., & Duffus, W. A. (2016). Chronic health conditions in Medicare beneficiaries 65 years and older with HIV infection. *AIDS (London, England), 30*(16), 2529-2536. doi:10.1097/QAD.0000000000001215

Fujiwara, S., Masunari, N., Suzuki, G., & Ross, P. D. (2001). Performance of osteoporosis risk indices in a Japanese population. *Current Therapeutic Research, 62*(8), 586-594. doi:http://dx.doi.org/10.1016/S0011-393X(01)80065-5

Fux, C. A., Christen, A., Zgraggen, S., Mohaupt, M. G., & Furrer, H. (2007). Effect of tenofovir on renal glomerular and tubular function. *Aids, 21*(11), 1483-1485. doi:10.1097/QAD.0b013e328216f15b

Gallant, J. E., Staszewski, S., Pozniak, A. L., DeJesus, E., Suleiman, J. M. A. H., Miller, M. D., . . . Cheng, A. K. (2004). Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: A 3-year randomized trial. *Journal of the American Medical Association, 292*(2), 191-201.

Garcia Aparicio, A. M., Munoz Fernandez, S., Gonzalez, J., Arribas, J. R., Pena, J. M., Vazquez, J. J., . . . Martin Mola, E. (2006). Abnormalities in the bone mineral metabolism in HIV-infected patients. *Clinical Rheumatology, 25*(4), 537-539. doi:10.1007/s10067-005-0028-x

Gastaldello, R., Gallego, S., Isa, M. B., Nates, S., & Medeot, S. (1999). Efficiency of indirect immunofluorescence assay as a confirmatory test for the diagnosis of human retrovirus infection (HIV-1 and HTLV-I/II) in different at risk populations. *Revista do Instituto de Medicina Tropical de São Paulo, 41*, 159-164.

Gazzola, L., Comi, L., Savoldi, A., Tagliabue, L., Del Sole, A., Pietrogrande, L., . . . Marchetti, G. (2010). Use of the FRAX Equation as First-Line Screening of Bone Metabolism Alteration in the HIV-Infected Population. *The Journal of Infectious Diseases, 202*(2), 330-331. doi:10.1086/653584

Geater, S., Leelawattana, R., & Geater, A. (2004). Validation of the OSTA index for discriminating between high and low probability of femoral neck and lumbar spine osteoporosis among Thai postmenopausal women. *J Med Assoc Thai, 87*(11), 1286-1292.

Gehlbach, S., Saag, K. G., Adachi, J. D., Hooven, F. H., Flahive, J., Boonen, S., . . . Lindsay, R. (2012). Previous Fractures at Multiple Sites Increase the Risk for Subsequent Fractures: The Global Longitudinal Study of Osteoporosis in Women. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 27*(3), 645-653. doi:10.1002/jbmr.1476

Gershon, R. R., Vlahov, D., & Nelson, K. E. (1990). The risk of transmission of HIV-1 through non-percutaneous, non-sexual modes--a review. *Aids, 4*(7), 645-650.

Geusens, P., Hochberg, M. C., van der Voort, D. J., Pols, H., van der Klift, M., Siris, E., . . . Ross, P. (2002). Performance of risk indices for identifying low bone density in postmenopausal women. *Mayo Clin Proc, 77*(7), 629-637.

Gilsanz , V., Roe , T. F., Mora , S., Costin , G., & Goodman , W. G. (1991). Changes in Vertebral Bone Density in Black Girls and White Girls during Childhood and Puberty. *New England Journal of Medicine, 325*(23), 1597-1600. doi:10.1056/nejm199112053252302

Gloth, F. M., 3rd, & Tobin, J. D. (1995). Vitamin D deficiency in older people. *J Am Geriatr Soc, 43*(7), 822-828.

Goh, S. S. L., Lai, P. S. M., Tan, A. T. B., & Ponnampalavanar, S. (2018). Reduced bone mineral density in human immunodeficiency virus-infected individuals: a meta-analysis of its prevalence and risk factors. *Osteoporos Int, 29*(3), 595-613. doi:10.1007/s00198-017-4305-8

Gourlay, M. L., Miller, W. C., Richy, F., Garrett, J. M., Hanson, L. C., & Reginster, J. Y. (2005). Performance of osteoporosis risk assessment tools in postmenopausal women aged 45–64 years. *Osteoporosis International, 16*(8), 921-927. doi:10.1007/s00198-004-1775-2

Grant, P. M., Kitch, D., McComsey, G. A., Dube, M. P., Haubrich, R., Huang, J., . . . Brown, T. T. (2013). Low Baseline CD4+ Count Is Associated With Greater Bone Mineral Density Loss After Antiretroviral Therapy Initiation. *Clinical Infectious Diseases, 57*(10), 1483-1488. doi:10.1093/cid/cit538

Grijsen, M. L., Vrouenraets, S. M., Wit, F. W., Stolte, I. G., Prins, M., Lips, P., . . . Prins, J. M. (2013). Low bone mineral density, regardless of HIV status, in men who have sex with men. *J Infect Dis, 207*(3), 386-391.

Guaraldi, G., Orlando, G., Zona, S., Menozzi, M., Carli, F., Garlassi, E., . . . Palella, F. (2011). Premature Age-Related Comorbidities Among HIV-Infected Persons Compared With the General Population. *Clinical Infectious Diseases, 53*(11), 1120-1126. doi:10.1093/cid/cir627

Guerri-Fernandez, R., Vestergaard, P., Carbonell, C., Knobel, H., Aviles, F. F., Castro, A. S., . . . Diez-Perez, A. (2013). HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. *J Bone Miner Res, 28*(6), 1259-1263. doi:10.1002/jbmr.1874

Günthard, H. F., Saag, M. S., Benson, C. A., & et al. (2016). Antiretroviral drugs for treatment and prevention of hiv infection in adults: 2016 recommendations of the international antiviral society–usa panel. *JAMA, 316*(2), 191-210. doi:10.1001/jama.2016.8900

Gutierrez, F., & Masia, M. (2011). The Role of HIV and Antiretroviral Therapy in Bone Disease. *Aids Reviews, 13*(2), 109-118.

Gyllensten, K., Josephson, F., Lidman, K., & Saaf, M. (2006). Severe vitamin D deficiency diagnosed after introduction of antiretroviral therapy including efavirenz in a patient living at latitude 59 degrees N. *Aids, 20*(14), 1906-1907. doi:10.1097/01.aids.0000244216.08327.39

Hadigan, C., Meigs, J. B., Corcoran, C., Rietschel, P., Piecuch, S., Basgoz, N., . . . Grinspoon, S. (2001). Metabolic Abnormalities and Cardiovascular Disease Risk Factors in Adults with Human Immunodeficiency Virus Infection and Lipodystrophy. *Clinical Infectious Diseases, 32*(1), 130-139. doi:10.1086/317541

Hansen, A. B., Obel, N., Nielsen, H., Pedersen, C., & Gerstoft, J. (2011). Bone mineral density changes in protease inhibitor-sparing vs. nucleoside reverse transcriptase inhibitor-sparing highly active antiretroviral therapy: Data from a randomized trial. *HIV Medicine, 12*(3), 157-165.

Hariparsad, N., Nallani, S. C., Sane, R. S., Buckley, D. J., Buckley, A. R., & Desai, P. B. (2004). Induction of CYP3A4 by Efavirenz in Primary Human Hepatocytes: Comparison With Rifampin and Phenobarbital. *The Journal of Clinical Pharmacology, 44*(11), 1273-1281. doi:10.1177/0091270004269142

Harris, R. P., Helfand, M., Woolf, S. H., Lohr, K. N., Mulrow, C. D., Teutsch, S. M., & Atkins, D. (2001). Current methods of the US Preventive Services Task Force: a review of the process. *Am J Prev Med, 20*(3 Suppl), 21-35.

Haskelberg, H., Hoy, J. F., Amin, J., Ebeling, P. R., Emery, S., Carr, A., . . . Woolley, I. (2012). Changes in bone turnover and bone loss in HIV-infected patients changing treatment to tenofovir-emtricitabine or abacavir-lamivudine. *PLoS One, 7*(6), 1-9.

Heaney, R. P., Abrams, S., Dawson-Hughes, B., Looker, A., Looker, A., Marcus, R., . . . Weaver, C. (2000). Peak Bone Mass. *Osteoporosis International, 11*(12), 985-1009. doi:10.1007/s001980070020

Hidalgo, J. A., Macarthur, R. D., & Crane, L. R. (2000). An Overview Of Hiv Infection And Aids: Etiology, Pathogenesis, Diagnosis, Epidemiology, And Occupational Exposure. *Seminars in Thoracic and Cardiovascular Surgery, 12*(2), 130-139. doi:http://dx.doi.org/10.1053/ct.2000.7128

Hofbauer, L. C., Hamann, C., & Ebeling, P. R. (2010). Approach to the patient with secondary osteoporosis. *Eur J Endocrinol, 162*(6), 1009-1020. doi:10.1530/eje-10-0015

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., . . . Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab, 96*(7), 1911-1930. doi:10.1210/jc.2011-0385

Howe, T. E., Shea, B., Dawson, L. J., Downie, F., Murray, A., Ross, C., . . . Creed, G. (2011). Exercise for preventing and treating osteoporosis in postmenopausal women. *Cochrane Database Syst Rev*(7), Cd000333. doi:10.1002/14651858.CD000333.pub2

Huang, J. S., Mulkern, R. V., & Grinspoon, S. (2002). Reduced intravertebral bone marrow fat in HIV-infected men. *Aids, 16*(9), 1265-1269. doi:10.1097/00002030-200206140-00009

Ingole, N. A., Sarkate, P. P., Paranjpe, S. M., Shinde, S. D., Lall, S. S., & Mehta, P. R. (2013). HIV-2 Infection: Where Are We Today? *Journal of Global Infectious Diseases, 5*(3), 110-113. doi:10.4103/0974-777X.116872

Institute of Medicine. (2011). The National Academies Collection: Reports funded by National Institutes of Health. In Ross, A. C., Taylor, C. L., Yaktine, A. L., & Del Valle, H. B. (Eds.), *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US)

National Academy of Sciences.

International Osteoporosis Foundation. (2015). International Osteoporosis Foundation. Retrieved from https://www.iofbonehealth.org/what-is-osteoporosis

International Society for Clinical Densitometry. (2013). International Society for Clinical Densitometry. 2013 Official Positions—Adult. Retrieved from http://www.iscd.org/official-positions/2013-iscd-official-positions-adult/

Iqbal, J., Sun, L., & Zaidi, M. (2010). Denosumab for the Treatment of Osteoporosis. *Current Osteoporosis Reports, 8*(4), 163-167. doi:10.1007/s11914-010-0034-z

Jain, R. G., & Lenhard, J. M. (2002). Select HIV protease inhibitors alter bone and fat metabolism ex vivo. *J Biol Chem, 277*(22), 19247-19250. doi:10.1074/jbc.C200069200

Jang, H., Koo, F. K., Ke, L., Clemson, L., Cant, R., Fraser, D. R., . . . Brock, K. (2013). Culture and sun exposure in immigrant East Asian women living in Australia. *Women Health, 53*(5), 504-518. doi:10.1080/03630242.2013.806386

Janssen, H. C., Samson, M. M., & Verhaar, H. J. (2002). Vitamin D deficiency, muscle function, and falls in elderly people. *Am J Clin Nutr, 75*(4), 611-615.

Ji, M. X., & Yu, Q. (2015). Primary osteoporosis in postmenopausal women. *Chronic Diseases and Translational Medicine, 1*(1), 9-13. doi:http://dx.doi.org/10.1016/j.cdtm.2015.02.006

Jin, F., Jansson, J., Law, M., Prestage, G. P., Zablotska, I., Imrie, J. C. G., . . . Wilson, D. P. (2010). Per-contact probability of HIV transmission in homosexual men in Sydney in the era of HAART. *AIDS (London, England), 24*(6), 907-913. doi:10.1097/QAD.0b013e3283372d90

Jones, S., Restrepo, D., Kasowitz, A., Korenstein, D., Wallenstein, S., Schneider, A., & Keller, M. J. (2008). Risk factors for decreased bone density and effects of HIV on bone in the elderly. *Osteoporosis International, 19*(7), 913-918. doi:10.1007/s00198-007-0524-8

Jonville-Béra, A. P., Crickx, B., Aaron, L., Hartingh, I., & Autret-Leca, E. (2009). Strontium ranelate-induced DRESS syndrome: first two case reports. *Allergy, 64*(4), 658-659. doi:10.1111/j.1398-9995.2009.01940.x

Kanis, J. A., Johansson, H., Johnell, O., Oden, A., De Laet, C., Eisman, J. A., . . . Tenenhouse, A. (2005). Alcohol intake as a risk factor for fracture. *Osteoporosis International, 16*(7), 737-742. doi:10.1007/s00198-004-1734-y

Kanis, J. A., Johansson, H., Oden, A., Johnell, O., De Laet, C., Eisman, J. A., . . . Tenenhouse, A. (2004). A family history of fracture and fracture risk: a meta-analysis. *Bone, 35*(5), 1029-1037. doi:10.1016/j.bone.2004.06.017

Kanis, J. A., Johnell, O., Oden, A., Johansson, H., De Laet, C., Eisman, J. A., . . . Tenenhouse, A. (2005). Smoking and fracture risk: a meta-analysis. *Osteoporos Int, 16*(2), 155-162. doi:10.1007/s00198-004-1640-3

Kanis, J. A., Melton, L. J., 3rd, Christiansen, C., Johnston, C. C., & Khaltaev, N. (1994). The diagnosis of osteoporosis. *J Bone Miner Res, 9*(8), 1137-1141. doi:10.1002/jbmr.5650090802

Kanis, J. A., Oden, A., Johansson, H., Borgstrom, F., Strom, O., & McCloskey, E. (2009). FRAX and its applications to clinical practice. *Bone, 44*(5), 734-743. doi:10.1016/j.bone.2009.01.373

Kaplan, E. H. (1989). Needles That Kill: Modeling Human Immunodeficiency Virus Transmission via Shared Drug Injection Equipment in Shooting Galleries. *Reviews of Infectious Diseases, 11*(2), 289-298. doi:10.1093/clinids/11.2.289

Keen, R. W., Hart, D. J., Arden, N. K., Doyle, D. V., & Spector, T. D. (1999). Family history of appendicular fracture and risk of osteoporosis: a population-based study. *Osteoporos Int, 10*(2), 161-166. doi:10.1007/s001980050211

Kennel, K. A., & Drake, M. T. (2009). Adverse Effects of Bisphosphonates: Implications for Osteoporosis Management. *Mayo Clin Proc, 84*(7), 632-638.

Khosla, S. (2010). Update in Male Osteoporosis. *The Journal of clinical endocrinology and metabolism, 95*(1), 3-10. doi:10.1210/jc.2009-1740

Kiptoo, M. K., Mpoke, S. S., & Ng'ang'a, Z. W. (2004). New indirect immunofluorescence assay as a confirmatory test for human immunodeficiency virus type 1. *East Afr Med J, 81*(5), 222-225.

Kling, J. M., Clarke, B. L., & Sandhu, N. P. (2014). Osteoporosis Prevention, Screening, and Treatment: A Review. *Journal of Women's Health, 23*(7), 563-572. doi:10.1089/jwh.2013.4611

Knobel, H., Guelar, A., Vallecillo, G., Nogues, X., & Diez, A. (2001). Osteopenia in HIV-infected patients: Is it the disease or is it the treatment? *Aids, 15*(6), 807-808.

Kruger, M. J., & Nell, T. A. (2017). Bone mineral density in people living with HIV: a narrative review of the literature. *AIDS Research and Therapy, 14*(1), 35. doi:10.1186/s12981-017-0162-y

Kuchuk, N. O., Pluijm, S. M., van Schoor, N. M., Looman, C. W., Smit, J. H., & Lips, P. (2009). Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. *J Clin Endocrinol Metab, 94*(4), 1244-1250. doi:10.1210/jc.2008-1832

Kung, A. W., Ho, A. Y., Sedrine, W. B., Reginster, J. Y., & Ross, P. D. (2003). Comparison of a simple clinical risk index and quantitative bone ultrasound for identifying women at increased risk of osteoporosis. *Osteoporos Int, 14*(9), 716-721. doi:10.1007/s00198-003-1428-x

Kung, A. W. C., Chao, H. T., Huang, K. E., Need, A. G., Taechakraichana, N., Loh, F. H., . . . Thiebaud, D. (2003). Efficacy and Safety of Raloxifene 60 Milligrams/Day in Postmenopausal Asian Women. *The Journal of Clinical Endocrinology & Metabolism, 88*(7), 3130-3136. doi:10.1210/jc.2002-021855

Kwan, C. K., Eckhardt, B., Baghdadi, J., & Aberg, J. A. (2012). Hyperparathyroidism and complications associated with vitamin D deficiency in HIV-infected adults in New York City, New York. *AIDS Res Hum Retroviruses, 28*(9), 1025-1032. doi:10.1089/aid.2011.0325

Lake, J. E., & Adams, J. S. (2011). Vitamin D in HIV-Infected Patients. *Curr HIV/AIDS Rep, 8*(3), 133-141. doi:10.1007/s11904-011-0082-8

Laskowski, L. K., Goldfarb, D. S., Howland, M. A., Kavcsak, K., Lugassy, D. M., & Smith, S. W. (2016). A RANKL Wrinkle: Denosumab-Induced Hypocalcemia. *Journal of Medical Toxicology, 12*(3), 305-308. doi:10.1007/s13181-016-0543-y

Lau, E., Ong, K., Kurtz, S., Schmier, J., & Edidin, A. (2008). Mortality following the diagnosis of a vertebral compression fracture in the Medicare population. *J Bone Joint Surg Am, 90*(7), 1479-1486. doi:10.2106/jbjs.g.00675

Leali, P. T., Muresu, F., Melis, A., Ruggiu, A., Zachos, A., & Doria, C. (2011). Skeletal fragility definition. *Clinical Cases in Mineral and Bone Metabolism, 8*(2), 11-13.

Lee, J. K., & Khir, A. S. M. (2007). The incidence of hip fracture in Malaysians above 50 years of age: variation in different ethnic groups. *APLAR Journal of Rheumatology, 10*(4), 300-305. doi:10.1111/j.1479-8077.2007.00314.x

Leibson, C. L., Tosteson, A. N. A., Gabriel, S. E., Ransom, J. E., & Melton, L. J. (2002). Mortality, Disability, and Nursing Home Use for Persons with and without Hip Fracture: A Population-Based Study. *Journal of the American Geriatrics Society, 50*(10), 1644-1650. doi:10.1046/j.1532-5415.2002.50455.x

Lerma, E., Molas, M. E., Montero, M. M., Guelar, A., González, A., Villar, J., . . . Knobel, H. (2012). Prevalence and Factors Associated with Vitamin D Deficiency and Hyperparathyroidism in HIV-Infected Patients Treated in Barcelona. *ISRN AIDS, 2012*, 5. doi:10.5402/2012/485307

Levenson, D. I., & Bockman, R. S. (1994). A review of calcium preparations. *Nutr Rev, 52*(7), 221-232.

Levy, J. A. (1993). Pathogenesis of human immunodeficiency virus infection. *Microbiological Reviews, 57*(1), 183-289.

Li-Yu, J. T., Llamado, L. J. Q., & Torralba, T. P. (2005). Validation of OSTA among Filipinos. *Osteoporosis International, 16*(12), 1789-1793. doi:10.1007/s00198-005-1929-x

Li, E. P. H., Min, H. J., & Belk, R. W. (2008). Skin lightening and beauty in four Asian cultures. *ACR North American Advances*.

Li, X. L., & Xu, J. H. (2013). Coffee consumption and hip fracture risk: a meta-analysis. *Journal of Nutritional Science, 2*, e23. doi:10.1017/jns.2013.13

Li, Y. M. (2008). Concordance of a Self Assessment Tool and Measurement of Bone Mineral Density in Identifying the Risk of Osteoporosis in Elderly Taiwanese Women. *Tzu Chi Medical Journal, 20*(3), 206-212. doi:http://dx.doi.org/10.1016/S1016-3190(08)60037-3

Libois, A., Clumeck, N., Kabeya, K., Gerard, M., De Wit, S., Poll, B., . . . Rozenberg, S. (2010). Risk factors of osteopenia in HIV-infected women: No role of antiretroviral therapy. *Maturitas, 65*(1), 51-54.

Lim, P. S., Ong, F. B., Suniza, S. S., & Adeeb, N. (2011). Developing a Malaysian Osteoporosis Screening Tool (MOST) for early osteoporosis detection in Malaysian women. *Sex Reprod Healthc, 2*(2), 77-82. doi:10.1016/j.srhc.2010.11.004

Llibre, J. M., Walmsley, S., & Gatell, J. M. (2016). Backbones versus core agents in initial ART regimens: one game, two players. *Journal of Antimicrobial Chemotherapy, 71*(4), 856-861. doi:10.1093/jac/dkv429

Loiseau-Pérès, S., Delaunay, C., Poupon, S., Lespessailles, E., Ballouche, N., Arsac, P., & Benhamou, C. L. (2002). Osteopenia in patients infected by the human immunodeficiency virus. A case control study. *Joint Bone Spine, 69*(5), 482-485. doi:http://dx.doi.org/10.1016/S1297-319X(02)00433-5

Low, A., Gavriilidis, G., Larke, N., MR, B. L., Drouin, O., Stover, J., . . . Easterbrook, P. (2016). Incidence of Opportunistic Infections and the Impact of Antiretroviral Therapy Among HIV-Infected Adults in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. *Clin Infect Dis, 62*(12), 1595-1603. doi:10.1093/cid/ciw125

Lydick, E., Cook, K., Turpin, J., Melton, M., Stine, R., & Byrnes, C. (1998). Development and validation of a simple questionnaire to facilitate identification of women likely to have low bone density. *Am J Manag Care, 4*(1), 37-48.

Madeddu, G., Spanu, A., Solinas, P., Calia, G. M., Lovigu, C., Chessa, F., . . . Mura, M. S. (2004). Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. *Quarterly Journal of Nuclear Medicine and Molecular Imaging, 48*(1), 39-48.

Madureira, M. M., Ciconelli, R. M., & Pereira, R. M. R. (2012). Quality of life measurements in patients with osteoporosis and fractures. *Clinics, 67*(11), 1315-1320. doi:10.6061/clinics/2012(11)16

Mahy, M., Autenrieth, C. S., Stanecki, K., & Wynd, S. (2014). Increasing trends in HIV prevalence among people aged 50 years and older: evidence from estimates and survey data. *AIDS (London, England), 28*(4), S453-S459. doi:10.1097/QAD.0000000000000479

Mallon, P. W. G. (2010). HIV and bone mineral density. *Current Opinion in Infectious Diseases, 23*(1), 1-8. doi:10.1097/QCO.0b013e328334fe9a

Manolagas, S. C., O'Brien, C. A., & Almeida, M. (2013). The role of estrogen and androgen receptors in bone health and disease. *Nature reviews. Endocrinology, 9*(12), 699-712. doi:10.1038/nrendo.2013.179

Mansueto, P., Seidita, A., Vitale, G., Gangemi, S., Iaria, C., & Cascio, A. (2015). Vitamin D Deficiency in HIV Infection: Not Only a Bone Disorder. *2015*, 735615. doi:10.1155/2015/735615

Marks, G., Crepaz, N., & Janssen, R. S. (2006). Estimating sexual transmission of HIV from persons aware and unaware that they are infected with the virus in the USA. *Aids, 20*(10), 1447-1450. doi:10.1097/01.aids.0000233579.79714.8d

Matovu, F. K., Wattanachanya, L., Beksinska, M., Pettifor, J. M., & Ruxrungtham, K. (2016). Bone health and HIV in resource-limited settings: a scoping review. *Current Opinion in HIV and AIDS, 11*(3), 306-325. doi:10.1097/coh.0000000000000274

Mazokopakis, E. E., & Starakis, L. K. (2011). Recommendations for Diagnosis and Management of Osteoporosis in COPD Men. *ISRN Rheumatology, 2011*, 901416. doi:10.5402/2011/901416

McComsey, G. A., Tebas, P., Shane, E., Yin, M. T., Overton, E. T., Huang, J. S., . . . Brown, T. T. (2010). Bone Disease in HIV Infection: A Practical Review and Recommendations for HIV Care Providers. *Clinical Infectious Diseases, 51*(8), 937-946. doi:10.1086/656412

Melton, L. J., Achenbach, S. J., Gebhart, J. B., Babalola, E. O., Atkinson, E. J., & Bharucha, A. E. (2007). Influence of Hysterectomy on Long-Term Fracture Risk. *Fertility and sterility, 88*(1), 156-162. doi:10.1016/j.fertnstert.2006.11.080

Meunier , P. J., Roux , C., Seeman , E., Ortolani , S., Badurski , J. E., Spector , T. D., . . . Reginster , J. Y. (2004). The Effects of Strontium Ranelate on the Risk of Vertebral Fracture in Women with Postmenopausal Osteoporosis. *New England Journal of Medicine, 350*(5), 459-468. doi:doi:10.1056/NEJMoa022436

Michaelsson, K., Bergstrom, R., Mallmin, H., Holmberg, L., Wolk, A., & Ljunghall, S. (1996). Screening for osteopenia and osteoporosis: Selection by body composition. *Maturitas, 25*(1), 79. doi:http://dx.doi.org/10.1016/0378-5122(96)81663-X

Miller, K. D., Masur, H., Jones, E. C., Joe, G. O., Rick, M. E., Kelly, G. G., . . . Blackwelder, W. C. (2002). High prevalence of osteonecrosis of the femoral head in HIV-infected adults. *Annals of Internal Medicine, 137*(1), 17-25.

Ministry of Health. (2012). *Clinical Guidance on Management of Osteoporosis 2012*.

Ministry of Health. (2015). Global AIDS Response Progress Report Malaysia 2015.

Ministry of Health. (2016a). Consensus Guidelines on Antiretroviral Therapy 2016 (Draft document).

Ministry of Health. (2016b). Global AIDS Response Progress Report 2016.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med, 151*(4), 264-269, w264.

Mondy, K., Yarasheski, K., Powderly, W. G., Whyte, M., Claxton, S., DeMarco, D., . . . Tebas, P. (2003). Longitudinal evolution of bone mineral density and bone markers in human immunodeficiency virus-infected individuals. *Clinical Infectious Diseases, 36*(4), 482-490. doi:10.1086/367569

Morgan, S. L. (2001). Calcium and vitamin D in osteoporosis. *Rheum Dis Clin North Am, 27*(1), 101-130.

Moyer, V. A. (2013). Menopausal hormone therapy for the primary prevention of chronic conditions: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med, 158*(1), 47-54. doi:10.7326/0003-4819-158-1-201301010-00553

Murata, H., Hruz, P. W., & Mueckler, M. (2000). The mechanism of insulin resistance caused by HIV protease inhibitor therapy. *Journal of Biological Chemistry, 275*(27), 20251-20254.

Murphy, G., & Aitken, C. (2011). HIV testing--the perspective from across the pond. *J Clin Virol, 52 Suppl 1*, S71-76. doi:10.1016/j.jcv.2011.09.027

Mylonakis, E., Paliou, M., Lally, M., Flanigan, T. P., & Rich, J. D. (2000). Laboratory testing for infection with the human immunodeficiency virus: established and novel approaches. *Am J Med, 109*(7), 568-576.

Nair, R., & Maseeh, A. (2012). Vitamin D: The "sunshine" vitamin. *Journal of Pharmacology & Pharmacotherapeutics, 3*(2), 118-126. doi:10.4103/0976-500X.95506

Nair, S. (2010). Vitamin D Deficiency and Liver Disease. *Gastroenterology & Hepatology, 6*(8), 491-493.

Narayanan, P. (2013). Denosumab: A comprehensive review. *South Asian Journal of Cancer, 2*(4), 272-277. doi:10.4103/2278-330X.119895

National Clinical Guideline Centre. (2012). National Institute for Health and Clinical Excellence: Guidance *Osteoporosis: Fragility Fracture Risk: Osteoporosis: Assessing the Risk of Fragility Fracture*. London: Royal College of Physicians (UK), National Clinical Guideline Centre.

National Coordinating Commitee on Food and Nutrition. (2005). *Recommended Nutrient Intakesfor Malaysia : A Report of the Technical Working Group on Nutritional Guidelines*. Putrajaya, Malaysia Retrieved from http://www.moh.gov.my/images/gallery/rni/insert.pdf.

National Institutes of Health. (2001). Osteoporosis prevention, diagnosis, and therapy: N. I. H. Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. *JAMA, 285*(6), 785-795. doi:10.1001/jama.285.6.785

National Institutes of Health. (2014). Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies *National Institutes of Health*.

National Statistical Office. (2011). *Executive Summary: The 2011 Survey on Status of Society and Culture*. Retrieved from http://web.nso.go.th/stat_theme_socpop.htm.

Ndour, M., Sow, P. S., Coll-Seck, A. M., Badiane, S., Ndour, C. T., Diakhate, N., . . . Colebunders, R. (2000). AIDS caused by HIV1 and HIV2 infection: are there clinical differences? Results of AIDS surveillance 1986-97 at Fann Hospital in Dakar, Senegal. *Trop Med Int Health, 5*(10), 687-691.

Negin, J., Martiniuk, A., Cumming, R. G., Naidoo, N., Phaswana-Mafuya, N., Madurai, L., . . . Kowal, P. (2012). Prevalence of HIV and chronic comorbidities among older adults. *AIDS (London, England), 26*(0 1), S55-S63. doi:10.1097/QAD.0b013e3283558459

Negredo, E., Domingo, P., Ferrer, E., Estrada, V., Curran, A., Navarro, A., . . . Clotet, B. (2014). Peak Bone Mass in Young HIV-Infected Patients Compared With Healthy Controls. *Jaids-Journal of Acquired Immune Deficiency Syndromes, 65*(2), 207-212. doi:10.1097/01.qai.0000435598.20104.d6

Neumann-Haefelin, C., Blum, H. E., Chisari, F. V., & Thimme, R. (2005). T cell response in hepatitis C virus infection. *Journal of Clinical Virology, 32*(2), 75-85. doi:10.1016/j.jcv.2004.05.008

Nguyen, N. D., Frost, S. A., Center, J. R., Eisman, J. A., & Nguyen, T. V. (2008). Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. *Osteoporosis International, 19*(10), 1431-1444. doi:10.1007/s00198-008-0588-0

Nimitphong, H., & Holick, M. F. (2013). Vitamin D status and sun exposure in southeast Asia. *Dermato-endocrinology, 5*(1), 34-37. doi:10.4161/derm.24054

Nyamweya, S., Hegedus, A., Jaye, A., Rowland-Jones, S., Flanagan, K. L., & Macallan, D. C. (2013). Comparing HIV-1 and HIV-2 infection: Lessons for viral immunopathogenesis. *Rev Med Virol, 23*(4), 221-240. doi:10.1002/rmv.1739

Ofotokun, I., & Weitzmann, M. N. (2010). HIV-1 infection and antiretroviral therapies: risk factors for osteoporosis and bone fracture. *Curr Opin Endocrinol Diabetes Obes, 17*(6), 523-529.

Ofotokun, I., & Weitzmann, M. N. (2011). HIV and Bone Metabolism. *Discovery medicine, 11*(60), 385-393.

Onen, N. F., Overton, E. T., Seyfried, W., Stumm, E. R., Snell, M., Mondy, K., & Tebas, P. (2010). Aging and HIV infection: a comparison between older HIV-infected persons and the general population. *HIV Clin Trials, 11*(2), 100-109. doi:10.1310/hct1102-100

Open Source Epidemiologic Statistics for Public Health. (2013, 6/4/2013). Version 3.01. Retrieved from http://www.openepi.com/Menu/OE_Menu.htm

Osborne, V., Layton, D., Perrio, M., Wilton, L., & Shakir, S. A. W. (2010). Incidence of Venous Thromboembolism in Users of Strontium Ranelate. *Drug Safety, 33*(7), 579-591. doi:10.2165/11533770-000000000-00000

Pacifici, R. (2010). T CELLS: CRITICAL BONE REGULATORS IN HEALTH AND DISEASE. *Bone, 47*(3), 461-471. doi:10.1016/j.bone.2010.04.611

Painter, S. E., Kleerekoper, M., & Camacho, P. M. (2006). Secondary osteoporosis: a review of the recent evidence. *Endocr Pract, 12*(4), 436-445. doi:10.4158/ep.12.4.436

Palella , F. J. J., Delaney , K. M., Moorman , A. C., Loveless , M. O., Fuhrer , J., Satten , G. A., . . . Investigators, t. H. O. S. (1998). Declining Morbidity and Mortality among Patients with Advanced Human Immunodeficiency Virus Infection. *New England Journal of Medicine, 338*(13), 853-860. doi:doi:10.1056/NEJM199803263381301

Pan, G., Wu, X., McKenna, M. A., Feng, X., Nagy, T. R., & McDonald, J. M. (2004). AZT enhances osteoclastogenesis and bone loss. *AIDS Res Hum Retroviruses, 20*(6), 608-620. doi:10.1089/0889222041217482

Pandhi, D., & Ailawadi, P. (2014). Initiation of antiretroviral therapy. *Indian Journal of Sexually Transmitted Diseases, 35*(1), 1-11. doi:10.4103/0253-7184.132399

Park, H. J., Park, K. H., Park, G. M., Paek, Y. J., & Cho, J. J. (2003). Evaluation of Simple Tool as a Screening Test for Osteoporosis and Osteopenia in Korean Postmenopausal Women. *Korean J Fam Med 24*, 702-708.

Pinzone, M. R., Moreno, S., Cacopardo, B., & Nunnari, G. (2014). Is there enough evidence to use bisphosphonates in HIV-infected patients? A systematic review and meta-analysis. *AIDS Rev, 16*(4), 213-222.

Pirrone, V., Libon, D. J., Sell, C., Lerner, C. A., Nonnemacher, M. R., & Wigdahl, B. (2013). Impact of age on markers of HIV-1 disease. *Future virology, 8*(1), 81-101. doi:10.2217/fvl.12.127

Poulsen, A. G., Aaby, P., Gottschau, A., Kvinesdal, B. B., Dias, F., Molbak, K., & Lauritzen, E. (1993). HIV-2 infection in Bissau, West Africa, 1987-1989: incidence, prevalences, and routes of transmission. *J Acquir Immune Defic Syndr, 6*(8), 941-948.

Pruzansky, M. E., Turano, M., Luckey, M., & Senie, R. (1989). Low body weight as a risk factor for hip fracture in both black and white women. *J Orthop Res, 7*(2), 192-197. doi:10.1002/jor.1100070206

Qaqish, R. B., & Sims, K. A. (2004). Bone disorders associated with the human immunodeficiency virus: Pathogenesis and management. *Pharmacotherapy, 24*(10), 1331-1346. doi:10.1592/phco.24.14.1331.43150

Rajasuriar, R., Chong, M. L., Ahmad Bashah, N. S., Abdul Aziz, S. A., McStea, M., Lee, E. C., . . . Kamarulzaman, A. (2017). Significant health impact of accelerated aging in young HIV-infected individuals on antiretroviral therapy in Malaysia. *Aids*. doi:10.1097/qad.0000000000001475

Reginster, J. Y. (2014). Cardiac concerns associated with strontium ranelate. *Expert Opinion on Drug Safety, 13*(9), 1209-1213. doi:10.1517/14740338.2014.939169

Reginster, J. Y., Sedrine, W. B., Viethel, P., Micheletti, M. C., Chevallier, T., & Audran, M. (2004). Validation of OSIRIS®, a prescreening tool for the identification of women with an increased risk of osteoporosis. *Gynecological Endocrinology, 18*(1), 3-8. doi:10.1080/09513590310001651713

Richy, F., Gourlay, M., Ross, P. D., Sen, S. S., Radican, L., De Ceulaer, F., . . . Reginster, J. Y. (2004). Validation and comparative evaluation of the osteoporosis self-assessment tool (OST) in a Caucasian population from Belgium. *Qjm, 97*(1), 39-46.

Riggs, B. L., & Melton, L. J. (1986). Involutional osteoporosis. *N Engl J Med, 314*(26), 1676-1686. doi:10.1056/nejm198606263142605

Rodriguez, M., Daniels, B., Gunawardene, S., & Robbins, G. K. (2009). High frequency of vitamin D deficiency in ambulatory HIV-Positive patients. *AIDS Res Hum Retroviruses, 25*(1), 9-14. doi:10.1089/aid.2008.0183

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., . . . Shapses, S. A. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab, 96*(1), 53-58. doi:10.1210/jc.2010-2704

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., . . . Ockene, J. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA, 288*(3), 321-333.

Rwebembera, A., Sudfeld, C. R., Manji, K. P., Duggan, C., Aboud, S., & Fawzi, W. W. (2013). Prevalence and Risk Factors for Vitamin D Deficiency Among Tanzanian HIV-Exposed Uninfected Infants. *Journal of Tropical Pediatrics, 59*(5), 426-429. doi:10.1093/tropej/fmt028

Saccomanno, M. F., & Ammassari, A. (2011). Bone disease in HIV infection. *Clinical Cases in Mineral and Bone Metabolism, 8*(1), 33-36.

Saetung, S., Ongphiphadhanakul, B., & Rajatanavin, R. (2008). The relationship of an Asian-specific screening tool for osteoporosis to vertebral deformity and osteoporosis. *Journal of Bone and Mineral Metabolism, 26*(1), 47-52. doi:10.1007/s00774-007-0796-2

Schambelan, M., Benson, C. A., Carr, A., Currier, J. S., Dubé, M. P., Gerber, J. G., . . . Mulligan, K. (2002). Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr, 31*(3), 257-275.

Schouten, J., Wit, F. W., Stolte, I. G., Kootstra, N. A., van der Valk, M., Geerlings, S. E., . . . Reiss, P. (2014). Cross-sectional Comparison of the Prevalence of Age-Associated Comorbidities and Their Risk Factors Between HIV-Infected and Uninfected Individuals: The AGEhIV Cohort Study. *Clinical Infectious Diseases, 59*(12), 1787-1797. doi:10.1093/cid/ciu701

Sedrine, W. B., Chevallier, T., Zegels, B., Kvasz, A., Micheletti, M. C., Gelas, B., & Reginster, J. Y. (2002). Development and assessment of the Osteoporosis Index of Risk (OSIRIS) to facilitate selection of women for bone densitometry. *Gynecological Endocrinology, 16*(3), 245-250. doi:10.1080/gye.16.3.245.250

Seeman, E. (2001). Sexual Dimorphism in Skeletal Size, Density, and Strength. *The Journal of Clinical Endocrinology & Metabolism, 86*(10), 4576-4584. doi:10.1210/jcem.86.10.7960

Shafinaz, I. S., & Moy, F. M. (2016). Vitamin D level and its association with adiposity among multi-ethnic adults in Kuala Lumpur, Malaysia: a cross sectional study. *BMC Public Health, 16*(1), 232. doi:10.1186/s12889-016-2924-1

Shapses, S. A., & Sukumar, D. (2012). Bone Metabolism in Obesity and Weight Loss. *Annual review of nutrition, 32*, 287-309. doi:10.1146/annurev.nutr.012809.104655

Sharma, U., & Stevermer, J. J. (2009). Bisphosphonate therapy: When not to monitor BMD. *The Journal of Family Practice, 58*(11), 594-596.

Sheu, A., & Diamond, T. (2016). Secondary osteoporosis. *Australian Prescriber, 39*(3), 85-87. doi:10.18773/austprescr.2016.038

Shiau, S., Broun, E. C., Arpadi, S. M., & Yin, M. T. (2013). Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. *AIDS (London, England), 27*(12), 1949-1957. doi:10.1097/QAD.0b013e328361d241

Short, C. E. S., Shaw, S. G., Fisher, M. J., Gilleece, Y. C., & Walker-Bone, K. (2014). Comparison of peripheral forearm DXA and clinical risk factor screening using FRAX® to assess the risk of HIV-associated osteoporosis: a cross-sectional study. *Archives of osteoporosis, 9*, 181-181. doi:10.1007/s11657-014-0181-4

Sierra, S., Kupfer, B., & Kaiser, R. (2005). Basics of the virology of HIV-1 and its replication. *Journal of Clinical Virology, 34*(4), 233-244. doi:https://doi.org/10.1016/j.jcv.2005.09.004

Stellbrink, H. J., Orkin, C., Arribas, J. R., Compston, J., Gerstoft, J., Van Wijngaerden, E., . . . Pearce, H. (2010). Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. *Clin Infect Dis, 51*(8), 963-972. doi:10.1086/656417

Stepan, J. J. (2002). Techniques for measuring bone mineral density. *International Congress Series, 1229*, 63-68. doi:http://dx.doi.org/10.1016/S0531-5131(01)00477-0

Stephens, K. I., Rubinsztain, L., Payan, J., Rentsch, C., Rimland, D., & Tangpricha, V. (2016). Dual-energy X-ray absorptiometry and calcualted FRAX risk scores may underestimate osteoporotic fracture risk in vitamin D-deficient veterans with HIV infection. *Endocr Pract, 22*(4), 440-446. doi:10.4158/ep15958.or

Sterne, J. A. C., Gavaghan, D., & Egger, M. (2000). Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *Journal of clinical epidemiology, 53*(11), 1119-1129.

Subbiah, V., Madsen, V. S., Raymond, A. K., Benjamin, R. S., & Ludwig, J. A. (2010). Of mice and men: divergent risks of teriparatide-induced osteosarcoma. *Osteoporosis International, 21*(6), 1041-1045. doi:10.1007/s00198-009-1004-0

Sunyecz, J. A. (2008). The use of calcium and vitamin D in the management of osteoporosis. *Therapeutics and Clinical Risk Management, 4*(4), 827-836.

Sweet, D. E. (2005). Metabolic complications of antiretroviral therapy. *Top HIV Med, 13*(2), 70-74.

Tajeu, G. S., Delzell, E., Smith, W., Arora, T., Curtis, J. R., Saag, K. G., . . . Kilgore, M. L. (2014). Death, Debility, and Destitution Following Hip Fracture. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 69A*(3), 346-353. doi:10.1093/gerona/glt105

Tangpricha, V. (2007). Vitamin D Deficiency in the Southern United States. *Southern medical journal, 100*(4), 384-385.

Taylor, A., & Rogers, M. HIV treatments and the skeleton: Do NRTIs directly effect bone cells? *Bone, 46*, S56. doi:10.1016/j.bone.2010.01.131

Tebas, P., Powderly, W. G., Claxton, S., Marin, D., Tantisiriwat, W., Teitelbaum, S. L., & Yarasheski, K. E. (2000). Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. *Aids, 14*(4), F63-67.

Teichmann, J., Lange, U., Discher, T., Lohmeyer, J., Stracke, H., & Bretzel, R. G. (2009). Bone mineral density in human immunodeficiency virus-1 infected men with hypogonadism prior to highly-active-antiretroviral-therapy (HAART). *Eur J Med Res, 14*, 59-64.

Teichmann, J., Stephan, E., Lange, U., Discher, T., Friese, G., Lohmeyer, J., . . . Bretzel, R. G. (2003). Osteopenia in HIV-infected Women Prior to Highly Active Antiretroviral Therapy. *Journal of Infection, 46*(4), 221-227. doi:http://dx.doi.org/10.1053/jinf.2002.1109

Tomazic, J., Ul, K., Volcansek, G., Gorensek, S., Pfeifer, M., Karner, P., . . . Vidmar, L. (2007). Prevalence and risk factors for osteopenia/osteoporosis in an HIV-infected male population. *Wiener Klinische Wochenschrift, 119*(21-22), 639-646. doi:10.1007/s00508-007-0844-x

Tseng, A., Foisy, M., Hughes, C. A., Kelly, D., Chan, S., Dayneka, N., . . . Yoong, D. (2012). Role of the Pharmacist in Caring for Patients with HIV/AIDS: Clinical Practice Guidelines. *The Canadian Journal of Hospital Pharmacy, 65*(2), 125-145.

Tung, W. C. (2012). Osteoporosis Among Asian American Women. *Home Health Care Management & Practice, 24*(4), 205-207. doi:10.1177/1084822312441702

Tuppurainen, M., Kroger, H., Saarikoski, S., Honkanen, R., & Alhava, E. (1995). The effect of gynecological risk factors on lumbar and femoral bone mineral density in peri- and postmenopausal women. *Maturitas, 21*(2), 137-145.

Tweeten, S. S. M., & Rickman, L. S. (1998). Infectious Complications of Body Piercing. *Clinical Infectious Diseases, 26*(3), 735-740. doi:10.1086/514586

UNAIDS. (2016a). Fact sheet - Latest statistics on the status of the AIDS epidemic. Retrieved from http://www.unaids.org/en/resources/fact-sheet

UNAIDS. (2016b). Global AIDS Update. Retrieved from http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016

UNAIDS. (2016c). Prevention gap report. Retrieved from http://www.unaids.org/en/resources/documents/2016/prevention-gap

United States Centers for Disease Control and Prevention. (1993). 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults.

US Department of Health Human Services. (2004). Bone health and osteoporosis: a report of the Surgeon General. *Rockville, MD: US Department of Health and Human Services, Office of the Surgeon General, 87*.

Vahle, J. L., Sato, M., Long, G. G., Young, J. K., Francis, P. C., Engelhardt, J. A., . . . Nold, J. B. (2002). Skeletal Changes in Rats Given Daily Subcutaneous Injections of Recombinant Human Parathyroid Hormone (1-34) for 2 Years and Relevance to Human Safety. *Toxicologic Pathology, 30*(3), 312-321. doi:doi:10.1080/01926230252929882

van den Bergh, J. P. W., van Geel, T. A. C. M., Lems, W. F., & Geusens, P. P. (2010). Assessment of Individual Fracture Risk: FRAX and Beyond. *Current Osteoporosis Reports, 8*(3), 131-137. doi:10.1007/s11914-010-0022-3

Vochteloo, A. J., Moerman, S., Tuinebreijer, W. E., Maier, A. B., de Vries, M. R., Bloem, R. M., . . . Pilot, P. (2013). More than half of hip fracture patients do not regain mobility in the first postoperative year. *Geriatr Gerontol Int, 13*(2), 334-341. doi:10.1111/j.1447-0594.2012.00904.x

Vuori, I. M. (2001). Dose-response of physical activity and low back pain, osteoarthritis, and osteoporosis. *Med Sci Sports Exerc, 33*(6 Suppl), S551-586; discussion 609-510.

Wacker, M., & Holick, M. F. (2013). Sunlight and Vitamin D: A global perspective for health. *Dermato-endocrinology, 5*(1), 51-108. doi:10.4161/derm.24494

Walker Harris, V., & Brown, T. T. (2012). Bone Loss in the HIV-Infected Patient: Evidence, Clinical Implications, and Treatment Strategies. *The Journal of Infectious Diseases, 205*(Suppl 3), S391-S398. doi:10.1093/infdis/jis199

Wang, M. W., Wei, S., Faccio, R., Takeshita, S., Tebas, P., Powderly, W. G., . . . Ross, F. P. (2004). The HIV protease inhibitor ritonavir blocks osteoclastogenesis and function by impairing RANKL-induced signaling. *J Clin Invest, 114*(2), 206-213. doi:10.1172/jci15797

Wasserman, P., & Rubin, D. S. (2010). Highly prevalent vitamin D deficiency and insufficiency in an urban cohort of HIV-infected men under care. *AIDS Patient Care STDS, 24*(4), 223-227. doi:10.1089/apc.2009.0241

Weber, J. (2001). The pathogenesis of HIV-1 infection. *British Medical Bulletin, 58*(1), 61-72. doi:10.1093/bmb/58.1.61

Weinstein, L., & Ullery, B. (2000). Identification of at-risk women for osteoporosis screening. *American Journal of Obstetrics and Gynecology, 183*(3), 547-549. doi:http://dx.doi.org/10.1067/mob.2000.106594

Weitzmann, M. N., & Pacifici, R. (2006). Estrogen deficiency and bone loss: an inflammatory tale. *Journal of Clinical Investigation, 116*(5), 1186-1194. doi:10.1172/JCI28550

Welz, T., Childs, K., Ibrahim, F., Poulton, M., Taylor, C. B., Moniz, C. F., & Post, F. A. (2010). Efavirenz is associated with severe vitamin D deficiency and increased

alkaline phosphatase. *Aids, 24*(12), 1923-1928. doi:10.1097/QAD.0b013e32833c3281

Wheeler, A. L., Tien, P. C., Grunfeld, C., & Schafer, A. L. (2015). Teriparatide treatment of osteoporosis in an HIV-infected man: a case report and literature review. *AIDS (London, England), 29*(2), 245-246. doi:10.1097/QAD.0000000000000529

White, A. (2001). Mitochondrial toxicity and HIV therapy. *Sexually Transmitted Infections, 77*(3), 158-173. doi:10.1136/sti.77.3.158

Wiboonchutikul, S., Sungkanuparph, S., Kiertiburanakul, S., Chailurkit, L. O., Charoenyingwattana, A., Wangsomboonsiri, W., . . . Ongphiphadhanakul, B. (2012). Vitamin D insufficiency and deficiency among HIV-1-infected patients in a tropical setting. *J Int Assoc Physicians AIDS Care (Chic), 11*(5), 305-310. doi:10.1177/1545109711432142

Wing, E. J. (2016). HIV and aging. *International Journal of Infectious Diseases*. doi:10.1016/j.ijid.2016.10.004

Woolf, A. D., & Akesson, K. (2003). Preventing fractures in elderly people. *BMJ, 327*(7406), 89-95. doi:10.1136/bmj.327.7406.89

World Health Organisation. (1994). Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser, 843*, 1-129.

World Health Organisation. (2006). WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children.

World Health Organisation. (2010). PMTCT Strategic Vision 2010–2015: Preventing mother-to-child transmission of HIV to reach the UNGASS and Millennium Development Goals.

World Health Organisation. (2013). Consolidated Guidelines on the use of Antiretroviral Drugs For Treating and Preventing HIV infection. Recommendation for a public health approach.

World Health Organisation. (2015). Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV.

World Health Organization. (2012). *Guidance on Pre-Exposure Oral Prophylaxis (PrEP) for Serodiscordant Couples, Men and Transgender Women Who Have Sex with Men at High Risk of HIV: Recommendations for Use in the Context of Demonstration Projects.* Geneva.

Wu, P. Y., Chen, M. Y., Hsieh, S. M., Sun, H. Y., Tsai, M. S., Lee, K. Y., . . . Hung, C. C. (2014). Comorbidities among the HIV-Infected Patients Aged 40 Years or Older in Taiwan. *PLoS One, 9*(8), e104945. doi:10.1371/journal.pone.0104945

Yin, M., Dobkin, J., Brudney, K., Becker, C., Zadel, J. L., Manandhar, M., . . . Shane, E. (2005). Bone mass and mineral metabolism in HIV plus postmenopausal women. *Osteoporosis International, 16*(11), 1345-1352. doi:10.1007/s00198-005-1845-0

Yin, M. T., McMahon, D. J., Ferris, D. C., Zhang, C. A., Shu, A., Staron, R., . . . Shane, E. (2010). Low Bone Mass and High Bone Turnover in Postmenopausal Human Immunodeficiency Virus-Infected Women. *The Journal of clinical endocrinology and metabolism, 95*(2), 620-629. doi:10.1210/jc.2009-0708

Yin, M. T., Shiau, S., Rimland, D., Gibert, C. L., Bedimo, R. J., Rodriguez-Barradas, M. C., . . . Womack, J. A. (2016). Fracture Prediction With Modified-FRAX in Older HIV-Infected and Uninfected Men. *J Acquir Immune Defic Syndr, 72*(5), 513-520. doi:10.1097/qai.0000000000000998

Yoon, V., Maalouf, N. M., & Sakhaee, K. (2012). The effects of smoking on bone metabolism. *Osteoporos Int, 23*(8), 2081-2092. doi:10.1007/s00198-012-1940-y

Young, F. E. (1988). The role of the FDA in the effort against AIDS. *Public Health Rep, 103*(3), 242-245.

Zhang, Z., Ou, Y., Sheng, Z., & Liao, E. (2014). How to decide intervention thresholds based on FRAX in central south Chinese postmenopausal women. *Endocrine, 45*(2), 195-197. doi:10.1007/s12020-013-0076-y

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Journal publications

1. Goh, S. S. L., Lai P. S. M., Tan, A. T. B., Ponnampalavanar, S (2018). "Osteopenia or osteoporosis in human immunodeficiency virus-infected individuals: A meta-analysis of its prevalence and risk factors," *Osteoporosis International 29*(3), 595-613. doi:10.1007/s00198-017-4305-8 (Submitted:23 March 2017: Published: 20 November 2017.

Abstract publications in national and international conferences

1. Goh, S. S. L., Lai, P. S. M., Ponnampalavanar, S., Tan, A.T.B., Rajasuriar, R., Raja Azwa, R. I. S., Syed Omar. S., Sulaiman, H., Ahmad Bashah, N. S., Chong, M. L.,Kamaruzzaman, S. B.,Kamarulzaman, A.(2015) "Prevalence of osteoporosis in men and women infected with the human immunodeficiency virus compared to healthy community dwelling men and women: Preliminary results, " *The 5th Asia Pacific Primary Care Research Conference 2015, Putrajaya, Malaysia*

2. Goh, S. S. L., Lai, P. S. M., Ponnampalavanar, S., Tan, A.T.B., Rajasuriar, R., Raja Azwa, R. I. S., Syed Omar. S., Sulaiman, H., Ahmad Bashah, N. S., Chong, M. L.,Kamaruzzaman, S. B.,Kamarulzaman, A.(2016) "Prevalence of reduced bone mineral density in human immunodeficiency virus (HIV) infected compared to uninfected individuals: Preliminary results, " *National HIV Treatment Update 2016, Sungai Buloh, Malaysia*

3. Goh, S. S. L., Lai, P. S. M., Tan, A.T.B., Ponnampalavanar, S. (2016)."Reduced bone mineral density in human immunodeficiency virus-infected individuals: A meta-

analysis of its prevalence and risk factors, ” *International Osteoporosis Foundation (IOF) Regionals 6th Asia-Pacific Osteoporosis Meeting 2016, Singapore*

4. Goh, S. S. L., Lai, P. S. M., Ponnampalavanar, S., Tan, A.T.B., Rajasuriar, R., Raja Azwa, R. I. S., Syed Omar. S., Sulaiman, H., Ahmad Bashah, N. S., Chong, M. L., Mcstea, M., Kamaruzzaman, S. B.,Kamarulzaman, A.(2017). “Prevalence of reduced bone mineral density in human immunodeficiency virus (HIV)-infected compared to uninfected individuals in Malaysia,” *The joint meeting of the Australian and New Zealand Bone and Mineral Society (ANZBMS) and the International Federation of Musculoskeletal Research Societies (IFMRS), in conjunction with the Japanese Society for Bone and Mineral Research (JSBMR), Brisbane, Australia.*

5. Goh, S. S. L., Lai, P. S. M., Tan, A.T.B., Ponnampalavanar, S. (2017).“Reduced bone mineral density in human immunodeficiency virus-infected individuals: A meta-analysis of its prevalence and risk factors,” *The joint meeting of the Australian and New Zealand Bone and Mineral Society (ANZBMS) and the International Federation of Musculoskeletal Research Societies (IFMRS), in conjunction with the Japanese Society for Bone and Mineral Research (JSBMR), Brisbane, Australia.*

GRANTS RECEIVED

To fund for study research:

1) University of Malaya High Impact Research Grants for Malaysian Elderly Longitudinal Research group, MELOR (UM0000099/HIR.C3)
2) University of Malaya High Impact Research Grantsfor Malaysian Elders Longitudinal Research (MELoR) - Health & Wellbeing: HIV & Aging (MHIVA) (H-20001-00-E000091)
3) Postgraduate Research Grant (PG197-2015B)

To fund for tuition fees:

1) Program Pembiayaan MyMaster (MyBrain 15) scholarship

To fund for travel to attend conference

1) Travel grant: University of Malaya Specialist Centre (UMSC) CA.RE fund
   Conference: International Osteoporosis Foundation (IOF) Regionals 6th Asia Pacific Osteoporosis Meeting 2016, Singapore
2) Travel grant award: Australian and New Zealand Bone and Mineral Society (ANZBMS) Travel Grant Award, 2017
   Conference: The joint meeting of the ANZBMS and the International Federation of Musculoskeletal Research Societies (IFMRS) 2017, Brisbane, Australia.