BONE HEALTH AND VITAMIN D STATUS AMONG PERINATALLY HIV-INFECTED ASIAN CHILDREN AND ADOLESCENTS WITH VIROLOGICAL SUPPRESSION

by
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ABSTRACT

Background: Pathogenesis of reduced bone mass and its relationships with hypovitaminosis D in HIV-infected populations are largely unknown. The goals of this dissertation were to determine the prevalence of low bone mass and its pathogenesis, along with the prevalence of hypovitaminosis D and its effects on bone turnover and bone density among HIV-infected Asian adolescents.

Methods: A multicenter, cross-sectional study was conducted at four pediatric HIV centers in Thailand and Indonesia. Perinatally HIV-infected adolescents aged 10-18 years receiving antiretroviral therapy with virologic suppression (HIV RNA <400 copies/ml) were enrolled. Study assessments included lumbar spine dual-energy x-ray absorptiometry, 25-hydroxyvitamin D (25-OHD), intact parathyroid hormone (iPTH), bone turnover markers [C-terminal cross-linked telopeptide of type I collagen (CTX) and procollagen type I amino-terminal propeptide (PINP)]. Bone mineral density (BMD) and bone mineral apparent density (BMAD) Z-scores were calculated based on Thai normative reference. Z-scores $\leq$-2 was defined as low bone mass. The 25-OHD <20 ng/ml and iPTH >65 pg/ml were defined as hypovitaminosis D and hyperparathyroidism, respectively.

Results: Of 396 participants, 57% were female and median age (IQR) was 15.0 (13.3-16.9) years. The prevalence of lumbar spine BMD and BMAD Z-scores $\leq$-2 were 16.4% and 8.3%, respectively. Z-scores were lower with older age, female sex, body mass index <5th percentile, protease inhibitor exposure and CD4^{+} <15% before ART initiation. Increased bone turnover markers were inversely associated with BMD/BMAD Z-scores.

Among 394 adolescents who had 25-OHD results, the prevalence of hypovitaminosis D, hyperparathyroidism, and both conditions were 21% (95%CI: 17-25%), 17% (95%CI: 13-20%) and 5% (95%CI: 3-7%), respectively. Adolescents with hypovitaminosis D and secondary hyperparathyroidism had the highest median bone resorption (CTX: 1610 vs. 1270 ng/l; *P*=0.04) and bone formation (PINP: 572 vs. 330 μg/l; *P*=0.02) markers, and the greatest proportion of low BMD (42 vs. 15%; *P*=0.01) compared to the rest of the cohort.

Conclusion: Low bone mass was not uncommon in our adolescents. Bone turnover dysregulation was associated with reduced bone mass. Hypovitaminosis D complicated with secondary hyperparathyroidism was associated with increased bone turnover and bone demineralization. Monitoring of bone mass and vitamin D status may be important for this population.

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CHAPTER I

INTRODUCTION AND OVERVIEW

Introduction

Advances in antiretroviral therapy (ART) over the past twenty years have led to a dramatic decline in disease-associated morbidity and mortality in HIV-infected populations, including infant and children perinatally infected with HIV [1-3]. In countries where ART is available and accessible, the face of the HIV epidemic has changed from a disease with high mortality to that of a chronic illness [4]. Most patients achieve sustainable and possibly lifelong viral suppression. As a consequence, the numbers of perinatally HIV-infected infants and children entering adolescence and young adulthood have been expanding worldwide [5,6]. However, a considerable improvement in life expectancy has been accompanied by a growing incidence of non-communicable diseases (NCDs) associated with long-term HIV infection and its therapies, including adverse bone health, neurocognitive dysfunction, renal diseases, metabolic disorders and endocrine diseases [7-15]. The changing spectrum of HIV-associated diseases is a new challenge and a major concern in the medical management of this unique population. Among the important roles of healthcare providers and clinical researchers are to find ways to help these children and adolescents to be able to grow up with HIV with a high or comparable quality of life to their healthy peers.

The pathogenesis and underlying mechanisms of NCDs in the setting of HIV infection remain unclear. Both HIV *per se* and ART have been reported to be associated with these complications [7,10-12,16-18]. In addition, persistent immune activation and inflammation due to HIV infection may be another contributing factor even in virally suppressed patients [19-21]. Importantly, the impact of these factors on NCDs may be magnified in adolescents with perinatal HIV infection because they have been exposed to

HIV infection since birth and have experienced ART for a long duration compared to HIV-infected adults who acquire HIV infection later in life.

Although there are several NCDs reported in perinatally HIV-infected adolescents [7-15], adverse bone health is a specific concern. Generally, adverse bone health is a term used to describe skeletal disorders which are characterized by reduced bone mass, deterioration of bone tissue, disruption of bone microarchitecture and compromised bone strength [22,23]. Unlike adults, the term osteoporosis in adolescents is defined as having low bone mass with a clinically significant evidence of bone fracture [24]. Therefore, to prevent the delayed diagnosis and treatment, most pediatricians and researchers usually evaluate adverse bone health problem in adolescents by focusing on individuals who have low bone mass rather than osteoporosis [24].

Low bone mass, which is usually evaluated by Dual-energy X-ray absorptiometry (DXA) technique and is defined as bone density Z-score less than or equal to -2.0 standard deviation [24], has been documented in perinatally HIV-infected children and adolescents [7-10,25]. Nevertheless, most of the studies were conducted in resource-rich and developed countries, with limited data from resource-constrained settings.

Bone is a dynamic, viable and highly organized connective tissue that is continually being remodeled throughout life to maintain the strength and integrity. Bone remodeling is a highly coordinated process which requires the synchronized activities of 2 specialized bone cells, including osteoblasts for bone formation and osteoclasts for bone resorption [26,27]. The imbalance activities between these two cells may result in the dysregulated bone turnover and bone mass loss [27]. In the setting of HIV infection, several factors can derail this important physiological process, including traditional

factors, e.g., malnutrition, hypovitminosis D, physical inactivity; and HIV-related factors, e.g., advanced disease, poor virologic status and some specific ART medications, as well as persistent immune activation and inflammation associated with HIV infection [7-10,25,28-37]. Therefore, all of these factors may result in the adverse bone outcomes in HIV-infected population. Unfortunately, the pathogenesis of low bone mass among people living with HIV, particularly HIV-infected adolescents, has not been carefully investigated.

Hypovitaminosis D is a global epidemic and health problem that has been documented in healthy and HIV-infected populations in all age groups and all settings [38-41]. Populations who live in countries with low latitude where sunshine is plenty all year long, as well as individuals who live in developed countries where vitamin D fortified foods are available, demonstrate a high prevalence of hypovitaminosis D [38]. Importantly, this condition has been reported to be associated with numerous adverse health outcomes, including osteoporosis, cardiovascular disease, diabetes, cancer, autoimmune diseases and some infectious diseases, e.g., tuberculosis [42-48]. Furthermore, in HIV-infected populations, deficiency of vitamin D has demonstrated the association with poor immune responses to ART which may accelerate disease progression to AIDS and increase all-cause mortality [49-53]. However, there are limited studies evaluating vitamin D status and its adverse consequences among HIV-infected adolescents, especially in resource-constrained settings, even though this population is at high risk of hypovitaminosis D.

There are several factors contributing to hypovitaminosis D in people living with HIV. Similar to the general population, biological factors implicated with

hypovitaminosis D include age, sex, race and ethnicity, skin pigmentation and body mass index; physical factors such as clothing, glass shielding and sunscreens; and environmental factors such as geographic location (latitude) and seasonality can influence vitamin D status in individuals [47,54-59]. Additionally, HIV-related factors, including clinical stage, immunological status, specific types of ART, e.g., efavirenz and boosted protease inhibitors, can affect vitamin D synthesis and metabolism [47,57-59]. Since previous studies were mostly conducted in HIV-infected youth who live in Western or industrialized countries [56,58], inferences may unfortunately not be able to generalize to individuals who live in other settings, such as in resource-constrained countries or tropical regions where, besides geographic location and seasons, there may be important dissimilarities in lifestyles, cultures as well as foods and dietary supplements that must be considered.

Hypovitaminosis D is considered to be a risk factor for adverse bone health. It can result in poor bone mineralization, progressive bone loss, and increased osteoporosis risk [54]. Although a study in HIV-infected adults found the association between vitamin D deficiency and bone loss [42], several studies in adolescents living with HIV did not demonstrate a correlation [9,29]. Moreover, there is lack of research study investigating the effects of hypovitaminosis D on the pathogenesis of reduced bone mass among this population.

Normally, when vitamin D levels are deficient or insufficient, calcium absorption from the gastrointestinal tract is inadequate to meet the calcium requirements to maintain normal physiological functions, which include bone metabolism, neuromuscular activity and other important metabolic functions [54]. Through a positive feedback mechanism,

the decreased serum calcium concentration causes an increased in parathyroid hormone (PTH) secretion by parathyroid glands, which is called secondary hyperparathyroidism, in order to restore calcium homeostasis [54]. The main effects of elevated serum PTH concentration are to enhance the release of calcium from the large reservoir in bone matrix which is indirectly mediated by osteoclasts and results in bone resorption; accelerate the active tubular reabsorption of calcium in the kidney; and increase the renal production of calcitriol (active form of vitamin D) to enhance the intestinal absorption of calcium [54,60]. Therefore, with these mechanisms, hypovitaminosis D along with secondary hyperparathyroidism may potentially cause increased bone turnover, bone demineralization and bone loss as the adverse consequences [54,60]. However, to date, there is limited information regarding the pathologic impacts of hypovitaminosis D and secondary hyperparathyroidism on bone turnover and bone mass among HIV-infected adolescents, especially in individuals who live in resource-constrained countries.

Because a great deal of bone mineral deposition and a lifetime peak bone mass (PBM) are normally attained during adolescence [61-63] and, importantly, up to 60% of the lifetime risk of osteoporosis is attributable to the amount of bone mineral accrued through the first two decades of life [64], any harmful factor occurring during this critical period may have tremendous adverse effects on bone mass and bone health of these individuals. As a consequence, perinatally HIV-infected adolescents who have been exposed to factors negatively influencing bone health during puberty are more likely to have reduced bone density and compromised adult PBM, a key determinant of bone health [30]. Furthermore, these individuals may experience serious detrimental bone consequences, particularly osteoporosis and bone fracture, when they enter their later

decades of life [65-68]. Therefore, feasible interventions to prevent bone loss and promote bone health for these adolescents should be promptly implemented before the unfavorable outcomes occur.

Goals of the dissertation

Despite the clear clinical significance and public health importance, the information regarding the extent of adverse bone health and hypovitaminosis D among perinatally HIV-infected adolescents living in resource-constrained countries are limited. Moreover, the pathogenesis of reduced bone mass, the associated factors of vitamin D status, and the pathological effects of hypovitaminosis D on bone turnover and bone mass among this population have not been intensively explored.

To fill gaps in current knowledge, the goals of this dissertation were to determine the prevalence of low bone mass and its pathogenesis, including bone turnover dysregulation and chronic immune activation and inflammation by HIV infection, in perinatally HIV-infected adolescents. In addition, this dissertation also aimed to assess the prevalence of hypovitaminosis D, secondary hyperparathyroidism and their pathological effects on bone turnover and bone mass in this population. By focusing the study on Asian adolescents who perinatally acquired HIV infection, this dissertation would be able to provide useful scientific information that can raise awareness about adverse bone health and hypovitaminosis D problems among pediatricians and healthcare providers who are taking care of adolescents living with HIV in their clinical settings, and would be beneficial for them to develop the strategies to screen and monitor bone health and vitamin D status in individuals who are at high risk for bone demineralization and vitamin D deficiency, as well as to implement the interventions to prevent bone deterioration and improve vitamin D status before the irreversible consequences develop.

Specific aims

Accordingly, the specific aims of this dissertation are:

AIM 1: To determine the prevalence of low bone mass and evaluate its pathogenesis among perinatally HIV-infected Asian adolescents with virologic suppression.

Perinatally HIV-infected Asian adolescents with virologic suppression on ART have a high prevalence of low bone mass. Their bone density is much lower than that of their HIV-uninfected peers. Dysregulation of bone turnover, reflected in the increases in bone resorption and formation markers, and chronic immune activation and inflammation due to HIV infection are potential mechanisms of bone demineralization among this population.

AIM 2: To assess the prevalence of hypovitaminosis D and hyperparathyroidism, and identify their effects on bone turnover and bone mineral density among perinatally HIV-infected Asian adolescents with virologic suppression.

The prevalence of hypovitaminosis D and hyperparathyroidism among perinatally HIV-infected Asian adolescents with virologic suppression on ART are high. Hypovitaminosis D complicated with secondary hyperparathyroidism is associated with increased bone turnover and decreased bone mineral density in this population.

Conceptual framework

Among perinatally HIV-infected adolescents with virologic suppression on ART, HIV-specific factors associated with low bone mass include WHO clinical status, immunological status, some specific types of ART and duration of ART use. Similar to general populations, traditional factors which include hypovitaminosis D, advanced age, female sex, low BMI and physical inactivity are potentially associated with reduced bone mass. Furthermore, hypovitaminosis D can cause secondary hyperparathyroidism through a positive feedback mechanism to maintain calcium homeostasis, which thereafter may result in dysregulation of bone turnover and acceleration of bone demineralization among this population. A residual chronic immune activation and inflammation by HIV infection, which usually persists even in individuals with successful virologic suppression on ART, may also aggravate bone turnover dysregulation that may enhance bone mass deterioration. The combination of these factors, traditional factors, HIV-specific factors and HIV itself, on low bone mass may consequently result in the decreased adult PBM and the increased risk of osteoporosis and bone fracture when these adolescents enter their fourth or fifth decades of life. The conceptual framework for this dissertation is summarized in Figure 1.

Organization of dissertation

This dissertation is composed of five chapters. The first chapter (this chapter) provides an introduction and overview of the dissertation by addressing the significance of adverse bone health and hypovitaminosis D problems among perinatally HIV-infected adolescents, pointing out the gaps in knowledge in this field, summarizes the goals of the dissertation, describes the specific aims of the study, and presents a conceptual framework for this dissertation.

The second chapter reviews the literatures on adverse bone health among children and adolescents growing up with HIV. This chapter describes the extent of adverse bone health problem among perinatally HIV-infected children and adolescents in different settings, explains the pathogenesis, illustrates the risk factors, and discusses the consequences of adverse bone health among this population. Furthermore, the second chapter summarizes both standard and advanced measurements to assess bone health, and updates the management of bone demineralization for this specific population.

The third chapter presents a study on adverse bone health and abnormal bone turnover among perinatally HIV-infected Asian adolescents with virologic suppression that aims to determine the prevalence of low bone mass and evaluate its pathogenesis in this population. In addition, this chapter describes the association between dysregulated bone turnover and poor bone mass and the relationships of chronic immune activation and inflammation with abnormal bone turnover.

The fourth chapter presents a study on hypovitaminosis D and hyperparathyroidism among perinatally HIV-infected Asian adolescents with virologic

suppression which aims to assess their prevalence, as well as pathological impacts on bone turnover and bone density in this population.

The fifth chapter, which is the last chapter for this dissertation, provides the summary of the dissertation findings, points out the lessons learned, addresses the public health significance of this dissertation, and proposes the future directions for research in this field.

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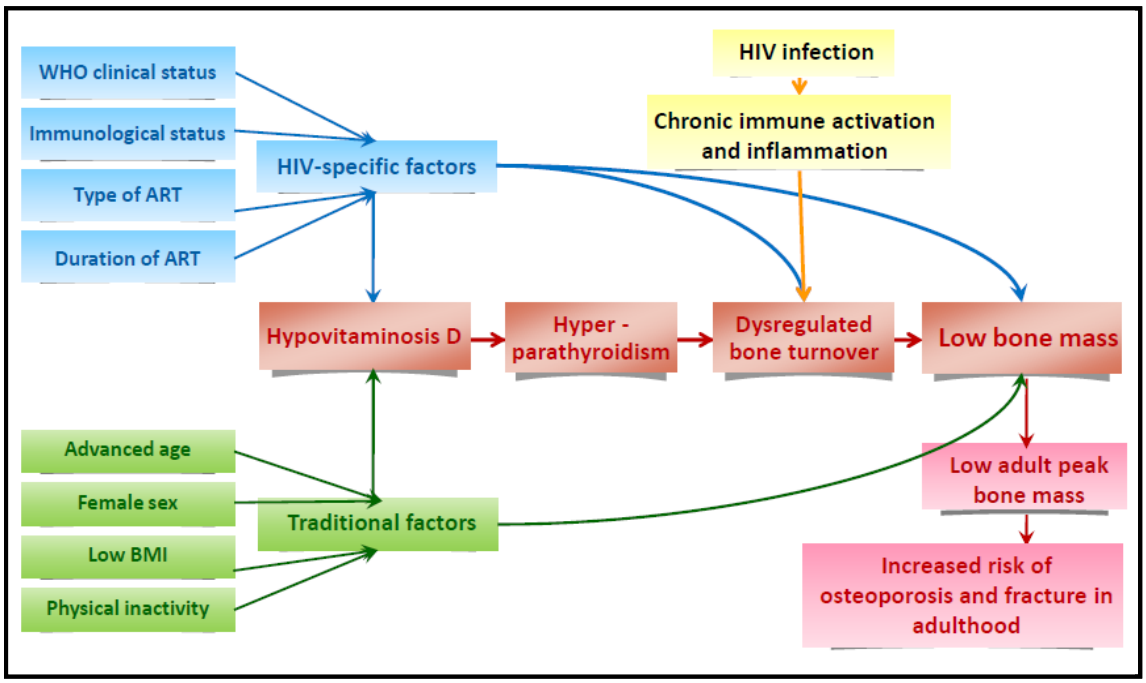
Figure 1. Conceptual framework of the dissertation.



Abbreviations: ART, antiretroviral therapy; BMI, body mass index; WHO, World Health Organization.

CHAPTER II

ADVERSE BONE HEALTH AMONG CHILDREN AND ADOLESCENTS GROWING UP WITH HIV

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ABSTRACT

Adverse bone health is one of the important non-communicable conditions during the course of life-long HIV treatment. Adolescence is the critical period of bone mineral acquisition for attaining adult peak bone mass. With traditional and HIV-related risk factors, adolescents growing with HIV have a greater chance of having impaired bone mineral density (BMD). Prevalence of low BMD has been reported in 16-32% of HIV-infected adolescents from middle-income countries. The deep interaction between the immune and skeletal systems, called immunoskeletal interface, is proposed as one of the underlying mechanisms of adverse bone health in HIV-infected individuals. Dual-energy X-ray absorptiometry (DXA) is a standard tool to assess BMD among HIV-infected adolescents. Non-invasive imaging techniques such as quantitative computerized tomography (QCT) and quantitative magnetic resonance imaging (QMRI) provide more information on true volumetric density and bone microarchitecture. To date, there are no paediatric recommendations on the treatment and prevention of adverse bone health. Having healthy lifestyle, routine weight-bearing exercises and adequate dietary intake are the standard approaches to optimise bone health. There are several ongoing randomised clinical trials using pharmacologic treatment options, for example vitamin D, calcium and alendronate to improve bone health among this population.

Introduction

The success of antiretroviral therapy (ART) has turned HIV/AIDS from a disease with a high mortality rate to a manageable chronic life-long illness. Treatment with ART can lead to restoration of immune function and sustained viral suppression, which in turn increases life expectancy for perinatally HIV-infected children and adolescents [1,2]. Currently, HIV-infected children are able to grow up and enter adolescence and young adulthood similarly to their healthy peers [3]. Yet, these individuals do experience several long-term, non-AIDS-related complications that are obstacles to the goal of normal life expectancy and quality of life. The emerging non-communicable diseases (NCDs) associated with HIV infection and antiretroviral treatment include adverse bone health, cardiovascular, liver and renal diseases, and other metabolic and endocrinological disorders [3-5]. Among these complications, adverse bone health has been recognised as an important area of investigation during the past decade in children and adolescents [6]. Since the maximum bone mineral accrual occurs during the first two decades of life [7], reduced bone deposition and increased bone resorption during these critical periods can lead to serious consequences, in particular, osteoporosis and bone fragility later in life [8,9]. This review focuses on current knowledge regarding adverse bone health among children and adolescents living with HIV, including the magnitude of problem, immunopathogenesis and factors responsible for reduced bone mass, assessment of bone health in clinical practices and up-to-date research studies of management strategies to prevent bone loss and optimise bone health.

Prevalence of low bone mineral density among perinatally HIV-infected children and adolescents

Dual energy X-ray absorptiometry (DXA) is a commonly used technique to assess total body and lumbar spine bone mineral density (BMD) in children and adolescents. Because adults have already reached peak bone mass (PBM) [7], their BMD is assessed using T-scores. In contrast, BMD measurements in growing children need to be compared to healthy age-, sex- and race-matched population norms and are reported as Z-scores. BMD Z-scores less than or equal to -2 are regarded as low bone mass [10]. The prevalence of low BMD among perinatally HIV-infected children and adolescents is much higher in studies conducted in middle-income countries than that observed in resource-rich countries. The variation in prevalence might be explained by the differences in HIV clinical staging at time of ART initiation, duration of ART, lifestyles, nutritional status, food intake and dietary supplements across studies (Figure 1) [11-16].

Resource-rich countries

In a longitudinal study of 66 HIV-infected Dutch children with a median age of 6.7 years, almost all children (96%) were on ART for a median duration of 3.4 years and 58% had undetectable plasma viral load. The prevalence of lumbar spine and femoral neck BMD Z-scores below -2 were 8% and 4%, respectively. The median BMD Z-scores were 0.9 [interquartile range (IQR) -1.6 to 0.1] for lumbar spine and 0.5 (IQR -0.2 to 1.2) for femoral neck [11]. A large cross-sectional study was conducted among 350 HIV-infected adolescents living in the United States and Puerto Rico, with a median age of 12.6 years, median duration of ART 9.5 years and 55% with plasma HIV RNA <400

copies/ml. Similar to the Dutch study, the prevalence of low BMD was 7% for total body and 4% for lumbar spine. This was higher than the low BMD prevalence in their uninfected peers of 2% for total body and 1% for lumbar spine [12]. Likewise, the Pediatric AIDS Clinical Trial Group (PACTG) 1045 found that post-pubertal HIV-infected adolescent males had significantly lower total body BMD (adjusted difference -0.10 g/cm^2; 95% CI -0.16 to -0.04 g/cm^2) and lumbar spine BMD (adjusted difference -0.13 g/cm^2; 95% CI -0.23 to -0.04 g/cm^2), compared with HIV-uninfected males at similar Tanner stage [17].

Middle-income countries

There are four reports from Thailand [13,14] and Brazil [15,16]. The prevalence of low lumbar spine BMD among HIV-infected Thais was 16–24%. The median ages of Thai participants in these studies were 14.3–15.0 years. All of them were receiving stable ART for a median duration of 7.0–9.3 years; of whom 90–96% had virological suppression [13,14]. In a cross-sectional study among 48 HIV-infected Brazilian adolescents with a mean age of 12.7 years, the prevalence of low BMD was 17% for total body less head. Almost all participants (96%) were on ART, of whom 58% had plasma HIV RNA <50 copies/ml [15]. Another cross-sectional Brazilian study was found a 32% prevalence of low BMD (total body and/or lumbar spine) among 74 HIV-infected adolescents with a mean age of 17.3 years. Approximately 91% of them received ART with a mean duration of 11 years, of whom 48% had undetectable virus [16].

Pathogenesis of adverse bone health among HIV-infected population

Bone is a specialised supporting and protecting structure of the body. It contains two major components: calcium phosphate, a mineral compound that gives bone strength and rigidity; and collagen, a protein that provides a flexible framework [18,19]. Bone is not a static structure, but one that constantly undergoes longitudinal and radial growth, rebuilding and remodelling throughout the life [18,20].

Bone remodelling involves bone resorption, by osteoclasts, and bone formation, by osteoblasts [18,20]. In general, bone formation predominates bone resorption during childhood and young adulthood [20]. Thus, bone mass increases over time and reaches its peak during the third decade of life. Subsequently, bone remodelling becomes balanced between bone formation and resorption, resulting in stable bone mass with small variations [20]. Around 50 years of age, the rate of bone resorption begins to outpace that of bone formation, and thus bone mass declines [20].

Changes in bone mass with the course of HIV disease

In HIV-infected individuals, the physiological regulation of bone remodelling can be disrupted by several factors: HIV itself, ART and other HIV-related factors [21-23]. Bone mass changes with the course of HIV disease. During the pre-treatment period, many conditions, for example wasting syndrome, disrupt immune system function through loss of $CD4^+$ T cells and B cells [24,25]. Together with chronic systemic inflammation [26], this disturbs bone homeostasis. Untreated individuals tend to have raised inflammatory markers and dysregulated bone turnover compared with healthy individuals [27-29]. This finding supports the linkage between systemic inflammation

and bone turnover imbalance, a likely cause of bone demineralisation in HIV-infected persons.

After ART initiation, HIV-infected persons usually have improved health status and restored immunological system function. Weight is regained and systemic inflammation is reduced [30]. However, such individuals may experience transient reduced bone mass and worsening of bone health during the first 1-2 years of ART [31-33]. Possible explanations are poor health status and wasting before ART initiation [21], ART causing imbalance of bone turnover [34,35], and time lag between ART initiation and improvement of BMD [21,31]. Accelerated bone loss, as much as 2-6%, has been demonstrated in HIV-infected individuals on various ART regimens, including tenofovir disoproxil fumarate (TDF) [31], efavirenz (EFV) [32], nevirapine [33] and boosted protease inhibitors (PIs) [32,33]. In HIV-infected adults, both TDF and PIs, have been associated with a 33% increased in osteocalcin (OC), a bone formation biomarker [34]. Another study demonstrated that switching to TDF vs. staying on zidovudine resulted in a significant increase in both bone resorption marker; C-terminal cross-linked telopeptide of type I collagen (CTX), and bone formation markers; OC and procollagen type I amino-terminal propeptide (PINP), which correlate with reductions in lumbar spine BMD [35].

Immunoskeletal interface on bone health

In the context of untreated HIV infection, there are two concurrent and important alterations in bone health: progressive loss of immune function and accelerated bone resorption. Emerging evidences suggests that the immune and skeletal systems are deeply intertwined as a result of a centralisation of common cell types and cytokine mediators

[22]. This is called the immunoskeletal interface (Figure 2). There are well-established evidence of the association between immune and skeletal systems observed in many inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythrematosus [36-38]. These immune alterations accelerate the natural skeletal ageing process contributing to adverse bone health.

The immunoskeletal interface can be divided into two aspects: the interactions of immune cells with osteoblasts and with osteoclasts. Osteoblastic cells, derived from osteoprogenitor mesenchymal stem cells, are able to modulate the immune system by regulating the hematopoietic stem cell microenvironment in which immune cells are derived [39]. Immune cells can produce cytokine mediators, such as tumor necrotic factor-alpha (TNF-α), that functions as a potent inhibitor of osteoblast differentiation and activity [40]. Osteoclasts are derived from mature cells of monocyte and macrophage lineage. Osteoclastogenesis requires the presence of RANK ligand (RANKL) and RANK interactions. RANKL is a receptor activator of nuclear factor κβ ligand expressed by osteoblastic lineage cells, while RANK is a receptor activator of nuclear factor κβ presented on the surface of osteoclast precursors and mature osteoclasts [41]. RANKL is recognised as a key osteoclastogenic cytokine and the final effectors of osteoclast formation and activity [42-45]. Additionally, it is considered to have important immunological functions as a mediator for T cell proliferation and dendritic cell function [46,47]. The interactions between RANKL with RANK (RANKL-RANK system) induce the formation and differentiation of osteoclast precursors into pre-osteoclasts, which then fuse together to form the mature osteoclasts [42,48]. The essential regulatory component of the RANKL-RANK system is osteoprotegerin (OPG), a member of the TNF receptor

superfamily and a RANKL decoy receptor. OPG modulates RANKL activity by binding itself to RANKL and prevents the RANKL-RANK interaction, resulting in the inhibition of osteoclast formation and maturation [42-44,48,49]. A new cytokine, secreted osteoclastogenic factor of activated T cells (SOFAT), was recently identified and observed to potently induce osteoblastic IL-6 production [50], which in turn stimulates osteoclastogenesis independently of RANKL [51].

B and T cells are critical for preserving bone homeostatis [52]. B cells produce OPG in response to T cell co-stimulation [53]. Activated T cells, through the CD40 ligand and its receptor CD40 on B cells, can promote B lineage OPG production *in vivo* [52]. However, under inflammatory conditions, B cells and activated T cells turn into a significant source of RANKL and SOFAT production [50,54-57]. In addition, several inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-7 and TNF-α, overexpressed by immune cells during inflammation are able to drive up RANKL-dependent osteoclastogensis [58,59]. Taken all together, the osteoclastic bone resorption is enhanced (Figure 2).

Risk factors for adverse bone health among perinatally HIV-infected children and adolescents

The causes of adverse bone health among perinatally HIV-infected children and adolescents are multifactorial. They can be classified into traditional risk factors and HIV-specific factors (Figure 2). The traditional risk factors for low bone mass include malnutrition, short stature, low body mass index, delayed puberty, vitamin D deficiency, inadequate calcium intake, physical inactivity, smoking and steroid exposure [12-14,60,61]. HIV-specific factors, including HIV itself, advanced HIV disease, poor immunological status, uncontrolled viraemia, exposure to some specific types of ART, as well as persistent immune activation and chronic systemic inflammation also play a significant role in driving bone loss among this population [11-17,62-70].

Traditional risk factors

Weight and height are independently associated with bone mass in healthy individuals; however, the effects may be exaggerated in the HIV-infected population because they usually have significantly delayed linear growth and poor weight gains [71-74]. Although low vitamin D has been considered a traditional risk factor for poor bone health, there is no well-established evidence demonstrating the significant direct association between vitamin D deficiency or inadequate vitamin D intake and adverse bone health in HIV-infected adolescents [13,14,60]. Similarly, the evidence of inadequate calcium intake as a determinant of reduced bone mass is limited and controversial in this population [14,60,61]. A cross-sectional study among 19 HIV-infected girls whose calcium intake was 20-50% lower than the recommended daily allowance hypothesised

that suboptimal calcium intake might lead to the increased bone resorption and impaired bone mineral acquisition [61]. In contrast, two cross-sectional studies carried out in Brazil and the United States did not find such an association [15,60]. Calcium supplementation may be only beneficial to individuals with insufficient calcium intake.

The relationship between weight-bearing physical activity and bone health among HIV-infected youth was demonstrated in a few studies [12,15,62]. The challenge of these studies is the best measure for physical activity level. Self-report and questionnaires are used by some [12,62], while an accelerometer is used by others [15].

HIV-specific factors

HIV *per se* is one of the factors for bone abnormalities. Higher prevalence of bone demineralisation was reported among treatment-naïve HIV-infected adults compared with HIV uninfected controls [75]. HIV infection is related to a high bone turnover state, as demonstrated by the disturbances of histomorphometric parameters and/or the dysregulation of biochemical markers of bone formation and bone resorption [28,29,76].

Advanced HIV disease, uncontrolled viraemia and poor immunological status are predictors of adverse bone health among HIV-infected children and adolescents [11-16,63,64]. Individuals with severe clinical symptoms of HIV had a significant impairment of BMD, evaluated by DXA [14] and quantitative high-frequency ultrasound (QUS) techniques [63]. High plasma HIV RNA also correlated with low bone mass [11,12]. A positive correlation between current $CD4^+$ percentage and BMD/ bone mineral content (BMC) was observed in HIV-infected youth [11,64].

Several studies demonstrated a significant linkage between ART exposure and reduced bone mass [12,13,15-17,62,66-70]. These were either performed in treatment-naïve patients [31-33,62,65] or treatment-experienced patients who switch treatment because of poorly controlled viraemia [69]. Although early loss of bone mass within the first 1-2 years of ART initiation has been observed in treatment-naive adults and adolescents [31-33,62,65], there are no data in young children. Significant bone abnormalities after ART switching have also been demonstrated in children and adolescents [69]. Early bone loss is observed during the first 24-48 weeks of TDF but remained stable thereafter. Such an effect is, however, not detected among treatment-experienced children with stable clinical status and controlled viraemia prior to switch to TDF [77]. Tenofovir alafenamide (TAF), a prodrug of TDF, has a more favorable bone safety profile. Two randomised clinical trials in HIV-infected adults demonstrated significantly less bone toxicity (mean change of lumbar spine BMD -1.3 vs. -2.9%, P <0.0001; total hip BMD -0.7 vs. -3.0%, P <0.0001) among adults receiving TAF vs. TDF in combination with elvitegravir/cobicistat/emtricitabine [78].

Consequences of adverse bone health in perinatally HIV-infected children and adolescents

Childhood and adolescence represent the critical periods for bone mineralisation and maturation, and at least 90% of final adult bone mass is achieved during these periods [79]. Any factor impairing bone mineral acquisition may diminish bone gain, induce bone loss and compromise adult PBM [7,80]. From two meta-analyses, the prevalence of osteopenia and osteoporosis among HIV-infected adults was 52-67% and 15%, respectively [81,82]. A study conducted in a large United States reported higher prevalence of overall fracture in the HIV-infected group compared with uninfected controls (2.87 vs. 1.77 patients with fractures per 100 persons) [8]. Furthermore, the HIV Outpatient Study revealed that the adjusted fracture rate among HIV-infected adults increased from 57.7 to 89.9 per 10000 population between the years 2000 and 2008, respectively and that it was 49.6-72.9% higher than in uninfected population over the study period [9]. However, an increased risk of fracture events among HIV-infected children and adolescent has not been documented. A prospective cohort PACTG219/219C study, which followed more than 1000 HIV-infected children for a median of 5 years showed similar incidence rate of fracture among HIV-infected compared with HIV-exposed but uninfected children (1.2 vs. 1.1 per 1000 person-years) [83]. Since fracture is a long-term complication of adverse bone health, these populations may be too young to demonstrate the increase in the incidence of this condition.

Measurements to assess bone health in HIV-infected children and adolescents

Standard measurement in clinical practice

DXA is a non-invasive imaging technique that uses two X-ray beams with different photon energy levels aimed at the bone to be measured. The BMD can be determined from the absorption of each beam by bone once soft tissue absorption is subtracted. DXA is the most commonly used bone densitometric technique for children and adolescents throughout the world [10]. The measurements provided include bone mineral content (BMC, grams) and areal bone mineral density (BMD, g/cm^2). The most appropriate skeletal sites for performing densitometry in children and adolescents are posterior-anterior (PA) lumbar spine and total body less head [10]. Compared with conventional radiographs, measurement of the spine provides more information about the trabecular bone status, while total body measurement focuses more on cortical bone status [84]. In children with short stature or delayed growth, the areal BMD and BMC should be adjusted in order to eliminate the influences of bone size and skeletal dimension. For total body less head, adjustment should be performed using the height Z-score. For the spine, adjustment can be made by using either bone mineral apparent density (BMAD) or the height Z-score [10]. According to the 2013 International Society for Clinical Densitometry (ISCD) Pediatric Official Positions, an areal BMD Z-score less than or equal to -2.0 SD is described as low bone mass or BMD [10,85].

Advanced measurements in research settings

During the past decade, the concept of bone strength has encompassed a number of bone characteristics, including trabecular and cortical architectures, bone turnover,

mineralisation and cellularity, aspects of called bone quality [86-88]. Summary of advanced techniques for assessing bone health are shown in Table 1.

Bone histomorphometry is a histological examination of bone biopsy specimens to obtain qualitative and quantitative information on *in vivo* bone structure and remodelling. It is the gold standard for bone metabolic and mineralisation evaluation [89,90]. Currently, there are non-invasive imaging techniques using three-dimensional reconstruction for bone microstructure and microarchitecture analysis [91]; therefore bone biopsy is frequently avoided.

Quantitative ultrasonography (QUS) is a non-invasive method using high frequency sound waves that are transmitted through bone to assess the bone quality and strength. The longitudinal sound wave transmitted through calcaneus is the accepted measurement to determine bone health status [92].

Quantitative computerized tomography (QCT) is a three-dimensional imaging technique to assess true volumetric density (mg/cm^3) without the overlapping of other tissues [93]. This technique ranges from a volumetric QCT to advanced imaging modalities such as high-resolution CT (hrCT) and microCT. Currently, there is no preferred QCT method for clinical evaluation in children and adolescents [94]. Most QCT studies in children investigated peripheral sites, primarily the radius and tibia, because of radiation exposure concerns [94]. The advantage of QCT over DXA is that it provides a separate analysis of trabecular or cortical components of bone.

Quantitative magnetic resonance imaging (QMRI) is a non-invasive, non-ionising radiation technique that provides three-dimensional imaging of trabecular bone architecture. Magnetic resonance is based on the application of a strong magnetic fields

and a series of radiofrequency waves to generate three-dimensional images of the hydrogen protons in the water within skeletal tissues [88].

Biochemical markers of bone turnover are helpful research tools to reflect the ongoing bone remodelling processes. Currently available markers are classified into biochemical markers of bone formation and resorption [95-98]. Biomarkers of bone formation are products of active osteoblasts expressed during different developmental stages, including: (i) osteoblast-specific enzymes such as bone-specific alkaline phosphatase (BALP); (ii) osteoblast-related protein such as OC; and (iii) by-products of collagen synthesis such as PINP. All can be measured in serum or plasma [95-98]. The biomarkers for bone resorption are classified into: (i) osteoclast-specific enzymes such as tartrate-resistant acid phosphatase (TRACP) 5b; and (ii) collagen degradation products such as hydroxyproline, pyridinoline, deoxypyridinoline, CTX, and N-terminal cross-linked telopeptide of type I collagen (NTX) [95-98]. The measurements can be performed from blood or urine.

Bone health assessment in HIV-infected children and adolescents in clinical practice

The recommendations for the evaluation and management of bone disease in HIV-infected adults have recently been developed by HIV specialists from 16 countries [103]. The screening for adverse bone health depends on an individual's risk for fragility fracture. For individuals with major risk factors, including a previous history of fragility fracture; receipt of glucocorticoid treatment for more than 3 months; and at high risk for falls, BMD assessed by DXA should be performed. Additionally, DXA, if available, is recommended for all men age >50 years, postmenopausal women and individuals with a 10-year risk of major osteoporotic fracture >10% by the Fracture Risk Assessment Tool (FRAX) score. For those without major risk factors, including men age 40-49 years and premenopausal women age ≥40 years, FRAX (without DXA) is the recommended assessment [103]. FRAX is a prediction tool for assessing an individual's fracture risk and is applicable for people aged between 40 and 90 years. The model incorporates several components such as age, race, sex, body mass index, smoking, alcohol consumption, long-term use of glucocorticoids, vitamin D deficiency, prior fragility fracture and parenteral history of hip fracture into the calculation [103]. Therapeutic management guidelines vary by country and are based on the availability, as well as cost of diagnostic tools and medications. In the United States, anti-osteoporosis treatments are prescribed in individuals presenting with hip or vertebral fracture, osteoporosis (T-score no more than -2.5), or osteopenia (T-score between -1.0 and -2.5) with a 10-year probability of hip fracture ≥3% or major osteoporosis-related fracture ≥20% based on FRAX [104]. The follow-up interval of DXA should be adjusted according to degree of bone demineralization, repeated after 1-2 years for those with advanced osteopenia (T-

score between -2.0 and -2.5) and after 5 years for those with mild to moderate osteopenia (T-score between -1 and -2) [103].

The objective of bone health assessment in the paediatric population is to screen children who fail to achieve the expected gains in bone size, mass and strength, which leaves them vulnerable to fracture as they age. The ISCD 2013 Statement recommends that DXA should be considered only in children and adolescents who may benefit from interventions and those whose DXA results will influence management [105]. Among chronic diseases, cystic fibrosis has a well-established recommendation for bone health assessment and monitoring. The European Cystic Fibrosis Mineralisation Guidelines recommend the first routine bone density scans at age around 8-10 years, to be repeated every 5 years if the BMD Z-score is above -1; every 2 years if the Z-score is between -1 and -2; and every year if the Z-score is below -2 [106]. However, to date, there is no specific recommendation for DXA screening among HIV-infected children and adolescents in any national or international guidelines. In settings where DXA is available and accessible, bone density scans may be performed at 6-12 months after ART initiation since transient reduction in bone mass may be occurring, with repeat measurements every year if BMD Z-score is less than or equal -2. In settings where access to DXA is limited, one may consider performing bone density scans only in individuals who have a combination of multiple risk factors for bone demineralisation, for example history of wasting or stunting, advanced HIV disease, use of TDF with ritonavir boosted PIs, and vitamin D deficiency.

Management of adverse bone health in perinatally HIV-infected children and adolescents

As adverse bone health during childhood and adolescence may result in adult osteoporosis and bone fragility, several approaches, primarily prevent bone loss and optimise bone health, should be implemented during these critical periods.

General management

Promoting a healthy lifestyle

Healthy lifestyle choices include avoiding smoking and heavy alcohol consumption. Smoking is a major lifestyle risk factor for osteoporosis. Studies in twins have provided a powerful study design by controlling for age, sex, genetic background to identify the effects of smoking on bone health [107-109]. A cross-sectional study of 41 pairs of female twins found that smoking one pack of cigarettes per day throughout adulthood would reduce BMD by approximately 5–10%, thus increasing the risk for osteoporosis by the time of menopause [107]. Similarly, a study of 146 female twin pairs showed that a discordance of 10 pack-years smoking was related to a 2.3–3.3% decrease in BMD at the lumbar spine, proximal femur and total body [108]. Furthermore, meta-analyses indicated that smoking substantially increased hip fracture by 31–60% when comparing current smokers with non-smokers [110-112].

Alcohol consumption negatively impacts bone health in several ways. First, excessive alcohol consumption causes hypovitaminosis D, which in turn reduces calcium reserves [113,114]. Second, chronic heavy alcohol consumption can disturb testosterone

production, a male hormone linked to the production of osteoblasts [115], while, cortisol, a hormone that arrests osteoblast differentiation, is increased [116].

Exercise

Weight-bearing and muscle strengthening exercises are important for building and maintaining bone density. Weight-bearing exercise can be either high impact, such as dancing, running, jumping, gymnastics, soccer, basketball or low impact, such as fast walking or low-impact aerobics. Muscle strengthening exercises include weight lifting, using elastic exercise bands or weight machine, or functional movements. Previous studies showed that children who usually participate in high-impact activities have higher bone mass compared with individuals who are less active or frequently engage in non-weight bearing exercises [117-120]. The American College of Sports Medicine recommends exercising for 10-20 minutes per day, at least 3 days per week [117]. These exercise prescriptions could improve bone strength in children and adolescents.

Nutrition

Key bone nutrition includes calcium and vitamin D [121]. The Institute of Medicine (IOM) recommendation for daily calcium intake for children and adolescents age 9-18 years is 1300 mg per day [122]. In clinical practice, diet should be the primary source for calcium. Calcium supplementation should be provided to individuals who unable to obtain adequate calcium from their diet and who are at high risk for adverse bone health. Common dietary sources of calcium are dairy products, soymilk, soybeans, dark leaf greens and sardines. According to the IOM, the recommended vitamin D intake

for children and adolescents age 9-25 years is 600 IU per day [122]. The most common source of vitamin D is sunlight. However, in countries without year-round sunlight, foods containing vitamin D such as fatty fish (e.g., salmon, tuna and mackerel) and fish oils are among the best sources. Vitamin D-fortified foods may be available in resource-rich countries [123], but they are not available in resource-limited settings.

A high prevalence of vitamin D deficiency and insufficiency among HIV-infected children and adolescents has been reported, ranging from 71% to 96% [124-127]. Vitamin D deficiency may diminish calcium absorption in the gastrointestinal tract. Therefore, if 25-hydroxyvitamin D (25-OHD), a surrogate for vitamin D levels, is lower than 30 ng/ml, supplementation should be initiated. However, in settings where 25-OHD measurement is not available, supplementation should be considered based on history of vitamin D intake and clinician decision. However, evidence demonstrating the benefit of calcium and vitamin D supplementation on bone health among HIV-infected children and adolescents is limited and controversial [128,129]. A randomised clinical trial assessing the effects of calcium (1 g per day) and vitamin D3 (1600 IU per day) supplementation for 2 years on bone mineral accrual among HIV-infected children and adolescents with normal baseline BMD in the United States found no significant difference in BMD when compared with placebo groups [128]. In contrast, a recent small observational study supplementing 1200 mg calcium and 400 IU vitamin D3 daily for 6 months showed a significant improvement of lumbar spine BMD and BMD Z-scores among HIV-infected Thai adolescents with low BMD at baseline [129]. Currently, there are on-going randomised clinical trials determining the impact of calcium and/or vitamin D supplementation on BMD among HIV-infected adolescents (Table 2).

Pharmacological interventions

There are several agents used in the treatment of low BMD in HIV-infected populations, including anti-resorptive therapies (bisphosphanates, serum oestrogen receptor modulators or SERMs, and monoclonal antibody to RANKL), strontium ranelate and peptides of the parathyroid hormone family [130]. Among all treatment options, bisphosphonates are the longest established therapy of osteoporosis. Bisphosphonates are derivatives of inorganic pyrophosphate that have a high affinity for bone minerals. These agents are preferentially incorporated into sites of active bone remodelling and accelerated bone turnover and inhibit hydroxyapatite breakdown, which in turn suppresses bone resorption [131]. This property results in their utility as clinical agents for osteoporosis treatment. Alendronate and zolendronate are the only two agents recommended for HIV-infected adults with osteoporosis [103]. However, clinical evidence for efficacy of these medications in HIV-infected individuals is scarce and no studies in children and adolescents have taken place. Previous randomised clinical trials of the bisphophonates, alendronate (weekly) or zolendronate (annually), in HIV-infected adults with low bone density demonstrated significant improvement of BMD compared to placebo groups [132-137]. Before bisphosphonates can be recommended as an anti-osteoporosis treatment, larger studies with longer follow up periods should be performed. Currently, there are several ongoing randomised clinical trials that aim to determine the efficacy of alendronate on bone density among HIV-infected adolescents and young adults (Table 2).

Conclusions

Adverse bone health is common in perinatally HIV-infected children and adolescents, particularly those living in middle-income countries. The pathogenesis of low BMD is complex, and is related to HIV disease course and systemic inflammation (immunoskeletal interface). Many factors, both traditional and HIV specific, can lead to adverse bone health. An important consequence of low bone density during childhood and adolescence is compromised PBM, which may result in osteoporosis and bone fracture later in life. Refraining from smoking and heavy alcohol consumption, performing regular weight-bearing exercises and adequate dietary intake of calcium are basic health education messages for patients to optimise bone health. More data are required to support the efficacy of calcium and vitamin D supplementation, and bisphosphonates in restoring bone mineralisation and preventing bone loss among HIV-infected children and adolescents.

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Table 1. Methods for assessment of bone health in HIV-infected children and adolescents.

Method of assessment	Advantages	Disadvantages	Clinical data from HIV-infected children and adolescents
Bone mineral density (BMD) and bone mineral content (BMC)			
Dual-energy X-ray Absorptiometry (DXA)	• Widely available • Safe • Excellent precision • High reproducibility • Examination time 5 minutes	• Subject to systematic errors • Cannot differentiate cortical and trabecular bones • Limited paediatric normative data references	• HIV-infected adolescents had high prevalence of BMD Z-score $\leq$-2 (16-32%) in middle-income countries [13-16] • BMD and BMC of HIV-infected adolescents is significantly lower than healthy controls [12,14]
Speed of sound (SOS) and broadband ultrasound attenuation (BUA)			
Quantitative ultrasonography (QUS)	• Radiation free • Portable and simple to operate • Correlates well with DXA • Cost-effective	• Limited skeletal site of measurement • Lack of paediatric normative data for interpretation	• HIV-infected children with severe clinical symptoms had lower calcaneal BUA Z-score [63] and phalangeal SOS [99] compared with healthy controls • Tibial and radial SOS were associated with lumbar spine BMC and BMD, and total body BMC and BMD [100]
True volumetric bone density and bone microarchitecture			
Quantitative computerized tomography (QCT)	• More accurate assessment of BMD than DXA • Provide separate analysis of cortical and trabecular bones • Not susceptible to degenerative changes of bone calcifications	• High radiation dose • High cost • Hard to access • Lack of paediatric normative data for interpretation	• Similar vertebral volumetric bone density in HIV-infected children compared with controls [101] • DXA Z-scores were significantly lower than QCT Z-scores in HIV-infected children [101] • Cortical BMD (peripheral QCT) was positively associated with NNRTI use, but negatively associated with PI use [64]
Quantitative magnetic resonance imaging (QMRI)	• Lack of ionising radiation • Ability to investigate marrow fat content, marrow diffusion and marrow perfusion	• Long acquisition time • Requires specialised machine • High cost • Lack of paediatric reference data	• None

Table 1. Methods for assessment of bone health in HIV-infected children and adolescents (cont.)

Method of assessment	Advantages	Disadvantages	Clinical data from HIV-infected children and adolescents
Bone turnover rate, osteoclast and osteoblast activity			
Bone biochemical markers	• Non-invasive • Can be performed from blood and urine specimens • Helpful tools in diagnosis and treatment assessment of bone health and diseases	• Diurnal variation • Limited paediatric normative data and cut-off levels	• Higher serum BALP and urine NTX in ART-experienced HIV-infected children compared with untreated children and healthy controls [102] • Significantly reduced osteocalcin and urinary deoxypyridinoline in HIV-infected children with severe clinical symptoms compared with healthy controls [63] • CTX and PINP levels were not different between HIV-infected adolescents with and without low BMD, but PINP was significantly inversely correlated with BMD Z-score [13]

Abbreviations: BALP: bone-specific alkaline phosphatase; CTX: C-terminal cross-linked telopeptide of type I collagen; NNRTI: non-nucleoside reverse transcriptase inhibitors; NTX: N-terminal cross-linked telopeptide of type I collagen; PI: protease inhibitor; PINP: procollagen type I amino-terminal propeptide.

Table 2. Ongoing randomised clinical trials on the interventions for improve bone health among HIV-infected adolescents and young adults.

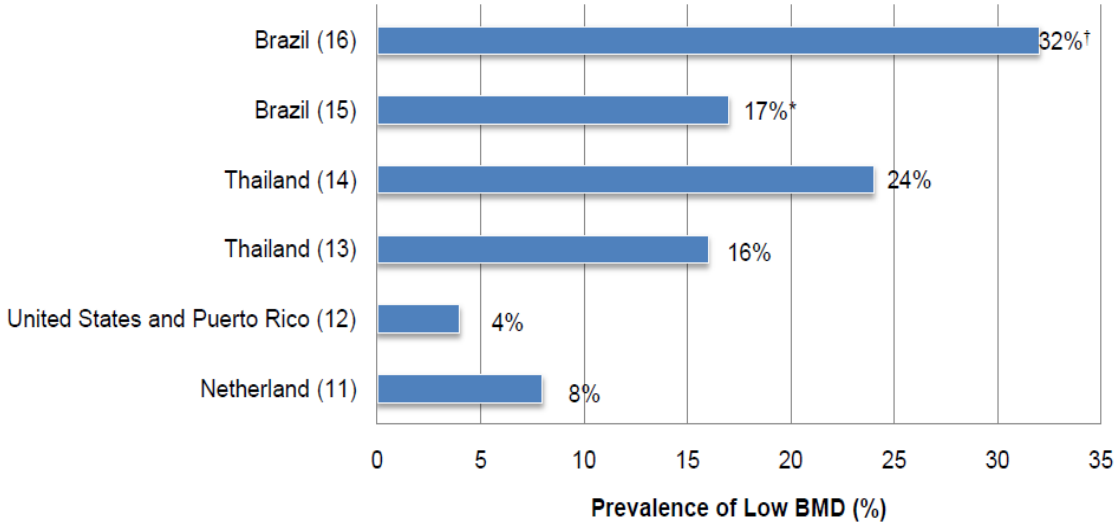
Investigator, study name, (Trials ID)	Study location	Study population	Intervention	Primary outcome
Siberry GK *et al.* IMPAACT P1076 (NCT00921557)	United States, Brazil, Puerto Rico	Age 11-24 years Lumbar spine BMD Z-score <-1.5 or history of fragility fracture	**ARM 1:** Oral alendronate 70 mg weekly for 96 weeks **ARM 2:** Oral alendronate 70 mg weekly for 48 weeks plus placebo for 48 weeks **ARM 3:** Placebo for 96 weeks	Changes of LS BMD after 24 and 48 weeks of alendronate treatment versus placebo
Havens P *et al.* ATN109 (NCT01751646)	United States Puerto Rico	Age 16-24 years receiving TDF-containing ART	**ARM 1:** Vitamin D3 50000 IU orally every 4 weeks for 48 weeks **ARM 2:** Placebo for 48 weeks	Changes in LS BMD after 48 weeks of supplementation
Sudjaritruk T *et al.* CAL-D (NCT02426840)	Thailand	Adolescents age 10-20 years receiving stable ART	**ARM 1:** Co-formulated oral calcium (600 mg elemental calcium)/vitamin D3 (200 IU) twice daily for 48 weeks **ARM 2:** Co-formulated oral calcium/vitamin D3 twice daily plus vitamin D2 (20000 IU/cap) once weekly for 48 weeks	Changes in LS BMD after 48 weeks of supplementation
Tan D *et al.* BATARI (NCT01968850)	Canada	ART-naïve age >18 years with low fracture risk (FRAX 10-year fracture risk scores <10%)	**ARM 1:** Standard of care **ARM 2:** Co-formulated oral alendronate (70 mg)/vitamin D3 (5600 IU) weekly started at time of ART initiation for 24 weeks **ARM 3:** Co-formulated oral alendronate (70 mg)/vitamin D3 (5600 IU) weekly started at week 24-48 of ART	Changes in LS and proximal femur BMD at week 48

Table 2. Ongoing randomised clinical trials on the interventions for improve bone health among HIV-infected adolescents and young adults (cont.)

Investigator, study name, (Trials ID)	Study location	Study population	Intervention	Primary outcome
Mallon PW *et al.* APART (NCT02322099)	Ireland	ART-naïve adults age >30 years initiated with TDF/FTC	**ARM 1:** Oral alendronate (70 mg) weekly plus daily calcium (500 mg elemental calcium)/vitamin D3 (400 IU) for 14 weeks **ARM 2:** Placebo plus calcium (500 mg elemental calcium)/vitamin D3 (400 IU) for 14 weeks	Between-group differences in the change in total hip, LS, femoral neck BMD and body composition to week 50

Abbreviations: ART: antiretroviral therapy; BMD: bone mineral density; FRAX: fracture risk assessment tool; FTC: emtricitabine; LS: lumbar spine; TDF: tenofovir disoproxil fumarate.

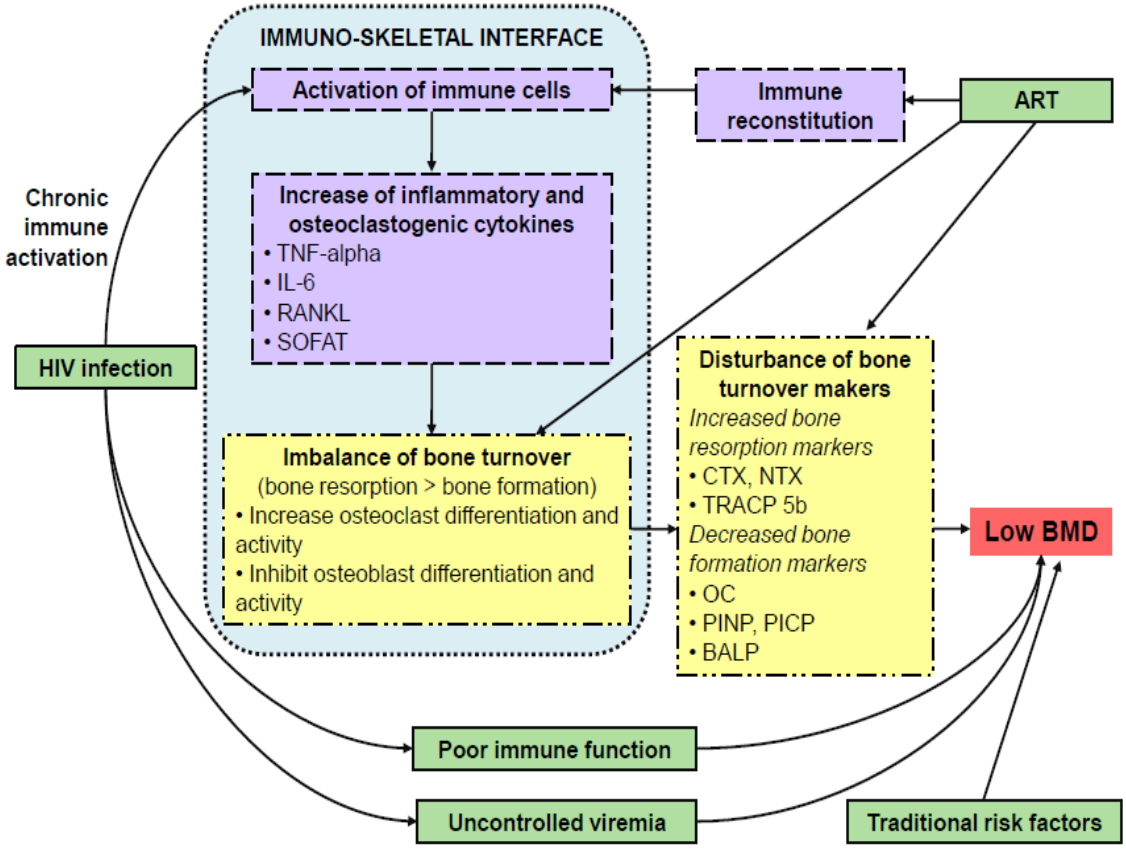
Figure 1. The prevalence of low bone mineral density among perinatally HIV-infected children and adolescents.



Note: Low bone mineral density (BMD) is defined as BMD Z-score ≤-2.
The BMD measurements shown are taken at lumbar spine, except where otherwise indicated.

*Subtotal BMD; †Total body BMD and/or lumbar spine BMD.

Figure 2. The immunopathogenesis and risk factors of low BMD among HIV-infected children and adolescents.



Note: Green textboxes with solid border represent important risk factors of low BMD. Purple textboxes with dashed border represent components of the immune system. Yellow textboxes with dashed and dotted border represent components of the skeletal system. A blue textbox with dotted border represents the immunoskeletal interface.

Abbreviations: ART: antiretroviral treatment; BALP: bone-specific alkaline phosphatase; BMD: bone mineral density; CTX: C-terminal cross-linked telopeptide of type I collagen; IL-6: interleukin-6; NTX: N-terminal cross-linked telopeptide of type I collagen; OC: osteocalcin; PICP: procollagen type I carboxy-terminal propeptide; PINP: procollagen type I amino-terminal propeptide; RANKL: receptor activator of nuclear factor κβ ligand; SOFAT: secreted osteoclastogenic factor of activated T-cells; TNF-alpha: tumor necrotic factor-alpha; TRACP 5b: tartrate-resistant acid phosphatase 5b.

CHAPTER III

ADVERSE BONE HEALTH AND ABNORMAL BONE TURNOVER AMONG PERINATALLY HIV-INFECTED ASIAN ADOLESCENTS WITH VIROLOGIC SUPPRESSION

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ABSTRACT

Objectives: This study aimed to determine the prevalence of low bone mass and assess its relationship with abnormal bone turnover among HIV-infected Asian adolescents.

Methods: A multicenter, cross-sectional study was conducted at 4 pediatric HIV centers in Thailand and Indonesia. Perinatally HIV-infected adolescents aged 10-18 years receiving antiretroviral therapy (ART) with virologic suppression (HIV RNA <400 copies/ml) were enrolled. Study assessments included lumbar spine (L2-L4) dual-energy X-ray absorptiometry, serum 25-hydroxyvitamin D, bone turnover markers and pro-inflammatory cytokines. Bone mineral density (BMD) and bone mineral apparent density (BMAD) Z-scores were calculated based on Thai normative age- and sex-matched references. Low bone mass was defined as BMD or BMAD Z-scores $\leq$-2.

Results: Of 396 participants, 57% were female. The median age [interquartile range (IQR)] was 15.0 (13.3-16.9) years, and 73% were in Tanner stage 3-5. At enrollment, median $CD4^+$ (IQR) was 734 (581-907) cells/mm^3, and 37% were on protease inhibitor (PI)-based regimens. Overall prevalence of lumbar spine BMD and BMAD Z-scores $\leq$-2 were 16.4% and 8.3%, respectively. Z-scores were lower with older age, female sex, body mass index (BMI) <5^{th} percentile, PI exposure and $CD4^+$ <15% before ART initiation. Increased bone turnover markers were inversely associated with BMD and BMAD Z-scores. Pro-inflammatory cytokines tended to have a positive but not significant association with bone turnover markers.

Conclusion: Low bone mass was linked to advanced age, female sex, low BMI and PI exposure, and poor immunological status before ART commencement in our cohort.

Dysregulation of bone turnover was associated with bone demineralization, while chronic immune activation showed a trend toward a positive association with bone turnover.

Introduction

Significant success in expanding access to effective antiretroviral therapy (ART) has turned HIV infection into a chronic life-long illness. Perinatally infected infants and children now survive into adolescence and young adulthood similar to their healthy peers [1]. However, long-term, non-AIDS-related complications associated with HIV infection and ART have been emerging [1-3]. Adverse bone health is a specific concern in this population undergoing normal physical development [3-5]. Low bone mass, evaluated by dual-energy X-ray absorptiometry (DXA), has been reported in HIV-infected children and adolescents [6-10]. The prevalence of low bone mass, which is defined as bone mineral density (BMD) Z-score $\leq$-2.0, has been observed to be higher in middle-income (17-32%) [6-8] compared with high-income countries (4-8%) [9,10]. Importantly, bone mass in this population has appeared to be much lower than their age- and sex-matched HIV-exposed but uninfected [9], and healthy controls [11-13].

There are multiple factors associated with decreased bone mass in HIV-infected individuals [6-20]. Hypovitaminosis D, inadequate calcium intake, limited physical activity, cigarette smoking, corticosteroid use, malnutrition, low lean body mass, short stature and delayed puberty are among the important traditional risk factors [7,9-12]. In addition, HIV-specific factors, including HIV itself, advanced HIV disease, impaired immunologic and virologic status, specific antiretroviral drugs, as well as chronic immune activation and inflammation due to HIV infection are the major determinants accelerating bone demineralization [6-10,13-20].

Because bone is a dynamic tissue, it continuously undergoes remodeling, which consists of bone resorption (mediated by osteoclasts) and formation (mediated by osteoblasts) throughout life to maintain its strength and mineral homeostasis [21,22]. Uncoupling between these two processes can result in increased bone turnover, a net decline in bone mass, and the development of bone disorders [22].

Since a great deal of bone mineral accrual occurs during childhood and adolescence and approximately 90% of final adult bone mass is attained by 18 years of age [23], the impairment of bone mineralization during these critical periods can contribute to serious consequences, particularly osteoporosis and bone fracture, later in life [24,25]. However, information on the extent of bone demineralization and its pathogenesis are limited in resource-constrained settings, where factors affecting bone may be different, for example, race and ethnicity, seasonality, lifestyles, foods and dietary supplements. This study aimed to determine the prevalence and important associated factors of low bone mass, as well as its relationship with abnormal bone turnover among perinatally HIV-infected Asian adolescents.

Methods and measurements

Study design and participants

This multicenter, cross-sectional study was conducted in four clinical research sites in Asia: 3 in Thailand [HIV Netherland Australia Thailand (HIV-NAT), Bangkok; the Faculty of Medicine and Research Institute for Health Sciences (RIHES), Chiang Mai; Srinagarind Hospital, Khon Kaen University (KKU), Khon Kaen], and one in Indonesia [Cipto Mangunkusumo General Hospital (CMGH), Jakarta]. Eligible participants were perinatally HIV-infected adolescents aged 10 to 18 years who were on ART and had a documented history of virologic suppression (plasma HIV-1 RNA <400 copies/ml) within the past 6 months. Participants were excluded if they had underlying bone disease or established risks factors for adverse bone health, such as renal failure or endocrine diseases, or received medications for treatment bone demineralization or other medications that affect bone health (e.g., corticosteroids). The study was approved by the institutional review boards at all research sites. All participants and their caregivers provided written informed consent and assent, as appropriate, before taking part enrolling in this study.

Data collection and clinical assessments

Clinical data collection of HIV-specific characteristics, including World Health Organization (WHO) clinical stage, $CD4^+$ T-cell percentage, plasma HIV-1 RNA, ART history and duration of treatment were abstracted from medical records. Physical examination, anthropometric measurement and Tanner stage evaluation were conducted.

Physical activity levels, sun exposure duration and daily dietary intake were obtained by interview using standard questionnaires in Thai and Bahasa.

Weight and height measurements were converted into age- and sex-standardized Z-scores, using a Thai normative reference [26]. Body mass index (BMI) was calculated [weight (kg) / height (m^2)] and transformed into BMI percentile based on the US Centers for Disease Control and Prevention (CDC) reference [27]. Physical activity levels were assessed and reported in the form of physical activity scores (PAQ), ranging from 1 (low) to 5 (high) [28]. The average duration of sun exposure was calculated and described as hours per day. Daily calories (kcal/day) and calcium (mg/day) intake were analyzed using the Institute of Nutrition, Mahidol University Calculation (INMUCAL)-Nutrients Program, version 3.0 (Institute of Nutrition, Mahidol University) [29].

Blood sample collection and laboratory analysis

At enrollment, blood samples for calcium, phosphate, 25-hydroxyvitamin D (25-OHD), intact parathyroid hormone (iPTH), bone turnover markers and pro-inflammatory cytokines were collected. All participants were asked to fast overnight for at least 12 hours before blood sampling. Phlebotomy was performed by trained nurses in the morning between 8:00 to 10:00 a.m. to avoid diurnal variation in bone turnover markers. Blood tubes were centrifuged for 10 minutes (1200 x g at room temperature) to obtained serum, which was stored at -70°C until laboratory analysis.

The 25-OHD, iPTH, and bone turnover markers which included C-terminal cross-linked telopeptide of type I collagen (CTX; bone resorption marker) and procollagen type I amino-terminal propeptide (PINP; bone formation marker) were determined using

automated electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany) on Cobas® e411 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). Pro-inflammatory cytokines, including interleukin (IL)-1α, IL-1β, IL-6 and tumor necrotic factor (TNF)-α were quantified using sandwich enzyme-linked immunosorbant assay (ELISA) with DuoSet® ELISA development system (R&D Systems, Minneapolis, Minnesota, USA) on microplate reader (Varioskan Flash, version 4.00.53). The standard curves were performed in duplicate and generate reproducible optical density. Laboratory procedures were performed according to the manufacturer's instructions. Blood specimens for each lab were analyzed by a single technician at a single reference site at RIHES, Chiang Mai University (Chiang Mai, Thailand) for 25-OHD, iPTH, and bone turnover markers; and the HIV-NAT, Thai Red Cross AIDS Research Centre (Bangkok, Thailand) for pro-inflammatory cytokines. The other laboratory measurements were conducted locally using routine laboratory analyses. The normal level of 25-OHD was 30 ng/ml or above [30], and the lower and upper limit of normal for iPTH were 10 and 65 pg/ml [31]. The normal range of calcium was 8.4-10.2 mg/dl, and phosphorus ranges were 3.3-5.4 mg/dl (age 10-15 years) and 2.4-4.4 mg/dl (age >15 years). The normal range for alkaline phosphatase levels were 100-390 U/l (male) and 100-320 U/l (female), respectively.

Bone health evaluations

Lumbar spine (L2-L4) bone mass was evaluated by DXA technique using scanners from the same manufacturer (Lunar, General Electric [GE] Healthcare, Madison, Wisconsin, USA) at all sites. The measurements were obtained by well-trained

radiologic technologists according to standard methods. The machines were calibrated on a daily basis following the standard quality assurance procedures provided by the manufacturer. Additionally, a spine quality control phantom was scanned weekly according to the manufacturer recommendations to monitor the performance of a scanner over time. The scans were analyzed using enCORE software version 14.1 (enCORE, GE Healthcare, Madison, Wisconsin, USA).

Lumbar spine bone mass measurements for all participants were reported as BMD Z-scores, which standardizes the absolute BMD results against the average results expected for a Thai adolescent of similar age and sex [32]. Additionally, bone mineral apparent density (BMAD) was calculated by using the formula BMAD (g/cm^3) = BMD (g/cm^2) × (4 / [¶ × width of lumbar spine measurement area]) in order to eliminate the effects of height and skeletal size on bone mass measurement. BMAD Z-score was also computed based on a Thai normative reference [32]. This normal reference for BMD and BMAD was conducted in 381 healthy Thai children and adolescents aged between 5-18 years who resided in urban areas in Bangkok. There were 114 male and 127 female adolescents (about 10-15 adolescents per each 1-year age category) aged between 10-18 years whom were used as age- and sex-matched controls for our participants [32]. According to the 2013 Pediatric Official Positions of International Society for Clinical Densitometry (ISCD), lumbar spine BMD and BMAD Z-scores $\leq$-2 were described as indicating low bone mass [33].

Sample size and power

In previous studies conducted in middle-income countries, the prevalence of low bone mass (BMD Z-score ≤-2) among perinatally HIV-infected adolescents varied from 23.8-32.4% [7,8]. We had hypothesized that the prevalence among our adolescents would be 30%. Therefore, in order to have a study that contained 80% power with 2-sided significance level of 0.05, we needed to enroll approximately 390 adolescents.

Statistical analysis

Characteristics and laboratory results of adolescents with BMD Z-score ≤-2 and >-2 were compared using chi-square and Wilcoxon rank sum tests for categorical and continuous variables, respectively. Bone mass, including BMD and BMAD, of HIV-infected individuals were compared with matched healthy peers by age (1-year class interval) and sex (male vs. female) categories, using the Student *t* test. Univariate linear regression analyses were performed to identify factors associated with BMD and BMAD Z-scores. Covariates were retained in a multivariable model if they were associated with a $P < 0.1$ in univariate analysis. Variables suspected to be potential effect modifiers of the associations were assessed. The magnitudes of association were summarized with mean difference and adjusted mean difference, together with their 95% confidence intervals (CI), in univariate and multivariable analyses, respectively.

In the models determining the association between bone turnover and bone mass, bone biochemical markers (CTX and PINP) were adjusted by significant factors in multivariable analysis. Each marker was transformed into their natural logarithms in order to allow the data to fit a standard normal distribution. The results of analyses on

log-transformed variables were back-transformed and then interpreted on the original scale. The magnitudes of association were reported as mean differences and adjusted mean differences per 10% increase in bone biomarker levels. To assess the association of chronic immune activation and inflammation and bone turnover, multivariable analyses were performed. Pro-inflammatory cytokines (IL-1α, IL-1β, IL-6 and TNF-α) and bone biochemical markers were transformed into their natural logarithms during analysis, and were back-transformed into the original scale for interpretation. The magnitudes of association were reported as percentage change in geometric mean of each bone biomarker per 10% increase in each cytokine.

A two-sided P <0.05 was considered to indicate statistical significance. All statistical analyses were performed using Stata statistical software, version 13. (StataCorp LP, College Station, Texas, USA).

Results

Participant characteristics

Between April and December 2014, 396 perinatally HIV-infected adolescents were enrolled, of whom approximately half (57%) were female. The median age was 15.0 years, and 73% were in Tanner Stage 3-5. Prior to ART initiation, 167 adolescents (43.7%) were in WHO clinical stage 3-4, with median $CD4^+$ T-cell percentage of 12.8. At enrollment, two-thirds were taking non-nucleoside reverse transcriptase inhibitor-based treatment, and one-third, protease inhibitor (PI)-based treatment, with a median duration of ART use of 9.3 years and median $CD4^+$ T-cell count of 734 cells/mm^3. All had HIV RNA <400 copies/ml and 96% had HIV RNA <50 copies/ml. Among adolescents with available data, the median PAQ scores was 1.8 out of 5 (n = 363), and sun exposure duration was 0.4 hours per day (n = 246) (Table 1).

Laboratory results and bone turnover markers

The median serum 25-OHD level was 26.3 ng/ml, and iPTH level was 41.7 pg/ml. The median serum calcium level was 9.5 mg/dl. For bone turnover markers, the median CTX and PINP levels were 1270 ng/L and 337 μ/l, respectively. The other laboratory results related to bone metabolism are shown in Table 2.

Lumbar spine bone mass of HIV-infected adolescents

Of 396 adolescents, 65 had lumbar spine BMD Z-scores ≤-2, representing a prevalence of low bone mass of 16.4% (95% CI: 12.7-20.0%). Thirty-three adolescents had lumbar spine BMAD Z-scores ≤-2, which reflects a prevalence of 8.3% (95% CI:

5.6-11.1%). The mean BMD (standard deviation) of HIV-infected adolescents was 0.899 (0.170) g/cm^2, which was significantly lower than that of age- and sex-matched HIV-uninfected controls [0.943 (0.166) g/cm^2, $P = 0.001$]. The mean BMAD for those with HIV was less than that of healthy controls, but the difference was not statistically significant (0.319 vs. 0.322 g/cm^3; $P = 0.48$). The comparison of lumbar spine bone mass between HIV-infected and healthy adolescents stratified by sex is demonstrated in Figure 1.

Associated factors of low bone mass

In the multivariable analysis for BMD, older age, BMI <5th percentile, boosted PI exposure and CD4$^+$ T-cell <15% prior to ART initiation were significantly associated with lower BMD Z-scores (Table 3). In the multivariable analysis for BMAD, in addition to the above, female sex was also an independent factor for lower BMAD Z-score (Table 3). Daily calcium intake was not significantly associated with BMD or BMAD Z-scores ($P >0.05$).

Association between bone turnover and bone mass

In the multivariable analysis for BMD, PINP was inversely associated with BMD Z-score, while CTX showed a trend of negative association that did not reach statistical significance. In the multivariable analysis for BMAD, PINP and CTX were negatively associated with BMAD Z-score (Supplemental Table 1).

Associations of chronic immune activation and inflammation with bone turnover

In univariate analysis, IL-1α and IL-1β demonstrated a significant positive association with CTX ($P = 0.02$ and 0.04, respectively), while IL-6 and TNF-α showed a trend of positive association that did not reach statistical significance. After controlling for confounders (age, sex, TDF exposure, duration of ART and iPTH level), IL-1α and IL-1β were no longer significantly associated with CTX (Supplemental Table 2).

In the univariate but not the multivariable analysis, there was a trend towards a positive association between IL-1β and PINP ($P = 0.09$) (Supplemental Table 2).

Discussion

Sixteen percent of our perinatally HIV-infected Asian adolescents had evidence of adverse bone health defined as having lumbar spine BMD Z-scores ≤-2. Bone mass for these individuals were much lower than that of their age- and sex-matched healthy peers. Older age, female sex, low BMI, boosted PI exposure, and low $CD4^+$ prior to ART initiation were associated with decreased bone mass. The dysregulation of bone turnover, demonstrated by increased bone formation and resorption markers, correlated with low bone mass. In addition, there was a trend towards a positive association between levels of pro-inflammatory cytokines and bone turnover markers.

The 16.4% prevalence of low bone mass (lumbar spine BMD Z-score ≤-2) among our Asian adolescents was comparable to that observed in middle-income countries (17-32% in Brazil) [6,8], but much higher than those documented in resource-rich countries (4% in United States and 8% in the Netherlands) [9,10]. This may be because the age, anthropometric parameters and HIV disease status are more similar in our and the Brazilian participants [6,8], but not the US and Dutch participants who were younger, had better nutritional status and less severe HIV disease [9,10]. These key demographic, growth and HIV disease parameters likely contributed to differences in prevalence of low bone mass in resource-rich vs. limited settings.

As recommended by the ISCD, BMD results for children and adolescents can be affected by chronic diseases, including HIV/AIDS, which lead to short stature or delayed sexual maturation. Therefore, BMAD may be a preferred endpoint in those infected by HIV, as it is adjusted for height, bone size and skeletal dimension [33]. In this study, we calculated BMAD Z-score based on Thai normative age- and sex-matched reference [32],

and found that the prevalence of low bone mass (lumbar spine BMAD Z-score ≤-2) declined to 8.3%. Since about one-fourth of our adolescents were short-for-age [height-for-age Z-score (HAZ) <-1.5], using BMAD Z-score to determine the prevalence of low bone mass might be more appropriate than using BMD Z-score, and this made our prevalence comparable to those documented in resource-rich countries. Additionally, the ISCD recommends employing HAZ adjustment to remove the confounding effect of height on bone density measurement [33]. This rationale has been supported by Zemel *et al,* who found that the HAZ adjustment is the least biased method compared with other approaches [34]. Unfortunately, this study could not assess the HAZ-adjusted BMD Z-score because there are no normative Asian reference data. Moreover, the publicly available web-based analysis tool is developed for US children based on BMD measured using the Hologic DXA system that is different to ours.

In this study, we found that HIV-specific factors, including boosted PI exposure and low $CD4^+$ prior to ART initiation, and traditional risk factors, including older age, female sex and low BMI (a combination of weight and height) were associated with lower bone mass. PI regimens and poor immunological status have been reported as important predictors for adverse bone health [9,10,13,34,35]. In addition, several studies reported that delayed linear growth and poor weight gain were independently associated with compromised bone mass in HIV-infected adolescents [7-9,12]. We used Thai normative reference to calculated height-for-age and weight-for-age Z-scores for all participants in this study, but since anthropometric parameters for Thai and Indonesian adolescents were comparable, this would not impact our results [26,36]. Because there were no Asian norms for BMI, our use of the US CDC reference to compute BMI

percentile might have inflated the number of adolescents who had low BMI (BMI <5th percentile) and magnified its association with decreased bone mass. We showed no association between daily calcium intake and bone density, which was similar to a previous study conducted in HIV-infected adults living in Italy where calcium-rich foods are commonly available [37]. Unfortunately, the missing data on sun exposure and physical activity prohibited accurate assessment of their relationships with poor bone mass in our study.

Disturbance of bone turnover process can lead to a net reduction in bone mass [38]. In this study, we observed that the high bone turnover, reflected in increased bone biomarkers, correlated with reduced bone mass, suggesting that dysregulation of bone turnover was accelerating bone loss in this population.

Chronic immune activation in HIV is associated with increased AIDS and non-AIDS morbidities and mortalities [39-43]. Bone loss, osteopenia and osteoporosis are among the consequences of residual HIV-associated immune hyperactivation [44]. Previous studies have demonstrated the associations between pro-inflammatory cytokines, such as IL-6 and TNF-α, and bone turnover dysregulation, reflected in increased and/or uncoupled bone resorption and formation markers [45-47]. Here we observed a trend toward a positive association between pro-inflammatory cytokines and bone turnover markers, suggesting that this may be a potential mechanism provoking bone demineralization in people living with HIV.

To the best of our knowledge, this is the largest cohort evaluating bone health status among perinatally HIV-infected adolescents in resource-limited settings. Other strengths of our study include the use of calculated BMAD and BMAD Z-scores as

endpoints that we believe to be a more accurate assessment of bone mass in adolescents with HIV than the BMD and BMD Z-scores. We were able to use DXA scanners from the same manufacturer (Lunar-GE) for all participating sites. We also performed the special laboratory measurements, including bone biochemical markers and pro-inflammatory cytokines at a single central laboratory. Our study is limited by its cross-sectional design. Furthermore, the number of adolescents with low bone mass was not large and likely contributed to the lack of strong associations between bone turnover and immune activation markers. Our bone densitometry was performed on the lumbar spine, which is mostly trabecular bone, therefore, the results may not be applicable to other parts of the body, particularly the hip or proximal femur, which are mostly cortical bone. Performing total body bone densitometry may have overcome this limitation, but it would have been very costly, and importantly, exposed these young people to greater amounts of radiation. Finally, we had to use Thai norms for Indonesian adolescents because lack of Indonesian norms, but they constituted a small proportion (6%) of the participants.

In conclusion, adverse bone health affects about 10% of Asian children and adolescents perinatally infected with HIV. Individuals with older age, female sex, low BMI, boosted PI exposure, and poor immunological status before ART initiation were at higher risk of low bone mass despite effective treatment. Dysregulation of bone turnover was associated with bone demineralization, while there was a trend towards a positive association between immune activation and bone turnover markers. Prospective research would be needed to monitor for long-term consequences of bone mass deterioration, particularly bone fracture, which may occur in the fourth to fifth decades of life. Furthermore, screening for low bone mass should be implemented in order to identify at-

risk young people with HIV who might benefit from interventions to promote long-term bone health.

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Table 1. Characteristics of perinatally HIV-infected Asian adolescents by bone mass status.

Characteristics[a]	Total (n = 396)	BMD Z-score ≤-2 (n = 65)	BMD Z-score >-2 (n = 331)	P^b
Demographic characteristics				
Age (year)	15.0 (13.3-16.9)	16.1 (14.5-18)	14.7 (13.1-16.8)	<0.001
Female sex	226 (57.1)	33 (50.8)	193 (58.3)	0.26
Anthropometric parameters				
WAZ	-0.9 (-1.5 to 0.1)	-1.7 (-2.3 to -1)	-0.7 (-1.4 to 0.3)	<0.001
WAZ <-1.5	104 (26.3)	39 (60)	65 (19.6)	<0.001
HAZ	-0.8 (-1.6 to -0.1)	-1.4 (-2.3 to 0.61)	-0.7 (-1.4 to 0.1)	<0.001
HAZ <-1.5	102 (25.8)	30 (46.2)	72 (21.8)	<0.001
BMI (kg/m^2)	17.9 (16.15-20)	16.7 (15.3-18.3)	18.1 (16.3-20.5)	<0.001
BMI <5th Percentile	87 (22)	33 (50.8)	54 (16.3)	<0.001
Tanner stage 3-5	290 (73.2)	47 (72.3)	243 (73.4)	0.85
HIV-specific characteristics				
WHO clinical stage prior to ART				0.16
Stage 1-2	215 (56.3)	29 (46.8)	186 (58.1)	
Stage 3	100 (26.2)	22 (35.5)	78 (24.4)	
Stage 4	67 (17.5)	11 (17.7)	56 (17.5)	
CD4$^+$ percentage prior to ART (%)	12.8 (3.0-20.1)	5 (1.0-14.0)	14 (4.3-21.0)	<0.001
History of ART				
Ever received TDF	139 (35.1)	26 (40)	113 (34.1)	0.36
Ever received boosted PI	160 (40.4)	30 (46.2)	130 (39.3)	0.30
Current ART regimen				0.21
NNRTI-based	234 (60.9)	36 (56.3)	198 (61.9)	
Boosted PI-based	141 (36.7)	28 (43.8)	113 (35.3)	
Both NNRTI-based and PI-based	9 (2.3)	0 (0)	9 (2.8)	
Duration of ART (year)	9.3 (6.9-11.5)	9.9 (7.2-11.9)	9.3 (6.9-11.4)	0.34
Duration of NNRTI (year)	6.2 (3.0-9.4)	5.5 (2.5-9.9)	6.4 (3.1-9.4)	0.27
Duration of PI (year)	7.0 (4.3-9.8)	7.4 (6.0-10.5)	6.9 (4.2-9.8)	0.33
Current CD4$^+$ T-cell count (cells/mm^3)	734 (581-907)	711 (550-858)	735 (587-917)	0.14
Lifestyle characteristics				
PAQ score[c] (out of 5)	1.8 (1.4-2.5)	1.8 (1.4-2.5)	1.8 (1.4-2.5)	0.89
Sun exposure duration[d] (hour/day)	0.4 (0.2-0.8)	0.4 (0.2-0.8)	0.4 (0.2-0.8)	0.62

Table 1. Characteristics of perinatally HIV-infected Asian adolescents by bone mass status (cont.)

Characteristics[a]	Total (n = 396)	BMD Z-score ≤-2 (n = 65)	BMD Z-score >-2 (n = 331)	P^b
Dietary intake				
Total calory (kcal/day)	1,191 (949-1,544)	1,328 (1,013-1,545)	1,180 (934-1,542)	0.36
Total calcium (mg/day)	239 (149-365)	248 (131-352)	229 (152-371)	0.79
Ever smoking	21 (5.3)	4 (6.2)	17 (5.2)	0.75
Ever drinking alcohol	16 (4.1)	1 (1.5)	15 (4.6)	0.26

Abbreviations: ART, antiretroviral therapy; BMD, bone mineral density; BMI, body mass index; EFV, efavirenz; HAZ, height for age Z-score; NNRTI, non-nucleoside reverse transcriptase inhibitor; PAQ, physical activity questionnaire; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate; WAZ; weight for age Z-score; WHO, World Health Organization.

[a]Data were presented as n (%) for categorical data and median (IQR) for continuous data.
[b]The comparisons were performed using Chi-square and Wilcoxon rank sum tests for categorical and continuous data, respectively.
[c] The analysis was based on 363 adolescents whose data were available.
[d]The analysis was based on 246 adolescents whose data were available.

Table 2. Laboratory results and bone turnover markers among perinatally HIV-infected Asian adolescents.

Laboratory parameters[a]	Total (n = 396)	BMD Z-score ≤-2 (n = 65)	BMD Z-score >-2 (n = 331)	P^b
Laboratory tests related to bone metabolism				
25-OHD (ng/ml)	26.3 (20.8-33.0)	24.9 (20.0-33.3)	26.7 (21.0-32.8)	0.63
iPTH (pg/ml)	41.7 (33.2-55.8)	41.7 (32.9-53.1)	41.4 (33.3-56.3)	0.78
Alkaline phosphatase (U/l)	178 (116-263)	213 (124-281)	173 (116-262)	0.20
Calcium (mg/dl)	9.5 (9.2-9.8)	9.5 (9.3-9.9)	9.5 (9.2-9.8)	0.04
Phosphorus (mg/dl)	4.3 (3.9-4.9)	4.4 (3.8-4.9)	4.3 (3.9-4.8)	0.74
Bone turnover markers				
CTX (ng/l)	1270 (860-1,810)	1300 (890-1,880)	1270 (850-1,790)	0.26
PINP (μg/l)	337 (153-621)	407 (153-678)	328 (153-611)	0.44

Abbreviations: 25-OHD, 25-hydroxyvitamin D; BMD, bone mineral density; CTX, C-terminal cross-linked telopeptide of type I collagen (bone resorption marker); iPTH, intact parathyroid hormone; PINP, procollagen type I amino-terminal propeptide (bone formation marker).

[a]Data were presented as n (%) for categorical data and median (IQR) for continuous data.
[b]The comparisons were performed using Chi-square and Wilcoxon rank sum tests for categorical and continuous data, respectively.

Table 3. Factors associated with low bone mass among perinatally HIV-infected Asian adolescents.

Characteristics	BMD Z-scores				BMAD Z-scores			
	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis	
	Mean difference (95% CI)	*P*	Adjusted mean difference (95% CI)	*P*	Mean difference (95% CI)	*P*	Adjusted mean difference (95% CI)	*P*
Age (per 1 year increased)	-0.08 (-0.15, -0.02)	0.01	-0.07 (-0.13,-0.02)	<0.001	-0.07 (-0.12, -0.02)	0.01	-0.06 (-0.11,-0.01)	0.02
Female sex	0.02 (-0.27, 0.32)	0.88	-	-	-0.45 (-0.69, -0.21)	<0.001	-0.53 (-0.77,-0.30)	<0.001
BMI <5th percentile	-1.31 (-1.64,-0.99)	<0.001	-1.24 (-1.55,-0.93)	<0.001	-0.77 (-1.05,-0.5)	<0.001	-0.81 (-1.09,-0.54)	<0.001
Tanner stage 3-5 (vs. 1-2)	0.48 (0.15, 0.80)	<0.001	-	-	0.11 (-0.16, 0.38)	0.44	-	-
WHO clinical stage 4 prior to ART (vs. 1-3)	-0.41 (-0.69, -0.12)	0.01	-0.29 (-0.6,0.03)	0.09	-0.25 (-0.49, -0.01)	0.04	-0.16 (-0.39,0.08)	0.19
Ever received boosted PIs	-0.34 (-0.63, -0.04)	0.03	-0.28 (-0.55,-0.01)	0.04	-0.15 (-0.39, 0.10)	0.24	-	-
Duration of ART (per 1 year increased)	0.03 (-0.01, 0.08)	0.14	-	-	0.02 (-0.01, 0.06)	0.22	-	-
CD4^{+} prior to ART <15%	-0.51 (-0.75,-0.27)	<0.001	-0.59 (-0.85,-0.32)	<0.001	-0.69 (-0.97,-0.4)	<0.001	-0.47 (-0.71,-0.23)	<0.001

Table 3. Factors associated with low bone mass among perinatally HIV-infected Asian adolescents (cont.)

Characteristics	BMD Z-scores				BMAD Z-scores			
	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis	
	Mean difference (95% CI)	*P*	Adjusted mean difference (95% CI)	*P*	Mean difference (95% CI)	*P*	Adjusted mean difference (95% CI)	*P*
Daily calcium intake (per 1000 mg increased)	-0.02 (-0.68, 0.72)	0.95	-	-	-0.06 (-0.64, 0.52)	0.83	-	-

Abbreviations: ART, antiretroviral treatment; BMAD, bone mineral apparent density; BMD, body mineral density; BMI, body mass index; 95% CI, 95% confidence interval; PI, protease inhibitor; WHO, World Health Organization.

Supplemental Table 1. Association between bone turnover markers and bone mass among perinatally HIV-infected Asian adolescents.

Bone turnover markers (per 10% increase)	BMD Z-score				BMAD Z-score			
	Mean difference (95% CI)	***P***	**Adjusted mean difference (95% CI)[a]**	***P***	**Mean difference (95% CI)**	***P***	**Adjusted mean difference (95% CI)[b]**	***P***
CTX	-0.026 (-0.052, 0.002)	0.07	-0.029 (-0.048, 0.003)	0.08	0.0013 (-0.0120, 0.0145)	0.99	-0.0286 (-0.0572, -0.0095)	0.02
PINP	-0.015 (-0.031, 0.001)	0.07	-0.029 (-0.038, -0.004)	0.02	0.0002 (-0.0225, 0.0230)	0.85	-0.0191 (-0.0381, -0.0095)	0.001

Abbreviations: BMD, bone mineral density; BMAD, bone mineral apparent density; CTX, C-terminal cross-linked telopeptide of type I collagen (bone resorption marker); PINP, procollagen type I amino-terminal propeptide (bone formation marker).

[a]Multivariable model was adjusted for age, BMI, history of receiving boosted PI, $CD4^+$ percentage prior to ART.
[b]Multivariable model was adjusted for age, sex, BMI, $CD4^+$ percentage to prior to ART.

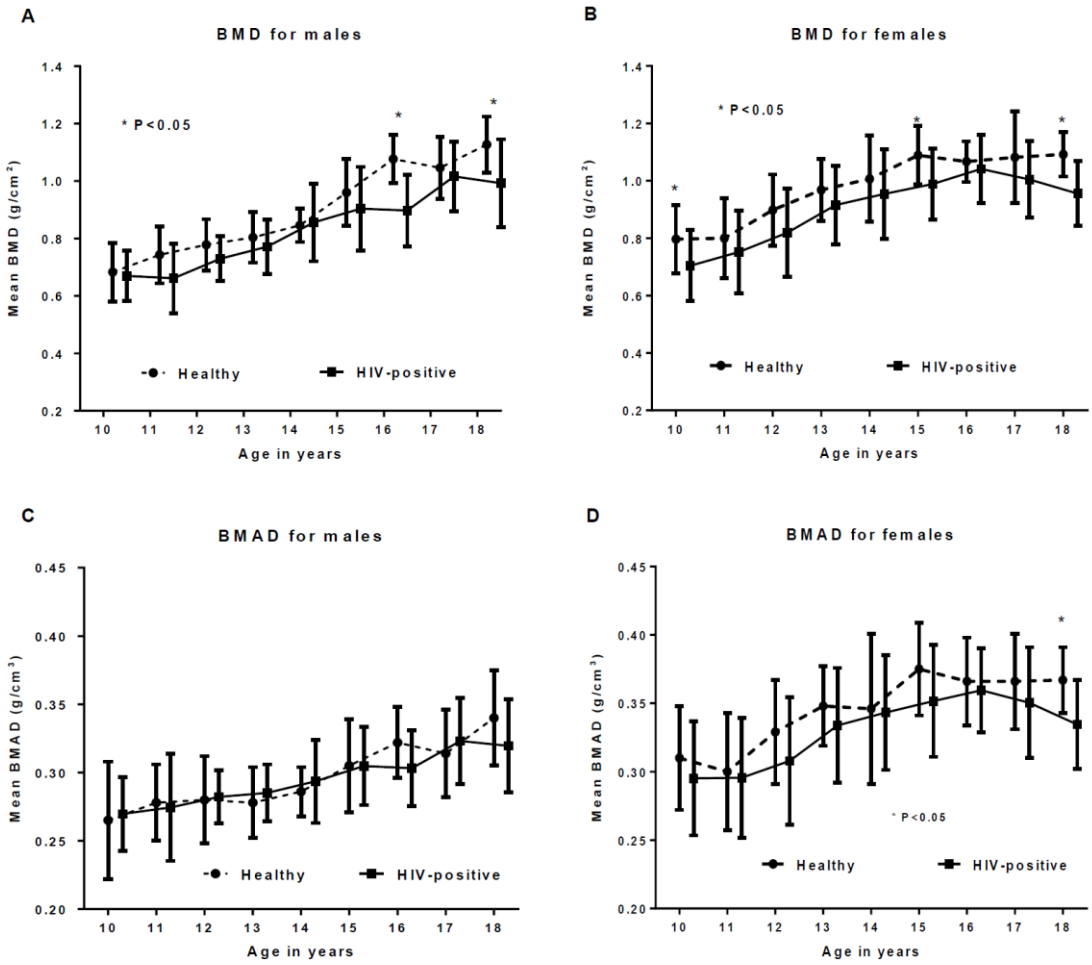
Supplemental Table 2. Association between pro-inflammatory cytokines and bone turnover markers among perinatally HIV-infected Asian adolescents.

Pro-inflammatory cytokines (per 10% increase)	CTX				PINP			
	Percentage change in geometric mean (95% CI)	***P***	**Adjusted percentage change in geometric mean (95% CI)[a]**	***P***	**Percentage change in geometric mean (95% CI)**	***P***	**Adjusted percentage change in geometric mean (95% CI)[b]**	***P***
Interleukin-1α	0.40 (0.06, 0.76)	0.02	0.14 (-0.15, 0.42)	0.35	0.42 (-0.18, 1.04)	0.17	-	-
Interleukin-1β	0.30 (0.01, 0.57)	0.04	0.04 (-0.19, 0.27)	0.76	0.42 (-0.07, 0.91)	0.09	0.02 (-0.35, 0.39)	0.90
Interleukin-6	0.20 (-0.19, 0.58)	0.32	-	-	0.50 (-0.18, 1.17)	0.15	-	-
Tumor necrotic factor-α	0.16 (-0.04, 0.37)	0.11	-	-	0.24 (-0.11, 0.60)	0.19	-	-

Abbreviations: CTX, C-terminal cross-linked telopeptide of type I collagen (bone resorption marker); PINP, procollagen type I amino-terminal propeptide (bone formation marker).

[a]Multivariable model was adjusted for age, sex, history of receiving tenofovir disoproxil fumarate, duration of antiretroviral treatment and intact parathyroid hormone level.
[b]Multivariable model was adjusted for age, sex, physical activity level, duration of antiretroviral treatment and intact parathyroid hormone level.

Figure 1. Comparisons of lumbar spine bone mineral density and bone mineral apparent density for HIV-infected and healthy adolescents, stratified by sex.



Note: (A) represents the comparison of BMD (g/cm^2) for HIV-infected male adolescents with healthy males; (B) represents the comparison of BMD (g/cm^2) for HIV-infected female adolescents with healthy females; (C) represents the comparison of BMAD (g/cm^3) for HIV-infected male adolescents with healthy males; and (D) represents the comparison of BMAD (g/cm^3) for HIV-infected female adolescents with healthy females.

Data from healthy adolescents are from Nakavachara *et al*. PLoS One. 2014;9(5):e97218.

CHAPTER IV

HYPOVITAMINOSIS D AND HYPERPARATHYROIDISM: EFFECTS ON BONE TURNOVER AND BONE MINERAL DENSITY AMONG PERINATALLY HIV-INFECTED ADOLESCENTS

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ABSTRACT

Objectives: The impact of hypovitaminosis D and secondary hyperparathyroidism on bone mineral density (BMD) in the setting of pediatric HIV infection remains unclear. This study aimed to determine the prevalence of hypovitaminosis D and hyperparathyroidism and their effects on bone turnover and BMD among HIV-infected adolescents in Southeast Asia.

Design: A multicenter, cross-sectional study evaluating bone health and vitamin D metabolism in HIV-infected adolescents in Thailand and Indonesia.

Methods: Perinatally HIV-infected adolescents aged 10-18 years on antiretroviral therapy with virologic suppression were enrolled. Serum 25-hydroxyvitamin D, intact parathyroid hormone, and bone turnover markers (C-terminal cross-linked telopeptide of type I collagen and procollagen type I amino-terminal propeptide) were assessed; 25-hydroxyvitamin D less than 20 ng/ml and intact parathyroid hormone more than 65 pg/ml were defined as hypovitaminosis D and hyperparathyroidism, respectively. Lumbar spine (L2-L4) BMD Z-score -2 or less was defined as low BMD.

Results: Of 394 adolescents, 57% were women. The median age [interquartile range (IQR)] was 15.0 (13.3-16.9) years. The prevalence of hypovitaminosis D, hyperparathyroidism, and both conditions were 21% [95% confidence interval (CI): 17-25%), 17% (95% CI: 13-20%) and 5% (95% CI: 3-7%), respectively. Adolescents with hypovitaminosis D and secondary hyperparathyroidism had the highest median bone resorption (C-terminal cross-linked telopeptide of type I collagen: 1610 vs. 1270 ng/l; P=0.04) and bone formation (procollagen type I amino-terminal propeptide: 572 vs. 330

μg/l; *P*=0.02) markers, and the greatest proportion of low BMD (42 vs. 15%; *P*=0.01) compared with the rest of the cohort.

Conclusion: Hypovitaminosis D complicated with secondary hyperparathyroidism was associated with increased bone turnover and bone loss. Early treatment of hypovitaminosis D before hyperparathyroidism occurs may be important to prevent bone mass deterioration.

Introduction

In recent years, hypovitaminosis D, as measured by serum 25-hydroxyvitamin D (25-OHD) below 20 ng/ml, has emerged as a research priority among HIV-infected populations because of its association with HIV disease progression [1] and all-cause mortality [2]. In addition, this condition plays a critical role in the pathogenesis of several long-term, non-AIDS-related complications, including adverse bone health, cardiovascular disease, insulin resistance and diabetes mellitus [3-7]. However, most studies were conducted in Western and developed countries, and such conditions in resource-limited settings have been poorly studied.

High prevalence of hypovitaminosis D among HIV-infected individuals, both adults (13-69%) [8] and youth (25-77%), have been observed [9-11]. Numerous factors could contribute to this condition, including traditional factors such as female sex, dark skin pigmentation, high body mass index (BMI), physical inactivity, reduced sun exposure and winter season [6,8,10,12-14], as well as HIV-related factors such as advanced stage of disease, exposure to certain antiretroviral drugs, for example, efavirenz and ritonavir-boosted protease inhibitors, and immune activation [6,8,12,14]. Yet, it is usually difficult to differentiate the direct effects of each factor on vitamin D status in HIV-infected persons.

Vitamin D is critical for calcium homeostasis and bone metabolism. It promotes calcium absorption from the gut, preserves adequate calcium in blood circulation, aids bone growth and remodeling, and maintains normal bone mineralization and strength [15,16]. Low vitamin D level can result in low serum calcium levels [17], which stimulate parathyroid glands to release parathyroid hormone (PTH), causing secondary hyperparathyroidism, through a positive feedback mechanism [16]. To maintain calcium

levels in the blood, increased PTH levels thereafter accelerate calcium mobilization from the labile bone pool via osteoclast activation, together with other mechanisms [15,16]. Thus, hypovitaminosis D can contribute to elevated PTH that may result in bone demineralization, and with time, bone loss [15,16]. This study aimed to investigate the prevalence hypovitaminosis D and secondary hyperparathyroidism, as well as their pathological effects, on bone turnover and bone mineral density (BMD) among HIV-infected adolescents from Asia.

Methods and measurements

Study design and participants

This is a substudy of a multicenter, cross-sectional study evaluating bone health outcomes and vitamin D metabolism in perinatally HIV-infected Asian adolescents. The study was conducted at four pediatric HIV centers in Thailand (latitude 13.75°N) and Indonesia (latitude 6.18°S), including the HIV Netherlands Australia Thailand collaboration in Bangkok (BKK), the Faculty of Medicine and Research Institute for Health Sciences in Chiang Mai, and Srinagarind Hospital, Khon Kaen University in Khon Kaen; the Cipto Mangunkusumo General Hospital in Jakarta. Since BKK and Jakarta are capital cities, and Chiang Mai and Khon Kaen are provincial cities, we categorized these sites as urban (BKK and Jakarta) and rural (Chiang Mai and Khon Kaen) regions. Perinatally HIV-infected adolescents (aged 10-18 years) who had virologic suppression (plasma HIV-1 RNA <400 copies/ml, within the past 6 months) on antiretroviral therapy (ART) were enrolled. Adolescents with previous bone abnormality or having other diseases that affect bone health were excluded. We also precluded adolescents who were on treatment of low bone mass, reported taking vitamin D supplements, or received medications affecting bone density and metabolism. Written informed assent and consent were obtained from adolescents and their caregivers as appropriate. The study was reviewed and approved by the local institutional review boards at all sites.

Data collection and clinical assessments

Demographic characteristics and HIV-related parameters of each participant were retrieved from medical records. Anthropometric measurements, Tanner stage evaluation, and complete physical examination were performed at study entry. Weight-for-age and

height-for-age Z-scores were calculated using a Thai normative reference [18], and BMI percentile was computed based on the US Centers for Disease Control and Prevention reference [19]. Information regarding sun exposure duration (h/ day) and physical activity levels [score 1 (low) to 5 (high)] were assessed using standard questionnaires [20].

Laboratory measurements

Overnight fasting blood samples for 25-OHD, intact parathyroid hormone (iPTH), calcium, phosphorus, alkaline phosphatase and bone turnover markers were collected between 8:00 – 10:00 a.m. to prevent circadian variation of biochemical markers of bone turnover. Serum was obtained and stored at -70°C until laboratory analysis. An automated electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), measured on a Cobas® e411 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany), was used to quantify 25-OHD, iPTH, bone resroption marker (C-terminal cross-linked telopeptide of type I collagen; CTX), and bone formation marker (procollagen type I amino-terminal propeptide; PINP). These bone metabolism-related markers were analyzed at a single reference site, the Research Institute for Health Sciences, Chiang Mai University (Chiang Mai, Thailand) by a single technician. The other laboratory parameters were measured at each local site. According to the Endocrine Society, vitamin D insufficiency (VDI) and hypovitaminosis D were defined as 25-OHD level between 20 and 29.9 ng/ml and below 20 ng/ml, respectively [21]. Serum iPTH level above 65 pg/ml was considered to be high and defined as hyperparathyroidism [22]. The normal ranges for alkaline phosphatase level were 100-390 U/l (men) and 100-320

U/l (women). The normal range of calcium was 8.4-10.2 mg/dl, and phosphorus was 3.3-5.4 mg/dl (age 10-15 years) and 2.4-4.4 mg/dl (age >15 years).

Bone mineral density assessment

BMD of the lumbar spine (L2 to L4) was evaluated by dual-energy X-ray absorptiometry technique (Lunar; General Electric Healthcare, Madison, Wisconsin, USA) by radiology technologists at all sites. The scans were analyzed using enCORE software version 14.1 (encore, General Electric Healthcare, Madison, Wisconsin, USA). BMD Z-scores comparing the absolute BMD results of participants to the average results of Thai adolescents of the same age and sex were calculated [23]. A Z-score -2 or less was defined as low BMD according to the 2013 Pediatric Official Positions of the International Society for Clinical Densitometry [24].

Statistical analysis

The prevalence of hypovitaminosis D (25-OHD <20 ng/ml) and hyperparathyroidism (iPTH >65 pg/ml), together with 95% confidence intervals (CI), were calculated. The comparisons of prevalence of hypovitaminosis D between regions (urban vs. rural) and across seasons [hot (April to June) vs. rainy (July to October) vs. cool (November to December)] were performed using chi-square test. Participant characteristics, stratified by 25-OHD levels (25-OHD <20; 20-29.9; and ≥30 ng/ml), were evaluated by chi-square and Kruskal-Wallis tests for categorical and continuous variables, respectively. Univariate linear regression was performed to identify factors associated with 25-OHD levels. Covariates that were significant at P <0.1 in a univariate

model were included in the multivariable analysis. Variables suspected to be potential effect modifiers of the associations were evaluated. The magnitudes of associations were summarized with mean differences and adjusted mean differences for univariate and multivariable analyses, respectively.

To determine the effects of hypovitaminosis D and secondary hyperparathyroidism on bone turnover and BMD, we classified our adolescents into six subgroups according to their vitamin D and PTH statuses, as adolescents with hypovitaminosis D and hyperparathyroidism; hypovitaminosis D and normal iPTH level; VDI and hyperparathyroidism; VDI and normal iPTH level; normal vitamin D status and hyperparathyroidism; and normal vitamin D and PTH statuses. The comparisons of laboratory results, bone turnover markers and BMD across subgroups were conducted using chi-square test for categorical variables and Kruskal-Wallis test (comparing across six subgroups) or Wilcoxon rank sum test (comparing two specific subgroups) for continuous variables, as appropriate.

All statistical analyses were performed using Stata statistical software, version 13. (StataCorp LP, College Station, Texas, USA). A two-sided $P < 0.05$ was considered statistically significant.

Results

Participant characteristics

There were 394 adolescents, of whom 57.1% were women, with a median age of 15.0 years, enrolled between April and December 2014. The enrollment occurred during the hot (*n* = 75; 19.0%), rainy (*n* = 186; 47.2%), and cool (*n* = 133; 33.8%) seasons. Approximately three- quarters of adolescents (73.1%) reached Tanner stage 3-5. Nearly half (43.6%) were in WHO clinical stage 4, with a median $CD4^+$ T-cell percentage of 13 before ART initiation. At entry, 140 adolescents (35.5%) were on boosted protease inhibitor-based regimens, of which 81% were lopinavir/ritonavir based. The median duration of ART use was 9.3 years and the median $CD4^+$ T-cell count was 734 cells/mm^3. Among those with available data (*n* = 245), the median duration of sun exposure was 0.4 h/ day that was different between individuals with hypovitaminosis D, VDI, and normal vitamin D status (*P* = 0.03). The characteristics of participants stratified by vitamin D status are summarized in Table 1.

Prevalence of hypovitaminosis D and hyperparathyroidism

The median 25-OHD level was 26.3 ng/ml. Overall, hypovitaminosis D (25-OHD <20ng/ml) was documented in 83 adolescents (21.1%; 95% CI: 17.0-25.1%) and approximately half (44.2%; 95% CI: 39.2-49.1%) had VDI (25-OHD = 20-29.9 ng/ml). The prevalence of hypovitaminosis D did not vary between regions (urban: 23.6%; rural: 19.3%; *P* = 0.31) or across seasons (hot: 18.7%; rainy: 21.0%, cool: 22.6%; *P* = 0.80). The median iPTH level was 41.7 pg/ml, and 66 adolescents (16.8%; 95% CI: 13.1-20.1%) had hyperparathyroidism (iPTH >65 pg/ml). Nineteen adolescents (22.9%) in the

hypovitaminosis D group and 36 (20.7%) in VDI group developed secondary hyperparathyroidism.

Associated factors of vitamin D level and its relationship with parathyroid hormone

In the univariate analysis, boosted protease inhibitor regimens (P <0.001) and current $CD4^+$ T-cell count (P = 0.01) were positively associated with 25-OHD level, whereas negative associations were observed for female sex (P <0.001) and BMI (P <0.001). In the multivariable analysis, boosted protease inhibitor regimens remained independently and positively associated with 25-OHD level (P <0.001), whereas female sex (P <0.001) and higher BMI (P = 0.04) were independently and negatively associated with 25-OHD level. The iPTH was inversely correlated with 25-OHD level in both the univariate (P <0.001), and multivariable analyses (P = 0.001) (Table 2).

The effects of vitamin D and parathyroid hormone on bone turnover

Adolescents with hypovitaminosis D (25-OHD <20 ng/ml) and secondary hyperparathyroidism (iPTH >65 pg/ml) had the highest median levels of bone resorption (CTX: 1610 vs. 1270 ng/l; P = 0.04) and bone formation (PINP: 572 vs. 330 μg/l; P = 0.02) markers compared with the rest of the cohort (Figure 1).

The comparison of laboratory results related to bone metabolism within each vitamin D group are summarized in Table 3. Among adolescents with hypovitaminosis D, individuals with secondary hyperparathyroidism had higher alkaline phosphatase (253 vs. 173 U/l; P = 0.02) and phosphorus levels (4.8 vs. 4.3 mg/dl, P = 0.03), but maintained calcium levels (9.4 vs. 9.5 mg/dl, P = 0.42) similar to those with normal iPTH level

(Table 3). In addition, biomarkers for bone turnover, both CTX (1610 vs. 1160 ng/l; P = 0.02) and PINP (572 vs. 289 μg/l; P = 0.01) were higher in adolescents with secondary hyperparathyroidism in comparison with individuals without this condition. Similarly, among adolescents with VDI (25-OHD 20-29.9 ng/ml), individuals with secondary hyperparathyroidism had greater levels of bone turnover markers compared with those with normal PTH status (CTX: 1610 vs. 1200 ng/l; P = 0.01; PINP: 430 vs. 342 μg/l; P = 0.04) (Table 3).

The effects of vitamin D and parathyroid hormone on bone mineral density

The proportion of low BMD varied considerably across six subgroups (P = 0.03), with the highest percentage in the group with hypovitaminosis D and secondary hyperparathyroidism (42.1%) (Figure 2). Additionally, the median BMD Z-score was lowest in this group as compared with the rest of the cohort (-1.2 vs. -0.7; P = 0.10) (Table 3).

When comparing within each vitamin D group, the proportion of low BMD in the group with hypovitaminosis D was greater in adolescents who had secondary hyperparathyroidism compared with those without this condition (42.1 vs. 12.7%; P = 0.01). However, such difference was not observed in the groups with VDI and normal vitamin D status (Table 3).

Discussion

One-fifth of the perinatally HIV-infected adolescents in this cross-sectional cohort had hypovitaminosis D. Boosted protease inhibitor regimens was positively associated with 25-OHD levels, whereas female sex, high BMI and elevated iPTH were negatively associated. Adolescents with hypovitaminosis D along with secondary hyperparathyroidism had significantly elevated bone turnover markers and substantially great proportion of low BMD, whereas adolescents with VDI and secondary hyperparathyroidism had increased bone turnover markers that did not result in reductions in BMD Z-scores.

Although our study settings, Thailand and Indonesia, are located in tropical climate zones, the prevalence of hypovitaminosis D (25-OHD <20 ng/ml) among our adolescents was relatively high (21%) and comparable with those of healthy, HIV-uninfected children and adolescents living in Thailand (20%) and Indonesia (15%) [25,26]. However, when compared with HIV-infected children and adolescents living in Western countries, the prevalence of hypovitaminosis D documented in our study was much lower [12,13]. The dissimilarity in latitude and seasons of study settings, race/ethnicity of study populations, definition of hypovitaminosis D, and assay techniques for 25-OHD might contribute to the difference in prevalence of hypovitaminosis D between ours and the Western studies. A US study (Philadelphia; latitude 39.96°N) revealed that the prevalence of hypovitaminosis D (25-OHD <11 ng/ml) among their HIV-infected children and adolescents was 36%, which was significantly greater than that seen in healthy controls from the same geographic area (15%, P <0.001). However, the proportion of African-American participants in the HIV-

infected group was significantly higher than in the HIV-uninfected group (83 vs. 37%; *P* <0.001) [12]. A French study (Paris; latitude 48.50°N), where the majority of participants (68%) were of African or African-French origin and the study period was during their winter/spring, reported a 25% prevalence of hypovitaminosis D (25-OHD <10 ng/ml) among their children and young adults living with HIV. Yet, this number was significantly lower than that observed in healthy controls living in the same area with similar skin phototype (54%, *P* <0.001) [13].

Important traditional factors associated with hypovitaminosis D among HIV individuals include Black race or Hispanic ethnicity, older age, female sex, high BMI, physical inactivity and low exposure to sunlight [10,14,27-29]. In this study, we demonstrated the significant associations between female sex and high BMI with low 25-OHD levels, whereas physical activity levels showed a trend of positive association that did not reach statistical significance when adjusting for confounders. Although we observed a difference in duration of sun exposure between adolescents with hypovitaminosis D, VDI and normal vitamin D status, we could not assess the association between short duration of sun exposure and decreased 25-OHD levels in this study because of missing data.

Several antiretroviral medications might also be associated with vitamin D status [28,30-32]. In this study, we found a positive association between ritonavir-boosted protease inhibitors and 25-OHD levels. A Dutch study noted that White HIV-infected adults on protease inhibitors had higher levels of 25-OHD compared with individuals exposed to nonnucleoside reverse transcriptase inhibitors or treatment-naïve persons [33]. This may be attributed to ritonavir blocking 1α-hydroxylase enzyme required for the

bioactivation of 25-OHD to 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] in the kidneys [34]. Therefore, individuals on ritonavir-boosted protease inhibitors may appear to have adequate 25-OHD levels, but they generally have decreased 1,25-$(OH)_2$D levels. Unfortunately, we were not able to measure 1,25-$(OH)_2$D.

We found that 17% of our adolescents had hyperparathyroidism (iPTH >65 pg/ml), of which 5% co-occurred with hypovitaminosis D; 9% with VDI; and 3% with normal vitamin D status. Hyperparathyroidism in our adolescents was inversely associated with vitamin D level as has been shown in other studies [9,35,36]. Hypovitaminosis D is one of the common causes of secondary hyperparathyroidism [37,38]. The possible explanation for the hyperparathyroidism independent from hypovitaminosis D and VDI found in our study may be related to inadequate calcium intake. The median daily calcium intake in adolescents with this condition (205 mg/day; data not shown) was much lower than the recommended dietary allowance for calcium for this age group (1300 mg/day) [39]. Other possible contributing factors include poor calcium absorption from the gut and other comorbidities, particularly chronic renal failure. However, we excluded participants with documented kidney diseases from this study and are unable to further assess calcium absorption.

Interestingly, we found dose-response relationships of vitamin D and PTH status on bone turnover markers and BMD in this study. To clarify, we found that, among adolescents with hypovitaminosis D, individuals with secondary hyperparathyroidism had significantly higher bone resorption and formation markers, and a greater proportion had low BMD (BMD Z-score $\leq$-2) compared with those without hyperparathyroidism. These findings were consistent with previous studies conducted in HIV-uninfected

populations [40,41]. A study of postmenopausal British women older than 60 years with established osteoporosis found that individuals with hypovitaminosis D and secondary hyperparathyroidism had significantly higher bone formation (bone alkaline phosphatase and osteocalcin) and bone resorption (urine-free deoxypyridinoline) markers, and considerably lower hip BMD compared with those who had hypovitaminosis D without hyperparathyroidism, and those without hypovitaminosis D [40]. Another study conducted in Indo-Asian middle-aged adults with vitamin D deficiency living in the United Kingdom revealed that individuals who had secondary hyperparathyroidism had significantly lower femoral neck and distal radius BMD Z and T-scores compared with those without hyperparathriodism [41]. Therefore, our findings support that hypovitaminosis D and secondary hyperparathyroidism were associated with acceleration of bone turnover that may aggravate reduction in bone density in HIV-infected population.

Furthermore, among adolescents who had VDI, we observed that those with secondary hyperparathyroidism had a significant elevation in bone turnover markers, but did not demonstrate higher prevalence of low BMD in comparison with the group without hyperparathyroidism. We hypothesized that these individuals might be able to keep net change of bone remodeling process in their skeletal system or the level of vitamin D might not be low enough to cause reduced BMD.

This study contained some limitations. First, our study was conducted only in tropical regions, and the findings may have limited generalizability to settings in different geographic locations. Second, although we found several significant associated factors for hypovitaminosis D, this was a cross-sectional study and our ability to assess causality

is limited. Nevertheless, our study had several strengths. To our knowledge, this study is among the first studies investigating the effects of hypovitaminosis D and secondary hyperparathyroidism on bone metabolism and bone density among perinatally HIV-infected adolescents in Asia. Furthermore, laboratory measures related to bone metabolisms, including 25-OHD, iPTH and bone biochemical markers, were measured at a single laboratory center. Therefore, the consistency and comparability of the results can be ensured.

In conclusion, among perinatally infected with HIV adolescents living in tropical countries, hypovitaminosis D is relatively high. The deficiency of vitamin D complicated by secondary hyperparathyroidism was associated with accelerated bone turnover and bone demineralization. Monitoring for vitamin D and PTH status may be important for individuals at high risk for hypovitaminosis D. Vitamin D supplementation in persons with deficiency of vitamin D before secondary hyperparathyroidism develops may be necessary for preventing future bone demineralization. The experimental study to confirm the potential benefit of this strategy is warranted.

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Table 1. Characteristics of perinatally HIV-infected Asian adolescents by vitamin D status.

Characteristics[a]	**Total** (*n* = 394)	**25-OHD <20 ng/ml** (*n* = 83)	**25-OHD 20-29.9 ng/ml** (*n* = 174)	**25-OHD ≥30 ng/ml** (*n* = 137)	***P***[b]
Demographic characteristics					
Age (year)	15.0 (13.3-16.9)	14.9 (12.9-17.2)	15.0 (13.6-16.7)	15.2 (13.3-16.9)	0.90
Female sex	225 (57.1)	61 (73.5)	103 (59.2)	61 (44.5)	<0.001
Urban regions	161 (40.9)	38 (45.8)	76 (43.7)	47 (34.3)	0.15
Anthropometric parameters					
WAZ	-0.9 (-1.6 to 0.1)	-0.5 (-1.6 to 0.4)	-0.8 (-1.4 to 0.1)	-1.0 (-1.8 to -0.3)	0.01
WAZ <-1.5	104 (26.4)	21 (25.3)	42 (24.1)	41 (29.9)	0.50
HAZ	-0.8 (-1.6 to -0.1)	-0.8 (-1.5 to 0.1)	-0.8 (-1.4 to 0.1)	-0.9 (-1.7 to -0.2)	0.22
HAZ <-1.5	102 (25.9)	20 (24.1)	41 (23.6)	41 (29.9)	0.40
BMI (kg/m^2)	17.9 (16.1-20.0)	18.3 (16.1-21.5)	18.3 (16.6-20.2)	17.5 (15.8-19.0)	0.02
BMI <5th Percentile	87 (22.1)	18 (21.7)	32 (18.4)	37 (27.0)	0.19
Tanner stage 3-5	288 (73.1)	60 (72.3)	129 (74.1)	99 (72.3)	0.92
HIV-specific characteristics					
WHO clinical stage prior to ART					0.74
Stage 1-3	215 (56.4)	47 (58.8)	90 (54.2)	78 (57.8)	
Stage 4	166 (43.6)	33 (41.3)	76 (45.8)	57 (42.2)	
$CD4^+$ percentage prior to ART	13 (3-20)	11 (3-22)	13 (3-21)	14 (4-20)	0.87
Current ART regimen					<0.001
NNRTI based	233 (59.1)	65 (78.3)	99 (58.9)	69 (51.1)	
PI based	140 (35.5)	14 (16.9)	64 (38.1)	62 (45.9)	
Both NNRTI based and PI based	21 (5.4)	4 (4.8)	5 (3.0)	4 (3.0)	
Duration of ART (year)	9.3 (6.9-11.5)	8.8 (6.3-11.6)	9.3 (7.4-11.4)	9.8 (6.7-11.5)	0.69
Current $CD4^+$ T-cell count ($cell/mm^3$)	734 (581-907)	681 (534-889)	723 (583-903)	759 (628-929)	0.08

Table 1. Characteristics of perinatally HIV-infected Asian adolescents by vitamin D status (cont.)

Characteristics[a]	**Total** (*n* = 394)	**25-OHD <20 ng/ml** (*n* = 83)	**25-OHD 20-29.9 ng/ml** (*n* = 174)	**25-OHD ≥30 ng/ml** (*n* = 137)	***P***[b]
Lifestyle characteristics					
Sun exposure duration[c] (hour/day)	0.4 (0.2-0.8)	0.3 (0.2-0.6)	0.4 (0.2-0.8)	0.5 (0.2-1.0)	0.03
PAQ score[d] (out of 5)	1.8 (1.4-2.5)	1.7 (1.3-2.4)	1.8 (1.4-2.5)	1.9 (1.4-2.6)	0.37

Abbreviations: 25-OHD, 25-hydroxyvitamin D; ART, antiretroviral therapy; HAZ, height for age Z-score; NNRTI, non-nucleoside reverse transcriptase inhibitor; PAQ, physical activity questionnaire; PI, protease inhibitor; WAZ; weight for age Z-score.

[a]Data were presented as *n* (%) for categorical data and median (IQR) for continuous data.
[b]The comparisons were performed using Chi-square and Kruskal-Wallis tests for categorical and continuous data, respectively.
[c]The analysis was based on 245 adolescents whose data were available.
[d] The analysis was based on 363 adolescents whose data were available.

Table 2. Factors associated with 25-hydroxyvitamin D levels among perinatally HIV-infected adolescents.

Characteristics	Univariate analysis		Multivariable analysis	
	Mean difference (95% CI)	*P*	Adjusted mean difference (95% CI)	*P*
Age (per 1 year increased)	0.03 (-0.38, 0.43)	0.90	-	-
Female sex	-5.18 (-7.06, -3.30)	<0.001	-4.82 (-6.84, -2.80)	<0.001
BMI	-0.43 (-0.69, -0.17)	<0.001	-0.31 (-0.59, -0.02)	0.04
PAQ score (per 1 score increased)	2.38 (-0.39, 5.14)	0.09	1.14 (-1.61, 3.89)	0.42
WHO stage 4 prior to ART (vs. stage 1-3)	-0.24 (-2.23, 1.75)	0.81	-	-
Current ART regimen (boosted PI vs. NNRTI/others)	3.97 (2.02, 5.92)	<0.001	3.62 (1.63, 5.60)	<0.001
Duration of ART (per 1 year increased)	0.14 (-0.15, 0.43)	0.34	-	-
$CD4^+$ prior to ART <15%	0.03 (-0.07, 0.13)	0.57	-	-
Current $CD4^+$ T-cell count	0.005 (0.001, 0.009)	0.01	0.003 (-0.001, 0.007)	0.09
iPTH level	-0.09 (-0.13, -0.05)	<0.001	-0.08 (-0.13, -0.04)	0.001

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; iPTH, intact parathyroid hormone; NNRTI, non-nucleoside reverse transcriptase inhibitor; PAQ; physical activity questionnaire; PI, protease inhibitor.

Table 3. Laboratory results related to bone metabolism and bone mineral density among perinatally HIV-infected adolescents.

Laboratory tests[a]	25-OHD <20 ng/ml[b]			25-OHD 20-29.9 ng/ml			25-OHD ≥30 ng/ml			Overall *P*[d]
	iPTH >65 pg/ml (*n* = 19)	iPTH ≤65 pg/ml (*n* = 63)	*P*[c]	iPTH >65 pg/ml (*n* = 36)	iPTH ≤65 pg/ml (*n* = 138)	*P*[c]	iPTH >65 pg/ml (*n* = 11)	iPTH ≤65 pg/ml (*n* = 126)	*P*[c]	
Blood chemistries										
25-OHD (ng/ml)	15.7 (13.0-17.7)	15.9 (13.1-18.6)	0.52	24.6 (22.4-27.9)	24.7 (21.9-27.6)	0.82	38.0 (34.7-42.4)	36.9 (32.6-41.6)	0.41	<0.001
iPTH (pg/ml)	86.4 (75.1-97.7)	41.4 (34.4-48.6)	<0.001	82.0 (73.8-102.0)	40.0 (32.6-47.2)	<0.001	71.1 (67.6-83.6)	35.9 (28.5-45.1)	<0.001	<0.001
Alkaline phosphatase (U/l)	253 (180-290)	173 (107-245)	0.02	219 (133-330)	177 (109-263)	0.09	148 (101-208)	172 (125-253)	0.38	0.13
Calcium (mg/dl)	9.4 (9.1-9.7)	9.5 (9.2-9.7)	0.42	9.4 (9.1-9.6)	9.5 (9.2-9.9)	0.03	9.3 (9.0-9.6)	9.6 (9.3-9.9)	0.03	0.03
Phosphorus (mg/dl)	4.8 (4.2-5.2)	4.3 (3.9-4.7)	0.03	4.6 (4.0-5.1)	4.3 (3.8-4.8)	0.15	4.3 (3.4-4.6)	4.3 (3.9-4.8)	0.40	0.11
Bone turnover markers										
CTX (ng/l)	1,610 (1,240-2,170)	1,160 (750-1,530)	0.02	1,610 (1,100-2,330)	1,200 (850-1,710)	0.01	1,060 (910-2,280)	1,290 (860-1,810)	0.80	0.01
PINP (µg/l)	572 (309-791)	289 (126-576)	0.01	430 (229-1,033)	342 (136-591)	0.04	254 (149-751)	335 (176-586)	0.43	0.04

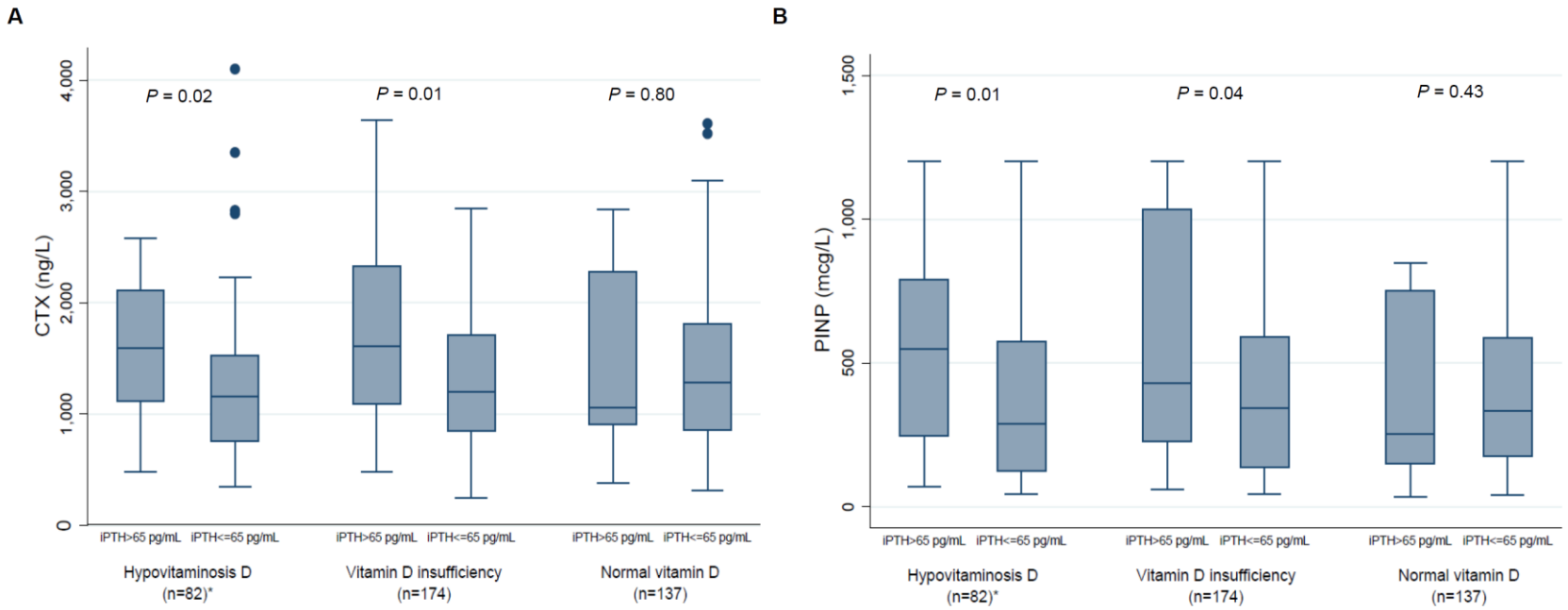
Table 3. Laboratory results related to bone metabolism and bone mineral density among perinatally HIV-infected adolescents (cont.)

Laboratory tests[a]	25-OHD <20 ng/ml[b]			25-OHD 20-29.9 ng/ml			25-OHD ≥30 ng/ml			Overall *P*[d]
	iPTH >65 pg/ml (*n* = 19)	iPTH ≤65 pg/ml (*n* = 63)	*P*[c]	iPTH >65 pg/ml (*n* = 36)	iPTH ≤65 pg/ml (*n* = 138)	*P*[c]	iPTH >65 pg/ml (*n* = 11)	iPTH ≤65 pg/ml (*n* = 126)	*P*[c]	
Bone mineral density										
BMD Z-score	-1.2 (-2.4 to -0.3)	-0.4 (-1.6 to 0.5)	0.08	-0.3 (-1.2 to 0.7)	-0.8 (-1.6 to 0.2)	0.06	-0.4 (-1.3 to 0.2)	-0.7 (-1.6 to 0.0)	0.50	0.17
BMD Z-score ≤-2	8 (42.1)	8 (12.7)	0.01	3 (8.3)	25 (18.1)	0.16	1 (9.1)	20 (15.9)	0.55	0.03

Abbreviations: 25-OHD, 25-hydroxyvitamin D; BMD, bone mineral density; CTX, C-terminal cross-linked telopeptide of type I collagen (bone resorption marker); iPTH, intact parathyroid hormone; PINP, procollagen type I amino-terminal propeptide (bone formation marker).

[a]Data were presented as *n* (%) for categorical data and median (IQR) for continuous data.
[b]One adolescents missed iPTH result.
[c]Chi-square and Wilcoxson rank sum tests were used to compare categorical and continuous data, respectively.
[d]Chi-square and Kruskal-Wallis tests were used to compare categorical and continuous data, respectively.

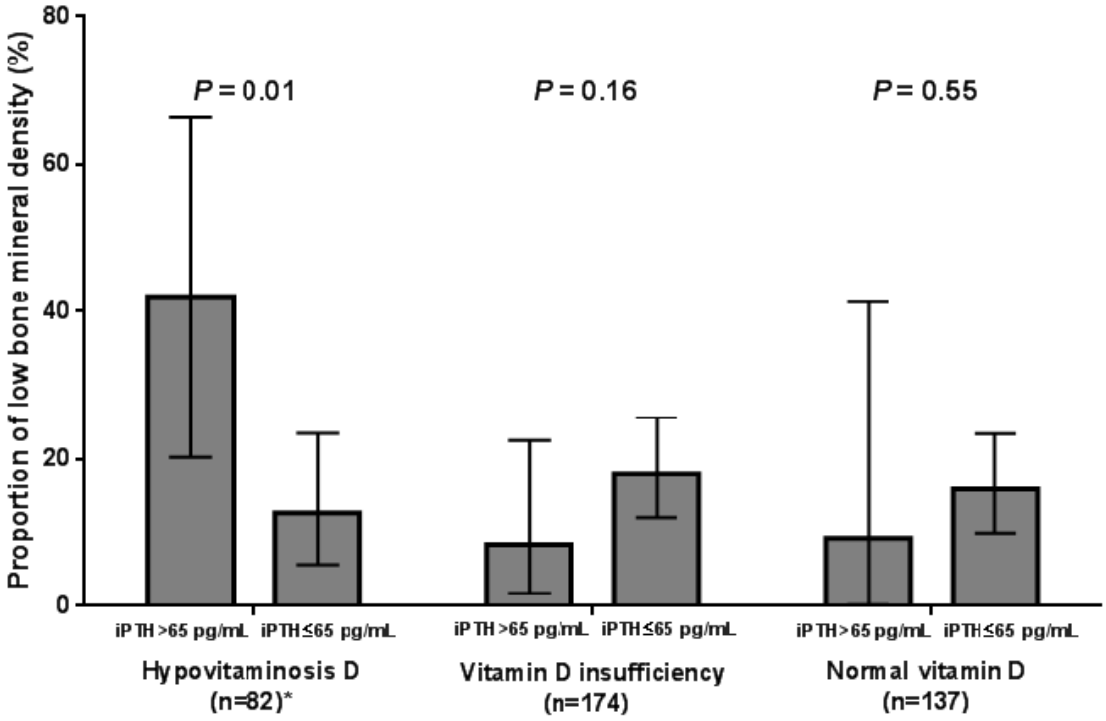
Figure 1. Comparison of bone turnover markers among perinatally HIV-infected adolescents by vitamin D and parathyroid hormone status.



Note: (A) represents the comparison of C-terminal cross-linked telopeptide of type I collagen (CTX; bone resorption marker) of perinatally HIV-infected adolescents according to their vitamin D and parathyroid hormone statuses; and (B) represents the comparison of procollagen type I amino-terminal propeptide (PINP; bone formation marker) of perinatally HIV-infected adolescents according to their vitamin D and parathyroid hormone statuses.

*One adolescent missed iPTH result.

Figure 2. Comparison of the proportion of low bone mineral density among perinatally HIV-infected adolescents by vitamin D and parathyroid hormone status



Note: Low bone mineral density was defined as lumbar spine BMD Z-score -2 or less.

*One adolescents missed iPTH result.

CHAPTER V

CONCLUSION

Summary of findings

Adverse bone health is an important non-communicable disease (NCD) among perinatally HIV-infected adolescents receiving antiretroviral treatment (ART) [1,2]. Since adolescence is a time of the maximum rapid growth and bone mineral accrual [3,4], the impairment of bone mineral acquisition and mineralization during this critical period may lead to several serious consequences, particularly osteoporosis and bone fracture, later in life [5-7]. In the setting of HIV infection, the pathogenesis of bone mass loss seems to be much more complicated than what has been demonstrated in general populations. Hypovitaminosis D complicated with secondary hyperparathyroidism can aggravate bone demineralization by causing the dysregulation of bone turnover [8]. Moreover, residual chronic immune activation and systemic inflammation due to HIV itself may also hasten bone turnover which subsequently results in the loss of bone mass [9]. As a consequence, all of these traditional risk factors along with HIV-related factors can accelerate the rate of bone demineralization and bone mass deterioration in HIV-infected individuals compared with their healthy peers [10]. During the past few years, adverse bone health problems among people living with HIV have been extensively investigated, but there is a dearth of studies specifically focusing on perinatally HIV-infected adolescents living in resource-constrained settings. Therefore, the main purpose of this dissertation stemmed from the need to assess information regarding the extent of adverse bone health and its pathogenesis, together with the magnitude and severity of hypovitaminosis D and its pathological impacts on bone mass among perinatally HIV-infected adolescents living in low resource countries who are at high risk for these comorbidities.

Chapter 3 assessed the prevalence of low bone mass and its relationship with abnormal bone turnover among perinatally HIV-infected adolescents with virologic suppression on ART. Using dual-energy X-ray absorptiometry (DXA) scan to evaluate bone mass, we noted that the prevalence of low bone mass [lumbar spine bone mineral density (BMD) Z-score $\leq$-2] among adolescents in our cohort was approximately 16%. Since about one-fourth of our adolescents were short-for-age (height-for-age Z-score <-1.5), we adjusted their BMD results by calculating bone mineral apparent density (BMAD) to eliminate the effects of height and skeletal size, and found that the prevalence of low bone mass (BMAD Z-score $\leq$-2) declined to approximately 8%. Additionally, we demonstrated that bone mass of our adolescents was much lower than those of their age- and sex-matched healthy peers.

Furthermore, in our analysis, the traditional factors that included advanced age, female sex and low body mass index (BMI), together with HIV-specific factors which included boosted protease inhibitor (PI) exposure and low $CD4^+$ prior to ART initiation showed a significant association with decreased bone mass in our adolescents. We also found that the dysregulation of bone turnover, reflected in the increased in bone resorption and formation markers, were independently associated with bone mass reduction. Although the results did not attain statistical significance, residual chronic immune activation and inflammation by HIV infection, demonstrated by the elevation in pro-inflammatory cytokines, showed a trend of positive correlation with the increase in bone turnover among our population.

In summary, our findings highlighted that adverse bone health is not uncommon in perinatally HIV-infected adolescents receiving ART. Therefore, bone mineral status of

these individuals should be routinely monitored, especially for those at high risk of bone demineralization. In addition, the appropriate measures to augment bone mineral accretion should be encouraged among this population to prevent the future unfavorable and irreversible consequences.

In Chapter 4, we explored the prevalence of hypovitaminosis D and hyperparathyroidism, as well as their pathological effects on bone turnover and bone mass among perinatally HIV-infected adolescents with virologic suppression on ART. Although our study settings are located in a tropical climate zone where sunshine is considered to be adequate all year long, we demonstrated that approximately 21% and 44% of our adolescents had hypovitaminosis D [25-hydroxyvitamin D (25-OHD) <20 ng/ml] and vitamin D insufficiency (VDI; 25-OHD = 20-29.9 ng/ml), respectively. In addition, the prevalence of hypovitaminosis D did not vary by season or study setting. Of note, about 23% of adolescents with hypovitaminosis D and 21% of those with VDI developed secondary hyperparathyroidism [intact parathyroid hormone (iPTH) >65 pg/ml].

In the analysis, female sex, high BMI and elevated iPTH demonstrated negative associations, while boosted PI regimens showed a positive association with vitamin D levels. Interestingly, we found that adolescents with hypovitaminosis D and secondary hyperparathyroidism had the highest median bone resorption and bone formation markers, as well as the greatest proportion of low bone mass (lumbar spine BMD Z-score $\leq$-2) compared to the rest of the cohort. Furthermore, we noted dose-response relationships of vitamin D and parathyroid hormone (PTH) status on bone turnover and BMD in this study. Among adolescents with hypovitaminosis D, individuals with

secondary hyperparathyroidism had higher bone turnover (both bone resorption and bone formation markers), and a greater proportion of low bone mass compared to those without hyperparathyroidism. Additionally, among adolescents with VDI, those with secondary hyperparathyroidism had greater bone turnover, but did not demonstrate a higher proportion of low bone mass compared to individuals with normal PTH status. For adolescents with normal vitamin D status (25-OHD $\geq$30 ng/ml), the levels of bone turnover markers and the proportion of low bone mass were not different between those with and without hyperparathyroidism.

In conclusion, we demonstrated that hypovitaminosis D was prevalent among perinatally HIV-infected adolescents, even living in tropical region. The deficiency of vitamin D complicated with secondary hyperparathyroidism showed the adverse effects on bone turnover and bone mass among these individuals. Thus, the regular monitoring of vitamin D and PTH status, especially for those at high risk for hypovitaminosis D, may be necessary. Additionally, vitamin D supplements for those with deficiencies, before secondary hyperparathyroidism develops, may be important to prevent bone demineralization and bone loss.

Lesson learned

From this dissertation, it is apparent that low bone mass is one of the important NCDs among HIV-infected adolescents with perinatally acquired HIV infection. We found a marked reduction of bone mass in our adolescents in comparison to healthy individuals at the same age and sex. Because, in general, bone mass deterioration is considered as an aging-associated condition that commonly causes problem in elderly

populations, most pediatricians and healthcare providers are usually not concerned with this condition in children and adolescents under their care. However, in an HIV-infected population, particularly in perinatally HIV-infected adolescents who have experienced HIV infection since birth and have been exposed to ART for a long duration, these individuals may face the earlier occurrence of age-related co-morbidities compared to their HIV-uninfected peers. Therefore, the information derived from this dissertation would help raise awareness of pediatricians and healthcare providers to evaluate and monitor bone health status among their adolescents in clinical practice that would allow them to provide earlier diagnosis and promptly treatment if the problem occurs. However, because of a cross-sectional study design of this dissertation, the longitudinal trajectory of bone mass in this population cannot be assessed. A prospective cohort study may be an appropriate study design to address this research question.

With the limitation of DXA measurement, which is currently considered a standard tool of bone mass measurement in children and adolescents, regarding the issues of height and skeletal size, the 2013 International Society for Clinical Densitometry recommends that BMD measurements in individuals with short stature or growth delay should be adjusted by using BMAD [11]. In this dissertation, we calculated BMAD and noticed that the prevalence of low bone mass was declined from 16% (using the definition of BMD Z-score $\leq$-2) to 8% (using the definition of BMAD Z-score $\leq$-2) which was comparable to those reported in resource-rich countries [12,13]. This information suggests that HIV-infected children and adolescents who are usually short-for age, the use BMD results to determine low bone mass may underestimate their actual (volumetric) BMD and overestimate the prevalence of low bone mass. Hence, the

prevalence of low bone mass should be evaluated based on BMAD rather than BMD to reflect the actual extent of the problem in HIV-infected population. In addition, the measurement of true volumetric BMD using quantitative computed tomography (qCT) or quantitative magnetic resonance imaging (qMRI) may be better approaches evaluating bone health status in this population [14-16].

It is interesting that while Thailand and Indonesia are sunny countries, nearly three-fourths of our HIV-infected adolescents had vitamin D levels lower than the normal range (25-OHD <30 ng/ml). Changes in lifestyle, e.g., avoiding direct sunlight exposure, using sunscreen and reducing outdoor activities, lack of food fortification with vitamin D, and high levels of air pollutions that influence the amount of ultraviolet B radiation may be potential factors that increase the risk of vitamin D deficiency among our adolescents. Thus, the monitoring of vitamin D status, using 25-OHD measurements, may be necessary to be implemented in clinical practice for this population, especially for those at high risk for hypovitaminosis D.

Additionally, with the information gained from this dissertation, PTH plays a significant role in determining bone mass status. We noted that among our HIV-infected adolescents with hypovitaminosis D, those with secondary hyperparathyroidism had higher bone turnover markers and greater prevalence of low bone mass in comparison to those without this condition. Therefore, monitoring of PTH along with 25-OHD levels may help identify individuals at high risk of reduced bone mass. However, more evidence to confirm the causal relationship of between these conditions is needed.

Public health significance

As the knowledge regarding adverse bone health among adolescents with perinatally acquired HIV infection in resource-constrained countries is scarce, this dissertation provides meaningful scientific information on the extent of low bone mass problem in this population. Additionally, because our data revealed that low bone mass is not uncommon in HIV-infected adolescents, this helps raise awareness and draws clinical attention to pediatricians and healthcare providers who might not be familiar with adverse bone health problems, a long-term non-AIDS-related comorbidity facing adolescents growing up with HIV in the ART era. Furthermore, we also demonstrated significant associated factors and possible related mechanisms for bone demineralization in this population. Understanding the role of these factors and mechanisms may help healthcare providers and policy makers to develop more appropriate strategies to prevent bone loss, as well as the feasible interventions to promote bone health for these individuals in their public health practice.

In addition, this dissertation demonstrated the high prevalence of hypovitaminosis D and VDI in our HIV-infected adolescents. This information highlights that, despite living in tropical countries where there is abundant sunlight all year round, vitamin D inadequacy still exists as a public health problem. Moreover, because hypovitaminosis D complicated with secondary hyperparathyroidism appears to be associated with an increase in bone turnover and the loss of bone mass in our study, routine screening for vitamin D and PTH statuses among these adolescents in public health practice may be beneficial for pediatricians and healthcare providers to promptly diagnose any laboratory abnormalities, identify individuals at high risk for adverse bone health earlier, and

possibly prevent long-term unfavorable skeletal outcomes. Nevertheless, additional cost-effectiveness analyses are required to confirm the benefits of this action plan before widespread implementation.

Lastly, this dissertation may serve as a preliminary study to efficiently move toward the more comprehensive researches to confirm the causal relationships, as well as to explore the other concealed factors and mechanisms related to adverse bone health among adolescents living with HIV in the near future.

Future directions

This dissertation emphasizes the importance of adverse bone health problem among adolescents with perinatally acquired HIV infection as they survive into adulthood. However, because of the cross-sectional study design, it is difficult to extrapolate our findings to predict the longitudinal change of bone mass in these individuals over time. Thus, a prospective cohort study is required to determine whether, without any intervention, bone mass of these adolescents will be able to catch up to a normal range at the end of puberty as compared to that observed in their healthy peers, or if it will be worsened and progress to more severe bone disease such as osteoporosis or pathological fracture as they enter their adulthood. Moreover, a study exploring the within-person association between poor bone mass developed during adolescence and later osteoporosis experienced during adulthood is also important to provide healthcare providers and researchers with a better understanding of the trajectory of bone mass change in people living with HIV.

Although this dissertation demonstrated several factors, both traditional and HIV-specific, associated with reduced bone mass, we cannot infer causation from these associations due to the limitation of the study design. Additionally, since the pathogenesis of low bone mass in HIV-infected population is likely more complicated than that documented in general populations, the identification of the direct effects of each factor on bone mass deterioration may be challenging. Therefore, a prospective cohort study is an appropriate study design to provide more detailed information on causative factors of low bone mass among these adolescents.

In this dissertation, we used DXA scan to measure bone mass in our adolescents. However, this DXA technique is based on the two-dimensional x-ray projected area of a three-dimensional structure, and thus DXA-derived BMD (areal BMD) is not a true volumetric measure [11]. This might allow us to underestimate BMD of each individual, and overestimate the extent of low bone mass problem in our population. Therefore, a future research may have to consider the alternative approaches, such as qCT or qMRI, for bone density measurements in order to avoid measurement errors. To date, the evidence on safety and efficacy of these procedures in children and adolescents remain limited. Furthermore, because of the other limitations of the DXA technique, including the requirement of calibration of the machine and well-trained technicians, lack of normative reference data for children and adolescents in low resource settings, cost of procedure and radiation exposure [11,17], the researchers may have to think carefully about surrogate markers which may be beneficial to screen for bone mass deterioration among this population. In addition, a study on the diagnostic accuracy of such markers is needed to evaluate their ability and performance compared with DXA scan which is

currently a standard procedure for measure bone mass in children and adolescents worldwide [11].

The results of this dissertation demonstrated the possible detrimental effects of hypovitaminosis D and secondary hyperparathyroidism on bone turnover and bone mass, suggesting that the routine monitoring for vitamin D and PTH statuses in perinatally HIV-infected adolescents, especially in those who are at high risk for vitamin D deficiency, may be necessary. Future research on cost-effective analyses may be required to assure the utility and worthiness of these tests before implementation. Moreover, with the limitation of the study design of this dissertation, we cannot draw any firm conclusions on the benefits of vitamin D supplementation on bone health status of these individuals. The experimental study, particularly a randomized clinical trial, is the best approach to address this important research question as to whether vitamin D supplements is a potential preventive measure for bone demineralization in adolescents with hypovitaminosis D or VDI. The results obtained from this study will provide very meaningful scientific evidence to healthcare providers and public health practitioners, and may eventually have profound impact on the public health practice and policy.

Final conclusions

Adverse bone health is an important NCD among HIV-infected adolescents with perinatally acquired HIV infection. Bone mass of these individuals is marked lower compared to their healthy peers. Apart from traditional risk factors, HIV-specific factors, dysregulated bone turnover and residual chronic immune activation and inflammation due to HIV infection may aggravate bone mass deterioration among these individuals.

The routine monitoring of bone health status for individuals at high risk of low bone mass should be encouraged. The long-term follow up on the sequelae of poor bone mass, particularly osteoporosis and bone fracture, in their later decades of life are needed.

Hypovitaminosis D is common in perinatally HIV-infected adolescents living in tropical countries. Several factors, both traditional and HIV-related factors, affect vitamin D status. The deficiency of vitamin D along with secondary hyperparathyroidism appears to be associated with abnormal bone turnover and bone demineralization in these individuals. The screening for vitamin D and PTH statuses may be helpful to identify individuals at high risk of having adverse bone health problem.

In summary, with the changes of HIV epidemic in the ART era, this dissertation underscores an important non-AIDS-related comorbidity, adverse bone health, which is a new challenge facing adolescents living with HIV as they survive into adulthood. All information derived from this dissertation provide necessary scientific evidence which to help filling the gaps in current medical knowledge, raising the awareness among pediatricians and healthcare providers, allowing for a novel and well-designed study characterizing the problem, and, ultimately, tailored interventions to prevent the long-term consequences of this comorbidity.

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10. Puthanakit T, Saksawad R, Bunupuradah T, et al. Prevalence and risk factors of low bone mineral density among perinatally HIV-infected Thai adolescents receiving antiretroviral therapy. *J Acquir Immune Defic Syndr* 2012;**61**:477-83.

11. Crabtree NJ, Arabi A, Bachrach LK, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. *J Clin Densitom* 2014;**17**:225-42.

12. Dimeglio LA, Wang J, Siberry GK, et al. Bone mineral density in children and adolescents with HIV infection. *AIDS* 2013;**27**:211-20.

13. Bunders MJ, Frinking O, Scherpbier HJ, et al. Bone mineral density increases in HIV-infected children treated with long-term combination antiretroviral therapy. *Clin Infect Dis* 2013;**56**:583-6.

14. Donnelly E. Methods for assessing bone quality: a review. *Clin Orthop Relat Res* 2011;**469**:2128-38.

15. Adams JE. Quantitative computed tomography. *Eur J Radiol* 2009;**71**:415-24.

16. Adams JE, Engelke K, Zemel BS, Ward KA; International Society of Clinical Densitometry. Quantitative computer tomography in children and adolescents: the 2013 ISCD Pediatric Official Positions. *J Clin Densitom* 2014;**17**:258-74.

17. Wildman SS, Henwood-Finley MJ. Pediatric DXA: a review of proper technique and correct interpretation. *J Am Osteopath Coll Radiol* 2012;**1**:17-26.

CURRICULUM VITAE

TAVITIYA SUDJARITRUK, M.D., Sc.M.

Born February 15, 1981
Chiang Mai, Thailand

PERSONAL DATA

Home Address

330/1 Changklan Road
Tumbon Changklan
Amphur Muang
Chiang Mai 50100 THAILAND

Business Address

Department of Pediatrics, Faculty of Medicine and
Research Institute for Health Sciences
Chiang Mai University
110 Intavaroros Road
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EDUCATION AND TRAINING

2016	Doctor of Philosophy (Ph.D.) candidate in Epidemiology Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States
2013	Master of Science (Sc.M.) in Epidemiology Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States
2004	Doctor of Medicine (M.D.), First class honor Chiang Mai University, Chiang Mai, Thailand

Medical Licensure

2004	Unrestricted Medical License No. 30493 (The Medical Council of Thailand)

Medical Board and Other Certification

2005	Chiang Mai University, Chiang Mai, Thailand (Certification of Internship)
2008	Chiang Mai University, Chiang Mai, Thailand (Thai Board of Pediatrics)
2010	Chiang Mai University, Chiang Mai, Thailand (Thai Board of Pediatrics Infectious Disease)
2012	Johns Hopkins Bloomberg School of Public Health (Certification of Vaccine Clinical Trial and Good Clinical Practice)
2013	Johns Hopkins Bloomberg School of Public Health (Certification of Vaccine Science and Policy)
2014	Johns Hopkins Bloomberg School of Public Health (Certification of Healthcare Epidemiology and Infection Prevention and Control)

PROFESSIONAL EXPERIENCE

2010-present	Instructor, Division of Infectious Disease, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Description of job: Teaching of fourth-, fifth-, and sixth-year medical students about common pediatric infectious diseases; teaching of residents who are training in Pediatrics, including: bedside teaching about infectious diseases in the ward, outpatient clinic, and intensive care unit; teaching of fellows who are training in Pediatric Infectious Diseases, including: bed side teaching about infectious diseases in the ward, outpatient clinic, and intensive care unit; service rounds in infectious disease ward; service at special clinic for HIV infected children; Service at special clinic for TB infected children

2010-present	Clinical Researcher, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand

Description of job: Conducting clinical research studies focusing on HIV infection and its complications in children and adolescents

PROFESSIONAL ACTIVITIES

Society Membership

2004-present	Member of the Medical Council of Thailand
2008-present	Member of the Royal College of Pediatricians of Thailand
2009-present	Member of the Pediatric Infectious Diseases Society of Thailand

HONORS AND AWARDS

2004	First Class Honor, Doctor of Medicine, Chiang Mai University, Chiang Mai, Thailand
2007	Fogarty International Clinical Scholarship, National Institute of Health, Bethesda, Maryland, United States
2010	Young Investigator Awards, 17th Conference of Retrovirus and Opportunistic Infections, San Francisco, United States
2010	Pediatric Infectious Disease Society of Thailand (PIDST) Paper Presentation Award (First), The Pediatric Infectious Diseases Society of Thailand
2011	Ananda Mahidol Scholarship (King's Scholarship)
2012	Master tuition Scholarship, Johns Hopkins Bloomberg School of Public Health
2014	Collaborative Initiative for Paediatric HIV Education and Research (CIPHER) Grant Award, International AIDS Society (IAS), Melbourne, Australia
2014	The Best Researcher Award, Faculty of Medicine, Chiang Mai University
2014	HIV Drug Therapy Glasgow Scholarship Award
2015	IAS International Scholarship, Vancouver, Canada, July 2015
2015	Collaborative Initiative for Paediatric HIV Education and Research (CIPHER) Travel Award, International AIDS Society (IAS), Vancouver, Canada, July 2015

2015	The Best Researcher Award, Faculty of Medicine, Chiang Mai University
2016	Young Investigator Scholarship, 23rd Conference of Retrovirus and Opportunistic Infections, Boston, United States

PUBLICATIONS

Journal Articles

1. Seewijee T, **Sudjaritruk T**, Oberdorfer P. Atypical pneumonia: diagnosis and treatment. *Thai J Pediatr* 2009;**48**:193-9.

2. **Sudjaritruk T**, Udompornwattana S, Gromrat P, Oberdorfer P. Relapse neonatal HSV infection: 1 case report. *Thai J Pediatr* 2009;**48**:325-31.

3. Makonkawkeyoon K, **Sudjaritruk T,** Sirisanthana V, Silvilairat S. Fulminant enterovirus 71 infection: case report. *Ann Trop Pediatr* 2010:**30**:245-8. PMID: 20828460.

4. **Sudjaritruk T**, Sirisanthana T, Sirisanthana V. Antibody responses to hepatitis A virus vaccination in Thai HIV-infected children with immune recovery after antiretroviral therapy. *Pediatr Inf Dis J* 2011;**30**:256-9. PMID: 20856163.

5. **Sudjaritruk T**, Sirisanthana V. Successful treatment of a child with vascular pythiosis**.** *BMC Infect Dis* 2011;**11**:33. PMID: 21276255.

6. Phongsamart W, Sirisanthana V, Wittawatmongkol O, Maleesatharn A, **Sudjaritruk T**, Chearskul P, Aurpibul L, Sirisanthana T, Chokephaibulkit K. Immunogenicity and safety of monovalent influenza A (H1N1) 2009 in HIV-infected Thai children. *Vaccine* 2011;**29**:8705-11. PMID: 21893147.

7. **Sudjaritruk T**, Oberdorfer P. Clinical characteristics and outcomes of Dengue-infected children admitted to the Chiang Mai University Hospital during an outbreak in 2008. *Chiang Mai Med J* 2011;**50**:95-104.

8. **Sudjaritruk T**, Sirisanthana T, Sirisanthana V. Immune reconstitution inflammatory syndrome from Penicillium marneffei in an HIV-infected child: A case report and review of literature. *BMC Infect Dis* 2012;**12**:28. PMID: 22289885.

9. **Sudjaritruk T**, Oberdorfer P, Puthanakit T, Sirisanthana T, Sirisanthana V. Causes of hospitalization among 1121 HIV-infected children: comparison of the pre-*Pneumocystis jiroveci* pneumonia prophylaxis, pre-antiretroviral therapy, and antiretroviral therapy period. *Int J STD AIDS* 2012;**23**:335-9. PMID: 22648887.

10. Cressey TR, Aurpibul L, Narkbunnam T, Sirisanthana V, Phongsamart W, Oberdorfer P, Lapphra K, **Sudjaritruk T**, Chokephaibulkit K. Pharmacological assessment of efavirenz weight-band dosing recommendations in HIV-infected Thai children. *J Acquir Immune Defic Syndr* 2013;**62**:e27-9. PMID: 23262981.

11. Bunupuradah T, Puthanakit T, Fahey P, Kariminia A, Yusoff NK, Khanh TH, Sohn AH, Chokephaibulkit K, Lumbiganon P, Hansudewechakul R, Razali K, Kurniati N, Huy BV, **Sudjaritruk T**, Kumarasamy N, Fong SM, Saphonn V, Ananworanich J; TApHOD. Second-line protease inhibitor-based HAART after failing non-nucleoside reverse transcriptase inhibitor-based regimens in Asian HIV-infected children. *Antivir Ther* 2013;**18**:591-8. PMID: 23296119.

12. **Sudjaritruk T**, Maleesatharn A, Prasitseubsai W, et al. Prevalence, characteristics, management and outcome of pulmonary tuberculosis in HIV-infected children in the TREAT Asia Pediatric HIV Observational Database (TApHOD). *AIDS Patient Care STDS* 2013;**27**:649-56. PMID: 24206012.

13. Aurpibul L, Cressey TR, Sricharoenchai S, Wittawatmongkol O, Sirisanthana V, Phongsamart W, **Sudjaritruk T**, Chokephaibulkit K. Efficacy, Safety and pharmacokinetics of tenofovir disoproxil fumarate in virologic-suppressed HIV-infected children using weight band dosing. *Pediatr Inf Dis J* 2015;**34**:392-7. PMID: 25760566.

14. Aurpibul L, Puthanakit T, **Sudjaritruk T**, et al. The 10-year effectiveness of combination antiretroviral treatment in perinatally HIV-infected children participating in Thailand's National Access Program. *Antivir Ther* 2015 Jul 9. [Epub ahead of print] PMID: 26158783.

15. **Sudjaritruk T**, Puthanakit T. Adverse bone health among children and adolescents growing up with HIV. *J Virus Erad* 2015;**1**:159-67.

16. Prasitsuebsai W, Kerr SJ, Truong KH, Ananworanich J, Do VC, Nguyen LV, Kurniati N, Kosalaraksa P, **Sudjaritruk T**, et al. Using lopinavir concentrations in hair samples to assess treatment outcomes on second-line regimens among Asian children. *AIDS Res Hum Retroviruses* 2015;**31**:1009-14. PMID: 26200586.

17. Aurpibul L, Sricharoenchai S, Wittawatmongkol O, Sirisanthana V, Phongsamart W, **Sudjaritruk T**, Chokephaibulkit K. Vitamin D status in perinatally HIV-infected Thai children receiving antiretroviral therapy. *J Pediatr Endocrinol Metab* 2015 Nov 13. [Epub ahead of print] PMID: 26565540.

18. Boettiger DC, **Sudjaritruk T**, Nallusamy R, et al. Non-Nucleoside Reverse Transcriptase Inhibitor-Based Antiretroviral Therapy in Perinatally HIV-Infected, Treatment-Naïve Adolescents in Asia. *J Adolesc Health* 2016;**58**:451-9. PMID: 26803201.

19. **Sudjaritruk T**, Bunupuradah T, Aurpibul L, et al. Hypovitaminosis D and hyperparathyroidism: effects on bone turnover and bone mineral density among perinatally HIV-infected adolescents. *AIDS* 2016;**30**:1059-67. PMID: 26807972.

20. Prasitsuebsai W, Teeraananchai S, Singtoroj T, Truong KH, Ananworanich J, Do VC, Nguyen LV, Kosalaraksa P, Kurniati N, **Sudjaritruk T**, et al. Treatment outcomes and resistance patterns of children and adolescents on second-line antiretroviral therapy in Asia. The manuscript has been accepted for publication in *J Acquir Immune Defic Syndr* on February 9, 2016.

Chapters

1. **Sudjaritruk T**, Aurpibul L, Sirisanthana V. Guideline for vaccination in Thai HIV-infected children. In: Kerdpanich A, Lohlekha R, Watanaveeradej V, Chotpitayasunon T, eds. Update on Pediatric Infectious Diseases 2009. Bangkok, 2009:52-68.

2. **Sudjaritruk T**, Oberdorfer P. What's new in immunization for children and adolescents. In: Chartapisak W, Tanpaibul P, Dejkamron P, Unachak K, eds. What's in, what's out in Pediatrics. Chiang Mai, 2009:185-198.

3. **Sudjaritruk T**, Oberdorfer P. Treatment and care of HIV-infected adolescents . In: Kerdpanich P, Watanaveeradej V, Chotpitayasunon T, eds. Update on Pediatric Infectious Diseases 2011. Bangkok, 2011:212-228.

4. **Sudjaritruk T.** Current issues in adolescent immunization. In: Ukarapol N, Louthrenoo O, Chareonkwan P, Chartapisak W, eds. New era in Global Child Health. Chiang Mai, 2011;47-60.

5. **Sudjaritruk T.** Sexually transmitted infections in children and adolescents. In: Ukarapol N, Louthrenoo O, Chareonkwan P, Chartapisak W, eds. New era in Global Child Health. Chiang Mai, 2011:61-74.

6. **Sudjaritruk T.** Optimizing antimicrobial therapy in sepsis. In: Chareonkwan P, Silvilairat S, Natesirinilkul R, Louthrenoo O, Opastirakul S, eds. Pediatric Emergencies. Chiang Mai, 2013:191-201.

7. **Sudjaritruk T.** Updated practice guidelines for the management of HIV-infected children and adolescents. In: Chareonkwan P, Silvilairat S, Louthrenoo O, Kittisakmontri K, eds. Current practices and trend in pediatrics. Chiang Mai, 2015:21-36.

8. **Sudjaritruk T.** Updated practice guidelines on prevention of mother-to-child transmission (PMTCT). In: Chareonkwan P, Silvilairat S, Louthrenoo O, Kittisakmontri K, eds. Current practices and trend in pediatrics. Chiang Mai, 2015:21-51.

9. Oberdorfer P, **Sudjaritruk T**, Issaranggoon na ayuthaya S. Antimicrobial agents. In: Chareonkwan P, Silvilairat S, Louthrenoo O, eds. Pediatric dosage handbook. Chiang Mai, 2015: 51-122.

TEACHING

Advisees

Fifth Year Medical Students
2008-2011

Pediatric Resident
2009-2011 (Graduated)

Classroom Instruction
Faculty of Medicine, Chiang Mai University

2008-2010	Fever with rash (319.491), Instructor, 250-280 fourth-year medical students
2008-2011	Dengue (331.504), Instructor, 250-280 fifth-year medical students
2008-2011	Streptococcal Infections (331.504), Instructor, 250-280 fifth-year medical students
2011	Immunization for Children and Adolescents (331.301), Instructor, 250-280 third-year medical students
2011	Basic Knowledge in Epidemiology, Instructor, 30 pediatric residents

Other Significant Teaching
Johns Hopkins Bloomberg School of Public Health

2013	Principle of Epidemiology (340.601), Teaching Assistant, 60-70 graduate students (lab section)

RESEARCH GRANT PARTICIPATION

Active

National Research University, Chiang Mai University **March 2015 to February 2017**

Dr. Tavitiya Sudjaritruk, P.I.
Effect of calcium and high-dose vitamin D supplementation on bone mineral density among perinatally HIV-infected children and adolescents

CIPHER Grant Programme, IAS **August 2014 to July 2016**

Dr. Tavitiya Sudjaritruk, P.I.
HIVNAT 203: Nonalcoholic fatty liver disease and long-term metabolic complications among perinatal HIV-infected children and adolescents receiving antiretroviral therapy.

NIH/NIAID under The International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) **September 2009 to January 2016**

Professor Sirisanthana, P.I. (Dr. Sudjaritruk, Investigator)
IMPAACT 1077HS: HAART Standard Version of the PROMISE Study (Promoting Maternal and Infant Survival Everywhere).

NIH/NIAID under The International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) **February 2011 to January 2017**

Professor Sirisanthana, P.I. (Dr. Sudjaritruk, Investigator)
IMPAACT P1093: Phase I/II, Multi-Center, Open-Label Pharmacokinetic, Safety, Tolerability and Antiviral Activity of GSK1349572, a Novel Integrase Inhibitor, in Combination Regimens in HIV-1 Infected Infants, Children and Adolescents.

NIH/NIAID under The International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) **September 2011 to August 2018**

Professor Sirisanthana, P.I. (Dr. Sudjaritruk, Investigator)
A5279: Phase III Clinical Trial of Ultra-Short-Course Rifapentine/Isoniazid for the Prevention of Active Tuberculosis in HIV-Infected Individuals with Latent Tuberculosis Infection.

ACADEMIC SERVICE

2008-present Division of Infectious Diseases, Department of Pediatrics
Faculty of Medicine, Chiang Mai University
Chiang Mai, Thailand

PRESENTATIONS

Scientific Meetings

2009 Successful treatment of vascular pythiosis: A case report (Oral Presentation), 67th Annual Pediatrics meeting, Chonburi, Thailand.

2009 Causes of hospitalization in 880 HIV-infected children: effect of Thailand's national antiretroviral access program (Poster), The 1st International Workshop on HIV Pediatrics, Cape Town, South Africa.

2010 Causes of hospitalization in 1,112 HIV-infected children: comparison of the pre-*Pneumocystis jiroveci* pneumonia (PCP) prophylaxis, pre-antiretroviral therapy, and antiretroviral therapy (ART) era (Poster), The 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, United States of America.

2010 Antibody Responses to Hepatitis A Virus Vaccination in Thai HIV-infected Children with Immune Recovery after Antiretroviral Therapy (Oral Presentation), The 14th Annual meeting of Pediatric Infectious Disease Society of Thailand, Chonburi, Thailand.

2010 Antibody Responses to Hepatitis A Virus Vaccination in Thai HIV-infected Children with Immune Recovery after Antiretroviral Therapy (Poster), The 2nd International Workshop on HIV Pediatrics, Vienna, Austria.

2010 Antibody Responses to Hepatitis A Virus Vaccination in Thai HIV-infected Children with Immune Recovery after Antiretroviral Therapy (Poster), XVIII International AIDS Conference, Vienna.

2011 Five-Year Surveillance of antimicrobial-resistant pathogens among pediatric patients admitted to Chiang Mai University Hospital, Thailand (Poster), 8th International Symposium on Antimicrobial Agents and Resistance, Seoul, Korea.

2011 Prevalence of antimicrobial resistance among gram-negative isolates in a pediatric intensive care unit at Chiang Mai University (Poster), 8th International Symposium on Antimicrobial Agents and Resistance, Seoul, Korea.

2011 Second-line highly active antiretroviral therapy in Asian HIV-infected children (Poster), 3rd International Workshop on HIV Pediatrics, Rome, Italy.

2012 24-week safety of tenofovir when administered according to weight band dosing in HIV-infected children ≥ 15 kg as part of once-daily HAART regimen (Poster). ID week 2012, San Diego, United States of America.

2013 48-week Safety of Tenofovir When Administered According to Weight Band Dosing in HIV-infected Children >/= 15 Kg as Part of a Once-daily HAART Regimen (Poster). The 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, United States of America.

2014 The 10-year Effectiveness of Highly Active Antiretroviral Treatment in Perinatally HIV-infected Children Participating in Thailand´s National Access Program (Poster). The 6th International Workshop on HIV Pediatrics, Melbourne, Australia.

2014 The 10-year Effectiveness of Highly Active Antiretroviral Treatment in Perinatally HIV-infected Children Participating in Thailand´s National Access Program (Poster). The 20th International AIDS Conference, Melbourne, Australia.

2015 Therapeutic Drug Monitoring of Lopinavir in HIV-infected Children on Second-line ART (Poster). The 22nd Conference on Retroviruses and Opportunistic Infections, Seattle, United States of America.

2015 Prevalence and Associated Factors of Nonalcoholic Fatty Liver Disease and Liver Fibrosis among Perinatally HIV-infected Asian Adolescents (Oral Presentation). The 7th International Workshop on HIV Pediatrics, Vancouver, Canada.

2015 Adverse Bone Health among Perinatally HIV-infected Asian Adolescents with Virological Suppression (Poster). The 7th International Workshop on HIV Pediatrics, Vancouver, Canada.

2015 Higher 25-hydroxyvitamin D levels in perinatally HIV-infected Asian adolescents receiving boosted protease inhibitors (Poster). The 7th International Workshop on HIV Pediatrics, Vancouver, Canada.

2015 Dysregulated Bone Remodeling but Stable Bone Mineral Density over 4-year Tenofovir Disoproxil Fumarate Exposure among Perinatally HIV-infected Asian Adolescents (Poster). The 7th International Workshop on HIV Pediatrics, Vancouver, Canada.

2015 Prevalence of nonalcoholic fatty liver disease and liver fibrosis among perinatally HIV-infected Asian adolescents with history of transaminitis (Poster Discussion). The 8th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Vancouver, Canada.

2016 Incidence of virologic rebound in perinatally HIV-infected adolescents on stable cART (Poster). The 23rd Conference on Retroviruses and Opportunistic Infections, Boston, United States of America.

2016 Seroprevalence of Hepatitis B Among HIV-infected Children and Adolescents in Asia (Poster). The 23rd Conference on Retroviruses and Opportunistic Infections, Boston, United States of America.

Invited Seminars

2009 What's in, What's out in Pediatrics: What's new in immunization for children and adolescents. Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

2009 Grand round: Why vaccinate? Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

2010 One day pediatrics conference: What's new in the antimicrobial horizon? Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

2010 Speaker in Radio program (FM 100; Chiang Mai University): Dengue hemorrhagic fever

2010 Speaker in Radio program (FM 100; Chiang Mai University): Hand-Foot and Mouth disease

2010 Speaker in Television program (Channel 11): Dengue hemorrhagic fever: A dangerous disease

2011 Antibiotics Smart Use: Antibiotics Smart Use: in pediatrics clinical practice. Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

2011 Current issues in adolescent immunization. Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

2011 Empirical carbapenems in severe pediatric infectious diseases: pro and con. Cha-Am, Petchburi, Thailand

2011 Speaker in Radio program (FM 100; Chiang Mai University): *E. coli* outbreak

2011 *E. coli* outbreak. Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

2011 Speaker in Television program (Channel 11): Dengue hemorrhagic fever: A dangerous disease and prevention

2014 Resurgence of measles outbreaks and challenges to disease eradication. Children's hospital Los Angeles, Los Angeles, United States of America

2015 Adverse bone health in HIV-infected adolescents. The 17th Bangkok International *S*ymposium on HIV Medicine 2015, Bangkok, Thailand

2015 Current practices and trends in paediatrics: Pediatric HIV infection: treatment update in general setting. Mae Ping Hotel, Chiang Mai, Thailand

2015 Pediatric HIV infection: Update on Thai national treatment guidelines 2014. The HIV-NAT, Thai Red Cross, AIDS Research Center, Bangkok, Thailand

ADDITIONAL INFORMATION

Brief description of research interests

Dr. Tavitiya Sudjaritruk is a pediatrician who specializes in infectious diseases. As a physician-researcher with broad-based training in pediatrics, infectious diseases, epidemiology and public health, she has focused my research areas of interest on HIV/AIDS among pediatric population. She has involved in many clinical research studies in both HIV-infected and HIV-affected children, such as prevention of maternal-to-child transmission, pharmacokinetic profiles of new antiretroviral drugs, treatment failure and second-line therapy, vaccination and re-vaccination in HIV-infected children. Additionally, she has participated in the Therapeutic Research, Education and AIDS Training (TREAT) Asia Pediatric HIV Observational Database (TApHOD) research network since 2008, and currently is the site principle investigator of the Chiang Mai University pediatric site. She has conducted several clinical research studies focusing on the long-term, non-infectious medical complications (i.e., non-hepatitis liver disease, bone demineralization and metabolic syndrome) among perinatally HIV-infected children and adolescents who are growing up with HIV and have experienced long duration of antiretroviral therapy.

Keywords

Pediatrics, Infectious Diseases, HIV infection, AIDS, Epidemiology, Antiretroviral therapy, Prevention maternal to child transmission (PMTCT), Vaccine, Immunization, Revaccination, Long-term complications of antiretroviral treatment.