Walden University
ScholarWorks

Walden Dissertations and Doctoral Studies

Walden Dissertations and Doctoral Studies Collection

2015

Vitamin D Deficiency and Immune Function in African American, HIV-Infected Men

Rana H. Ismail
Walden University

Follow this and additional works at: https://scholarworks.waldenu.edu/dissertations

Part of the Epidemiology Commons, and the Public Health Education and Promotion Commons

This Dissertation is brought to you for free and open access by the Walden Dissertations and Doctoral Studies Collection at ScholarWorks. It has been accepted for inclusion in Walden Dissertations and Doctoral Studies by an authorized administrator of ScholarWorks. For more information, please contact ScholarWorks@waldenu.edu.

Walden University

College of Health Sciences

This is to certify that the doctoral dissertation by

Rana Ismail

has been found to be complete and satisfactory in all respects,
and that any and all revisions required by
the review committee have been made.

Review Committee
Dr. Sriya Krishnamoorthy, Committee Chairperson, Public Health Faculty
Dr. Ji Shen, Committee Member, Public Health Faculty
Dr. James Rohrer, University Reviewer, Public Health Faculty

Chief Academic Officer
Eric Riedel, Ph.D.

Walden University
2015

Abstract

Vitamin D Deficiency and Immune Function in African American, HIV-Infected Men

by

Rana H. Ismail

MSc., American University of Beirut, 1999

BS, American University of Beirut, 1995

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

Walden University

June 2015

Abstract

Vitamin D deficiency is common in individuals diagnosed with HIV and is known for its detrimental health effects. Its recognition as a potent immune-modulator with possible immune health implications in HIV disease progression was the main impetus for this study. The association between Vitamin D and CD4 count falls short of being consistent and is too weak to allow conclusions. Similarly, the literature is inconsistent with regard to the impact of Vitamin D supplementation on CD4. This observational, retrospective chart review study aimed to explore the relationship between Vitamin D deficiency and CD4 count/percent, and to evaluate whether changes in Vitamin D levels after supplementation corresponds with significant changes in CD4 count/percent in a cohort of African American, HIV-infected men who attended an HIV clinic in southeast Michigan ($N = 70$). The conceptual framework was based on the role of Vitamin D in regulating the immune responses through Vitamin D nuclear receptors on the CD4 cells. It postulated that an increase in Vitamin D level might enhance immune function, promote cellular anti-inflammatory state, and decelerate CD4 destruction. Data analysis included descriptive statistics, bivariate correlation, logistic and linear regression, *t* test, repeated measures ANOVA, and ANCOVA. Findings of the study did not support the hypotheses of significant correlation between Vitamin D and CD4 count ($p = 0.458$) and percent ($p = 0.776$), or of any impact of supplementation on CD4 count ($p = 0.216$) and percent ($p = 0.918$). Social change implications include providing health professionals, researchers, and policymakers with knowledge to tailor health promotion interventions aiming to reduce Vitamin D deficiency in favor of improving the overall health of HIV patients, especially high-risk groups such as African American HIV-infected patients.

Vitamin D Deficiency and Immune Function in African American, HIV-Infected Men

by

Rana H. Ismail

MSc., American University of Beirut, 1999

BS, American University of Beirut, 1995

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

Walden University

June 2015

Dedication

This dissertation is dedicated to my wonderful husband, Dr. Hassan Dakroub—the love of my life—whom I shall always be grateful for his unconditional and exceptional support, encouragement, confidence, and love over the years. I could not have done it without you for sure! This is also dedicated to my loving parents, my brothers Jamal and Ali, and my lovely kids, Jana, Jad, and my soon to-be-born – if God willing – baby Joelle. Thank you all for your support and faith in me; this achievement is for you! God bless you all!

Acknowledgments

I would like to thank Allah, the most gracious, merciful, and benevolent, for blessing me with the strength of seeking knowledge that improves the self and helps others. Thank you God for blessing me with more than I deserve. I owe all my success to you, dear God. The completion of this dissertation would not have been possible without the invaluable support, excellent guidance, remarkable positive energy, and great advice of my chairperson, Dr. Sriya Krishnamoorthy. Thank you Dr. K.! Your diligent efforts throughout this process have been enormous, and I will always be grateful. I would also like to extend many thanks to the committee member, Dr. Ji Shen, for his important contributions to this dissertation process and for all his efforts throughout. Many thanks to Dr. James Rohrer, the URR, for his careful revision of the whole dissertation and for his significant feedback that helped improve the quality of this work. Overall, I was extremely fortunate to have worked with all of you, thank you! A special thank you goes to the anonymous participants who suffer from HIV; hope this work makes a humble and tiny contribution to the betterment of your quality of life.

Finally, I would like to thank my family and friends for their tireless encouragement and prayers throughout this journey, and for their endless faith in me; I could not have succeeded without you. A special word of thanks goes to my best friend, and wonderful partner: My amazing husband Hassan; I am more grateful to you for your love and encouragement than you will ever know.

Table of Contents

List of Tables iii

List of Figures iv

Chapter 1: Introduction to the Study 1

Background 1

Overview on Vitamin D 5

Overview on Vitamin D and CD4 Cells in HIV 9

CD4 T-cells and Immune Response 10

Vitamin D as an Immune-Modulator in HIV 12

Overview on the Role of Vitamin D in HIV Disease Progression 14

Statement of the Problem 18

Purpose of the Study 21

Nature of the Study 21

Research Questions 24

Conceptual Background/Foundation 24

Definition of Terms 29

Assumptions 31

Limitations and Delimitations 32

Significance of the Study 33

Implications for Social Change 35

Summary 38

Chapter 2: Literature Review 40

Introduction....40

Search Strategy 40

Vitamin D: Definition and Function....42

Vitamin D Metabolism: 44

Vitamin D deficiency & Its General Health Implications 48

Natural History of HIV 50

Overview on Vitamin D Deficiency and HIV Disease Progression....54

Vitamin D Modulating Effects on Immune Function and in HIV....60

Vitamin D and the Innate Immune Response 63

Vitamin D and the Adaptive Immune Response: 68

Prevalence of Vitamin D Deficiency in HIV....72

Vitamin D deficiency and ART:.... 75

CD4 Cells in HIV Infection: Preamble to Vitamin D & CD4 Relationship....79

CD4 Count and Vitamin D: A Controversial Relationship 84

Vitamin D & CD4 Count: Correlation? No Correlation? 85

Vitamin D Supplementation 92

Conclusion 105

Chapter 3: Research Method....106

Introduction....106

Research Design and Approach....106

Setting and Sample 112

Data Collection and Analysis....120

Consideration for the Rights of Human Subjects 125
Conclusion 126
Chapter 4: Results 127
Introduction 127
Data Collection 127
Descriptive Statistics 128
Research Questions and Hypotheses 136
Summary and Conclusion 149
Chapter 5: Discussion, Conclusions, and Recommendations 150
Overview 150
Interpretations of the Findings 152
Limitations of the Study 162
Recommendations 163
Implications 164
Conclusions 166
References 168

List of Tables

Table 1. HIV/AIDS Statistics, Global And In The U.S. 2

Table 2. CDC Immune Stages According to CD4 Percentage & CD4 Count Groups 112

Table 3. List of Variables Included in The Study and Their Descriptive Statistics 121

Table 4. Baseline Descriptive and Demographic Characteristics of Study Population...129

Table 5. Group Comparison on Baseline Categorical Variables. 131

Table 6. Baseline Vitamin D Level and HIV Immune Function Markers of Study

Population 134

Table 7. Baseline HIV Immune Function Markers by Vitamin D Group 135

Table 8. Baseline Comparsion of Bivariate Correlations Between 25(OH)D

Levels and Immune Function Variables 138

Table 9. Vitamin D Levels Pre- Post - Supplementation Changes: Wilcoxon

Signed-Rank Test..142

Table 10. HIV Immune Parameters Changes: Pre- and Post- Supplementation

(T0-T1)...143

Table 11. HIV Immune Parameters Changes: Pre- and Post- Supplementation

(T0-T2)...144

Table 12. Changes in Vitamin D Levels and Immune Parameters in Supplemented

Group Versus Nonsupplemented Group: Baseline to T1 148

List of Figures

Figure 1. Theoretical framework about the effects of Vitamin D deficiency on overall HIV disease course and the postulated effects of Vitamin D supplementation on CD4. 26

Figure 2. "The history of the discovery of Vitamin D and its daughter steroid hormone." 45

Figure 3. "Vitamin D and musculoskeletal health." 65

Figure 4. "Vitamin D and molecular actions on the immune system: Modulation of innate and autoimmunity." 68

Figure 5. Main immune-modulating effects of bioactive Vitamin D ($1,25(OH)_2D_3$) on immune cells. 72

Figure 6. "Vitamin D and the immune system: New perspectives on an old theme." 73

Figure 7. Change in 25(OH)D level from pre–to post–supplementation in deficient group. 140

Chapter 1: Introduction to the Study

Background

Despite years of invaluable medical advances, and after more than 3 decades since the onset of the HIV/AIDS epidemic, HIV infection rates are still on the rise without indication of slowing down. Epidemiological trends have shown a quadruple increase in the number of people living with HIV from 1990 until the end of 2011, from 8 million to 34 million infected individuals globally (United Nations Programme on HIV/AIDS [UNAIDS], 2012; World Health Organization [WHO], 2011). HIV infection affects the lives of 1.1 million individuals in the United States (Centers for Disease Control and Prevention [CDC], 2012a). By the end of the year 2009, there were about 480,000 individual living with AIDS in the United States (CDC, 2012b). The AIDS death toll globally reached 1.7 million people in 2011 (WHO, 2011). In the United States, AIDS has so far killed more than 600,000 individuals since the beginning of the HIV/AIDS epidemic in the 1980s (CDC, 2012a). Even with unceasing advances in the field of HIV/AIDS testing, prevention, and management still more than 2.5 million individuals worldwide were diagnosed with HIV infection in the year 2011 alone (WHO, 2011). Nevertheless, the health efforts yielded at least a 20% reduction in the number of newly diagnosed HIV cases in 2001 due to the expanding availability and accessibility to treatment with antiretroviral therapy (ART) in many parts of the world (UNAIDS, 2012; see Table 1). Despite the current medical care and disease prevention achievements, the statistics on HIV are still alarming and reveal the need to tackle risk factors that might affect high-risk HIV patients and influence their disease course.

In the United States, the trends in HIV incidence (new infections) over the recent years have been steady at 50,000 cases per year, and the majority of cases are among high-risk subpopulations such as in men who have sex with men (MSM), followed by young African American adults (CDC, 2012a, 2012b). Between 1991 and 2008, the national HIV surveillance data showed that males constituted 75% of the 1.1 million people living with HIV, of which 65.7% were MSM (CDC, 2011a). At the other end, women accounted for 25% of all AIDS cases in the United States in 2011 and for 20% of total HIV incidence in 2010, of which more than 80% were due to heterosexual activity, especially among African American women (with a 20 times higher incidence rate as compared to other racial groups; CDC, 2012c).

Table 1

HIV/AIDS Statistics, Global and in the United States

HIV/AIDS Epidemiological Distribution	**Estimates**
People living with HIV/AIDS globally	34 million (50% women)
People living with HIV/AIDS in the U.S.	1.1 million (20-25% women)
Global AIDS death toll since the epidemic	30 million
Global AIDS death toll in 2011	1.7 million (25% decrease from 2005 rate of 2.3 million deaths)
Death toll from AIDS in the U.S. from 1980s to present	600,000
Global newly diagnosed HIV cases in 2011	2.5 million (20% lower than 2001)
Annual newly diagnosed HIV cases in the U.S. (steady in recent years)	50,000

Note. Data for HIV/AIDS epidemiological trends globally and in the United States from UNAIDS (2012); CDC (2012a); and WHO (2011).

The national trends in HIV/AIDS show a persistent racial disparity in the related mortality and morbidity statistics: More African Americans are affected compared to other racial and ethnic groups (AIDS/HIV Program-Wisconsin Department of Health Services, 2012). African Americans constitute about 44% of all HIV infected people in the United States (CDC, 2012b). Between 2005 and 2008, data from 37 states indicated that African Americans accounted for 50.3% of all the HIV cases diagnosed compared to whites who accounted for 29.4% of all HIV diagnoses in that period (CDC, 2011b).

In view of the high burden of HIV worldwide and nationwide, more researchers in recent years have evaluated the role of different factors that can influence the course of HIV infection, especially those that contribute to HIV disease progression. In this context, Vitamin D has been identified in the literature as one of the highly prevalent risk factors with potential physiologic and metabolic mechanisms capable of influencing the HIV disease process (Viard et al., 2011; Villamor, 2006). Particularly, a deficiency in Vitamin D constitutes a major contributing factor for a series of negative health outcomes (Giusti, Penco, & Pioli, 2011; Holick, 2004, 2006; Holick & Chen, 2008; Villamor, 2006). In these last 2 decades, there has been a great undertaking to examine the role of Vitamin D deficiency in relation to HIV. Major researchers on HIV-infected individuals from the United States (Adeyemi et al., 2011; Crutchley et al., 2012; Dao et al., 2011; Egan et al., 2008; Murphy et al., 2012; Overton & Yin, 2011; Rodriguez, Daniels, Gunawardene, & Robbins, 2009; Tseng et al., 2009), Africa (Mehta et al., 2010, 2011), and Europe (Bang et al., 2010; Mueller et al., 2010; Van Den Bout-Van Den Beukel et al., 2008; Viard et al., 2011) have indicated that Vitamin D deficiency was more

prominent among HIV populations and ranged from a prevalence of 45% to 87% as compared to the general population. More importantly, several studies and meta-analyses on HIV patients have also concluded that Vitamin D deficiency could be a key culprit associated with faster occurrence of poor health outcomes (e.g., opportunistic infections, chronic diseases, or multiple organ systems complications) and higher susceptibility for HIV disease progression towards AIDS or death (Campbell & Spector, 2012; Dao et al., 2011; Giusti et al., 2011; Griffin & Arnold, 2012; Mehta et al., 2011; 2010; Spector, 2011; Sudfeld et al., 2012; Vescini et al., 2011; Viard et al., 2011; Villamor, 2006).

Based on the aforementioned research, there has been a growing interest recently for exploring the mechanism through which Vitamin D influences the course of HIV infection (Overton & Yin, 2011). Since Vitamin D is quite well known for its classical role in calcium absorption and bone metabolism, most published studies on Vitamin D and HIV have focused on its associations with bone diseases (e.g., osteoporosis, osteomalacia, and osteopenia) or with HIV metabolic complications (Adams & Hewison, 2010; Villamor, 2006). However, the mere discovery of the presence of Vitamin D receptors (VDRs) in many tissues throughout the body and in the immune cells (e.g., T lymphocytes, macrophages, and dendritic cells) initiated a series of studies about the physiological mechanism through which Vitamin D could exert its modulatory effects on the immune system. Initially, these studies focused on Vitamin D in relation to tuberculosis, respiratory infections, autoimmune diseases, some diarrheal diseases, and thereafter to HIV (Holick, 2007; Norman, 2012; Villamor, 2006). This discovery highlighted a new, nonclassic outlook on Vitamin D and reintroduced it to the scientific

platform as an immune modulator capable of influencing and regulating immune responses and actions (Hart, Gorman, & Finlay-Jones, 2011; Khoo et al., 2012; Lang, Samaras, Samaras, & Aspinall, 2013; Miller & Gallo, 2010; Walker & Modlin, 2009; White, 2008). So far, the exact immunologic mechanism has proven to be very complex and has not been fully described. In the context of HIV, Vitamin D—besides its anti-inflammatory and antimicrobial role—is mostly considered for its immune modulator effects; it targets immune cells (especially CD4 T lymphocytes), influences their cellular differentiation and proliferation, and regulates hormone secretion at the cellular level (Giusti et al., 2011; Kamen & Tangpricha, 2010; Spector, 2010). The next sections present an overview on Vitamin D and how it influences the immune system in HIV.

Overview on Vitamin D

Vitamin D has been misidentified as a vitamin for a long time, but it is in reality a steroid hormone (sterol) as per its molecular structure, 1,25-hydroxyVitamin D3 (Norman, 1998, 2012). There are two types of Vitamin D: D2 and D3 (White, 2008); Vitamin D refers to both or either type. Vitamin D2 is synthetic and comes from diet, specifically from sun-exposed yeasts or plant sterols (ergosterols) while D3 is synthesized in the skin. Both are used in food and vitamin supplements, but D2 is used mostly in prescriptions (Holick, 2007). By definition, Vitamin D status can be obtained by measuring the blood level of the main circulating metabolite of Vitamin D, 25-hydroxyVitamin D or 25(OH)D, with normal level set point at 30 ng/µL and above (indicating sufficiency), and levels of 20–29 ng/µL and below 20 ng/µL indicating Vitamin D insufficiency and deficiency, respectively (Holick, 2003, 2007; Wacker &

Holick, 2013). Therefore, for the sake of this study, Vitamin D deficiency was designated by 25(OH)D level < 30 ng/μL.

Exposure to sunlight is responsible for about 90% of Vitamin D synthesis in the skin (Holick, 2003; 2004), and the remaining 10% comes from nutrition. It takes 10 to 15 minutes of whole body sun exposure in the summer to produce and release 10,000 to 20,000 IU of Vitamin D into blood circulation, providing, therefore, more Vitamin D than the nationally recommended Vitamin D dosage of 600 IU per day (Hollis, 2005). Holick (as cited in Hollis, 2005, p. 318) argued that excessive sun exposure never leads to Vitamin D intoxication because the body naturally adapts and regulates the overproduction by inactivating some of the biological precursors of Vitamin D.

It is hard to self-assess sufficiency in Vitamin D intake because it depends on many factors such as age, lifestyle, sunscreen use, clothing, race/skin pigmentation, genetics, geographic area and latitude, degree and amount of sunlight exposure (cloud coverage or smog), diet, and existing health conditions (Hollis, 2005; Neri, Miller, & Potter, 2012; Rosen, 2011). Besides the intensity and quantity of sunlight exposure and the influence of seasons and latitude on UVB penetration to the skin (and consequently on Vitamin D production), skin color pigment melanin (UVB light filter or blocker), is yet a predetermined and irreversible factor that blocks Vitamin D skin synthesis and places African Americans at higher risk for Vitamin D deficiency as compared to fair skinned people (Egan et al., 2008; Hannan et al., 2008; Holick & Chen, 2008; Murphy et al., 2012; Tseng et al., 2009). It is estimated that people with dark skin, such as African Americans, need between 10–12 times more UVB light radiation exposure (sunlight) as

compared to people with fair skin in order to produce the same amount of Vitamin D (Hollis, 2005).

Dark-skinned people living in highly sunny areas, such as the equator or Africa, are less likely to suffer from Vitamin D deficiency because of adequate and extensive sun exposure; however, they tend to develop Vitamin D deficiency once they live in a northern climate with limited sun exposure (Hollis, 2005). On the other hand, full-body exposure to ambient sunlight in northern climates with latitudes above 40 degrees during wintertime does not warrant sufficient cutaneous production of Vitamin D (Hollis, 2005; Kimlin, 2004). In winter, even in southern geographical areas with high latitude, the amount of UVB radiation in sunlight is not adequate to promote skin production of Vitamin D (Holick, 2003, 2004, 2007). Overall, at any latitude and under normal exposure to sun throughout the year (without Vitamin D supplementation), African Americans rarely reach Vitamin D sufficiency and only experience a negligible increase in their Vitamin D levels between winter and summer months (Harris, 2006). Hall et al. (2010) estimated that African Americans— assuming low sun exposure— need between 2100 and 3100 IU/day of Vitamin D in all seasons to achieve sufficiency.

Findings from NHANES surveys between 2001 and 2004 indicated more than 90% prevalence of Vitamin D insufficiency/deficiency, $25(OH)D < 30$ ng/µL, among African Americans as compared to other subpopulations; the same surveys also showed that the prevalence of insufficiency in the general population (adolescents and adults) approached 77% irrespective of race and ethnicity (Ginde, Liu, & Camargo, 2009). Beyond the genetic, racial, geographical, and seasonal differences, African Americans

tend to have an insufficient intake of foods containing Vitamin D (e.g., milk and dairy products, fish, eggs, and fortified orange juice) and below national Vitamin D dietary recommendation as shown in national surveys (NHANES) findings, the fact that amplifies their risk for Vitamin D deficiency even further as compared to the general population (Dawson-Hughes, 2004; Ginde et al., 2009; Harris, 2006; Holick, 2007). Moreover, obesity and high body mass index (BMI) that are very common among African Americans influence Vitamin D levels; there is an inverse relationship (or negative correlation) between BMI and Vitamin D levels, whereby obese people with high BMI (greater than 30 or 40 kg/m^2) tend to be Vitamin D deficient (Lagunova, Porojnicu, Lindberg, Hexeberg, & Moan, 2009). Some researchers have suggested that in addition to the socioeconomic factors that contribute to obesity in general, structural environmental factors such as the proximity of fast food restaurants to place of residence increases further the risk of obesity and high BMI (Reitzel et al., 2013). Such findings may complicate the challenge of correcting Vitamin D deficiency in this population and the need to rectify nutritional recommendations.

Several studies have pinpointed serious concerns about the lack of effective, nationally targeted, nutritional supplement Vitamin D recommendations for Vitamin D (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Garrett-Mayer, Wagner, Hollis, Kindy, & Gattoni-Celli, 2012; Holick & Chen, 2008; Hollis 2005; Vieth et al., 2007); some researchers have argued that the dietary allowance (RDA) of 600 IU per day recommended by the Institute of Medicine (IOM, 2011) for Vitamin D deficient adults is mediocre and incapable of sufficiently raising Vitamin D level to

optimal levels and sustaining it in the blood for a considerable period of time (Bischoff-Ferrari et al., 2006; Ginde et al., 2009; Hollis, 2005). Consequently, Garrett-Mayer et al. (2012) and Hollis (2005) supported the Endocrine Society Clinical Practice Guidelines (Holick et al., 2011) that encourage the use of supplementation not less than 4000 IU/day to remedy the deficiency epidemic, close the gap in racial disparity, and improve the general health of high-risk population groups.

African Americans with HIV, in every context, are at a greater disadvantage than other racial groups; many HIV studies revealed a significant correlation between black race and high prevalence of Vitamin D deficiency (Adeyemi et al., 2011; Cervero et al., 2013; Crutchley et al., 2012; Dao et al., 2011; Mueller et al., 2010; Stein et al., 2011; Wasserman & Rubin, 2010; Welz et al., 2010; Yin, 2012). Besides the previously discussed non-HIV factors that can contribute to Vitamin D deficiency, there are several HIV factors that can alter Vitamin D absorption, activation, or metabolism secondary to the course of HIV infection, the coexisting clinical conditions, and to the highly active antiretroviral treatment (HAART) side effects (Cozzolino et al., 2003). However, the main focus of this study remained on the influence of Vitamin D on the immune function as depicted by immune cells (mainly CD4) and viral load.

Overview on Vitamin D and CD4 Cells in HIV

CD4 T cells and immune response. CD4 cells are the primary targets of HIV. They represent the body’s first defense against pathogens, initiating antimicrobial or antiviral responses (Sant & McMichael, 2012). The CD4 cells subdivide or differentiate into two major T helper subsets (also known as effector cells responsible for killing

pathogens): Th1 and Th2 (BMJ, 2000); they also differentiate into T regulatory cells (Tregs), which have a main function to control immune suppression mechanism, mainly suppressing T-cell reproduction (Gunville, Mourani, & Ginde, 2013; Hewison, 2010). Upon pathogen invasion, Th1 takes over the role of intracellular defense, while Th2 is in charge of extracellular defense. Th1 senses foreign antigens on the surface of infected cells, instigates CD4 T cellular proliferation, and mounts a vast immune response through releasing cytokines, the hormonal messengers that sense danger and mediate immune responses upon detection of foreign agents in the body (British Medical Journal, 2000).

During HIV infection, the viruses go to the lymph nodes where the immune cells (mainly CD4 T lymphocytes and B lymphocytes) are programmed to recognize and destroy foreign invaders. Once the immune cells are alerted, they activate and reproduce rapidly to fight the infection through initiating a cell-mediated defense mechanism, secreting cytokines, and later through launching humoral or antibody-mediated immune response (i.e., antibodies secretion by B lymphocytes; Kestens, 2005). However, with the buildup of viral load, and due to the ever-changing mutation of the genetic make-up and structure of the proteins (antigens) on the envelope of the viruses (thus, creating different strains), the latter baffle the immune system's memory, build viral reservoir, and escape the antibodies' counterattack; eventually, this leads to gradual destruction of CD4 cells by highly replicating HIV and exhaustive immune activation (Kestens, 2005).

In HIV infection, immune responses are driven by T helper subsets activities. There are two types of immune responses: cell-mediated or humoral/antibody-mediated (Kestens, 2005). As part of cell-mediated immune response, Th1 cytokines are

proinflammatory (e.g., interferon, interleukin-2 or IL-2, and tumor necrosis factor-alpha or TNF-α), and therefore induce tissue inflammation and activate macrophages (M1) as their line of defense to attack microbes (Herbein & Varin, 2010; Kurts, 2008) and get rid of intracellular pathogens (viruses) or tumors (Cantorna, Zhu, Froicu, & Wittke, 2004). Th1 also fosters the cytotoxic activities of CD8 cells or the natural killers -NK (Fevrier, Dorgham, & Rebollo, 2011). Simultaneously, Th1 collaborate with Th17 (another subset of CD4 that releases IL-17) to stimulate the release of their proinflammatory cytokines (Kurts, 2008).

In HIV infection, the role of Th17 is not yet fully understood, and understandings remain controversial. However, it is believed that Th17 cytokines or interleukin 17 can lower the risk for development of opportunistic infections in HIV (Elhed & Unutmaz, 2010), presumably because of their additional potency against fungi and parasites (Kurts, 2008), especially in the guts where most HIV replication takes place (Fevrier et al., 2011). Th17 cytokines seem to get more depleted during HIV infection as compared to Th1. Consequently, this deficiency in Th17 CD4 subset contributes to faster disease progression through generalized or chronic immune activation of more infected cells, which can lead to further depletion of infected and uninfected (naïve) CD4 cells, and to increased risk for opportunistic infections (Elhed & Unutmaz, 2010).

Th2 instigates the activation of B-lymphocytes to release antibodies and therefore is responsible for humoral or antibody-mediated immune responses. The Th2 related cytokines (e.g., interleukins/ IL 4, 5, and 10) induce macrophages (M2) to promote anti-inflammatory responses against extracellular invaders (Cantorna et al., 2004; Gunville et

al., 2013), and indirectly counteract the Th1 proinflammatory cellular actions (British Medical Journal , 2000; Cantorna, 2011; Cantorna et al., 2004; Herbein & Varin, 2010). Since Th1 cytokines have detrimental effects and are more implicated in accelerating HIV disease progression (Elhed & Unutmaz, 2010; Kurts, 2008), Th2 cytokines buffer their effect and inhibit Th17 differentiation to balance immune response and halt autoimmunity.

The balance between Th1 and Th2 is essential for cellular homeostasis and for optimal immune responses to invaders; when such balance is disrupted, some diseases can erupt such as allergies and asthma (Th2 driven diseases) or autoimmune diseases such as multiple sclerosis and Type 1 diabetes (Th1 and Th17 driven; Cantorna, 2011; Cantorna et al., 2004). Of paramount importance, Vitamin D plays an indispensable role as an immunomodulator that regulates cytokines production and promotes shifting the immune cellular milieu from proinflammatory to anti-inflammatory (Boonstra et al., 2001; Prietl, Treiber, Pieber, & Amrein, 2013).

Vitamin D as an immune modulator in HIV. Vitamin D seems to have an influence on modulating the immune responses through targeting cytokines expression between Th1, Th2, Th17, and Tregs. It is postulated that Vitamin D is in its active hormonal form binds to Th1 and Th2, and activates CD4 cells (occasionally leading to excessive cellular proliferation). Since all immune cells express Vitamin D receptors (VDR) in their nuclei, Vitamin D can influence T helper cells functions and cytokines secretions through the VDR. Vitamin D first binds to these VDR and then attaches to Vitamin D response elements (VDRE) present on the genes in the nucleus (Bearden,

Beard, & Striker, 2011). This represents the vehicle through which Vitamin D activates immune cells and exhibits its enzymatic actions to regulate gene transcription (at the VDR–VDRE complex) and promote expression of T cell responses (Cantorna, 2011; Mathieu & Adorini, 2002). The activation of CD4 T helper cells, in its turn, induces an increase in the number of Vitamin D receptors VDR by five times (Kamen & Tangpricha, 2010; Mahon, Wittke, Weaver, & Cantorna, 2003).

Vitamin D inhibits the excessive production and action of Th1 and Th17 cytokines in order to prevent susceptibility for autoimmune diseases; at the same time, it indirectly instigates the differentiation of Tregs to decelerate the immune suppression action (autoimmunity) and counteract the proinflammatory milieu (Cantorna, 2011; Gunville et al., 2013; Hewison, 2010; Prietl et al., 2013). Eventually, suppressing Th1 cytokines can be translated in less antigen presentation of pathogens and less T-cell proliferation and activation. Vitamin D boosts Th2 cytokines expression and proliferation to counteract the effects of Th1 cytokines (Boonstra et al., 2001; Gunville et al., 2013; Kamen & Tangpricha, 2010; Mathieu & Adorini, 2002). Therefore, a deficiency in Vitamin D would allow Th1 to have a stronger control over CD4 T-cell compartment (Beard, Bearden, & Striker, 2011; Cantorna et al., 2004). The literature described the action of Vitamin D as pulling or skewing the adaptive immune system away from Th1 and moving towards supporting Th2 responses (Bearden et al., 2011; Mathieu & Adorini, 2002).

In brief, Vitamin D has been known for its anti-inflammatory actions since discovery; whether in tuberculosis, asthma, or other respiratory and nonrespiratory

diseases, Vitamin D has demonstrated its efficacy as an anti-inflammatory agent (Gunville et al., 2013). This characteristic has been further emphasized in its potent role as immune modulator. Therefore, the presence of Vitamin D on immune target T cells that express VDR and the consequent increased number of VDR can present a protective effect against infections and diseases (Baeke, Takiishi, Korf, Gysemans, & Mathieu; 2010c; Kamen & Tangpricha, 2010). Moreover, Vitamin D can provide a protective effect against diseases related to the immune system and secure T-cell homeostasis to control immune system responses (innate and adaptive) against invading pathogens. A deficiency in Vitamin D, and correspondingly in the number of VDR, might disrupt the delicate balance of T-helper cell homeostasis and lead to diseases such as autoimmune diseases (e.g., multiple sclerosis), diabetes, Type 1 diabetes, asthma, and inflammatory bowel disease–IBD (Cantorna, 2011). Since HIV presents a high state of inflammation, Vitamin D deficiency in HIV patients can accelerate and promote a proinflammatory status; thus, it can be implicated in faster immune suppression and disease progression (Villamor, 2006).

Overview on the Role of Vitamin D in HIV Disease Progression

The course of HIV infection is depicted by a progressive immune dysfunction and a gradual decline in CD4+ lymphocytes, the primary targets of HIV. This decline represents the best biomarker of disease severity and progression as it represents the hallmark of immune dysfunction (immune aging or weakness) and the failure to adapt and respond properly to pathogen invaders (CDC, 2011c; Grossman, Meier-Schellersheim, Sousa, Victorino, & Paul, 2002; Overton & Yin, 2011). During HIV

infection, millions of HIV particles are generated and replaced every day with a very short lifespan that ranges from 0.5 to 1.5 hours. These virions, when released into the body, attack the CD4 cells and use them to replicate and then destroy billions of them daily; luckily, the CD4 daily replacement process ensures that not all CD4 cells get killed (Ho, 1995).

There are different mechanisms that explain HIV disease progression and how the decline in CD4 count weakens the immune response and makes the patient immune-compromised and more openly susceptible to opportunistic infections and comorbidities (Grossman et al., 2002; Kestens, 2005; Overton & Yin, 2011). However, these mechanisms about CD4 loss are still debatable. Kovacs et al. (2001) and Mohri et al. (2001) reinforced the argument presented by Ho (1995) and asserted that the increased viral replication and the exaggerated rates of CD4 simultaneous proliferation and replacement exhaust the immune system and lead to CD4 loss through apoptosis (cell death). More studies have also adopted this argument; the overstimulation of the immune system and the excessive activation of CD4 T cells can lead to self-destruction/depletion or apoptosis if left uninhibited (Langford, Ananworanich, & Cooper, 2007; Spector, 2010). In this sense, antiretroviral treatment can be effective in reducing the loss of CD4 cells through slowing down their excessive division and apoptosis, not through increasing their production (Kovacs et al., 2001; Mohri et al., 2001). As a counterargument, some researchers refuse this idea of exhausted immune system leading to CD4 loss, and suggest that two factors lead to CD4 cell depletion and disease progression: the short lifespan of CD4 cells due to virus-induced apoptosis and the infection of CD4 precursor

cells that result in production and replacement shortage (Hellerstein et al., 1999; Meyaard & Miedema, 1997).

According to the CDC (2012d), significant reductions in CD4 counts below 200 cells/µL characterize HIV disease progression. The optimal CD4 count ranges from 500 to 1500 cells/µL (CDC, 2011c). Accordingly, a patient with a CD4 count below 200 cells/µL is thought of as immunosuppressed and merits an AIDS diagnosis, regardless of clinical symptoms or events development (CDC, 2011c). CD4 count is not only clinically considered the best indicator of HIV disease progression and severity, but is also used as a marker for when treatment should be initiated (Langford et al.,, 2007).

Despite the fact that the relationship between Vitamin D and CD4 is not clear-cut and has been inconsistent in the literature, findings of the significant effects of Vitamin D deficiency on HIV disease progression in some major studies (Mehta et al., 2010; Sudfeld et al., 2012; Viard et al., 2011; Villamor, 2006) constituted an impetus to further explore and study this relationship. In the same context and as discussed previously, Vitamin D modulates the immune system's main target cell CD4 through the following known actions: It influences antigen presentation; regulates immune responses (protective effect); intercepts CD4 T lymphocytes excessive proliferation; and counteracts the overzealous immune response to infections by promoting Th2 and blocking the induction of proinflammatory cytokines (Th1 and Th17). These latter cytokines usually promote HIV infection while Vitamin D seeks to diminish the inflammation environment and halt tissue destruction (Overton & Yin, 2011).

Consequently, in case of sufficiency, Vitamin D is capable of enhancing normal immune functioning through producing peptides to combat pathogens and to control autophagy (cellular disintegration and degradation) in infected CD4 cells and other immune cells such as macrophages. Hence, Vitamin D ultimately affects or delays progression through reducing CD4 activation and differentiation, and influencing cytokines expression (Spector, 2009). On the contrary, Vitamin D deficiency or insufficiency is hypothesized to disrupt the immune function and response and to hasten CD4 destruction and disease progression. In fact, there is abundance of research that correlated low CD4 counts with Vitamin D deficiency in HIV patients (Haug, Müller, Aukrust, & Frøland, 1994; Ross et al., 2011; Stein et al., 2011; Welz et al., 2010). Moreover, knowing that CD4 reserves and production are already low due to immune dysfunction in HIV, a deficiency in Vitamin D is linked to further reduction in immune function, with lower CD4 counts and reduced responsiveness against intracellular pathogens (Langford et al., 2007).

Although I did not discuss the association between Vitamin D deficiency and HIV disease progression in this study, I highlighted the relationship between Vitamin D and CD4 count and percent in the context of Vitamin D supplementation. Chapter 2 delves into the literature that explored low CD4 counts' association with high prevalence of Vitamin D deficiency among HIV patients (irrespective of the causes). Correspondingly, I speculated whether there would be any significant improvement in the immunological status (measured by CD4 count and CD4 percent) of HIV patients who received Vitamin D supplementation. There are limited studies that investigated the importance of Vitamin

D supplementation or looked into the impact of supplementation on CD4 as the main or proxy parameter for HIV disease severity and progression.

Statement of the Problem

Vitamin D deficiency is a global health problem that is often and easily overlooked in people's lives. NHANES statistics between 1988 and 2004 showed that 25–35% of the U.S. population has Vitamin D deficiency with 25(OH)D level below 20 ng/µL and up to 25% have sufficient Vitamin D level (> 30 ng/µL); the same surveys also showed that 10% of African Americans are sufficient, and up to 77% are Vitamin D deficient (Ginde et al., 2009). Interestingly, 100% of the HIV cases surveyed in the NHANES for the same period had Vitamin D deficiency (Lake & Adams, 2011). Vitamin D deficiency has been associated with many detrimental effects on chronic skeletal and nonskeletal diseases, including cancers, cardiovascular diseases, diabetes, and others. On the other hand, it is well documented that Vitamin D deficiency is common in patients with HIV and that it disproportionately affects more African American HIV patients as compared to other racial groups (Adeyemi et al., 2011). Major research on HIV-infected individuals (Adeyemi et al., 2011; Crutchley et al., 2012; Dao et al., 2011; Egan et al., 2008; Mehta et al., 2010; 2011; Murphy et al., 2012; Overton & Yin, 2011; Rodriguez, Daniels, Gunawardene, & Robbins, 2009; Tseng et al., 2009; Van Den Bout-Van Den Beukel et al., 2008; Viard et al., 2011) have indicated that Vitamin D deficiency is highly prevalent among this population; most of this research has also demonstrated that Vitamin D deficiency highly correlates with black race.

The significant effects of Vitamin D deficiency on HIV disease progression constitute the impetus to understand the association between Vitamin D deficiency and CD4 count (and percent)—the main clinical parameter that reflects the degree of HIV disease progression. In fact, most above-mentioned studies dealt with predictors of Vitamin D deficiency and showed high consistency and similar results, although the association between Vitamin D deficiency and CD4 count fell short of being consistent or too weak to allow conclusions (Giusti et al., 2011; Griffin & Arnold, 2012; Lake & Adams, 2011) despite evident links between Vitamin D deficiency and HIV disease progression (Bang et al., 2010; Dao et al., 2011; Egger et al., 2002; Fawzi et al., 2005; Haug et al., 1998; Hogg et al., 2001; Lake & Adams, 2011; Langford et al., 2007; Mehta et al., 2010; 2011; Mueller et al., 2010; Philips & Lundgren, 2006; Rodriguez et al., 2009; Van Den Bout-Van Den Beukle et al., 2008; Viard et al., 2011; Villamor, 2006; Welz et al., 2010).

Although Vitamin D is an immunomodulator that targets mainly CD4 T cells, the relationship between Vitamin D levels and CD4 count is not clearly established and has been less consistent across the many cross-sectional and observational studies. Although some researchers reported a significant association between Vitamin D and CD4 count (De Luis et al., 2002; Haug et al., 1998; 1994; Stein et al., 2010; Theodorou, Serste, Van Gossum, & Dewit, 2014; Teichmann et al., 2003; Villamor, 2006), other researchers failed to demonstrate such association (Arpadi et al., 2009; Bang et al., 2010; Crutchley et al., 2012; Dao et al., 2011; Mehta et al., 2010, 2011; Van Den Bout-Van Den Beukle et al., 2008). Very few researchers have looked at or evaluated the association between

Vitamin D deficiency and CD4 as a biomarker of disease severity with special consideration to before and after Vitamin D supplementation (De Luis et al., 2002; Kakalia et al., 2011; Poowuttikul, Thomas, Hart, & Secord, 2013; Williams et al., 2009). In tandem with the recognition that Vitamin D might have an influence on the course and outcome of HIV, most of these studies acknowledged the importance of implementing cost-effective Vitamin D supplementation routines in treating Vitamin D deficient/insufficient HIV patients (as an adjunct to ART) until randomized studies provide enough evidence on its protective effect (Mehta et al., 2010, 2011) and until appropriate research on optimal dosing becomes available (Kakalia et al., 2011). Despite the complex mechanisms that Vitamin D exert on the immune cells, specifically on CD4, this study provided additional knowledge about the relationship between Vitamin D and CD4 with special focus on the impact of Vitamin D supplementation on CD4 count/percent in the Vitamin D deficient HIV subpopulation.

Two main inspiring studies evaluated Vitamin D supplementation in deficient and insufficient HIV patients (children with preserved immunologic function or high CD4 counts to start with) and assessed the impact of supplementation on CD4 count. Despite using different dosing in supplementation, both studies concluded that Vitamin D supplementation was too low and did not correlate with CD4 count changes (Kakalia et al., 2011; Poowuttikul et al., 2013). Therefore, it was interesting to study Vitamin D supplementation and its impact on CD4 count/percent among HIV African-American adult patients with Vitamin D deficiency and insufficiency and with somehow less optimal immune function compared to the studies by Kakalia et al. (2011) and

Poowuttikul et al. (2013). Assessing whether Vitamin D supplementation in African Americans Vitamin D deficient HIV patients affected their disease process and markers (as depicted by changes in CD4 count/percent and viral load) was important, especially for clinics or resource-limited settings and in high-risk population, and was worth investigating to extend knowledge in the field and to present the groundwork for further studies.

Purpose of the Study

The immune impact of Vitamin D deficiency and thereafter of Vitamin D supplementation in a cohort of HIV patients on CD4 count/percent as a proxy outcome reflecting disease progression is understudied and merits investigation. Moreover, the relationship between Vitamin D deficiency (independent variable) and CD4 count/percent (dependent variables) reflecting immune function constitutes another reason for undertaking this study. The purpose of this quantitative, observational retrospective study was to establish whether or not Vitamin D deficiency was associated with CD4 count/percent, and to evaluate whether improvement in Vitamin D levels after supplementation with 50,000 IU per week (time frame from baseline to 6 and 12 months) corresponded with significant changes in CD4 count/percent (absolute changes pre–post for CD4 count/percent and viral load) in a group of African American HIV men on HAART who were attending an HIV clinic in southeast Michigan.

Nature of the Study

The study design relied on a quantitative approach using retrospective observation (chart review). Despite an abundance of possible limitations such as missing or

incomplete data, retrospective chart reviews provide cost-effective access to a wealth of medical data capable of generating new hypotheses. Data abstractions from medical records have been widely used in epidemiological and clinical research, in quality assessment and improvement studies, and in inpatient care studies (Gearing, Mian, Barber, & Ickowicz, 2006). The study population consisted of all HIV-infected African American men (aged 21 years and up) who attended a specialty HIV clinic in an underserved community in Southeast Michigan between 2010 and 2014. The same HIV physician followed up with all of the study participants.

All personal identifiers of patients were excluded from the data collection process in order to avoid breaching privacy regulations. The HIV specialist who ran the clinic helped ensure easier access to all African American patients under his care; he also offered some help in the abstraction process, especially verifying all collected data. An earlier ballpark estimate indicated that the clinic offered services to more than 200 African American males with HIV infection. Based on similar studies, one study had a sample size of 160 patients (children; Poowuttikul et al., 2013), with only 8 (5%) patients with normal Vitamin D level as compared to 71.9% and 23.1% with deficiency and insufficiency, respectively. Another study by Kakalia et al. (2011) evaluated only 54 children with HIV and randomized them into three groups: no supplementation, Vitamin D supplementation, and placebo. Kakalia et al. (2011) calculated the sample size needed for their study based on mean percent change of CD4 percent (or count) between the nonsupplemented group and the supplemented group (with 80% power and $\alpha = 0.025$) and obtained a sample of 54 participants.

This study used a standardized electronic database to retrospectively abstract all data from medical records. I used SPSS 21 software package database for data storage and analysis. A medical record abstraction method retrospectively collected data on the following: health history, lifestyle and sociodemographic characteristics, Vitamin D status 25-hydroxyVitamin D [25(OH)D] before and after supplementation, BMI, viral load, CD4 count/percent, CD8 percent, CD4/CD8 ratio, ART treatment, HIV duration since diagnosis, and other clinical HIV related complications or progression to AIDS.

Vitamin D level was assessed at baseline, and the first measurement recorded in the chart marked the initial date of entry into the study. Based on their baseline Vitamin D levels, the cohort was divided into two groups: a group of HIV infected African American men with Vitamin D levels of less than 30 ng/μL (Vitamin D deficiency cutoff point is set at $<$ 30 ng/μL and included insufficiency cutoff point at 21–29 ng/μL) and a group of HIV infected African American men with adequate or sufficient levels of Vitamin D ($\geq$ 30 ng/μL). Both deficient and nondeficient/sufficient groups underwent evaluation of their CD4 count/percent and viral load at baseline in accordance with the first Vitamin D level at study initiation.

The analysis assessed the changes in CD4 count/percent and viral load as major HIV immune parameters and biomarkers of disease course, and evaluated the effect of supplementation on Vitamin D levels. The outcome measures consisted of calculating the absolute change in CD4 count/percent and viral load before and after Vitamin D supplementation. The follow-up period consisted of a minimum of 3 months and went up to 14 months. Correlation analyses were run between Vitamin D levels and CD4 count

and CD4 percent at baseline and at follow-up visits. Comparative analyses from baseline to follow-up were conducted using *t* test, Mann-Whitney *U* test, paired *t* test, and Wilcoxon signed-rank test, in addition to chi square or Fisher's exact test. Linear regression was used to discern all factors associated with Vitamin D deficiency, and Spearman's rho to check for correlation between Vitamin D and CD4 count/percent. All data were analyzed using SPSS.

Research Questions

RQ1: Do Vitamin D levels significantly correlate with CD4 count/percent in this group of HIV-infected African American adult men? Time frame: 0, 6 months, and 12 months.

Null Hypothesis H_0: There is no statistically significant correlation between Vitamin D levels and CD4 count/percent.

RQ2 (Quantitative): Does Vitamin D supplementation have a statistically significant effect on CD4 count/percent in HIV-infected African American adult men in this study? Time frame: 6 and 12 months.

Null Hypothesis H_0: There is no statistically significant difference or change in CD4 count/percent after Vitamin D supplementation in HIV-infected African American men in this study.

Conceptual Background/Foundation

The interaction between Vitamin D deficiency and HIV is complex and ambiguous to a certain degree. The HIV literature showed that HIV patients were more likely to be Vitamin D deficient as compared to the general population; it also

demonstrated how Vitamin D deficiency was more prominent among patients with advanced HIV infection or AIDS (Beard et al., 2011; Conesa-Botella et al., 2010; Mehta et al., 2010; Mueller et al., 2010; Ross & McComsey, 2012). Over the last 2 decades, Vitamin D has been more and more recognized for its involvement in modulating the immune system, specifically CD4 T cells as main targets. However, the lack of clear-cut consensus on the association between Vitamin D and CD4 count/percent, in addition to the lack of consistency in defining cut-off levels for Vitamin D deficiency and the associated clinical endpoints, complicated matters even further (Lake & Adams, 2011).

Since CD4 count/percent are proxy measures of immune functioning, disease severity, and disease progression, this study utilized a conceptual framework that was based on existing knowledge of the interplay between CD4 and Vitamin D. Due to the complexity of Vitamin D cell-mediated mechanisms that entail several immunological, genomic, and physiological activities, the conceptual framework for this study offered a simpler overview about the overall role of Vitamin D as an immunomodulator of CD4 cells in HIV that eventually fit the context of the postulated hypotheses and the research questions (see Figure 1).

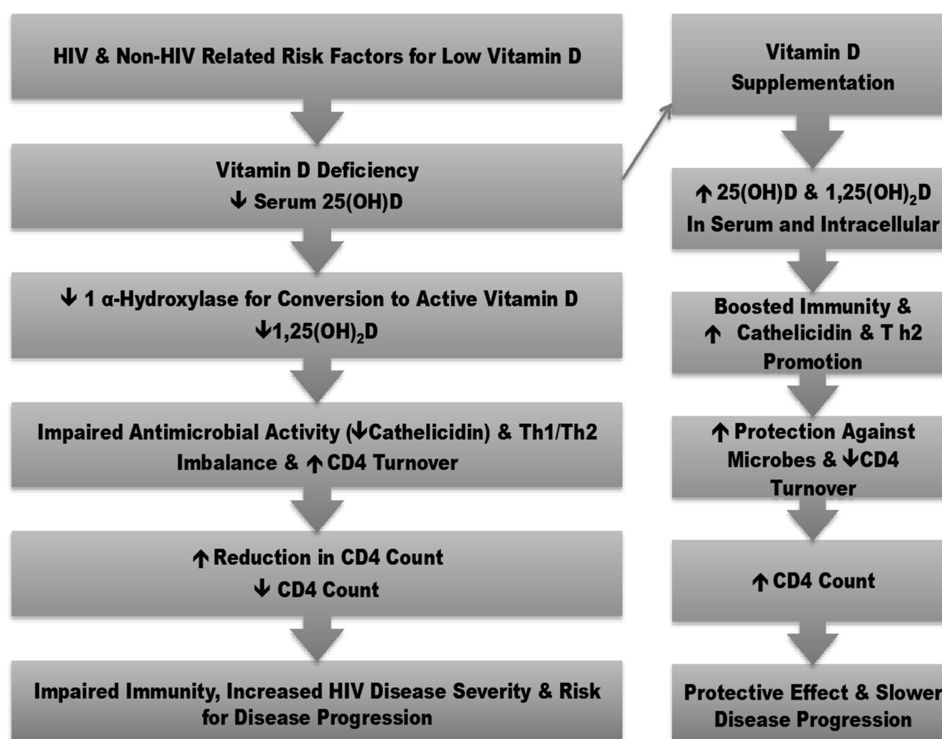

Figure 1. Theoretical framework about the effects of Vitamin D deficiency on overall HIV disease course and the postulated effects of Vitamin D supplementation on CD4.

While 25(OH)D reflects the total amount of Vitamin D available to the body, generated from both sun exposure and dietary/supplementary ingestion, $1,25(OH)_2D$, the metabolite of Vitamin D that results from hydroxylation in the liver and kidneys is considered the biologically active compound (hormonal form) known for its immune modulator effects on both the innate and the adaptive immune system (Kamen & Tangpricha, 2010). I will discuss the influence of Vitamin D on the innate and adaptive immune systems in detail in Chapter 2. After renal hydroxylation, Vitamin D in its active

hormonal form 1,25$(OH)_2$D binds to VDR (nuclear receptor) that are present on most body tissues and cells such as, monocytes, stimulated macrophages, dendritic cells, and, more importantly, on T and B lymphocytes (Mahon et al., 2003; Norman, 2006; White, 2008). The bioactive Vitamin D or 1,25$(OH)_2$D directly and indirectly activates CD4 T cells and insinuates a five-fold increase in VDR gene expression (Mahon et al., 2003). The amplified number of VDR allows Vitamin D to increase its modulating effect on CD4 T cells in a way that dictates regulation and expression of a number of genes (Mahon et al., 2003). Furthermore, Vitamin D influences the antigen presentation on the VDR of T cells and regulates immune response to pathogens by increasing the production of cathelicidin antimicrobial peptide (CAMP), the potent antimicrobial that fights invaders (Wang et al., 2004). Once Vitamin D attaches to the VDR, it enters the nucleus of cells, binds to VDRE on DNA, and starts regulating transcription of genes. It should be noted that Vitamin D could control more than 2000 genes (Holick, 2007).

Although the immune-modulatory effects of Vitamin D are spread across the different immune cells types, this study, first and foremost, focused on the known influences of Vitamin D on the immune response of activated CD4 T cells in HIV infection. The literature noted that low supply and production of Vitamin D could further promote and amplify proinflammatory cytokine (e.g., Th1 and Th17) generation and action (Lake & Adams, 2011). Since HIV infection depicts a constant inflammatory state characterized by a Th1-like response, Vitamin D deficiency is postulated to increase T lymphocytes turnover, increase cytokines activities, increase viral replication, distort inflammatory milieu, and eventually reduce CD4 counts (Lake & Adams, 2011).

Therefore, this study was concerned with the modulating influence of Vitamin D on the CD4 cell milieu and immune response. Upon VDR activation of CD4 T cells, Vitamin D induces a series of effects such as: responding to antigen (pathogen) presentation on the VDR of T cells by increasing the production of CAMP (Wang et al., 2004); intercepting T-lymphocytes proliferation and suppressing activation; altering cytokine secretion phenotype through inhibiting proinflammatory Th1 and Th17 cytokine production; inducing the production of anti-inflammatory cytokines Th2 to reduce inflammatory milieu (Kamen & Tangpricha, 2010; Lake & Adams, 2011); and promoting the generation of Tregs (Youssef et al., 2011). These Tregs are usually responsible for preventing development of autoimmune disorders and graft rejection posttransplantation (Baeke et al., 2007), but in HIV, they help circumvent CD4 T cell proliferation (Smolders et al., 2009).

This conceptual framework describes expected repletion of Vitamin D to alter the immune and metabolic milieu and improve the main immune parameters in HIV patients, specifically CD4 count/percent, in a way that would enhance immune functioning and slow progression towards AIDS. Restoration of serum 25(OH)D levels to normal could minimize ongoing inflammation and the complications of HIV (and that of ART) and could minimize severity of infections and malignancies associated with HIV disease course (Lake & Adams, 2011). The framework supported the hypothesis that Vitamin D active metabolite is capable of skewing T cells compartment towards anti-inflammatory Th2 pathways (Boonstra et al., 2001; Cantorna, 2011), therefore improving CD4 count/percent restoration and delaying progression towards AIDS.

Definitions of Terms

The following technical terms were defined with the use of the *Glossary of HIV/AIDS-Related Terms* (AIDSinfo, 2011), available online.

AIDS: Acquired Immunodeficiency Syndrome, a disease of the immune system due to HIV; this most advanced stage of HIV infection is characterized by the destruction of immune cells called CD4 T lymphocytes, leaving the patient vulnerable to opportunistic infections and cancers.

Antigen: A foreign body such as a pathogen (e.g., bacteria, viruses, and allergens) that triggers an immune response.

Antiretroviral (ARV): A drug that prevents the HIV retrovirus from replication.

Antiretroviral therapy (ART): also known as highly active retroviral therapy, HAART refers to the combination of two or more ARV drugs that stop HIV viral replication.

B lymphocytes: Also called B-cells, are immune cells that produce antibodies (e.g. immunoglobulin) to fight infection.

Cathelicidin: An antimicrobial peptide also known as CAMP produced by macrophages and monocytes. It is responsible for killing pathogens and reducing cellular inflammation. It also has antiviral properties and can play a role in HIV infection.

CD4 T lymphocytes: Also called CD4+ T cells, T Helper cells or just T lymphocytes. These CD4 cells regulate immune response to fight infection by stimulating other immune cells such as macrophages, B cells, and CD8 T lymphocytes. HIV weakens the immune system by depleting CD4 cells.

CD4 count: Also known as CD4 T lymphocytes count, the biomarker of immune function and the strongest predictor of HIV disease progression. Clinicians consider it as a decisive factor for starting ART and as a marker of ART effectiveness.

CD4 percentage: Percentage of white blood cells that are CD4 cells.

CD8 T lymphocyte: Also called CD8 cell, cytotoxic T lymphocytes, killer T cells. These cells identify cells with antigens (e.g., bacteria and viruses) and destroy them.

Cytokine: Proteins produced by immune cells that act as chemical messengers between cells in immune responses (e.g., cytokines are interferon-IFN and interleukin-IL).

HIV: Human immunodeficiency virus that causes AIDS.

HIV disease progression: Advance of HIV disease that can be measured by change in CD4 count below 200, and by occurrence of one or more AIDS defining illnesses.

Immune response: The actions of the immune system triggered against foreign pathogens.

Immune system cells: White blood cells (T and B lymphocytes), dendritic cells, monocytes, and macrophages.

Immunomodulator: A natural or synthetic substance (e.g., Vitamin D in this case) that influences the immune response by activating, enhancing, or suppressing actions.

Innate immunity: The immunity that is born with the individual; natural killer (NK) cells and toll-like receptors (TLRs) are part of innate immunity.

Macrophage: A white blood cell type that ingests foreign bodies, acts as antigen-presenting cells, and stimulates other immune cells to fight infection.

Opportunistic infections: Recurrent, severe infections that take advantage to attack patients when their immune systems are weak and compromised.

Viral load: The amount of HIV RNA copies in the blood. ART medications work on suppressing viral load to an undetectable level.

Viral replication: The process of viral multiplication.

Viremia: The state of viruses in the blood.

Assumptions

The literature supported the assumption that the participants have a high prevalence of Vitamin D deficiency, especially African American patients. Despite being observational and retrospective in nature, this study also assumed that the sample was highly representative of HIV male populations in the geographic area, based on close interaction and feedback from the HIV specialized physician in the clinic. It was important to note here that the majority of patients who presented to the clinic were under the Ryan White program umbrella. Based on a quick inquiry at the clinic and on the clinician feedback, the study assumed that testing for Vitamin D levels and treating deficiency with supplementation was formally initiated at the clinic in 2010. Testing and treating for Vitamin D deficiency was mainly based on the clinicians' astuteness with regard to recognizing a high prevalence of Vitamin D deficiency in HIV and in non-HIV patients. This study also assumed that the data documentation on Vitamin D levels and supplementation was available to a great extent for all HIV patients with Vitamin D

deficiency. Eventually, I assumed that correcting for Vitamin D deficiency was essential for HIV patients besides being considered a safe and a cost-effective practice capable of promoting good health.

Limitations and Delimitations

This study was observational and retrospective in nature, a fact that posed some inherent methodological limitations related to external and internal validity. The ultimate design for this study would have been a randomized controlled trial (RCT), which was beyond the scope of this dissertation study due to lack of time and resources to carry out a prospective intervention. Furthermore, due to time and cost limits for collecting data of qualitative nature, a mixed method approach was not considered feasible for this study. Moreover, the study was limited to African American adult men aged 21 and up presenting to one clinic in southeast Michigan serving an underserved community. The results of this study might not be generalized to other African American men at the broader geographical level beyond the clinic or at the state level. Study results should be interpreted cautiously and not be generalized to other busy urban settings even though the study site's clinic was considerably busy and caters mostly to African American HIV patients because of its location in metropolitan Detroit, Michigan. Being retrospective in nature, the accuracy of data with regard to compliance with Vitamin D supplementation treatment might not be available. Furthermore, some ART drugs could be considered as confounding variables because they might affect Vitamin D deficiency (Giusti et al., 2011; Mehta et al., 2010; Van Den Bout-Van Den Beukle et al., 2008) and might have affected CD4 counts/percent and Vitamin D levels. However, the study could not account

for the types of ART due to the longitudinal nature of the study and the complexity of collecting such data over time.

Significance of the Study

There is a scarcity of research that deals with HIV-positive African Americans, despite the fact that they are considered a high-risk group with innate biologic tendency for Vitamin D deficiency. Based on the aforementioned literature, there are racial disparities that are translated as higher prevalence of Vitamin D deficiency and consequent negative HIV outcomes among African Americans as compared to other racial groups (Egan et al., 2008; Kim et al., 2012; Murphy et al., 2009; Tseng et al., 2009). This fact justifies the need to correct Vitamin D status in order to reduce the gap and improve health outcomes in this subpopulation. The growing body of evidence in the literature about the health benefits of Vitamin D for the population at large suggests that adequate Vitamin D supplementation may be even more important for HIV patients as it can influence the course of disease with respect to rate of progression and CD4 counts (Lake & Adams, 2011; Mueller et al., 2010; Ross & McComsey, 2012; Rustein et al., 2011). Furthermore, some researchers found that Vitamin D supplementation may protect against a myriad of negative health outcomes related to Vitamin D deficiency (e.g., CVD, cancers, and autoimmune diseases), including potential protection against opportunistic infections (Hossein-Nezhad & Holick, 2012; Lake & Adams, 2011). Some researchers reported that correcting Vitamin D deficiency could lead to reduction in viral replication (Campbell & Spector, 2012; Conesa-Botella et al., 2010; Mehta et al., 2009, 2010). Practically, this study highlighted the prevalence of Vitamin D deficiency in a cohort of

African American, HIV-infected patients, but more importantly, it explored whether this deficiency correlated with the immune function biomarkers and whether replenishing Vitamin D level through supplementation could impact the course of HIV infection through affecting changes in the CD4 count/percent and viral load.

This observational study on Vitamin D supplementation should be looked at as a prelude to a RCT. Only RCTs could provide evidence-based results on the potential benefits of replenishing Vitamin D levels on HIV outcomes (Lake & Adams, 2011). Barbosa et al. (2014) carried out a systematic review about the immunological impact of Vitamin D in HIV (besides summarizing its role in osteoporosis). In this review, the authors compiled findings from different pertinent studies (RCTs, experimental, and observational) in the field of HIV; Barbosa et al. concluded that in view of the health benefits of sufficient Vitamin D on the course of HIV disease and on delaying the occurrence of chronic diseases, there was a need to expand the research efforts to emphasize the potential health gains from supplementation (preferably in large RCTs), rather than focusing on the negative implications of Vitamin D deficiency. The researchers concluded that Vitamin D supplementation can be viewed as a cost-effective and safe method for the general population and for HIV patients in specific who are prone for even more pronounced Vitamin D deficiency (Barbosa et al., 2014). Hence, my research also investigated whether Vitamin D testing and supplementation for highly-impacted HIV patients could be considered an important routine clinical practice in the course of managing this immunological disease and improving its outcomes, especially as far as the immune function of patients is concerned.

Based on the controversial results in the literature about the correlation between Vitamin D and CD4 (discussed thoroughly in Chapter 2), this research, unlike many cross-sectional studies in this topic, looked retrospectively at testing this correlation through analyzing longitudinal data on CD4 count/percent and Vitamin D levels measured concomitantly at each clinical visit up to 14 months follow-up. Moreover, this research performed correlation analysis on CD4 percent (in addition to CD4 count) because it has been reported to be a more stable measure than CD4 count (AIDS InfoNet, 2014). An advantage in this study was that the HIV physician at the study site used a uniform Vitamin D supplementation protocol (50,000 IU once per week) on all patients. This avoided discrepancy in analyzing Vitamin D results and allowed the observation of a possible dose dependent effect. Note that the literature on Vitamin D supplementation yielded inconsistent findings with regard to achieving optimal levels, possibly due to the use of different supplementation protocols.

In short, while this study cannot offer evidence-based findings about the impact of Vitamin D on CD4, the longitudinal, simultaneous data on both measures (Vitamin D and CD4) allowed observation of their relationship and assessment of whether modifying Vitamin D levels can have any significant influence on the main immune function parameters and in alleviating the course of HIV disease.

Implications for Social Change

Vitamin D deficiency is a worldwide epidemic that is linked to many adverse health outcomes, including all-cause mortality. The high prevalence of Vitamin D deficiency among HIV patients is a red flag that should alarm clinicians about the need to

screen for it and correct it. Based on the collected evidence to date, Vitamin D supplementation is viewed as a cost-effective, safe, and favorable intervention for the general population and for HIV patients in specific who are prone for even more pronounced deficiency (Holick, 2007; Pinzone et al., 2013; Prietl et al., 2013; Villamor, 2006). Replenishing Vitamin D can be viewed as an inexpensive and cost-effective therapeutic option that can help delay HIV progression and reduce risks for other comorbidities or chronic diseases in patients with HIV (Giusti et al., 2011; Haug et al., 1998; Mehta et al., 2010). Since HIV incidence and progression rates are still on the rise among young adults African Americans as compared to other HIV subpopulations, it is imperative to address their needs, not only through prevention efforts to reduce transmission and prevent infection, but also through embracing proper medical management after HIV diagnosis to slow down progression to AIDS. As a national response, the President's National HIV/AIDS Strategy-NHAS (The White House, 2010) has set an action plan to eliminate racial and ethnic health disparities in HIV morbidity and mortality. The strategy seeks to eliminate barriers to early HIV diagnosis and to increase access to HIV treatment in order to improve survival and HIV-related health outcomes among all HIV patients with special focus on high-risk and minority groups (e.g., African Americans).

The health literature offers a plethora of research that shows the protective and positive health effects of Vitamin D on a wide array of chronic diseases and its effects on the immune system (Holick, 2003, 2004; Holick et al., 2011; Hossein-Nezhad & Holick, 2013; Ross & McComsey, 2012; Rustein et al., 2011). Still, the mechanism through

which Vitamin D influences the immune system is very complex and needs further investigation as the field of immunology and HIV are both ever expanding. The risk reduction and preventive properties of Vitamin D (or Vitamin D supplementation) against major chronic diseases, including HIV, have earned Vitamin D a worldwide reputation beyond bone health; however, reputation remains founded largely on speculation if researchers do not conduct further studies.

Addressing Vitamin D deficiency should be observed from the public health perspective of improving the overall health of HIV patients (Giusti et al., 2011; Hossein-Nezhad & Holick, 2013) and offering an opportunity to close the racial disparity gap in HIV-related morbidity and mortality. Furthermore, in this study, I sought to promote public health efforts that highlight the health hazards of Vitamin D deficiency, a very common yet overlooked global health problem, through addressing and informing the general public, the patients, the health workers, the policy makers, and the clinicians about the importance of screening for Vitamin D deficiency and the potential benefits of correcting it. Yet, Vitamin D supplementation and impact on immune health in HIV remains understudied; there is a need to undertake large clinical and epidemiological studies to elucidate the overall immunological effects of Vitamin D. One of this study's contribution to the published research in the field of HIV is to emphasize the importance of modifying Vitamin D deficiency, a simple parameter which has the ability to influence the immune function in HIV patients and is hypothesized to have a potential positive impact in alleviating the course of HIV if properly managed and corrected.

Finally, in this current research, I did not seek to resolve the controversy of Vitamin D supplementation impact on CD4 count/percent; rather it explored the possible benefits of supplementation for the immune system with respect to HIV (Mehta et al., 2009; Ross & McComsey, 2012; Rustein et al., 2011). It also aimed to elucidate and challenge a less corroborated hypothesis about a possible correlation between Vitamin D and immune health in a disease that was primarily immunological in nature. This study explored whether Vitamin D deficiency is an influential or detrimental risk factor in HIV and sets the field for further research to verify the correlation between Vitamin D and CD4 count/percent. This study could bring about positive social change by providing public health professionals and HIV patients with the necessary knowledge to understand Vitamin D deficiency and its related health hazards in HIV, and by empowering them to use this knowledge to diminish the burden of HIV disease. The positive social change would not be complete without extending the knowledge to clinicians and policymakers to engage them in evaluating their medical decisions and their health legislations in the favor of their communities.

Summary

This chapter provided some background knowledge about the prevalence of Vitamin D deficiency in HIV-infected patients and its overall health consequences. It also discussed the immunomodulating effects of Vitamin D on the main target immune cells in HIV, the CD4 cells, and proposed a conceptual framework about the physiological mechanisms of Vitamin D exerted mainly on CD4 cells. The chapter highlighted the postulated beneficial role of Vitamin D in delaying HIV disease progression and reducing

mortality in HIV (Mehta et al., 2010; Viard et al., 2011). The discrepancies in the literature about the correlation between Vitamin D as an immune modulator and CD4 count/percent instigated the need to further investigate this issue and partly justified the need to conduct this study. Moreover, the shortage of studies on Vitamin D supplementation and its assumed benefits on the immune system in HIV further elicited the interest to explore the impact of supplementation on CD4 count/percent in HIV patients. Chapter 2 discusses the published literature in detail, and Chapter 3 goes over the methodology chosen for this research study.

Chapter 2: Literature Review

Introduction

In view of the gray area in research with regard to the nature of the association between CD4 count/percent, the main immune function parameter, and Vitamin D, there is a need to cover the topic extensively. This chapter examines the factors that lead to Vitamin D deficiency, followed by a review of Vitamin D deficiency and its health implications on the immune system and overall disease course in patients with HIV. There is special emphasis on covering the relationship between Vitamin D and CD4 and the extrapolated impact of deficiency on CD4 count/percent as a proxy measure of immune function. Finally, the literature review examines the role and impact of Vitamin D supplementation on CD4 count/percent through reviewing the most pertinent results of different studies in the field. A review of the effects of Vitamin D deficiency on HIV disease course was already discussed in Chapter 1, but I will revisited it in this chapter. Moreover, I explore some of the factors that influence both Vitamin D deficiency and CD4. Due to the scientific nature of the topic, the review of the literature exposed many conflicting results available to date, but it also highlighted the importance of undertaking more studies to add to the existing knowledge in the field.

Search Strategy

The literature search has identified related studies that are published in the last 10 years but with more emphasis on new research publications in the last 5 years between 2009 and 2015. I included some studies that dated back more than 10 years because they contained knowledge that is still pertinent to the field today. The search strategy

consisted of checking several online databases from Walden University library and other academic online libraries in addition to using Google Scholar. I also browsed peer-reviewed articles from specialized journals of relevance in the field of HIV/AIDS or infectious diseases. I also searched the ProQuest Dissertations and Theses database and databases with systematic reviews or meta-analyses such as Cochrane to identify similar, related, or relevant studies without any limits to patients' age, gender, ethnicity/race, language, or setting.

Eventually, I obtained the selected articles for this review from the following databases: Medline/Pubmed, Academic Search Premier, CINAHL, Cochrane, Psychinfo, and others. The main search terms or keywords used in the searches query were: *HIV* and/or *AIDS*, *human immunodeficiency virus*, *disease progession*, *CD4*, *CD4+ T cells*, *T lymphocytes*, *CD4 count*, *CD4 percent, Vitamin D*, *25(OH)D*, *Vitamin D deficiency*, *Vitamin D supplementation*, and *immune system.*

I searched some specific journals that publish articles in the field of HIV/AIDS using the same queries to ensure no relevant articles were missed by the search strategy. Websites related to HIV/AIDS, the CDC, and the World Health Organization (WHO) were also searched for relevant information and statistics. Moreover, I undertook careful examination of reference lists from review articles and from all of the primary articles to further identify relevant articles. I screened abstracts and articles for relevance and irrelevance, and articles that did not account for Vitamin D and CD4 or were not related to HIV population were excluded. For a more inclusive literature review, I selected the most frequently cited articles and some of the classical articles still considered of

particular relevance to the topic even if they were published more than 10 years ago. The literature review included studies that assessed and reported levels of Vitamin D as 25(OH)D. All quantitative study designs were eligible for this review, experimental and nonexperimental. The majority of the studies on this topic were observational in nature such as retrospective chart review, cross-sectional, prospective cohort studies, and case-control studies; there were few RCTs.

Vitamin D: Definition and Function

Vitamin D or the "sunshine vitamin" (Wacker & Holick, 2013) a steroid hormone that has far reaching effects on general health. Historically, Vitamin D gained recognition at the dawn of the British Industrial Revolution in the 1700s, specifically upon the manifestation of excessive incidences of rickets, a debilitating bone and skeletal deformities in children accompanied often with mental growth retardation; that condition exposed the presence of an epidemic of Vitamin D deficiency (Holick, 2003; National Academy of Sciences, 2000). It was not until the 19th century that scientists discovered that moderate exposure to UVB rays through sunlight or irradiation using a special mercury arc lamp and fortifying milk with Vitamin D actually prevented rickets rather than eradicated it (Holick, 2003; Holick & Chen, 2008; National Academy of Sciences, 2000). Later, in 1903, the discovery that increased Vitamin D production from exposure to UV light could cure a cutaneous form of tuberculosis (called lupus vulgaris) earned Niels Finsen the third Nobel Prize in Medicine (Moller, Kongshoj, Philipsen, Thomsen, & Wulf, 2005; Wacker & Holick, 2013).

Vitamin D has been linked to bone health and has been known to be responsible for enhancing calcium absorption through the intestines and bones (i.e., regulates calcium metabolism in accordance with parathyroid gland hormone; Holick, 2007; Holick & Chen, 2008; Kamen & Tangpricha, 2010). However, from the 1980s on, Vitamin D has been gaining more recognition for its nonclassical, rather noncalcemic roles, especially in the immune system, due to a major scientific finding in 1975. That specific year unveiled the discovery of VDR that bind Vitamin D to the nucleus of various body cells (Deluca & Cantorna, 2001; Kamen & Tangpricha, 2010). Far from immunology in infectious diseases, rather in a cancer research context, a Japanese researcher found that adding the hormonal active form of Vitamin D to immature leukemia cells with VDR stopped their growth by inducing cellular differentiation, maturation, and ultimately cellular death (National Academy of Science, 2000); this discovery was a crucial moment in cancer prevention research that led to further research on the genomic nature of Vitamin D actions since the nuclear VDR is involved in gene transcription and in the ability to modulate immune responses in most body cells/tissues (Deluca & Cantorna, 2001). Moreover, VDR are present on more than 800 genes (Williams et al., 2009); others claim that Vitamin D receptors regulate more than 2000 genes (Wacker & Holick, 2011).

Eventually, VDRs were found in almost all body tissues and cells, such as, immune cells T and B lymphocytes, macrophages, brain, adipose tissues, bone marrow, and cancer cells (Holick, 2007; Holick & Chen, 2008). This in fact put Vitamin D under the spotlight and instigated more research to explore the physiologic and metabolic implications of Vitamin D in many acute and chronic diseases, including skeletal and

nonskeletal diseases, autoimmune diseases, some types of cancer, cardiovascular diseases, infectious diseases, and HIV (Holick, 2007; Norman, 2012; Villamor, 2006; Wacker & Holick, 2013). Figure 2 shows the different roles and contributions of Vitamin D to human health as adapted from Norman (2012).

Vitamin D Metabolism

Most of Vitamin D production or synthesis occurs in the skin upon exposure to sunlight (UVB rays); in fact, exposure to sunlight is responsible for about 90% of Vitamin D synthesis (Holick, 2003) and the rest comes from nutritional intake. There are several factors that can influence the amount of Vitamin D production such as: the quantity and quality of UVB radiation, the season of the year, the geographical latitude, the concentration of melanin in the skin, older age, BMI, and the use of sunscreen (Holick & Chen, 2008). The topical application of a sunscreen with a 30 sun protection factor is capable of blocking most UVB radiation and of reducing the skin synthesis of Vitamin D by more than 95%, according to a study by Matsuoka et al. (1987). Melanin, the skin color pigment, acts as a UVB light filter that blocks Vitamin D3 synthesis, and therefore places dark skinned people such as African Americans at higher risk for Vitamin D deficiency as compared to fair skinned people (Hannan et al., 2008; Holick & Chen, 2008; Murphy et al., 2012; Tseng et al., 2009).

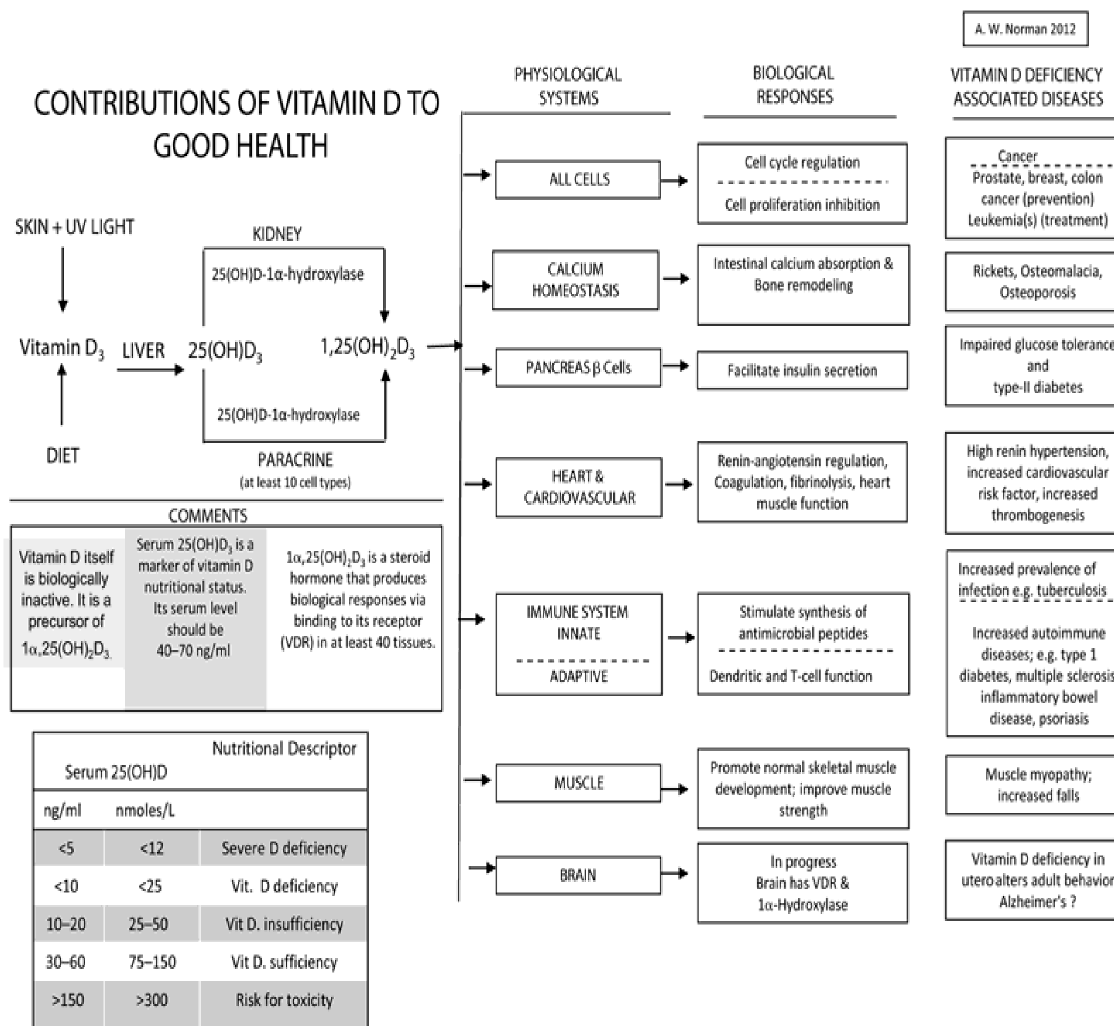

Figure 2. From "The history of the discovery of Vitamin D and its daughter steroid hormone," by A. W. Norman, 2012, *Annals of Nutrition and Metabolism, 61*, p. 204

Furthermore, sun exposure for most of the winter is not adequate for Vitamin D synthesis (Holick et al., 2011), especially in northern cold climates because the sun is too low in the sky (Mathieu & Van Der Schueren, 2011). On the other hand, exposing the whole body or parts of it to sunlight (for a short period of time 2–3 times weekly) and the occurrence of mild pinkish skin coloration (minimal erythemal dose [MED]) 24 hours

later is equivalent to Vitamin D_2 supplement intake of 10,000 to 25,000 IU (Holick, 2007; Holick et al., 2011; Holick & Chen, 2008; Wacker & Holick, 2011). However, although it is a tempting idea to produce Vitamin D from sun exposure (at least face and hand) for half an hour every day (Mathieu & Van Der Schueren, 2011), this also comes at a price because the UVB wavelength needed for Vitamin D production is the same as the one causing nonmelanoma skin cancer and skin aging (Reichrath, 2009). Based on thorough review of many cancer studies and UV radiation in relation to Vitamin D deficiency, Reichrath (2009) recommended sun protection and Vitamin D supplementation as a safe method to prevent Vitamin D deficiency and yet to protect against different types of cancers that may result from mutagenic solar UV-exposure. Holick (2007) reported that tanning beds discharge up to 6% of UVB radiation and can be used as a source of Vitamin D3 to prevent deficiency, provided it is used in moderation; tanning is especially recommended for people with fat or Vitamin D malabsorption.

Vitamin D, whether ingested or photosynthesized in the skin, is stored in fat cells and gets released and transported to the blood circulation via Vitamin D- binding protein (DBP; Holick, 2007). When UVB rays mediate the photochemical conversion of 7-dehydroxycholesterol in the skin, it converts it into pre-Vitamin D3 or 1,25-hydroxyVitamin D3 ($1,25(OH)_2D_3$) and later into Vitamin D3 (Holick, 2007; Kulie, Groff, Redmer, Hounshell, & Schrager, 2009). The lymphatic system transports the resultant Vitamin D3 into the circulation where it gets metabolized in the liver (hydroxylation) to produce 25-hydroxyVitamin D [25(OH)D], the main circulating form

of Vitamin D that is used clinically to measure Vitamin D status (Holick, 2007; Villamor, 2006). This 25(OH)D form of Vitamin D is considered biologically inactive until further hydroxylation and metabolizing in the kidneys (Deluca & Cantorna, 2001; Holick, 2007). However, the cumulative amount of 25(OH)D in the blood is very important because it will decide the amount of active form of Vitamin D available for multiple physiological functions (Kamen & Tangpricha, 2010).

The inactive 25(OH)D liver metabolite undergoes the second hydroxylation in the kidneys; it attaches to DBP and is carried to the kidneys where it is further metabolized through the enzyme 1-alpha-hydroxylase (1α-Oase) to produce $1,25(OH)_2D$, the active hormonal form of Vitamin D. This renal metabolite of Vitamin D ($1,25(OH)_2D$) enters target cells and binds to corresponding VDR in the small intestines, kidneys, and bone tissues to stimulate calcium absorption, and to exert other physiological effects (Holick, 2007). Any deactivation of this enzymatic conversion through the kidneys will lead to Vitamin D deficiency (Norman, 2012; Villamor, 2006). On the other hand, $1,25(OH)_2D$ is also responsible for many cellular actions such as regulating hormone secretion and immune function, and affecting cellular growth, proliferation and differentiation in musculoskeletal and extraskeletal tissues (Giusti et al., 2011; Holick, 2007; Norman, 1998, 2012).

More importantly, the discovery of the presence of the enzyme 1-alpha-hydroxylase (CYP27B1) in many cells (e.g., immune cells) outside the kidney was considered another major scientific breakthrough in the field because it indicated that these cells possessed their own enzymatic machinery to produce their own hormonal

form of Vitamin D (1,25$(OH)_2$D) or calcitriol (Holick, 2007). In other words, this implies that Vitamin D acts not only through endocrine (kidney-related) pathways but also through autocrine (intracellular and nonrenal) pathways to exert biological reactions; the latter enhance its ability to fight infectious diseases (Holick, 2006, 2007; Kamen & Tangpricha, 2010; Lappe, 2011). The ability of the immune cells to produce their own hormonal Vitamin D and then its binding to the VDRs would serve as an important pathway for a series of immune modulating actions, including gene transcription and cellular proliferation (Williams et al., 2009). In this context, serum Vitamin D deficiency would translate into reduced availability for cells and tissues to run enzymatic process (hydroxylation) and produce their own hormonal Vitamin D; ultimately, this would reduce the body ability to fight infection and to increased autoimmunity (Aranow, 2011).

Vitamin D Deficiency and Its General Health Implications

The evaluation of Vitamin D status has been based on the serum level of 25(OH)D, the main circulating metabolite of Vitamin D (Adeyemi et al., 2011; Holick; 2007; White, 2008). Vitamin D in its inactive form, 25(OH)D, has little metabolic activity but has high affinity for Vitamin D binding protein (DBP); that explains its relatively long half-life of 2 weeks in the bloodstream as compared to a very short half-life for active 1,25$(OH)_2$D that is present in very little amount in the blood and therefore cannot be used as a biomarker of Vitamin D adequacy (Jones, 2008). The production of 1,25$(OH)_2$D is greatly influenced by serum levels of other hormones or ions such as PTH (parathyroid hormone), calcium, and phosphorus (Monk & Bushinsky, 2011). It is known that 1,25$(OH)_2$D inhibits PTH secretion; therefore, elevated levels of PTH indicate

Vitamin D insufficiency or deficiency. As a result, Vitamin D supplementation will indirectly lead to reduction in PTH levels (Monk & Bushinsky, 2011).

As mentioned before and for the purpose of this study, severe Vitamin D deficiency, deficiency, and insufficiency status were considered in combination and referred to 25(OH)D levels below 30 ng/µL. A normal or sufficient Vitamin D level is any 25(OH)D value equal or more than 30 ng/µL (Holick, 2006, 2007). Vitamin D deficiency has been implicated in a series of morbidities such as bone diseases (Childs et al., 2012; Holick, 2003, 2007; Yin, 2012), cardiovascular diseases (Baz-Hecht & Goldfine, 2010; Holick, 2004; Lavie, Lee, & Milani, 2011), autoimmune diseases (Antico, Tampoia, Tozzoli, & Bizzaro, 2012), some types of cancers such as breast, prostate, and colon (Holick, 2004, 2007; Spina et al., 2006), diabetes (Baz-Hecht & Goldfine, 2010), tuberculosis (Campbell & Spector, 2012; Kibirige, Mutebi, Ssekitoleko, Worodria, & Mayanja-Kizza, 2013), hypertension (Judd, Nanes, Ziegler, Wilson, & Tangpricha, 2008), end-stage renal disease (Lavie, Lee, & Milani, 2011; Williams et al., 2009), depression (Ganji, Milone, Cody, McCarthy, & Wang, 2010), impaired lipid metabolism (Maki et al., 2009), and impaired immune and neurocognitive functions (Buell & Dawson-Hughes, 2008; Nimitphong & Holick, 2011). Even in utero, maternal Vitamin D deficiency can lead to fetal growth retardation and bone deformities with higher risk of fractures in the future (Holick, 2007; Wacker & Holick, 2013). In HIV, Vitamin D deficiency has not only been implicated in disease progression, it also affects the risk for HIV acquisition, as De La Torre et al. (2008) found in a recent study in HIV-

infected and noninfected drug users. The following sections discuss in detail the natural history of HIV and the role of Vitamin D as an immunomodulator in HIV.

Natural History of HIV

HIV refers to the retrovirus that attacks the immune cells (T and B lymphocytes) and damages them; AIDS refers to the advanced or progressed stage of HIV infection characterized by a severely weakened or compromised immune system (WHO, 2006). HIV is a retrovirus that belongs to the family of lentiviruses (slow viruses) that are characterized by a long latency period from time of infection to appearance of symptoms (National Institute of Health/National Institute of Allergy and Infectious Diseases-NIH/NIAID, 2012). Each HIV spherical viral particle (called virion) has an outer envelope layer or membrane made up of lipids with 72 spikes representing embedded proteins (glycoproteins or gp120 and gp41). The particle has a core (capsid) that mainly contains two strands of the HIV genetic material ribonucleic acid (RNA), along with three types of enzymes necessary for viral replication: reverse transcriptase, integrase, and protease (NIH/NIAID, 2012), hence exits different types of antiretroviral medications that work at the different levels of viral replication.

The natural course of HIV is depicted by a continuous and gradual damage to the immune system from time of infection (acute infection), to clinical latency (asymptomatic or chronic HIV infection), and until the manifestation of AIDS symptoms (marking progression). After transmission, the life cycle of HIV begins when the virus enters the human body and attaches itself through its viral envelope spikes of gp120 to the cell membrane of immune cells, specifically to a type of white blood cells called T-

helper cells – also known as T lymphocytes or CD4 (Barlett, 2010). Within weeks and up to six months post viral transmission, acute HIV infection or seroconversion takes place; most patients are not symptomatic at this stage but the virus has already started invading the lymphoid tissue (the main reservoir), disrupting the physical structure of the lymph nodes and entering into the blood stream to attack CD4 T lymphocytes (Barlett, 2010). The CD4 cells are responsible for fighting and destroying infected cells and germs throughout the body, and for initiating antiviral responses against viruses that are acquired through infection (or through vaccination) (Sant & McMichael, 2012).

Once attached to CD4, the HIV virus releases its content (the viral RNA and the enzymes) inside the CD4 cell and starts the damaging process to the immune system through viral replication and enzymatic processes (NIH/NIAID, 2012). For instance, the reverse transcriptase enzyme induces reverse transcription of viral RNA into viral DNA to make copies of the viral RNA (viral load buildup) but often makes random errors with copying, the fact that results in new strains of HIV that replicate at different rates and confuse the immune system. The integrase enzyme incorporates this viral DNA into the infected CD4 DNA (NIH/NIAID, 2012), thus, marking the seroconversion stage and clinical latency.

Upon seroconversion, the patients move to the second phase, called the clinical latency, whereby they do not witness symptoms but have chronic HIV infection with viral reproduction. This stage portrays a massive systemic immune response characterized by high T-cell activation and turnover (viral replication and viral antigens expression on the CD4) that cause a simultaneous excessive stimulation of B

lymphocytes (another type of immune cells) to release antibodies (NIH/NIAID, 2012). The excessive secretion and circulation of antibodies (immunoglobulins) by highly activated B-lymphocytes make the patient even more susceptible for opportunistic infections (Barlett, 2010). The immune system tries to maintain cellular balance as the CD8 T-killer cells lower the HIV viral load, the B-lymphocytes produce more antibodies, and the CD4 counts experience some rebound replacement and remain stable (NIH/NIAID, 2012). Antiviral treatment with HAART at this stage becomes an urgent necessity to partly restore immunity and reduce risk for opportunistic infections (Barlett, 2010).

A high viral load (high HIV RNA level) detected in the blood usually correlates with higher CD4 destruction (low CD4 counts) accompanied with a higher chance for disease progression- if patients lack appropriate immune responses to control the infection or do not get treatment on time (Barlett, 2010). Giorgi et al. (2002) found that viral load early in the course of infection can be predictive of progression to AIDS and that CD4 count has more prognostic value later in the course.

At this stage, CD4 lymphocytes continue to multiply to combat the infection; they are at the same time making more copies of HIV (viral replication). HIV protease enzyme plays an important role in helping the newly formed HIV viruses inside the CD4 nucleus to cleave or bud out from the cell surface using the CD4 cell machinery. The budding process can kill or destroy the infected CD4 cells and the released viruses proceed to infect more CD4 cells. The released copies of HIV spread throughout the body, they may hide and lie dormant away from the immune system, usually within the genome or DNA

of the infected cells (constituting latent reservoir for the HIV viruses) (NIH/NIAID, 2012).

Most of the time, when the CD4 cell machinery gets too beaten with HIV replication, CD4 can undergo apoptosis or programmed cell death. At some point, HIV may kill bystander-uninfected cells at a rapid pace (Spector, 2011) because the killer T cells –called CD8 (or memory cytotoxic T-cells) get activated and destroy these bystander lymphocytes due to the resemblance of some molecules on the CD4 cells (viral antigens expression) to the envelope proteins of HIV (NIH/NIAID, 2012). On the other hand, Spector (2011) emphasized the role played by the HIV virus in down-regulating autophagy in infected macrophages and CD4 cells in tandem with the process of apoptosis. His work showed that the virus does not only destroy the macrophages and the CD4 cells, but reduces both cells' cellular autophagy function in a way that would allow for more viral replication and longer survival of these infected cells as compared to uninfected bystander cells. Therefore, some drugs are used to induce autophagy and reduce viral replication. In that sense, Spector (2011), Campbell and Spector (2012), and Pirotte et al. (2013) suggested that active Vitamin D stimulate autophagy in macrophages, and therefore can enhance destruction of intracellular pathogens (i.e. autolysis of pathogens including HIV viruses) and reduce HIV viral replication (and ultimately, leading to HIV infection inhibition).

The HIV clinical latency stage is the longest; it can range from one year and up to more than 15 years. The probability of progression from HIV infection to AIDS diagnosis is estimated to be within 3 years after treatment; otherwise, if left untreated, HIV patients

can witness early progression to AIDS within one year of infection (May et al., 2010). Despite active replication to build-up viremia, still, patients can remain asymptomatic as long as the CD4 cellular destruction is in tandem with CD4 cellular replacement (Barlett, 2010). Consequently, an infected HIV patient may remain symptom-free for years during clinical latency until the immune system defenses start waning (immunosuppression) and the CD4 count goes down (NIH/NIAID, 2012) making way for disease progression towards AIDS, the last stage of HIV.

Overview on Vitamin D Deficiency and HIV Disease Progression

The HIV literature is inundating with studies that linked Vitamin D deficiency to HIV severity and disease progression. For instance, a large scale prospective European cohort study by Viard et al. (2011) reported a high prevalence of Vitamin D deficiency among 83% of HIV patients on ART ($N = 2000$); similarly, in another major prospective study, Mehta et al. (2011) provided a clearer picture on the potential correlation between Vitamin D deficiency and HIV disease progression; however, they only studied Tanzanian pregnant women with HIV ($N = 884$) and assessed their Vitamin D levels at baseline. All women were put on multivitamin supplementation that excluded Vitamin D and were then followed up for a median of 70 months, during which all clinical HIV and non-HIV outcomes were recorded. The authors used proportional hazards models to calculate risk of HIV disease progression (incidence rate ratios of AIDS related outcomes) based on baseline Vitamin D status. Their findings indicated that Vitamin D deficiency in these women significantly correlated with HIV disease progression, thus, Vitamin D deficiency could be considered a major risk factor in the incidence of HIV-

related complications and correcting deficiency status would offer a protection against these complications and against all-cause mortality. The authors acknowledged the importance of implementing cost-effective Vitamin D supplementation routine in treating Vitamin D deficient HIV patients once randomized studies provide enough evidence on its protective effect (Mehta et al., 2010, 2011).

Adeyemi et al. (2011) in their large scale study that compared Vitamin D deficiency among racially diverse HIV women and HIV uninfected women (total sample *N* = 1778; *n* (HIV+) = 1268); the authors found a higher prevalence of Vitamin D deficiency among HIV infected women (60%) as compared to non-infected HIV women and concluded that black race was the most significant and the most robust predictor of this deficiency. Adeyemi at al. (2011) also contributed another significant finding that high BMI level (more prevalent among AA women) highly correlated with Vitamin D deficiency; Egan et al. found a similar result earlier in 2008 whereby they compared non-HIV infected African American adults to their white counterparts, and BMI was a major predictor of Vitamin D deficiency. Similarly to other results in the literature (e.g., Kim et al., 2012; Egan et al., 2008; Van Den Bout-Van Den Beukel et al., 2008), they also found a high correlation between Vitamin D deficiency and high viral load, low CD4 count, and the use of special class of ART medications (non-nucleoside reverse transcriptase inhibitor-NNRTI). Only one study by Ormesher et al. (2011) had indicated that as compared to a matched cohort of HIV-uninfected African American men from the National Health and Nutrition Examination survey, a cohort of HIV-infected African

American men significantly exhibited a lower prevalence of Vitamin D deficiency (18 ng/μL versus 14 ng/μL, $p < 0.0001$).

Giusti et al. (2011) carried out a systematic review on Vitamin D deficiency in HIV-infected patients, whereby they compared and contrasted heterogeneous results on Vitamin D deficiency in HIV patients obtained mainly from 28 cross-sectional observational studies done in the United States and in Europe. Nine of these studies compared HIV patients to healthy subjects, and three of which used matched controls. Only nineteen studies gathered information on Vitamin D supplementation use (9 studies reported use that ranged from 2% to 49% among their participants). Due to the heterogeneity of the different studies' participants and to methodological discrepancies, the analysis showed inconsistent results and failed to provide a decisive answer on how Vitamin D deficiency affects HIV disease course; instead, the systematic review indicated various correlations between Vitamin D deficiency and CD4 T cell count (i.e., low Vitamin D correlates with low CD4 count), as well as with ART, viral load, and duration of HIV infection (Giusti et al., 2011).

Similarly, a review paper by Childs et al. (2012) identified some major HIV parameters that were found in the literature to be associated with Vitamin D deficiency; these included: intravenous drug use; length of HIV diagnosis; low CD4 cell count (below 200 cells/μL); current use of ART; and HIV RNA level (viral load). Childs et al. concluded that despite limited data on supplementation, still, Vitamin D repletion could have a significant impact on controlling the course of HIV infection and slowing its progression.

In the era of the revolutionary HIV/AIDS treatment using highly active retroviral therapy (HAART or ART), HIV infection stopped being the equivalence of a death sentence, especially in the developed countries where treatment is more accessible and available to the patients. ART modified the natural course of HIV infection epidemic by slowing the process of immunological suppression and deterioration depicted by major decline in CD4 counts, hence, halted viral replication and provided faster CD4 recovery in treated patients (restoring immune function) (Williams, Lima, & Gouws, 2011). As a result, HIV mortality rates diminished in the last decade due to HAART, but remained higher when compared to non-HIV population (Harrison, Song, & Zhang, 2010). Moreover, survival analysis from different studies showed that HIV patients witnessed a remarkable increase in their life expectancies (by more than 20 years) provided they started their treatment before HIV infection progressed to AIDS (i.e. CD4 count of less than 200 cells/μL) (BMJ, 2011; Harrison et al., 2010; Johnson et al., 2013).

Furthermore, in tandem with an increase in HIV patients' life expectancy and a decreased mortality secondary to advances in antiretroviral therapy, several studies have showed that HIV patients are at a higher risk for prematurely exhibiting serious aging co-morbidities such as osteoporosis, cardiovascular diseases, obesity, diabetes, neurocognitive impairment, autoimmune dysfunctions, inflammatory diseases, renal abnormalities, and cancer (Bhavan, Kampalath, & Overton, 2008; Overton & Yin, 2011). The risk for the aforementioned morbidities gets amplified when HIV patients suffer from Vitamin D deficiency.

At the International AIDS Society meeting in July 2013, Hogg et al. (2013) declared major strides in the life expectancy of a large cohort of American and Canadian HIV patients (*N* = 22,937; 62% white) diagnosed and treated with antiretroviral between 2000 and 2007. The researchers highlighted the importance of early treatment with ART before any drop of CD4 count below 350 cells/μL. Such approach would ensue a considerable increase in the life expectancy of treated HIV patients in a way that approximate that of the general population. According to their findings, a typical 20-year-old HIV patient who started ART with a CD4 count above 350 cells/μL is expected to live 68.6 more years as compared to 46.9 more years with ART started at a CD4 count below 350 cells/μL. While nonwhites and injection drug users also experienced a rise in their life expectancy (ranged between 29.7 to 48.4 years, as per baseline CD4 count at start), they were disproportionately more affected and their prognosis lagged behind. In a previous study, Hogg et al. (2001), accentuated the importance of CD4 as the most significant independent marker of severity and predictor of mortality in HIV; and they reported that in a cohort of 1219 patients on ART followed up over a median of 28 months, starting ART at a CD4 threshold of 200 cells/μL and up reflected lower cumulative mortality and better HIV prognosis.

Such longevity of HIV patients in the era of HAART and the expansive role of Vitamin D in regulating hormone secretion, cellular differentiation and proliferation, and other immune modulatory functions (besides its role in skeletal health) revealed that the consequences of Vitamin D deficiency should not be underestimated and should be treated. At the same token, while Vitamin D downplays the immune system (discussed

later in details), some HAART medications (e.g., nonnucleoside-reverse transcriptase inhibitor –NNRTI) may further reduce Vitamin D level and may exacerbate the effects of Vitamin D deficiency on the course of HIV disease (Conesa-Botella et al., 2010; Dao et al., 2011; Griffin & Arnold, 2012; Mueller et al., 2010; Rodriguez et al., 2009; Van Den Bout-Van Den Beukel et al., 2008; Viard et al., 2011). Examining the effects of some HAART medications on Vitamin D was beyond the scope of this study but would only be considered under the predictors of Vitamin D deficiency.

HIV disease progression into AIDS can vary from one patient to another depending on the immune responses of the host to HIV replication and on the timing of treatment since diagnosis (Langford et al., 2007). Progression can range from rapid (few years after infection), to intermediate (70-80% of patients belong to this category and present with symptoms within 6-10 years after infection), and late (about 5 percent of patients fall in this category and can remain symptom free for over 10 years) (Langford et al., 2007). Some HIV patients' immune system is capable of adjusting (mounting) the immune response to HIV antigens in a way that delays disease progression for over a decade or two without being on HAART and without manifesting any symptom; these patients are identified as long-term nonprogressors (Barlett, 2010; Langford et al., 2007). On the other hand, patients with advanced AIDS who present with a very low CD4 count below 50 cells/µL have poor prognosis with short survival even if they are receiving treatment (Barlett, 2010). In a nutshell, progression mainly depends on the rate of depletion of CD4 cell counts (as a cut off marker) and on the emergence of AIDS-related events or symptoms.

The CDC requires CD4 counts and occurrence of AIDS-related events to classify stages of disease progression from HIV to AIDS (CDC, 2012d). The CDC uses three HIV infection stages: Stage 1 refers to a CD4+T- lymphocytes count of > 500 cells/μL and the lack of AIDS-defining condition; stage 2 is characterized by CD4 count of 200-499 cells/μL and lack of AIDS events; and stage 3 is the AIDS stage whereby the CD4 count is ≤ 200 cells/μL and one or more AIDS defining condition is/are present. The CDC also uses a "stage unknown" term when there is lack of information on CD4 counts or AIDS –related events (CDC, 2012d).

Since CD4 cells constitute the main target for both HIV and Vitamin D, a deficiency in the latter may contribute to a modified immune response that is conducive to disease progression. It is important to look into the role of Vitamin D as modulator of the immune system and the potential benefits of adjunctive treatments such as Vitamin D supplementation that could go hand in hand with HAART to boost the immune functioning. Therefore, it is imperative to explain the role of Vitamin D in modulating the immune system in the course of HIV infection, with emphasis on its relation to CD4.

Vitamin D Modulating Effects on Immune Function and in HIV

It is known that the hormonal Vitamin D ($1,25(OH)_2D_3$ or calcitriol) has gained great reputation for its role in enhancing innate immunity in diseases such as tuberculosis (Aranow, 2012; Campbell & Spector, 2012; Heany, 2008; Wolff, Jones, & Hansen, 2008; Yuk et al., 2009). The favorable therapeutic role of Vitamin D in tuberculosis (TB) was an eye-opener due to its antiviral and antimicrobial responses (anti-inflammatory) at the intracellular level that influenced the course of tuberculosis, as well as some other

respiratory infectious diseases (Liu, Stenger, Tang, & Modlin, 2007). Of particular interest has been the role of antimicrobial peptide cathelicidin in fighting TB and a broad range of bacteria, viruses, and fungi (Kamen & Tangpricha, 2011). Likewise, the effects of low Vitamin D on the immune system in HIV started to emerge in the literature in the 1980s after the major discovery of VDRs in most body tissues and cells, particularly in immune cells (Deluca & Cantorna, 2001). Also, the presence of the extra-renal enzyme 1-alpha hydroxylase (called CYP27B1 enzyme) in immune cells and the subsequent synthesis of their own active Vitamin D locally contributed further to the body of knowledge (Beard et al., 2011; Holick, 2007). Eventually, VDRs and CYP27B1 in immune cells exert their immunomodulatory effects and give Vitamin D its reputation as a potent immune-modulator.

The presence of VDR (Vitamin D receptors) in immune cells is one of the most plausible physiological mechanisms of how Vitamin D influences pathogens' antigen presentation and regulates immune system responses (Beard et al., 2011; Deluca & Cantorna, 2001; Holick, 2007; Overton & Yin, 2011). The VDR is thought of as a natural ligand-activated transcription factor that binds to the Vitamin D related genetic material of immune cells and interferes with regulating their signaling pathways and their respective responses towards pathogens (Baeke et al., 2010b). Heany (2008) described the hormonal Vitamin D (1,25$(OH)_2D3$) or calcitriol as being "the right key to open up the locked stores of DNA information, allowing the cell to transcribe the plans and produce the proteins needed for tissue-specific responses" (p.1536). Hormonal Vitamin D can modulate the various genes in the cells with VDR by either turning on or off their

expression, just like Villamor (2006) described it as having paradoxical immunomodulating actions. Vitamin D is involved in regulating more than 2000 genes (Wacker & Holick, 2013); these genes control cellular growth and differentiation and overall innate response to pathogens (Holick, 2007; Wacker & Holick, 2007).

Bioactive Vitamin D ($1,25(OH)_2D3$) acts through its VDR on target cells and binds to DNA sequence elements VDRE located on the genes of these cells and encodes their DNA blueprints in order to initiate a series of molecular interactions that modulate and transcribe the gene expression in many tissues throughout the body (Lin & White, 2004). Vitamin D attaches to VDRs on the following immune cells: T lymphocytes (CD4 & CD8 T-cells), B-lymphocytes, neutrophils, monocytes, and antigen-presenting cells [APCs] such as macrophages and dendritic cells (Baeke, Takiishi, Korf, Gysemans, & Mathieu, 2010c). Furthermore, immune cells contain 1α-hydroxylase and when stimulated, they locally synthesize $1,25(OH)_2D$ through autocrine or paracrine pathways (Baeke et al., 2010c). As a potent immune modulator, Vitamin D intermediates the transcription of genes and the production of antimicrobial peptides in response to infectious agents, in particular the cathelicidin antimicrobial peptide or CAMP (Wang et al., 2004).

Without sufficient amount of hormonal Vitamin D produced locally in the cells, there will be limited antimicrobial gene transcription/expression and reduced ability to fight microbes (Lappe, 2011). Therefore, Vitamin D deficiency weakens and impairs the immune response. The amount of Vitamin D 25(OH)D available in the serum dictates or fuels the autocrine production of sufficient amount of active Vitamin D ($1,25(OH)_2D3$)

(Williams et al., 2009) and the successive synthesis of antimicrobial peptides (cathelicidin or CAMP) to kill pathogens through lysis i.e., degrading the microbe cell contents (Heany, 2008; Kamen & Tangpricha, 2010; Liu et al., 2006). The following section discusses the influence of Vitamin D on immune cells and separates the responses into innate and adaptive immune responses.

Vitamin D and the Innate Immune Response

Most of our understanding on the role of Vitamin D in innate immunity stemmed from research on *Mycobacterium* Tuberculosis, with special emphasis on monocytes and macrophages as key players and their corresponding pathogen recognition receptors (Liu et al., 2006; Liu et al., 2007). The innate immune system is genetically pre-programmed and is at the alert and ready to start a response even before pathogen attack; it acts as the first line of defense and as the main detector of invading pathogens through using pattern recognition receptors known as toll-like receptors (TLRs) (Miller & Gallo, 2010). These TLRs are capable of recognizing the pathogen-associated molecular patterns of foreign invaders in order to initiate an appropriate innate immune response and destroy them (Medzhitov, 2007; Trinchieri & Sher, 2007). An innate response is directly followed by an antigen-specific adaptive immune response as well (Medzhitov, 2007) that can be supportive (complementary), parallel (synergistic), or opposing (antagonistic) (Trinchieri & Sher, 2007). Therefore, it is essential to understand that TLRs modulate both innate and adaptive immune responses.

Vitamin D is an immune response enhancer, and in an innate response, it activates the toll-like receptors (TLRs) mainly on macrophages, monocytes, and dendritic cells

(Baeke et al., 2010b). These TLRs detect the molecular patterns of invading microbes and send signals to the immune system. When the microbe attaches to the TLRs, the complex formed induces local release or expression of 1-alpha hydroxylase (CYP27B1); this enzyme converts serum 25(OH)D into the biologically active hormonal form $1,25(OH)_2D3$ (Lappe, 2011). Once TLRs are triggered, $1,25(OH)_2D3$ binds to VDR and VDRE and initiates a series of antimicrobial activities, most important of which is encoding antimicrobial gene program that express antimicrobial peptides (cathelicidin/CAMP or defensin β) (See Figure 3*)* (Aranow, 2012; Campbell, Fantacone & Gombart, 2012; Gombart, O'Kelly, Saito, & Koeffler, 2007; Heany, 2008; Kamen & Tangpricha, 2010; Liu et al., 2007; Schwalfenberg, 2010; Wang et al., 2004; White, 2012; Wolff et al., 2008).

Cathelicidin is the only anti-microbial peptide present in human beings and it is sometimes referred to as hCAP18 (Kosciuczuk et al., 2012) or as LL-37 in its cleaved off peptide form (Beard et al., 2011; Bergman, Walter-Jallow, Broliden, Agerberth, & Soderlund, 2007). CAMP is considered the forerunner of innate immune response against bacterial invasion (Baeke, Van Etten, Overbergh, & Mathieu, 2007; Wang et al., 2004); it is often alluded to as an antibiotic protein (Wang et al., 2004). However, cathelicidin has antiviral properties too (Beard et al., 2011); for instance, it exerts its antiviral effects against HIV and impedes HIV viral replication (Bergman et al., 2007; Wang et al., 2004). CAMP and other peptides are highly present (and stored) in neutrophils and macrophages, and to a lesser extent in monocytes and lymphocytes (Gombart et al., 2007; Kosciuczuk et al., 2012; Schwalfenberg, 2010); they are also present in epithelial or skin

cells, respiratory cells, bone marrow cells, and gastrointestinal cells (Kosciuczuk et al., 2012).

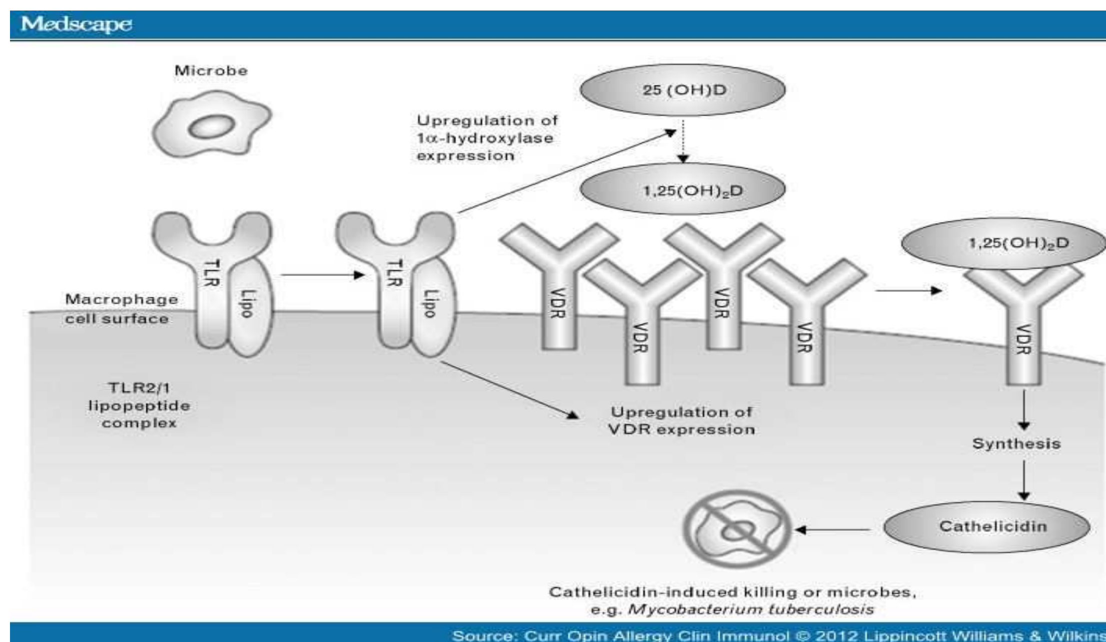

Figure 3. From Abuzeid, Akbar, & Zacharek (2012), available at http://www.medscape.com/viewarticle/757649_4, originally reprinted from "Vitamin D and musculoskeletal health" by Wolff, A.E., Jones, A.N., & Hansen, K.E. 2008, *Nature Clinical Practice Rheumatology*, *4(11),* p.585.

CAMP production and expression is highly reliant on the availability of sufficient serum Vitamin D, sufficient VDR, and CYP27B1 as prerequisites for conversion to bioactive Vitamin D that is necessary for optimal immune cellular functions (Beard et al., 2011; Liu et al., 2006; Wang et al., 2004)- most important of which is killing the microbes. A study by Jeng et al. (2009) demonstrated that critically ill patients with sepsis had high prevalence of Vitamin D deficiency/insufficiency that was associated with low levels of cathelicidin (LL-37); they concluded that such association between low LL-37 levels and low Vitamin D levels -(measured also by number of DBP- Vitamin

D binding protein, the carriers of 25(OH)D in the circulation), could reflect the importance of treating Vitamin D insufficiency/deficiency to prevent systemic inflammation in critically ill patients and to enhance their antimicrobial defenses.

Likewise, in response to invaders, macrophages make use of Vitamin D to synthesize cathelicidin – the only antimicrobial peptide synthesized in humans with wide spectrum against bacteria, fungus and viruses (Abuzeid, Akbar, & Zacharek, 2012). Neutrophils and some epithelial cells use Vitamin D to produce proteins (cytokines) needed for specific responses based on DNA transcription unlocked by Vitamin D response elements (VDREs) (Abuzeid et al., 2012). Moreover, the innate immune responses mediated by $1,25(OH)_2D$ include enhanced macrophage activity and phagocytosis (Wang et al., 2004; White, 2012). Vitamin D deficiency would disrupt the macrophages antimicrobial functions (reduced phagocytosis and tumor cell-cytotoxicity) and would suppress the innate immune response (Baeke et al., 2010b). Similarly to macrophages, dendritic cells (DCs) also have 1α-hydroxylase enzymes that locally produce $1,25(OH)_2D$, which in turn is capable of reducing the inflammation milieu and of regulating immune responses (Baeke et al., 2010b; Liu et al., 2006). Vitamin D-mediated cathelicidin production is responsible for modulating antigen presentation by macrophages, monocytes and dendritic cells, the so-called antigen presenting cells or APCs (which highly express VDR); eventually, sufficient Vitamin D or Vitamin D supplementation would lead to inhibition of DC maturation, suppression of antigen presentation, and promotion of T-cell responses (in adaptive responses) (Hewison, 2012).

Additionally, Liu et al. (2006) found that TLRs of activated monocytes increased the number of VDRs and CYP27B1 and expressed more CAMP- provided there was sufficient amount of circulating Vitamin D. The authors referred to a very important finding that, in African Americans, the activated monocytes and their TLRs produce less CAMP as compared to Caucasian counterparts. Such finding would reflect the indigenous low levels of Vitamin D or deficiency secondary to low skin synthesis of Vitamin D (melanin being a UV filter) and/or to poor dietary intake of Vitamin D rich foods. In brief, Vitamin D deficiency would result in lower CAMP production (Baeke et al., 2007) and would down-regulate immune response. Ultimately, supplementation can be considered an appealing and a promising method that can raise serum Vitamin D and improve antimicrobial action through instigating more CAMP production. Kamen and Tangpricha (2010) summarized the mechanism of Vitamin D on the innate immune system in the following figure (See Figure 4).

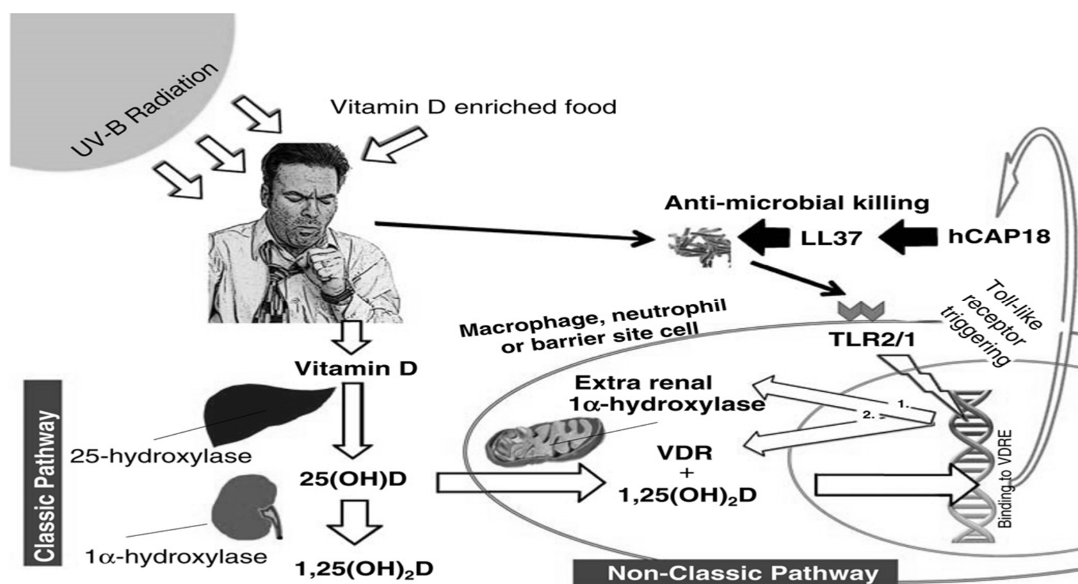

Figure 4. Adapted from “Vitamin D and molecular actions on the immune system: Modulation of innate and autoimmunity” by Kamen, D.L., & Tangpricha, V. (2010). *Journal of Molecular Medicine*, *88(5)*, p. 442. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2861286/?report=classic

Vitamin D and the Adaptive Immune Response:

In adaptive immunity, antigen presenting cells APCs, such as DCs and monocytes, are again considered targets for VDR compounds through which Vitamin D regulates T cell-mediated responses (with T cells subsets: Th1, Th2, Th17, & T regulatory cells; Gunville et al., 2013). T cells or lymphocytes refer to CD4 and CD8 cells. The T lymphocytes (CD4 & CD8), B-lymphocytes, and macrophages also express VDR (Kamen & Tangpricha, 2010). Deluca and Cantorna (2001) reported that CD4 T cells and macrophages possess small yet worthwhile quantity of VDR (at resting without stimulation), and mature CD8 have the highest VDR concentrations (B lymphocytes have minimal non-detectable concentration of VDR). Once activated, T and B cells increase their VDR expression that impacts their proliferation or differentiation (Mahon, Wittke,

Weaver, & Cantorna, 2003; Prietl et al., 2013). When Vitamin D is sufficient, the number of VDRs increases by five times to respond to excessive T cell differentiation (Kamen & Tangpricha, 2010).

The innate response regulates the adaptive response upon detecting danger. Once TLRs in the innate immune cells APCs detect invading antigens, these APCs (especially DCs) capture and present the antigens to T & B cells to start an adaptive response. As a result, the B-lymphocytes secrete immunoglubulins to kill the microbe antigens presented to them by APCs i.e., macrophages and dendritic cells (Lappe, 2011). On the other hand, the T lymphocytes get stimulated and differentiate into T helper cells, mainly Th1, Th2, and Th17 cells, and start releasing cytokines (Adams & Hewison, 2010; Baeke et al., 2010c; Beard et al., 2011). These cytokines have antigen receptors that sense the presence of pathogens and send signal to the immune system to start an antigen-specific immune reaction and protect the inside cellular environment (T-cellular compartment) against invaders (Beard et al., 2011; Cantorna et al., 2004).

Th1 cytokine is pro-inflammatory and releases interleukin IL-2, Interferon IFN γ, and tumor necrosis factor TNF-α; Th1 usually ensues cell-mediated responses related to intracellular invasion of viruses and tumors (Cantorna et al., 2004). On the contrary, Th2 cytokine is more anti-inflammatory (and anti-allergic) and it secretes interleukins IL-3, IL-4, IL-5, IL-10 (Adams & Hewison, 2008; Gunville et al., 2013) and it usually initiates antibody-mediated responses to extracellular pathogens such as bacteria and parasites, or antigens from the environment such as allergic antigens (Cantorna et al., 2004). Usually, Th1 and Th2 cell responses regulate each other in a way that secures a normal adaptive

response. However, upon provocative invasion to the immune system, any disequilibrium in Th-cell responses would result in either a Th2-driven good outcome (fighting infection) or a Th1 & Th17-driven negative outcome (e.g., higher susceptibility to developing autoimmunity, diabetes type-1, multiple sclerosis, & asthma) (Cantorna et al., 2004).

As an immunomodulator, 1,25$(OH)_2$D is thought of as a safeguard of immune homeostasis (Baeke et al., 2010a). However, in adaptive immune response, 1,25$(OH)_2$D is implicated in more suppressive or inhibitory functions as compared to its stimulating functions in innate responses (Hart et al., 2011). Since antigen stimulation to T-cells leads to increased differentiation into different Th cells phenotypes, 1,25$(OH)_2$D responds to inflammation by the following: suppressing excessive T lymphocytes proliferation; blocking or inhibiting the induction of inflammatory cytokines Th1 and Th17 while favoring the expression of cytokine phenotype Th2 and the development of T regulatory cells in DCs; inhibiting inflammation-mediated by monocytes, especially by tumor necrosis factor (TNF-α) and Th1 (Baeke et al., 2010b; Beard et al., 2011; Penna et al., 2005); and finally, intercepting B lymphocytes proliferation and differentiation into memory cells, blocking their immunoglobulin antibody production, and promoting their apoptosis (Baeke et al., 2010a; Chen et al., 2007; Youssef et al., 2011)- such actions are well appreciated in terms of preventing autoimmune diseases (Prietl et al., 2013).

At the same time, DCs play an important role in adaptive responses and remain direct targets for 1,25(OH)2D. Therefore, 1,25(OH)2D modulates DCs and inhibits their maturation and differentiation, and changes the DC-derived cytokine phenotype

expression through inhibiting Th1 & Th17 and elevating anti-inflammatory Th1 & IL-10 cytokines (Baeke et al., 2010a). These Vitamin D-modulated and IL-10- induced DCs develop T regulatory cells, which main function is to suppress and resolve infections (Baeke et al., 2010a) through halting cellular damage and diminishing the excessive immune responses and the pro-inflammatory milieu effects (Kamen & Tangpricha, 2010; Overton & Yin, 2011; Prietl et al., 2013). This is considered a triumphant role of Vitamin D in suppressing the overzealous adaptive immune responses and reflects its suppressive and protective mechanism against pathogens, especially in autoimmune diseases (Lappe, 2010; Prietl et al., 2013) and HIV (Spector, 2009; 2010). Figure 5 summarizes the main modulatory effects of Vitamin D on the adaptive immune system.

Vitamin D- or VDR-deficient hosts have elevated Th1 cell-associated responses and decreased Th2 cell-associated responses. Vitamin D sufficiency and the consequent presence of sufficient VDRs enable Vitamin D to skew the T cellular compartment's inflammatory status and to suppress T helper cells proliferation (e.g., Th1 and Th17) (Kamen & Tangpricha, 2010).

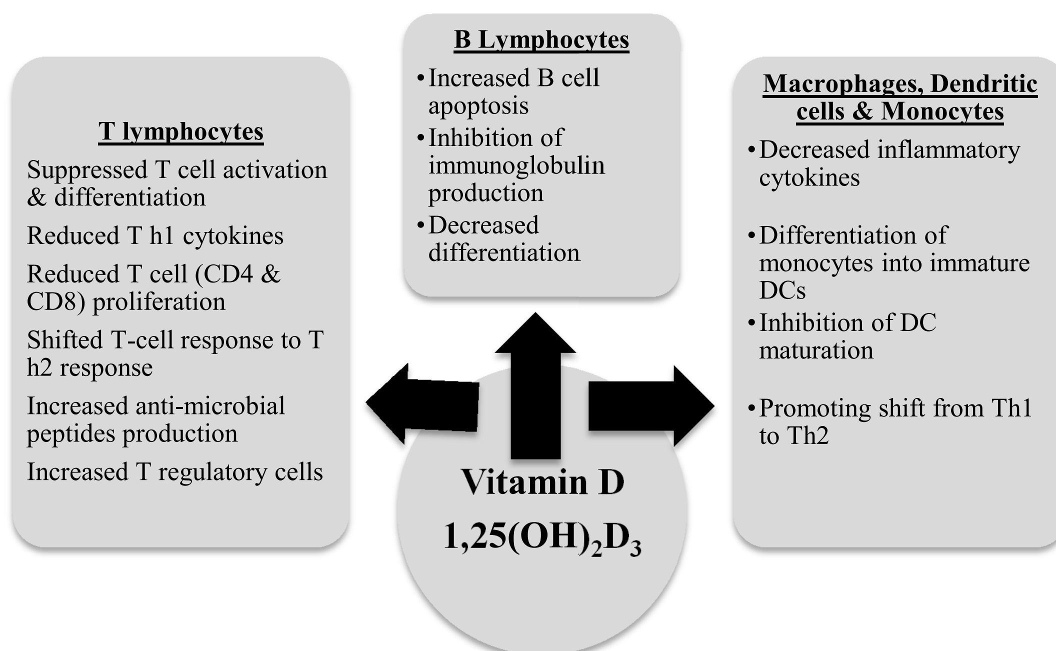

Figure 5. Main Immune-modulating Effects of Bioactive Vitamin D (1,25(OH)$_2$D$_3$) on Immune Cells.

Prevalence of Vitamin D Deficiency in HIV

Most published studies have focused on Vitamin D deficiency in HIV and its significant role in skeletal health. The negative implications of Vitamin D deficiency on bone diseases (e.g., osteoporosis, osteomalacia, and osteopenia) and the consequent reduced bone mineral density were highlighted in the literature as distinctive marks of HIV infection metabolic complications (Villamor, 2006). There are several factors that can alter Vitamin D metabolism and can contribute, consequently, to Vitamin D deficiency or insufficiency in HIV patients; these might include: limited sun exposure; low Vitamin D intake from food; altered Vitamin D absorption, activation, or metabolism secondary to coexisting clinical conditions; and the HIV highly active antiretroviral

treatment HAART side effects (Cozzolino et al., 2003), especially non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Griffin & Arnold, 2012).

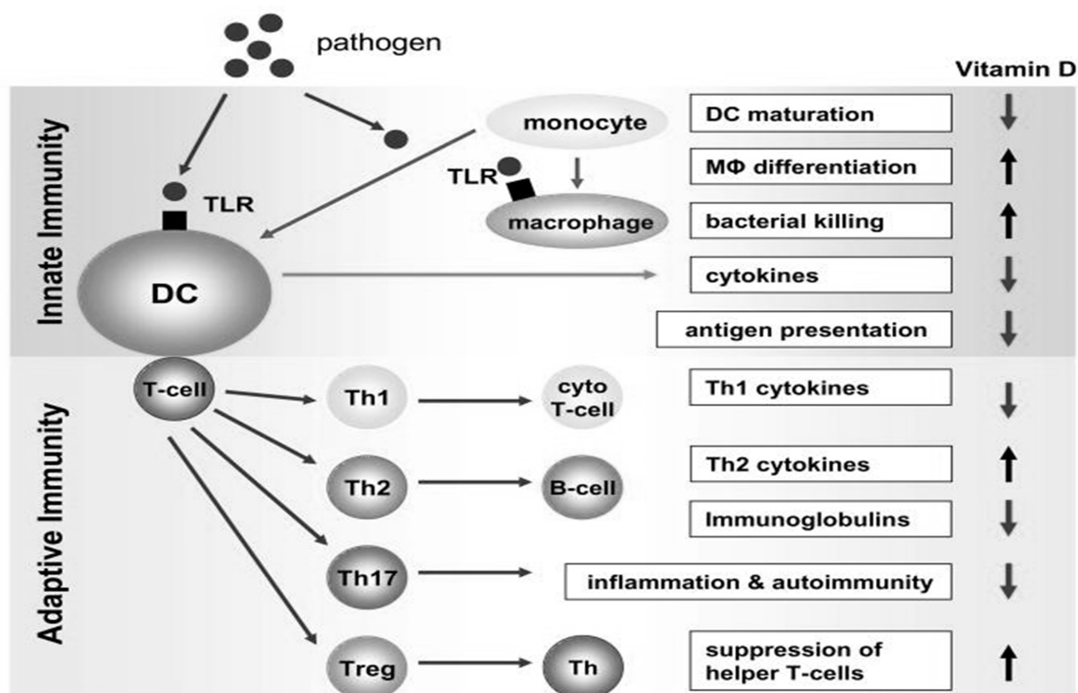

Figure 6. Adapted from “Vitamin D and the immune system: New perspectives on an old theme” by Hewison, M. (2010). *Endocrinol Metab Clin North Am*, *39(2)*, p. 379. Online at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2879394/pdf/nihms180153.pdf
Abbreviations: TLR, toll like receptor; DC, dendritic cell, Mφ, macrophage; T-cell, T-lymphocyte; cyto T-cell, cytotoxic T-cell; B-cell, B-lymphocyte; Treg, regulatory T-cell.

In-depth systematic reviews of several studies have demonstrated that Vitamin D deficiency status seems to be more pronounced among HIV patients (Giusti et al., 2011; Overton & Yin, 2011; Tafazoli & Khalili, 2013; Villamor, 2006). There is still ongoing research to understand why HIV patients have more Vitamin D deficiency compared to others- ranging from 70.3 to 89 percent (Dao et al., 2011; Viard et al., 2011). Besides the effects of non-HIV related factors (e.g., gender, older age, winter season, low Vitamin D dietary intake, dark skin, and reduced sun exposure), some studies pinpointed to a

possible defect in kidney hydroxylation of Vitamin D as a possible contributing factor to deficiency in HIV; the inadequate levels of renal 1-alpha hydroxylase enzyme (CYP27B1), essential to produce active Vitamin D metabolite, could derive from the influence of pro-inflammatory cytokines and/or from effects of ART intake (Haug et al., 1998; Mueller et al., 2010; Villamor, 2006; Welz et al., 2010).

Advanced HIV infection or progression to AIDS by itself may lead to an "inflammation-related impairment of 1-α hydroxylation" (Mueller et al., 2010, p. 1132) that can be associated with immunological hyperactivity as shown by Haug et al. (1994, 1998). Cervero et al. (2012) found a high prevalence of Vitamin D deficiency in a cohort of Spanish HIV-infected patients that exceeded by 16.4 percent that of non-HIV adults. A French study by Allavena et al. (2012) also found that 86.7% of their cohort (n = 2994) had Vitamin D deficiency (31.1%) or insufficiency (55.6%). In an Iranian study on adult HIV patients, Vitamin D deficiency reached 86.7 percent (Etminani-Esfahani et al., 2012). Bang et al. (2010) reported a 95 percent Vitamin D deficiency in a cohort of Danish male patients. Likewise, the EuroSIDA study, the largest on HIV in Europe, reported an 89 percent rate of Vitamin D deficiency (23.7% of which had severe deficiency < 10 ng/μL and 65.3% between 10 ng/μL & 30 ng/μL) in a cohort of 1985 individuals and associated such high prevalence with greater risk for AIDS and mortality (Viard et al., 2011).

In contrast, some studies found that this high prevalence was not any different than in the general population or in comparison groups. For instance, results from the SUN study (Study to Understand the Natural History of HIV and AIDS in the Era of

Effective Therapy) by Dao et al. (2011) showed a high prevalence of Vitamin D deficiency (70.3 percent, 95% CI, 68.1%-74.9%) among a cohort of HIV adults (*N* = 672) in the U.S. that is comparable to that of the U.S. general adult population based on NHANES data 2003-2006 (79.1 percent, 95% CI [76.7-81.3]). A study by Ormesher et al. (2011) reported that Vitamin D deficiency was less prevalent among HIV patients as compared to the general population, similar to the findings in a study by Yin et al. (2010) on postmenopausal women with HIV as compared to the general population. Likewise, the Women's Interagency HIV study (WIHS)- one of the largest prospective US study on women with HIV- found a Vitamin D deficiency prevalence of 60 percent among women (mostly African American) with HIV as compared to 72 percent among HIV negative women, $p < 0.001$ (Adeyemi et al., 2011). Interestingly, Adeyemi and colleagues' study (2011) found a positive association between Vitamin D deficiency and low CD4 count < 200 cells/μL. However, despite these contradicting results in diverse studies, meta-analyses and many reviews discussed throughout this study have confirmed one thing: Higher prevalence of Vitamin D deficiency in HIV populations as compared to non-HIV people.

Vitamin D deficiency and ART:

It is indisputable that ART has revolutionized the perception of HIV and changed the fatal scenario that used to be associated with HIV diagnosis; it has led to a significant decline in mortality, and resulted in prolonged longevity that made HIV another chronic disease readily modulated or influenced by Vitamin D (Mueller et al., 2010). Some ART impair Vitamin D metabolism and results in Vitamin D deficiency (Cozzolino et al.,

2003). For instance, protease inhibitors or PIs inhibit the two major enzymes activities in Vitamin D metabolism, 25-hydroxylase and 1-alpha-hydroxylase, in a reversible and dose-dependent manner resulting in reduced active Vitamin D production (1,25$(OH)_2D_3$) (Poowuttikul et al., 2013).

ART is presented here in this study as a potential contributing risk factor to Vitamin D deficiency. As far as treatment is concerned, Bang et al. (2010) and Van Den Bout-Van Den Beukel et al. (2008) found no difference in the Vitamin D deficiency status between HIV patients on ART and those naïve not-treated patients, in contrast to some cross-sectional studies that associated Vitamin D deficiency with some types of ART such as Efavirenz (Conesa-Botella et al., 2010; Dao et al., 2011; Fox et al., 2011; Pasquet et al., 2011; Welz et al., 2010; Wiboonchutikul et al., 2012). Paul et al. (2010) reported a higher prevalence of Vitamin D deficiency among Indian patients using HAART (74 percent) as compared to untreated patients (37 percent) or non-HIV controls (37 percent).

Efavirenz, a NNRTI, stimulates an increase in 24-hydroxylase enzyme or CYP450 (also called CYP24A1) that counteracts the effects of CYP27B1 or α-hydroxylase; this leads to increased catabolism of 25(OH)D and of active 1, 25$(OH)_2D_3$ and to their conversion into the inactive form 24,25$(OH)_2D$ that gets excreted in the urine (Overton & Yin, 2011; Van Den Bout-Van Den Beukel et al., 2008; Vescini et al., 2011; Welz et al., 2010)- thus leading to further Vitamin D deficiency. A type of ART called Tenofovir or TDF increases serum parathyroid hormone levels (PTH); it is known that Vitamin D deficiency coupled with high PTH are implicated in reducing bone density in

HIV patients on ART and increase their risk for fractures (Tafazoli & Khalili, 2013)- that makes a good reason why Vitamin D supplementation is essential for HIV patients on ART even beyond bone health.

In this context, the MONET trial found that patients exhibited higher levels of Vitamin D after stopping NNRTIs and shifting to PIs (Fox et al., 2011). Similarly, a large scale Spanish study by Cervero et al. (2013) reported that using boosted PI monotherapy was associated with lower risk for Vitamin D deficiency/insufficiency as compared to using NNRTIs or no treatment (OR = 0.08, 95% CI [0.01-0.6], p = 0.018). Interestingly, Mueller et al. (2010) admitted the high prevalence of Vitamin D deficiency among patients receiving combined ART (cART) but could not find conclusive results on the net clinical effects of using NNRTIs or PIs (Tenofovir-TDF) on Vitamin D levels and recommended further studying.

On the other hand, in a large Italian cohort (N = 810 patients that contributed 1408 Vitamin D measures), Vescini et al. (2011) reported higher Vitamin D deficiency prevalence among NNRTIs users as compared to PI users, and an overall higher deficiency among HIV patients as compared to the general population. The authors highlighted the hypothesis that the HIV virus and/or cART impair Vitamin D metabolism, in addition to the interference of confounding variables that differ between HIV positive and HIV negative subpopulations. It was noteworthy that Vescini et al. (2011) found that Vitamin D deficiency was predictive of HIV disease progression (AIDS), in addition to its association with the occurrence of negative health events (such as diabetes, cardiovascular or kidney diseases-related events). They also concurred with

Mueller et al. (2010) findings about the inflammation-related impairment of 1 α-hydroxylation phenomenon associated with the virus and/or with cART effects.

In view of the discrepancies in ART effects on Vitamin D deficiency, some studies could not find significant association between Vitamin D deficiency and ART regimen or HAART due to the heterogeneity of treatments, as shown in Rustein et al. (2011); however, others investigated the controversies about the deleterious effects of ART (Theodorou et al., 2013). In a retrospective cohort of 2044 HIV patients in Belgium, the researchers associated Vitamin D deficiency (89.2%, of which 32.4% < 10ng/mL) with longer duration of ART treatment, and with the use of sequential complex treatment modalities that include combinations of NNRTIs and PIs (Theodorou et al., 2013). Theodorou et al. (2013) reached the same conclusion as Mueller and colleagues' (2010) and all other studies in the literature that pinpointed (or incriminated) NNRTIs use as compared to PIs. Patients treated with a combination of two or three NNRTIs had significantly lower Vitamin D levels as those treated with a combination of NNRTIs plus PI; but untreated patients exhibited statistically significant higher Vitamin D levels as compared to treated patients (15.1 ng/μL vs. 12.8 ng/μL, respectively, $p = 0.0003$) (Theodorou et al., 2013). Similarly, Brown and McComsey (2010) found that within a year of ART initiation, there was a statistically significant reduction in serum Vitamin D levels among patients using Efavirenz (prevalence ratio of 1.8; $p = 0.007$) as compared to non-EFV users (mostly PI users).

In the famous large prospective SUN study, Dao et al. (2011) reported that the use of cART in general increased the odds of Vitamin D deficiency and insufficiency

especially among those using Efavirenz (*OR* =1.98, 95% CI [1.18-3.34]) as compared to patients not receiving ART. Pinzone et al. (2013) emphasized the need for large prospective studies that mainly aim to test the existence of a causal relationship between ART and Vitamin D deficiency instead of relying on controversial results from studies that used cross-sectional design. Additionally, there is a need to study the impact of Vitamin D supplementation on reversing the detrimental side effects of some ART drugs on Vitamin D levels.

CD4 Cells in HIV Infection: Preamble to Vitamin D and CD4 Relationship

The previous sections so far and some sections in Chapter 1 discussed the implications of Vitamin D deficiency in general and in HIV, and highlighted its modulating actions on the immune system and on the HIV course of infection. To recap, the HIV virus- despite treatment and continuous immune defenses- shows high resistance and persistence in lingering on immune cells (T and B lymphocytes), mainly on antigen-presenting cells or APCs such as dendritic cells, monocytes, and macrophages (Campbell & Spector, 2011; Deluca & Cantorna, 2001). In order to secure its survival, the virus itself sabotages the host cell machinery in a way to enhance its own replication and differentiation, and at the same time, it suppresses toll-like receptor (TLR) signaling and activation against its replication (Campbell & Spector, 2011). This ultimately results in increased viral replication and in gradual decline in the number of CD4 cells.

To recap, as discussed previously, Vitamin D induces the following main actions in HIV: producing cathelicidin to combat pathogens that can lead to inhibiting and delaying HIV infection events (Spector, 2009); suppressing viral replication; allowing

HIV infected CD4 to survive longer than bystander or uninfected CD4 cells (i.e., deferring their programmed cell death)- thus delaying HIV disease progression (Campbell & Spector, 2012; Spector, 2011); inhibiting dendritic cell maturation and differentiation; controlling autophagy (cellular disintegration or degradation) in infected CD4 T-cells; enhancing phagocytic activity of macrophages (such as increasing production and maturation of cytokines); and increasing natural killer cells (NK) to boost host's defense antiviral response and reduce HIV viral replication (Campbell & Spector, 2012; Walker & Modlin, 2009).

Of particular interest is the role of VDR in the course of HIV. Vitamin D as 25(OH)D has little or no interaction with VDRs, while 1,25(OH) $_{2D}$, the active metabolite, elicits the modulatory actions of Vitamin D and induces VDR-activated responses (Prentice, Goldberg, & Schoenmakers, 2008). Campbell and Spector (2011) were the first to demonstrate a novel role for Vitamin D and VDR-driven responses; their study demonstrated that the active Vitamin D metabolite could be an autophagy inducer in macrophages, which in turn, can inhibit viral replication in these macrophages. The authors called for studies to understand more this autophagy-dependent molecular mechanism through which Vitamin D mediated inhibition of viral replication in HIV. At the same time, structural mutations and polymorphism in VDR gene influences susceptibility to HIV infection (and to other infectious diseases such as TB or leprosy) by reducing VDR functions, inducing immune hyper-stimulation and Vitamin D signaling, and promoting disease progression (De la Torre et al., 2008; Nevado, Tenbaum, Castillo, Sanchez-Pacheco, & Aranda, 2007; Van Den Bout-Van Den Beukel et al., 2008).

Subsequently, Vitamin D deficient patients have diminished number of VDR, and that in fact cripples the VDR-driven responses in stimulated cells (Walker & Modlin, 2009). These HIV patients would be more likely to witness an increase in their susceptibility to opportunistic infections, earlier occurrence of chronic diseases, as well as higher incidence of multiple organ systems complications as compared to non-deficient patients (Giusti et al., 2011; Villamor, 2006).

In the course of HIV infection, the whole immunological mechanism on how Vitamin D deficiency affects HIV outcomes remains less clear and ambiguous, despite the numerous studies carried out in this field. A major study by Haug et al. in 1994 was the first to pinpoint to a correlation between low serum concentration of $1,25(OH)_2D$ and low CD4 counts and a corresponding shorter survival rate in HIV patients with advanced disease as compared to controls. Later, more in-vivo and in-vitro studies in 1980s and 1990s have highlighted the modulating effects of Vitamin D in HIV, and demonstrated how Vitamin D deficiency impaired cellular immunity and compromised patients' immune defenses (Haug et al., 1998). Such findings encouraged more research undertakings to explore to what extent $1,25(OH)_2D$ affect immune cells in diseases such as HIV and influence the course of disease.

As previously discussed, HIV virus mainly attacks macrophages, dendritic cells, and CD4 T-cells. Upon acquiring infection and transmission, the HIV viral glycoprotein (GP-120) binds to CD4 (cluster of differentiation 4) molecules on the surface membrane of dendritic cells, macrophages, and CD4 T cells. In fact, the CD4 molecules act as the primary target surface receptors and fuse with infected cells; this fusion creates a port of

entry that allows entrance of the virus into the cell membrane of immune cells. Once virus releases its viral core into the cell membrane, HIV starts replicating (building viral load) and spreading to lymph nodes and to blood circulation, followed by a spread to different body organs (Barlett, 2010; Simon, Ho, & Abdool Karim, 2006). After a series of immune T-cells activation and division, the virus destroys and depletes these CD4 receptors in T-cells expressing CD4- making way for disease to progress to AIDS (Giusti et al., 2011; Grossman et al., 2002). At the same time, besides destroying activated T cells, chronic immune activation in HIV also destroys, to a great extent, naïve (resting) CD4 and memory CD4 cells as a consequence of the high activation and turnover rate (Douek, Picker, & Koup, 2003; Hazenberg et al., 2003; Miedema et al., 2013; Simon et al., 2006) or burst-like activation of T cells (Grossman et al., 2002). As the CD4 cells react to their HIV infection by multiplying, they are making more copies of the virus itself, and paradoxically, they get gradually destroyed and depleted. Some of these cells die progressively from programmed activation-induced cell death or apoptosis (discussed earlier) (Grossman et al., 2002; Spector, 2010).

Accordingly, CD4 count (or CD4 percent) is considered the most plausible biological marker of the immune health in HIV (Hogg et al., 2001; Lodi et al., 2013; Miedema et al., 2013), reflecting the degree of CD4 cells destruction by HIV (immunodeficiency), and at the same time, the low degree of proliferation of CD4 T cells (Miedema et al., 2013). CD4 count is commonly used for HIV disease classification, along with emergence of AIDS-related clinical events (CDC, 2011c). The optimal CD4 count ranges from 500–1500 cells/μL. A CD4 count below this normal range reflects the

degree of damage to the immune system and extent of immunosuppression. According to the CDC guidelines (2011c), clinical HIV staging based on CD4 counts in the absence of AIDS-related events is the following: CD4 count ≥ 500 cells/μL (CD4 percent ≥ 29%) indicates a stage 1 HIV disease; a CD4 count of 200-499 cells/μL (CD4 percent 14-28%) represents stage 2 HIV disease or moderate immune suppression; while CD4 count < 200 cells/μL or a CD4 percent < 14% indicates stage 3 or disease progression and AIDS diagnosis (CDC, 2011c).

In 2013, a panel from the Department of Health and Human Services (DHHS) reviewed the guidelines with regard to ARV (same as ART) initiation and CD4 count. The panel recommended ART for all HIV patients with the goal of reducing risk for disease progression and preventing HIV transmission. It is urgently and highly recommended for patients with CD4 count < 350 cells/μL to start treatment; and CD4 count can be restored to normal values in treated patients (CDC, 2011c; DHHS, 2013).

Besides CD4 count, there are other immune parameters that are taken into consideration when evaluating HIV course of infection. Viral load or HIV RNA level denotes the number of viruses in the host's circulation. Giusti et al. (2011) presented a car analogy in order to simplify how CD4 and viral load operate together; the authors symbolized CD4 as the distance from immunosuppression or how far from AIDS, while viral load designated the speed of movement towards disease progression or AIDS. The higher the viral load, the faster the CD4 depletion; therefore, HAART is used to lower viral load and to slow down the infection process in a way to allow some CD4 recovery or repletion (Giusti et al., 2011).

Eventually, viral load can be used as a measure that predicts risk of viral transmission, knowing that viral load registers very high levels (10^6 to 10^7 copies per mL) in two instances: acute infection and advanced/progressed infection (Simon et al., 2006). Viral load is used as an adjunctive measure to CD4 to evaluate the degree of HIV suppression; both markers gauge disease severity and progression, and predict mortality and survival (Hogg et al., 2001). In addition, both CD4 count and HIV RNA levels are considered upon treatment initiation and are closely monitored to determine the effectiveness of HIV medical management using ART (Giusti et al., 2011; Hogg et al., 2001). Clinically, it is used to test the effectiveness of ART therapy in reducing the viral load to a non-detectable level (DHHS, 2013). The optimal undetectable viral load level should be less than 400 copies/mL (Giusti et al., 2011).

CD4 Count and Vitamin D: A Controversial Relationship

No one can doubt the immune modulatory effects of Vitamin D on the innate and adaptive immune system. However, a large body of HIV literature has presented contradicting results on the relationship between CD4 count -as the main biomarker of immune function- and Vitamin D. While some studies described an association or correlation, others failed to demonstrate any significant relationship. Most of the studies involving Vitamin D and CD4 counts aimed to assess HIV disease progression, such as the work done by Haug (1994), Hogg et al. (2001), Sudfeld et al. (2012), Viard et al. (2011), and Mehta et al. (2010). Most of these studies examined the association between Vitamin D deficiency and CD4 count in multivariate analyses not as an endpoint by itself, but as a mean to explain factors predisposing or contributing to HIV disease

progression. Consecutive studies focused more on testing the association between Vitamin D and CD4 count under the realm of Vitamin D supplementation. On the whole and regardless of their main objectives, most of the studies that examined Vitamin D deficiency and its association with CD4 count provided conflicting results, but were predominantly observational and subject to confounding of some sort.

Vitamin D & CD4 Count: Correlation? No Correlation?

A cross-sectional study by Stein et al. (2010) showed a weak but significant correlation between higher serum Vitamin D levels and higher CD4 counts in a convenience sample of HIV positive minority (AA) postmenopausal women on ART ($N = 68$; $r = 0.32$, $p < 0.01$). The authors emphasized that such correlation could underline a better immune competence and function (denoted by CD4 repletion) and better general health secondary to higher Vitamin D levels. The same study reported comparable results with regard to serum 25(OH)D and $1,25(OH)_2D$ levels among women on ART, women on different ART regimens, and women not on ART (control group). However, serum 25(OH)D level should be considered because it is a better indicator of Vitamin D available for later immune functions as compared to the short-lived hormone in the blood $1,25(OH)_2D$ (Holick et al., 2011). Knowing that higher 25(OH)D level does not necessarily reflect higher $1,25(OH)_2D$ level or vice versa (Rodriguez et al., 2009; Stephensen et al., 2006). In other words, Vitamin D deficiency has no effect on $1,25(OH)_2D$ levels (Childs et al., 2012).

In their quest to reveal the role of micronutrients in HIV disease progression, De Luis et al. (2002) did a study in Spain on 119 HIV patients (mean age 37.9 ± 9.9 years)

that aimed to compare the correlation between dietary micronutrient intake including Vitamin D and the immune status as denoted by CD4 count in Vitamin D deficient versus Vitamin D insufficient. The researchers found a significant positive association between high Vitamin D intake and high CD4 counts; the consecutive multivariate analysis (after adjusting for age, sex, energy and protein intake, and ART) showed that each one microgram of Vitamin D intake correlated with a 34 points increase in CD4 count (95% CI [5.81-167.3], $p < 0.001$). Such result clearly portrayed Vitamin D as an independent predictor of CD4 count. However, this study used a combination of vitamin A and D. Therefore, the small scale of the study and its cross-sectional design along with the accompanying threats to validity, warrant the need for further research investigation to delineate the distinctive effect of Vitamin D on CD4 count.

Another study by Ross et al. (2011) sought to explore the association between Vitamin D levels and CD4 count in a group of HIV patients on ART with Vitamin D insufficiency ($n = 149$). The researchers hypothesized that such association existed knowing the immune modulator effects of Vitamin D on the overall immune system and on CD4 cells. Their results indicated a positive association between an increase in Vitamin D level that corresponded with a significant change in CD4 counts (current CD4 count minus lowest or nadir CD4 count) ($p < 0.01$). From a clinical perspective, the authors emphasized the potential adjunctive role of Vitamin D supplementation in restoring the immune function (increasing CD4 count) of patients starting ART (Ross et al., 2011). There is a great need for further research to find an indisputable answer to the issue of association between Vitamin D status and CD4 count before or parallel to

embarking on studying the clinical benefits of Vitamin D supplementation in deficient HIV patients.

Upon examining the factors associated with Vitamin D deficiency in a large sample of HIV patients ($n = 2044$) in Belgium, Theodorou et al. (2013) compared median Vitamin D concentrations according to CD4 count, viral load, and ART type. It was quite interesting to find a significant correlation between Vitamin D deficiency and low CD4 counts (similar to Stein et al's study in 2010). Based on data from all Vitamin D deficient patients taking different treatment modalities, the analysis showed that a median 25(OH)D level of 11.5 ng/µL significantly correlated with a median CD4 count < 200 cells/µL as compared to median 25(OH)D of 14.1 ng/µL that correlated with median CD4 count > 200 cells/µL ($p = 0.0003$). The authors pinpointed to their major finding on severe Vitamin D deficiency (< 10 ng/mL) and its association with low CD4 counts (< 200 cells/µL) and called upon the need to consider Vitamin D supplementation to overcome and to prevent the detrimental effects of deficiency in HIV patients.

In a large-scale French study by Legeai et al. (2013), the researchers examined the immunological markers (in addition to metabolic and inflammatory markers) associated with Vitamin D deficiency in a cohort of 355 recently diagnosed young adults HIV patients (70 percent males; 43 percent Black) not yet on ART (ART naïve). In this cross-sectional COPANA study, the researchers reported a 93 percent prevalence rate of Vitamin D insufficiency (< 30 ng/µL), 67% of which referred to Vitamin D deficiency (< 20 ng/µL), and 24% referred to severe Vitamin D deficiency (< 10 ng/µL). The cohort median CD4 count at enrollment was 300 cells/µL (*IQR*: 173-463); however, median

CD4 counts were significantly lower among Black patients (245 cells/µL) as compared to Whites (344 cells/µL) ($p < 0.001$). Severe Vitamin D deficiency was associated with significant immune suppression in 18 percent of patients with a CD4 count < 100 cells/µL as compared to 10.7 percent of those with Vitamin D levels >10 ng/µL ($p < 0.04$). This study proved a significant association between severe Vitamin D deficiency and low CD4 count (< 100 cells/µL) without any metabolic influence from ART. However, although it provided further evidence that linked Vitamin D deficiency to HIV disease progression, still, this non-causative association diminished to a certain extent due to an intervening role of some inflammation markers in the analysis (e.g., high TNF-α & IL-6).

In a novel cross-sectional study by Aziz et al. (2013), the researchers investigated the influence of HAART initiation on CD4 recovery (immune reconstitution) in 204 women (60 percent Black) with advanced HIV participating in the Women's Interagency HIV Study (WIHS). About 89 percent of the women had Vitamin D insufficiency or deficiency < 3o ng/µL; these women were older than 38 years ($p = 0.04$), most likely Black ($p = 0.0001$), and had higher BMI ($p = 0.002$) as compared to women with Vitamin D sufficiency, but had comparable CD4 counts (nadir count or lowest registered) and viral load before HAART initiation ($p > 0.05$). After HAART initiation, the researchers investigated CD4 count recovery at six, twelve, and twenty-four months; they ran logistic regression that took into consideration Vitamin D status (< 30 & > 30 ng/µL), ethnicity, BMI, prior ART use (ART naïve or no), viral load pre-HAART, and undetectable viral load at 24 months. The difference in mean CD4 count gain or recovery from pre-HAART

to post-HAART according to Vitamin D status (insufficient/deficient versus sufficient) was not significant ($p > 0.05$), unlike the results of the study done by Ross et al. (2011), which could imply methodological discrepancies in measuring CD4 counts. It should be noted that the CD4 count results were compared against baseline pre-HAART Vitamin D levels, and the researchers justified that other studies of longitudinal nature also based their analyses on baseline Vitamin D levels. The authors concluded that the impaired CD4 count reconstitution after HAART associated with Vitamin D insufficiency/deficiency could be due to the impaired modulatory effects of Vitamin D that led to suppression of T-cell activation and production- especially of naïve CD4 cells (Aziz et al., 2013).

In their retrospective study aiming to examine the predictors of Vitamin D deficiency in a convenience sample of children and young adults with perinatal acquisition of HIV ($n = 81$; mean age 13.8 ± 4.1 years) seen at one clinic, Rustein et al. (2011) compared their Vitamin D status and associations with predictors against another sample of healthy subjects ($n = 372$). In the HIV sample, 83 percent were Black, 54 percent had advanced clinical stage (immunosuppression) denoted by nadir CD4 count < 200 cells/μL, a great majority were receiving HAART, and a little more than half had almost undetectable viral load (HIV RNA< 400 copies/mL). Like other studies, the prevalence of severe Vitamin D deficiency (marked at <11 ng/μL) was significantly more pronounced in the HIV group (36% vs. 15% in controls, $p < 0.0001$); about 56% of HIV patients had Vitamin D deficiency < 15 ng/μL as compared to 27% of the healthy participants. After adjusting for covariates, the researchers found a significant correlation

between low CD4 counts or poor immune status (not viral load) and black race in the HIV group; overall, low CD4 counts, black race, high BMI score, advanced clinical stage as per CDC classification, and Vitamin D obtained in Winter/Spring, were among the significant predisposing factors to Vitamin D deficiency (Rustein et al., 2011).

Similar to Viard et al. (2011), Mehta et al. (2009; 2010) whose imperative work underscored the association between Vitamin D insufficiency/deficiency and the increased risk for HIV disease progression and mortality, both studies could not find a significant association between Vitamin D levels and CD4 counts. The authors acclaimed the need for more studies to elucidate this controversy and to further inspect the potential beneficial role of Vitamin D supplementation in HIV patients with deficiency. Furthermore, Van Den Bout-Van Den Beukel et al. (2008) asserted the lack of association between Vitamin D insufficiency (25(OH)D levels (10-14 ng/µL) in middle-aged HIV patients (n = 252) and no subsequent association between Vitamin D status and CD4 recovery rate after initiating ART.

In a retrospective (chart review) cross-sectional study, Turett et al. (2013) reported a 90 percent prevalence of Vitamin D deficiency among HIV patients (n = 133) who attended a hospital-based clinic in New York City compared to 55.8 percent prevalence among HIV negative patients (n = 104) attending a private clinic within the same urban geographic location. The study ran multivariate analysis to compare the factors that correlate with Vitamin D deficiency in both groups. Their results showed lack of association between Vitamin D deficiency and CD4 count or viral load. In a descriptive cross-sectional Danish study, Bang et al. (2010) found that Vitamin D

insufficiency and deficiency was about 60 percent in 115 mostly Caucasian HIV males (median age 44 years), and about 62% the patients were on HAART. The study failed to find a significant correlation between Vitamin D level and CD4 count, even after comparing those on HAART versus those untreated (*Rho* = 0.232, *p* = 0.599). Low Vitamin D levels also did not correlate with viral load or with CDC class C advanced clinical stage in 32 patients (the rest of patients were class A and class B, 56 and 27, respectively). These results congregate with those from Wasserman and Rubin's study (2010), which also could not attain a correlation between CD4 count and Vitamin D insufficiency and deficiency (76.8 percent) in 62 men with some degree of immune competence (85 percent had viral load of < 200 copies/mL; median CD4 count 541 cells/μL).

In a cross-sectional study on a sample of 112 HIV patients (mean age 44.2 years) who volunteered to participate, Bearden et al. (2013) sought to elucidate the potential immune-modulating effects of Vitamin D through examining the associations of 25(OH)D and 1,25$(OH)_2$D with viral load and CD4 count. Their results showed that Vitamin D insufficiency/deficiency (< 30 ng/μL) was prevalent in 53% of the sample, and 22% had severe Vitamin D deficiency < 10 ng/μL); however, the study could not establish a significant association between Vitamin D and CD4 count. On the other hand, the authors found an interesting U-shaped relationship between low 1,25$(OH)_2$D and higher viral loads, but recommended further investigation of this finding. Lack of association between CD4 count and Vitamin D deficiency/insufficiency was also featured in a cross-sectional study from the UK (Gedala, Edwards, Benn, & Grant, 2013). The

authors argued that despite the high prevalence of Vitamin D deficiency (58.5 percent had 25(OH)D < 50 nmol/L) in their largely White male HIV patients, this did not even associate with viral load or clinical stage -knowing that patients were ART- naïve (not on treatment).

Vitamin D Supplementation

Based on the aforementioned thorough review about Vitamin D deficiency and its consequences on the immune system and on HIV course of disease, it is essential to present some of the major studies that actually assessed Vitamin D supplementation in HIV populations. Most of the studies examined Vitamin D deficiency and supplementation in relation to bone health; some limited studies have addressed Vitamin D supplementation in HIV patients with tuberculosis co-infection (Wejse et al., 2009), especially that the role of Vitamin D is known to halt replication of HIV and mycobacterium avium in infected macrophage cells (Campbell & Spector, 2012). Vitamin D insufficiency results in reduced monocytes and macrophage innate immunity to infectious agents such as mycobacterium TB, the leading cause of death in many parts of the world. However, in their double blind RCT, Wejse et al. (2009) could not establish any significant impact of Vitamin D supplementation on enhancing the TB clinical outcomes of HIV study population or on reducing its related-mortality; the researchers alluded to low supplementation dose as a possible explanation for the lack of effect.

Nevertheless, Vitamin D supplementation has some questionable metabolic outcomes. The Canadian and United States governments commissioned the Agency for Healthcare Research and Quality (AHRQ) to perform evidence-based systematic reviews

about what is the adequate or optimal level of Vitamin D; they reviewed bone health or skeletal health, and failed to obtain impressive results and to demonstrate causal benefit of Vitamin D supplementation on health outcomes (Bischoff-Ferrari et al., 2006). In general, 100 IU of Vitamin D is thought to raise serum levels of 25(OH)D by 1 ng/μL, although the increase may only be in fact 0.7 ng/μL (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). However, absorption of Vitamin D is not in a linear dose-dependent manner; the IOM (2011) concluded that in most children and adults, a total Vitamin D intake of 600 IU/day (for those older than 70, 800 IU/day) were adequate to raise the level to at least 20 ng/μL (50 nmol/L).

Vitamin D supplementation comes in two forms: Vitamin D2 or ergocalciferol and D3 as cholecalciferol. Both are used by the body same way. Each person gets a combination of both through ambient UV exposure (provides D3) and habitual dietary intake of D3 rich or fortified foods, and vitamin supplements (D2 or D3). D2 and D3 function as pro-hormones so they have no biological effect before liver and renal hydroxylation and conversion into active compounds (Tripkovic et al., 2012). Tripkovic et al. (2012) aimed to determine whether there was a difference in the efficacy of D2 versus D3 in raising serum Vitamin D, and for that reason, they led a systematic review (Medline, and Cochrane database, and clinicaltrials.gov) and a meta-analysis of all RCTs. Their review challenged to overthrow the old perception or presumption that D2 and D3 were equally efficacious in raising serum 25(OH)D, as Holick et al. (2008) and Biancuzzo et al. (2013) believed.

Despite having the same hydroxylation process and the same outcome $1,25(OH)_2D$ (calcitriol), most data have shown that there was a difference in the efficacy of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ in raising serum Vitamin D levels due to their dissimilar affinity to VDR (Houghton & Vieth, 2006). In their extensive study, Houghton and Vieth (2006) provided evidence on the different metabolic fates of D2 and D3 that indirectly affected the rate of D2 and D3 conversion to serum 25(OH)D and their affinity to VDR. While D3 retained a greater capacity to bind to VDR after kidney hydroxylation, D3 got deactivated biologically later than D2, and remained biologically active and maintained Vitamin D status for longer (greater bio-efficacy). This finding is similar to that in Mistretta et al. (2008) about the very short circulating plasma half-life of D2 and its lower affinity to bind to the Vitamin D biding protein and to VDR. D3 metabolite after hydroxylation had around 40% more affinity capability to bind to VDR that would allow it to generate significant biological activities with longer systemic influence (Houghton & Vieth, 2006). In conclusion, Houghton and Vieth (2006) called for disregarding D2 as supplementation or fortification to correct Vitamin D deficiency. Similarly, Armas et al. (2004) showed that D3 was three times more potent in correcting Vitamin D deficiency as compared to D2. Another study showed that on the long run and irrespective of dosage, whether daily or bolus (weekly or monthly), frequent or infrequent, still D3 was found better in evidence (Logan, Gray, Peddie, Harper, & Houghton, 2013).

In the same context, Tripkovic et al. (2012) found in their meta-analysis based on seven studies that the great absolute change in serum 25(OH)D from baseline favored cholecalciferol D3 intervention with a weighted mean difference of 15.23 nmol/L (95%

CI: 6.12-24.34; $p = 0.001$). Overall, total serum 25(OH)D concentrations were 21 nmol/L (95 % CI [14, 30]) lower in participants receiving Vitamin D2 compared to those receiving D3 ($p < 0.001$), among whom total serum 25(OH)D concentrations remained unchanged. Accordingly, a study by Mastaglia et al. (2006) reported that two and a half fold of D2 dosage is needed to achieve the same serum level achieved by D3. Tripkovic et al. (2012) asserted that despite the high heterogeneity among studies, separate analysis still showed that studies that used bolus single or multiple doses of D3 or D2, weekly or monthly like 50,000-300,000 IU with anywhere between 4 weeks to 1 year of follow-up, bolus doses with D3 had better results and increased serum 25(OH)D with a weighted mean difference of 34.1 ng/μL (95%CI [16.38-51.83]; $p = 0.0002$). On the other hand, there was no clear-cut differentiation between the two forms D2 or D3 in studies that used daily supplementation; a clear preference was shown for D3 with a weight mean difference of 4.83 ng/μL, but did not reach statistical significance.

In fact, the major prescription preparations of Vitamin D in the United States are in the form of D2 not D3. Most commonly used is the prescribed Vitamin D2 one pill of 50,000 IU. Armas et al. (2004) showed that it is true that D2 corrects deficiency with 50,000 IU, however, such dosage is equivalent to less than 15,000 IU of D3 and closer to 5000 IU D3. Serum D2 concentrations fell rapidly back to baseline after only 14 days, whereas 25(OH)D3 concentrations peaked and returned to baseline at the end of 28-day intervention. Recently, more companies in the US and Europe have been reformulating their products to contain Vitamin D in the form of D3. This should not lessen the

importance of D2 addition to milk and food and its role in eradicating rickets in 1930s (Houghton & Vieth, 2006).

Data are still lacking with regard to efficacy of Vitamin D supplementation and the outcomes in HIV patients. There are contrasting results from methodologically diverse studies and population samples; therefore, any kind of outcome, positive or negative, cannot be considered sufficiently evidence-based. Calling for well-designed randomized controlled studies to evaluate supplementation effects on HIV seem to be the most common recommendation in all studies. In HIV research, Vitamin D supplementation is speculated to have potential therapeutic effects. To consider supplementation, it sufficed to say that Vitamin D enhanced the immune system ability to fight microbial and viral infections, and that deficiency correlated with many unfavorable health outcomes (Kamen & Tangpricha, 2010). Moreover, Spector (2011) concluded that Vitamin D supplementation in HIV can reduce viral replication, increase CD4 counts, slow the rate of disease progression, improve control of opportunistic infections, reduce risk of HIV related neurocognitive impairment, and improve overall survival.

The most favorable effect of Vitamin D supplementation in patients with HIV is recognized under bone health. Few studies on Vitamin D supplementation in HIV populations that examined its effect on CD4 count and, therefore, provided some basis for this study (Arpadi et al., 2009; 2012; Bang et al., 2012; Giacomet et al., 2013; Havens et al., 2012; Kakalia et al., 2011; Poowuttikul et al., 2013; Van den Bout-Van den Beukel et al., 2008). Only the most pertinent for this current study are discussed in this chapter. While Vitamin D supplementation at different doses is considered safe and may result in

significant increases in serum Vitamin D concentrations in HIV patients (Arpadi et al., 2009; Van den Bout-Van den Beukel et al., 2008), Vitamin D supplementation does not directly increase the CD4 cell count in HIV patients as per two major studies by Kakalia et al. (2011) and Bang et al. (2012)- these two studies evaluated effect of Vitamin D on activation of CD4 lymphocytes. Potential beneficial effect of supplementation on CD4 count was suggested by cross-sectional studies on adults with HIV (see Haug et al., 1994; Teichmann et al., 2003). These potential benefits did not translate in the prospective cohort studies done by Van Den Bout-Van Den Beukel et al. (2008) in adults and Arpadi et al. (2009) in children.

Kakalia et al. (2011) study was very relevant to the current research because it evaluated in a randomized, non-blinded, controlled fashion the impact of Vitamin D supplementation on CD4 count and percent and other measures of Vitamin D homeostasis (25(OH)D and 1,25$(OH)_2$D) in children with stable HIV (n = 53). The children (age range 3-18 years; mean age 10.3 ± 3.9 years) consisted of 55 percent females and 64 percent African-Canadians. They were divided into three groups and were followed up for 6 months: no supplementation (Group 1), 5600 IU/week supplementation (Group 2), and 11,200 IU/week (Group 3). Once weekly dosing was used for more convenience and compliance. The study used liquid Vitamin D drops (D3) cholecalciferol. Adherence was assessed through measuring remaining Vitamin D liquid in the bottle. Patients in the placebo group were treated later. The study used a dietary questionnaire administered by a nurse or a physician before the initiation of supplementation to assess dietary intake of calcium and Vitamin D; though not formally

validated, this questionnaire was frequently used in the calcium bone clinic. Same questionnaire was reused at the end of the study to assess dietary changes. Vitamin D deficiency and insufficiency were defined as such: < 10 ng/μL and 10-30 ng/μL, respectively. Vitamin D levels and CD4 counts were measured at baseline, three and six months. The baseline results showed that the mean 25(OH)D was 53.1 ± 24.8 nmol/L; only 15% were Vitamin D sufficient at enrollment.

The three groups were comparable with no significant differences with respect to age, sex, BMI, ethnicity, CDC clinical or immunological category, receipt and duration of ART, CD4 percent, CD4 count, or viral load. Most importantly, there was no difference in 25(OH)D levels or $25(OH)_2D$ at baseline ($p = 0.1$ for both). Also, there was no significant difference in dietary Vitamin D intake ($p = 0.32$). Vitamin D insufficiency and deficiency exceeded 80 percent in all groups. Results showed significant increases in Vitamin D level in both supplemented groups; normalizing and achieving Vitamin D sufficiency level was significant in both groups: for Group 2 with 800 IU/day, there was an increase from 17% to 39% ($p = 0.0002$), and from 6% to 67% ($p < 0.0001$) for Group 3 with 1600 IU/day. This reflected some kind of dose-dependent increase in achieved level that corresponded with higher supplementation dose. There was no significant increase in Vitamin D level in the placebo group. The increase in 25(OH)D differed significantly between groups ($p = 0.0002$); in Group 2, 25(OH)D improved from baseline 49.9 nmol/L ($SD = 22.5$) to 76.5 nmol/L ($SD = 30.3$), and from 42.7 nmol/L ($SD = 18.1$) to 96.5 nmol/L ($SD = 41.9$) in Group 3.

There was no significant difference in the levels of 1,25$(OH)_2$D, CD4% ($p = 0.80$) or CD4 count ($p = 0.10$), and viral load log_{10} ($p = 0.99$). The lack of significant impact of Vitamin D supplementation on CD4 percent, CD4 count, or viral load (even after adjusting for potential confounding variables) incited the authors to run a separate analysis to examine the change in serum Vitamin D level and the association with CD4 count and percent irrespective of randomization group; they found a negative but significant association between change in serum Vitamin D level and change in CD4 count ($p = 0.02$). The variables that were associated with change in CD4 percent on univariate analysis included age ($p = 0.03$), duration of ART ($p = 0.03$), and BMI ($p = 0.06$). In summary, Vitamin D supplementation at both doses did not lead to an increase in CD4 count or percent in HIV positive children with somehow preserved immunologic function (mean 927 cells/µL ± 468), despite the fact that about 85 percent were Vitamin D deficient and insufficient and had significant increases during the course of study. However, the researchers raised the issue of investigating the need for higher supplementation dosage tailored for HIV patients, since about two-third of the children in the study failed to achieve the optimal serum level of 75 nmol/L. The researchers indirectly questioned the adequacy of 600 IU/day recommended dose by the Institute of Medicine in HIV patients; they recommended, instead, having a more appropriate dose such as 1000 to 2000 IU/day with monitoring.

The results of Kakalia's study (2011) resonate with those from Arpadi et al. (2009, 2012) study, especially that the latter's study population resembled Kakalia's, which also recruited healthy HIV-infected children and adolescents with a median

baseline CD4 percent of 30.6 ± 10.5, and CD4 count of 769 cells/μL ± 343. However, Arpadi et al. (2009) carried out their first study in 2009 before Kakalia and were quite content with their unique assessment of Vitamin D supplementation over a one-year period. The aim of Kakalia et al. (2011) study was to evaluate the effect of bimonthly (every two months) administration of oral cholecalciferol D3 100,000 IU plus 1 g/day of calcium (2 chews daily) on serum Vitamin D levels, serum and urine calcium, and on HIV disease progression during a 12-month period in HIV infected children and adolescents. The researchers assigned study personnel to dispense and administer Vitamin D or the placebo every two months during study visits (both personnel and participants were blinded to treatment allocation) to ensure adherence and remove bias.

Kakalia et al. (2011) recruited 59 children and adolescents with deficiency and insufficiency aged six to sixteen years from hospital-based pediatric HIV treatment programs in New York City between 2004-2005, but only 56 completed the 12-months study. For this randomized controlled study, subjects were randomly assigned through computer generated random numbers in SAS to receive Vitamin D supplementation D3 and calcium (VD+) or were double placebo (VD-). Stratified by gender, age, and study site, the final sample consisted of 29 in VD+ and 27 in VD-. At baseline, both groups VD+ and VD- were comparable. After 12 months, the mean monthly serum 25(OH)D level was significantly higher for VD+ (32.4 ng/μL ± 9.0) as compared to VD- (21.9 ng/μL ± 9.4), $p < 0.001$. By the end of the twelve months, only two subjects (6.7%) from the VD+ group remained deficient < 20 ng/μL, as compared to 14 (50%) of participants

from the VD- group. Optimal Vitamin D level ≥ 30 ng/μL was noted in 44.4% (n = 12) in the VD+ as compared to 11.1% (n = 3) from the VD-, (p < 0.02).

Regarding the immune effect of supplementation in Kakalia et al. (2011) study, there was no significant difference in markers of HIV disease progression between the two groups as measured by changes in CD4 count, CD4 percent, and vital load. For instance, in VD+, CD4 count at baseline was 771 ± 328 cells/μL and by the end of the 12 months, it registered 776 ± 359 cells/μL, while CD4 count decreased from a baseline of 719 ± 382 cells/μL to 661 ± 363 cells/μL at one year (p = 0.18). Despite the safe and well-tolerated supplementation regimen used in this study, the significant increase in Vitamin D level in the supplemented group was still below expectation and not up to par with bone health or with immune health. The researchers recommended caution with interpretation of results mainly due to the small sample size and suggested need for additional studies.

Kakalia's et al. (2011) and Arpadi's et al. (2009) results suggested that in case of relatively preserved immunological function in children as measured by CD4 count at baseline, Vitamin D supplementation in 800mg or 1600mg daily doses did not lead to significant increase in CD4 count; but this might not be generalized to children with more advanced HIV disease and with low baseline CD4 counts. In a randomized controlled study in Italy, Giacomet et al. (2013) studied 52 HIV+ youths (aged 8-26 years) with Vitamin D deficiency < 30 ng/μL; the study aimed to test whether 100,000 IU D3 administered every three months and over a one-year period (4 doses total) to a supplementation group (n = 26) - and withheld from or placebo group (n = 26) - would

lead to improvement in Vitamin D levels and T-cell phenotype (mainly CD4 count, plus T-lymphocyte VDR expression, Th1, Th2, Th17, and Treg lymphocytes). Both supplementation and placebo groups were comparable at baseline, more than 80% of patients in each group were receiving ART and had undetectable viral load; and both groups were similar in their immunological and Vitamin D profiles at baseline.

At the end of the study, Vitamin D levels increased considerably in the supplemented group with a mean difference of 27 ng/µL (95% CI [10 - 44], $p < 0.001$) as compared to placebo, while insufficiency persisted in only 20% as compared to 60% in the placebo group ($p = 0.007$). It was interesting to find that Vitamin D increase became more prominent after six months of supplementation; in this case, it coincided with the summer months and more sun exposure- indicating a possible effect on Vitamin D synthesis. Both 25(OH)D and 1,25(OH)2D increased but at different paces, the latter taking more time probably due to an acceptable degree of immune functioning. On the other hand, the study failed to show significant changes in CD4 counts in both groups. Knowing that the baseline CD4 counts were 663 cells/µL (95% CI [507 - 796]) in the supplemented group and 673 cells/µL (95% CI [601 – 773]), $p > 0.05$. Such result again concurred with Kakalia's et al. (2011) study and recommended studying subjects with less immune preservation in order to elucidate the link between Vitamin D supplementation and CD4 counts.

Perhaps, Poowuttikul et al. (2013) study is considered a real inspiration for the current study, especially with regard to the similarities in the location of the study, the type of participants who are predominantly dark skinned in a low sunlight area (Detroit,

Michigan), and the used methodology (retrospective chart review study). Similar to the aforementioned studies, Poowuttikul et al. (2013) aimed to examine the prevalence of Vitamin D deficiency in children and young adults with HIV (2 – 26 years old) and to assess whether Vitamin D supplementation would improve their immune disease markers, mainly viral load, CD4 counts, and CD4 percent. The researchers recruited 160 patients during routine clinic visits between 2010 and 2011. The sample was predominantly African American (152 out of 160 or 95 percent), and it consisted mainly of males (76.3 percent). Moreover, the proportion of young patients aged 21-26 years was about 47 percent, while those aged ≥ 10 years summed up to 8 percent. The majority of participants were on ARV, and only 28 percent were not. The investigators used ≤ 20 ng/μL and ≤ 35 ng/μL as thresholds for Vitamin D deficiency and insufficiency, respectively. At baseline, only eight children were Vitamin D sufficient, and therefore, were used in normal Vitamin D comparison group against insufficient and deficient groups. Out of the 152 with low Vitamin D, almost 72 percent (n = 115) had Vitamin D deficiency (≥ 20 ng/μL), 23 percent (n = 37) had insufficiency (21-35 ng/μL). It was kind of expected since the majority of patients were African Americans and lived in a cold climate area with low sun exposure. All patients whose Vitamin D level was ≤ 35 ng/μL received Vitamin D3 supplementation as part of routine care (cholecalciferol D3 1000 units/day), and Vitamin D testing was repeated every three months.

HIV plasma RNA (viral load), absolute CD4 counts, CD4 percent were compared between low Vitamin D subjects (n = 152) and the group with normal Vitamin D (n = 8) pre- and post-Vitamin D supplementation. At baseline, the results were comparable and

showed that HIV children aged ≤ 10 years had higher Vitamin D level (mean 24.8 ng/µL) as compared to adolescents aged 11-20 years (16.9 ng/µL) and young adults aged 21-26 years (mean = 17.6 ng/µL). Patients with severe deficiency (≤ 10 ng/µL) had lower mean absolute CD4 count of 574.41 (± 306.17) cells/µL compared to 701.15 (± 444.19) cells/µL among subjects with higher Vitamin D level ($p = 0.09$). After supplementation, only 39.5 percent ($n = 60$) of the 152 increased their Vitamin D level from a mean level of 13.7 (± 7) ng/µL pre-supplementation to 25.0 (± 13.3) ng/µL post-supplementation (only 10 patients reached normalization of 25(OH)D). About 45 percent ($n = 27$) had insufficiency (21-35 ng/µL), and 38.3 percent ($n = 23$) remained deficient (≤ 20 ng/µL). Similar to the aforementioned studies, and despite relative improvement in Vitamin D levels, mean CD4 counts post-supplementation 702.3 (± 446.7) cells/µL did not significantly differ from pre-supplementation mean count 734 (± 496.9) cells/µL, $p = 0.26$. It was quite interesting to find that mean viral load remained high and did not decrease significantly from pre-supplementation 22,310.77 (± 98,793.34) copies/mL to 10,209.65 (± 25,015.93) copies/mL post-supplementation, $p = 0.31$. The researchers stated that the low CD4 counts were most likely due to high viral load, and they referred to poor adherence to ARV that might have led to insignificant decrease in HIV viral load, rather than in response to the effect of Vitamin D. In the same sense, they suggested the need for more aggressive Vitamin D supplementation to change CD4 count and viral load. The fact that the majority of subjects remained deficient after supplementation might have reflected issues of poor adherence to Vitamin D, inadequate dose, poor diet, insufficient sun exposure, or some defects in Vitamin D metabolism from ARV.

Conclusion

Overall, the literature review emphasized that Vitamin D deficiency is associated with negative outcomes in HIV patients. It also gathered sufficient evidence regarding the risk factors of Vitamin D deficiency. There is evidence about the benefits of supplementation on bone health, but there is lack of sufficient data on its equal benefits on the immune system function in HIV, especially in view of the high volume of observational studies and a mediocre number of randomized clinical trials in HIV patients. Prevention of Vitamin D deficiency through attenuating the risk factors is highly needed. Vitamin D supplementation and impact on immune health remains substantially under-studied. There is a clear need for randomized controlled well-designed studies in order to establish a causative association between Vitamin D deficiency and CD4 count and to examine the real impact of supplementation on immune function. Armed with the postulation that Vitamin D supplementation might hold some promising results as far as boosting the immune system is concerned in HIV population, this current research did not seek to resolve the controversy of Vitamin D supplementation impact on CD4, rather it attempted to add further knowledge to the current public health literature in this complex and multidimensional field of HIV.

Chapter 3: Research Method

Introduction

This study explored the impact of Vitamin D supplementation on the immune function of a sample of African American, male HIV patients with Vitamin D deficiency. Vitamin D status categorization (normal, deficiency, or insufficiency) depended on Vitamin D [25(OH)D] level in the blood. The study also investigated the relationship between Vitamin D level and CD4 count and CD4 percentage. The study utilized secondary data obtained from reviewing patients' medical charts. This methodology chapter presented and discussed the following major sections: The study design; the setting and sample size; the ethical precautions; the types of data and variables; the data collection process and tools; and the analysis plan. Moreover, the chapter concluded with an overall description on the potential threats to validity (internal and external).

Research Design and Approach

The research design relied on a quantitative approach using retrospective observation (chart review), which is widely utilized in both public health and epidemiology, in addition to clinical research (Gearing et al., 2006). Retrospective chart review is generally underestimated and underutilized in the clinical and health care field and can be beneficial if researchers understand how to implement it correctly and how to minimize its limitations (Gearing et al., 2006). Retrospective chart review may not be the ideal research methodology that provides evidence of sound methodological standards due to the potential limitations in data completeness; still, it provides a cost-effective accessibility to readily available secondary data capable of answering most research

questions and of generating new hypotheses for future studies (Gearing et al., 2006). This study is observational/non-experimental and consists of following a cohort of HIV patients retrospectively from exposure to outcome. Moreover, the study is also considered analytical in nature (rather than descriptive) because it uses a comparison group and it seeks to test hypothesized association between exposure and outcome.

In this study, a standardized chart review data abstraction electronic form was used to collect data on specific demographic, socioeconomic, and clinical variables. An important methodological drawback in a retrospective chart review study, which is also known as medical record review (MRR), is when the abstractor lacks proper medical/clinical knowledge or training for understanding and dealing with the study variables (such as proper coding and using standardized abstraction forms) (Allisson et al., 2000). Failure to control for this drawback in the abstraction process jeopardizes the validity and reliability of data (Vassar & Holzmann, 2013; Worster, Bledsoe, Cleve, Fernandes, Upadhye, & Eva, 2005; Worster & Haines, 2004). Another methodological standard that should be guarded in the same context is the abstractor's blinding to the study research questions or hypothesis in order to remain objective and unbiased (Allisson et al., 2000; Gearing et al., 2006; Vatt & Holzmann, 2013). In view of the lack of financial and logistic resources to hire abstractors (Findley & Daum, 1989), this researcher, as a health professional with training in the medical and clinical field, abstracted data from charts into an electronic database and followed strict definition and coding manual for each variable to circumvent the lack of blinding to the study purpose (Allisson et al., 2000). Furthermore, inter-observer or inter-rater reliability testing can be

done to ensure reliability of collected data between abstractors but is totally beyond the scope of this study (Worster et al., 2005). On the other hand, Allisson et al. (2000) and Gearing et al. (2006) recommended an intra-rater reliability for the same abstractor, that is, reviewing same charts on two different occasions and measure a kappa intra-class correlation coefficient (ICC) to examine degree of resemblance and achieve more than 80% reliability score.

This retrospective design provided an opportunity to study a unique population at a single site and to capture all the necessary data. The study population consisted of all HIV-infected African American men (aged 21 years and up) who attended a specialty HIV clinic in an underserved community in Southeast Michigan between 2010 and 2014. Selecting a comparison group from the same clinic and HIV population was done to reduce sampling bias because both exposed and non-exposed groups (Vitamin D deficient and non-deficient/sufficient, respectively) assumingly share similar risk factors and characteristics, especially that they come from same geographic area and live in similar latitude. Retrospective studies are automatically free from information biases that result from recall bias, provided that the medical records have complete and accurate data (Gearing et al., 2006). In this case, chart review allows more reliance on specific objective biomarkers in the charts that significantly relate to the variables under study and the research hypotheses.

In this study, I adopted the Institute of Medicine (IOM) and the Endocrine Society's guidelines' definitions of the different Vitamin D statuses, whereby Vitamin D levels ≤ 20 ng/μL (equivalent to ≤ 50 nmol/L) represented deficiency status; 21-29 ng/μL

(equivalent to 51-74 nmol/L) represented insufficiency status; and levels ≥ 30 ng/µL sufficient (equivalent to 75 nmol/L) pertained to normal or sufficiency status (Holick et al., 2011; Holick & Chen, 2008; IOM, 2011). Vitamin D level at baseline was assessed and the first measurement recorded in the chart marked the initial date of entry into the study. Based on baseline Vitamin D levels, the cohort was divided into two groups: Group 1, deficient group, consisting of HIV infected African American men with Vitamin D levels of < 30 ng/µL (Vitamin D deficiency cutoff point is set at ≤ 20 ng/µL and insufficiency cutoff point at 21-29 ng/µL); Group 2, nondeficient or sufficient group, consisted of HIV infected African American men with adequate levels of Vitamin D (≥ 30 ng/µL). Both deficient and nondeficient groups underwent evaluation of their CD4 count/percent at baseline before supplementation in accordance with the first Vitamin D level at study initiation.

The study assessed CD4 count as the major biomarker reflecting the immune function. CD4 count test reflects the actual number of CD4 cells per microliter of blood sample (number of cells/µL is equivalent to number of cells in mm^3 of blood); the normal laboratory range for CD4 count is set between 500 and 1600 cells/µL (AIDS InfoNet, 2014). Moreover, the study assessed CD4 percentage (CD4%) as an additional immune function endpoint. The CD4% estimates the percentage of white blood cells or lymphocytes that are CD4 T-cells per microliter of blood. In fact, the HIV literature have shown the advantage of using both CD4 count and CD4 percent as equally important clinical markers of HIV disease progression and better assessors of overall immune function (Guiguet et al., 2009; Hulgan et al., 2007; Moore et al., 2006; Pirzada, Khuder,

& Donabedian, 2006). However, since CD4 count fluctuates in response to some factors such as stress level, diet, exercise, time of day the blood was drawn, presence of infection or illness, some HIV clinical literature have pinpointed to the importance of relying on CD4 percent in adjunct to CD4 count as another surrogate marker of immune function that is more stable and clinically accurate- provided patients are AIDS-free (i.e., with CD4 count > 200 cells/μL) (AIDS InfoNet, 2014; Guiguet et al., 2009; Hoffman, Van Griensven, Colebunders, & McKellar, 2010; Hulgan et al., 2007; Moore et al., 2006; Pirzada, Khuder, & Donabedian, 2006). On the other hand, when a patient experiences a transient and sudden drop in CD4 count that does not go hand in hand with a drop in CD4 percent, this could mean that this drop in CD4 count is most likely related to other factors (as mentioned above), and therefore, it is considered clinically insignificant since CD4 percent remained stable (Pirzada, Khuder, & Donabedian, 2006).

When CD4 count is evaluated, it is advised to take into consideration the trend or mean of several test results every 3-6 months for patients with CD4 count < 350 cells/μL, to capture the CD4 trend and the magnitude of immune cell recuperation/restoration (DHHS, 2012; 2014). According to the newest guidelines (DHHS, 2014), patients with CD4 count that ranges from 300 to 500 cells/μL and with controlled viral suppression can have a retesting of their CD4 count every 12 months; when patients experience a rebound in viremia (viral load increase) or clinical symptoms, then, frequent CD4 count testing should be resumed.

CD4 count and percentage follow the CDC (2008b) immune classification and staging system, whereby a CD4 count < 200 cells/μL is considered a severe immune

suppression and merits AIDS diagnosis, and CD4 count between 200 to 499 cells/μL is moderate immune suppression (refer to Table 2). CD4 count (or percent) follow-up on patients is essentially needed to monitor HIV disease course path and response to treatment, the need for ART commencement to restore immune function or halt immune deterioration, and to assess the need for prophylaxis against opportunistic infections (DHHS, 2012). On the other hand, recent clinical guidelines urge clinicians to start ART once patients' CD4 counts drop to 350 cells/μL and below, and recommend close examination of viral load to ensure viral suppression (DHHS, 2012). However, the most recent revised guidelines (DHHS, 2014) urge clinicians to proactively and aggressively start ART without waiting for the drop in CD4 count to 350 cells/μL. In addition to CD4 count and percentage, viral load is also accounted for in the immune function. HIV RNA or viral load is considered another important immunological marker that depicts response to ART and its effectiveness in suppressing viral replication or viremia (below 200 copies/mL) after initiating ART (DHHS, 2014).

Based on theoretical grounds discussed in the literature review, this study was guided by two predominant research questions and the following null hypotheses:

1. There is significant correlation between Vitamin D levels (independent variable measured as normal or deficient) and CD4 count and CD4 percent (dependent variables)- after adjusting for potential confounders in the sample.

2. There is a significant effect of supplementation as depicted by a statistically significant difference or change in CD4 counts and CD4 percent after supplementation.

Table 2

CDC Immune Stages According to CD4 Percentage & CD4 Count Groups

CDC Stage	CD4 Percentage (%)	CD4 Counts (cells/μL)
1	>29	>500
2	14-28	200-499
3 (AIDS)	<14	<200
Unknown	NA	NA

Source: CDC. (2008b). Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 months and for HIV infection and AIDS among children aged 18 months to <13 years—United States, 2008. *MMWR*, 57(RR10), 1-8.

The outcome measures consisted of running correlation analyses between Vitamin D level and CD4 count/percent, and of calculating the absolute change in Vitamin D levels, in addition to examining the changes in CD4 count, CD4 percent, and viral load from before to after Vitamin D supplementation (pre and post-supplementation). A randomized controlled trial with pretest and posttest could have been optimal but not logistically possible for the scope of the study and with regard to the retrospective nature of the study. As for ethical considerations, the IRB team at Walden University did not see the necessity to seek individual consent from each patient in the study as long as patients' describers were not gathered and reported, and as long as patients' privacy was secured in accordance with HIPPA regulations. The IRB approval number for this study is 01-08-15-0055963.

Setting and Sample

This study utilized data collected on HIV patients who presented to an underserved specialty clinic ACCESS in Dearborn, Michigan between 2010 and 2014. The convenience sample included only African American male HIV patients, aged 21

years and up, residing in Metropolitan Detroit area in Michigan and attending the same HIV clinic, under the care of the same physician. Besides treating patients who have health insurance, the ACCESS clinic also receives federal financial support from the Ryan White HIV/AIDS Program that funds treatment and medical care for uninsured or underinsured HIV/AIDS patients. The clinic has a high retention rate of patients that exceeds 95% a year with only one death that has been reported since initiation of services in 2008. The physician at the clinic has infectious diseases and HIV medical subspecialty and is the only physician who has been treating all HIV patients coming to the clinic. This HIV specialist at the clinic uses an aggressive approach in treating HIV, whereby almost all patients with CD4 counts below 350 cells/µL get treated.

The clinic started treating HIV patients since 2008 and has treated so far about 300 HIV patients (roughly estimated that more than 90% are males). However, regular checking for Vitamin D level on all patients started in 2010; therefore, only patients with baseline Vitamin D levels before supplementation were eligible for inclusion in the study. First Vitamin D measurement marks initial data of entry or time 0 (denoted as T0). This study recruited African American male HIV patients who were tested for Vitamin D at baseline as part of routine care and who only had subsequent Vitamin D levels at different follow-up visits. Professional phlebotomists at the on-site laboratory available at the clinic facility were responsible for withdrawing all blood specimens, and the Detroit Medical Center university laboratories in downtown Detroit performed the blood tests analyses.

A sampling frame was formed of all the HIV patients who presented to the clinic in 2010 and onward; only those who fulfilled the inclusion criteria and had baseline Vitamin D in records were considered. Unfortunately, the clinic has not yet established electronic medical records or EMR but it keeps a list of all HIV patients separate from other patients seen for other medical reasons at the clinic. The presence of an active outreach HIV screening and prevention program at the clinic constitutes a major hub for recruiting these HIV patients and bringing them into medical care. Therefore, all HIV patients who fit the eligibility criteria were selected and constituted the main sampling frame for the non-probability sample. As for inclusion criteria, all eligible male HIV patients should be African Americans and should have the following: age (21 years and up); baseline and consecutive (post-supplementation) tests on Vitamin D level, viral load, CD4 count, and CD4 percent; stability on ART regimen without medication change for at least three months before study initiation (i.e., first baseline Vitamin D level); no prior Vitamin D supplementation before study initiation; and stable clinical HIV disease. Exclusion criteria would contain presence of strong confounding factors such as subject already taking or took Vitamin D supplementation (there is two months wash out period), or having chronic comorbidities that would affect the validity of the data.

As mentioned earlier, based on their 25(OH)D level at baseline (T0), patients were divided into two groups: normal (sufficient or nondeficient) group and deficient group (insufficient and deficient). Only the deficient group who received Vitamin D supplementation was followed up for at least twelve months in retrospect to assess effect of supplementation on Vitamin D and CD4 count and percent. The supplementation

regimen consisted of a high dose 50,000 IU taken orally only once weekly to encourage more compliance; after 8-16 weeks, repeated 25(OH)D levels were reassessed again and collected within the follow-up period up to one year. On their clinical routine visits, all patients with Vitamin D deficiency were verbally encouraged by the physician to take their Vitamin D supplementation; there was only verbal follow-up on compliance through directly asking the patient on the medications taken on each follow-up visit.

In order to obtain an adequate number of participants, initially, all eligible patients under routine care were included (convenience sample). It was hard and impractical to select a random sample for this study and to avoid random sampling error that is characterized by a biased selection of participants (Suresh, Thomas, & Suresh, 2011). This would be a major drawback that may threaten external validity or generalizability of inferences and results to the greater population from which the sample is derived. However, the study will attempt to avoid the pitfalls of non-sampling errors throughout the processes of data collection, measurements, and analyses (e.g., through including potential confounders) (Suresh et al., 2011). Besides, selection bias can be reduced if both groups, deficient and non-deficient, displayed similar baseline characteristics. On the other hand, the statistical tests suggested to answer the proposed correlation and comparative research questions would dictate the suitable sample size.

Generally-speaking, in order to estimate the sample size needed, it would be essential to run power analysis to make sure that the selected statistical tests could improve the precision and certainty of results, along with increasing the likelihood to find an association between variables or to detect a treatment effect in comparison to a control

group (Findley & Daum, 1989; Suresh, Thomas, & Suresh, 2011). Once the statistical test is chosen (e.g., correlation r or *t* test), there are three elements that are essential for power analysis:

1. Alpha level or type I error, which is conventionally set at 5% or $p = 0.05$ level of significance; that means that there is only 5% chance that the researcher would incorrectly reject null hypothesis (Laureate, Education, Inc., 2010).

2. Effect size: usually, a predetermined effect size is used from previous research.

3. Sample size: in case there is lack of previous data in the literature, it will be wise to specify a Cohen's effect *d* (usually small effect or medium) and determine the corresponding sample size (Laureate, Education, Inc., 2010).

As far as effect size is concerned, it is important to take an idea or identify a clinically significant difference between treatment and comparison group from similar studies in the literature. Sometimes it is hard to find an effect size, the researcher can then deduce a clinically significant difference from using standard deviation units and subtracting highest and lowest mean values from the literature (Conroy, 2004). In fact, this is similar to the original work of Cohen (1988) on effect size *d*, in which d designates a statistical difference between two groups, and is calculated by dividing mean difference before and after treatment by standard deviation. Cohen (1988) specified the following effect sizes: small $d < .50$; medium $d = .50$ –.80; and large $d > .80$. In correlation studies, the effect size is depicted by the square of the correlation *R* (Laureate, Education, Inc., 2010).

In order to calculate any sample size, a power analysis is usually set between 80% and 95%, that is, the researcher has set a probability of 95% for finding a treatment effect size and there is no more than 5% margin of error (95% of the time expecting to get an effect) (Laureate, Education, Inc., 2010). While this study may fail to ensure the representativeness of the sample (affecting external validity) because of the use of a convenience sample, at least, an adequate sample size with sufficient statistical power might be able to detect effect, associations, and correlations with adequate precision (Suresh et al., 2011) and salvage internal validity to a certain extent.

Consequently, the researcher reviewed similar studies to examine indicators used in calculating sample size (e.g., effect size, or power analysis). From the literature, the most recent DHHS guidelines (2014), reported that a 30% change in the absolute CD4 count or a standard deviation difference between two CD4 count tests by two points – equivalent to a three percentage points difference in CD4 percent – was considered significant. Moreover, four methodologically similar studies influenced sample size determination for this study; all four dealt with Vitamin D deficiency in HIV population and assessed Vitamin D supplementation. In a retrospective chart review study by Poowuttikul et al. (2013), the researchers used routine visits in three clinics over one year from 2010 to 2011 and recruited 160 HIV-infected subjects (152 were African Americans), aged 2–26 years, with Vitamin D deficiency (≤ 35ng/μL). Only eight subjects had normal Vitamin D levels (nondeficient). The study did not mention sample size determination method. The researchers ran comparative analysis using a very small number of participants in the non-deficient group (n = 8), the fact that could have skewed

the results; therefore, in order to reduce this potential problem that ensued from this small number of non-deficient subjects, the researchers divided the deficient group into three subgroups: insufficient (37 subjects); deficient (74 subjects); and severely deficient (41 subjects). Consequently, in order to reduce between subjects variability, all three groups and the 4th non-deficient groups were compared pre and post supplementation for changes in Vitamin D, CD4 counts, and CD4%, and viral load.

The second study by Arpadi et al. (2009) studied the effect of bimonthly Vitamin D supplementation on Vitamin D levels and calcium levels in 56 HIV children and adolescents (aged 6–16 years) recruited from four hospital-based pediatric HIV treatment programs in New York. They specified that they randomly assigned subjects to supplementation group (VD+ = 29) or placebo (VD- = 27). The methodology did not specify how the statistician calculated the sample size, but rather discussed the randomization process to treatment and placebo. On the other hand, Kakalia et al. study (2011), the most resembling –methodologically– to the proposed study, provided all necessary details about sample size calculation. The purpose of Kakalia et al. study (2011) was to assess the impact of Vitamin D supplementation on CD4 count, percent, and Vitamin D levels in children with HIV. The researchers based their sample size calculation on previous data analysis done at their clinic that showed a CD4 percent mean of 20 ± 3 (*SD*); then they assumed a 3% CD4 percent change between Vitamin D levels in supplemented versus nonsupplemented with an alpha level of 0.025 and a power of 80%. Their final sample consisted of 54 subjects and was randomized into three groups for analyses. Lastly, Bang et al. (2012) in their placebo-controlled randomized study

sought to examine the impact of Vitamin D supplementation on CD4+ T cells and Tregs in HIV males; they determined a sample size equivalent to 50 patients, based on a power of 90% and an assumed increase in 25(OH)D level of 20 nmol/L following Vitamin D supplementation compared to no increase in the placebo group.

Based on the preceding sample size discussion, the study adopted Kakalia et al. (2011) published sample size calculation (n = 54). As previously mentioned, this study aimed to test the correlation between Vitamin D level and CD4 count or percent, and to examine the impact of Vitamin D supplementation on CD4 count and percent through comparing before and after levels and comparing between groups levels (deficient versus nondeficient). To answer the research question about comparing pre supplementation and post supplementation parameters, the researcher assumed a Cohen's effect size of $d = 0.5$ between the supplemented and nonsupplemented group at 80% power level for two-tailed alpha = 0.05; therefore, the researcher needed 95 participants for between groups comparison, and only 42 for within groups comparisons (Cohen, 1988). When comparing pre and post values or within subjects change values from baseline to follow-up, the variability in values as well as the standard deviation value is expected to be lower; this is expected to result in higher precision and power of the results as compared to between patients variability in the two groups comparison, and thus, would demand a smaller sample size (Shintani, 2008). On the other hand, if the sample size calculation was based on the other research question related to investigating the correlation between Vitamin D and CD4 count/percent, then, at 80% power level and a two-tailed alpha = 0.05, only 41 participants were needed in order to detect $r = 0.5$ (Cohen, 1988). Based on ballpark

estimate and the physician's opinion on the HIV population he treated at the clinic, about 100 patients would fit the inclusion criteria; therefore, this study originally sought to achieve a total sample size of 100 patients, but ended up recruiting only 70.

Data Collection and Analysis

I collected data and entered it manually into the electronic database of SPSS version 21.0 (SPSS Inc., Chicago) to perform statistical analysis. The total follow-up time to all patients ranged from six months to at least one year (maximum 14 months). The continuous data were presented as means and standard deviation, or median with interquartile range (IQR). All categorical or nominal variables were presented as frequency and percentages. The main dependent variables in this study were CD4 count, CD4 percent, and viral load. The independent variable is Vitamin D deficiency, 25(OH)D < 30 ng/μL. In order to test the main study hypotheses, the following variables (Table 3) were examined because of their potential direct bearing on the study: age; BMI; smoking; injection drug use; hepatitis C; time since HIV diagnosis or HIV duration; season of measurement; serum 25(OH)D; serum 25(OH)D category; ART; CD4 count; CD4 percent; CD8%, CD4/CD8 ratio; and HIV plasma RNA (viral load).

Table 3 provided a list of all the variables with their type of measurement and the corresponding type of results, parametric or non-parametric – depending on the distribution of data. Date of the first 25(OH)D level was used as time of entry into the study. Dates for all relevant laboratory results of all successive visits were entered to verify timeline between baseline and repeated measures, and to verify season of blood draw for Vitamin D. Only successive laboratory results within a one–year study time

frame were considered. A baseline and post-supplementation analyses were performed to check changes in parameters at baseline, after six months, and up to one year of supplementation. Participants were measured at T0 (baseline), T1 (six months), and T2 (12 months).

Table 3

List of Variables Included in The Study and Their Descriptive Statistics

Variables	**Statistic**
Age, years	Mean (*SD*) Median (range)
BMI Kg/m^2	Mean (*SD*) Median (*IQR*)
Smoking (yes, no)	Number (%)
Injection drug use (yes, no)	Number (%)
Chronic hepatitis C (yes, no)	Number (%)
Season of measurement: • Winter months (November- February) • Sunlight months (March-October)	Number (%)
HIV duration (years)	Mean (*SD*) Median (*IQR*)
ART	Number (%)
Serum 25(OH)D (ng/μL)	Mean (*SD*) Median (*IQR*)
Serum 25(OH)D category • Deficient • Sufficient/Non-deficient	Number (%)
CD4 cell count (cells/μL)	Mean (*SD*) Median (range)
CD4% (cells/μL)	Mean (*SD*) Median (range)
CD8% (cells/μL)	Mean (*SD*) Median (range)
CD4/CD8 ratio (cells/μL)	Mean (*SD*) Median (range)
Viral load (copies/mL)	%

The main focus of this study addressed the two aforementioned types of research questions: comparative and associational/correlational (Morgan & Harmon, 2000). Although the study was not descriptive in nature, it was still essential to run a preliminary descriptive analysis to learn about the characteristics of the sample at baseline through summarizing demographic and clinical data, and to examine whether both groups are similar at baseline. To check for normality of distribution of continuous data, Shapiro-Wilk test was used. In order to assess significant differences between groups and delineate effect of Vitamin D supplementation on Vitamin D levels and on CD4 count/percent, this study sought to answer one correlational research question (Vitamin D and CD4) and one major comparative pre–post question.

The first correlation question addressed whether there was a correlation between Vitamin D deficiency and CD4 count or CD4 percent. Pearson's correlation ("*r*" ranging from 0 to 1) or Spearman rho (r_s) were used when both variables were continuous; they also assessed linear association between both variables. A Pearson's or Spearman's correlation coefficient that is equal to 1 means perfect correlation, 0 means no correlation, and -1 means inverse correlation. When the data were skewed, the nonparametric Spearman rho correlation was used instead of Pearson's. Most of the time, both parametric and nonparametric correlation analyses were run even when the assumption of normality was not met. P value set at < 0.05 depicted statistical significance for all analyses.

1. **RQ1:** Do Vitamin D levels significantly correlate with CD4 count (percent)?

- Null H_0: There is no statistically significant correlation between Vitamin D levels and CD4 count (percent) in supplemented group versus nonsupplemented.
- Alternative H_1: There is statistically significant correlation between Vitamin D levels and CD4 count (percent) in supplemented group as compared to nonsupplemented.

2. **RQ2:** Does Vitamin D supplementation have a statistically significant effect on CD4 count/percent in HIV-infected African American adult men in this study [time frame: baseline, six, and twelve months]?
   - Null H_0: There is no statistically significant effect of Vitamin D supplementation on CD4 count/percent in HIV-infected African American men in this study.
   - Alternative H_1: There is a statistically significant effect of Vitamin D supplementation on CD4 count/percent in HIV-infected African American men in this study.

In order to answer the second research question that dealt with assessing effect of Vitamin D supplementation on CD4 count/percent in the supplemented group, the researcher performed statistical tests to examine changes in Vitamin D levels, and in absolute CD4 count and CD4 percent between pre and postsupplementation. The statistical tests that were used: paired *t* test, repeated measures ANOVA, or the non-parametric Wilcoxon signed rank test (for skewed or not normally distributed data) to compare data before and after. The best way to calculate the change in score was to subtract post-test CD4 count/percent from pre-test CD4 count/percent; when the change score got multiplied by 100, this displayed percent increase (or decrease) in CD4 count.

Same thing applied to CD4 percent and viral load. At the same time, RQ2 addressed the difference in CD4 count/percent change scores from baseline between supplemented group and non-supplemented group. For that reason, *t* test was used to compare means of the two groups when variables were continuous and their data were normally distributed; when data were skewed, the non-parametric Mann-Whitney *U* test was used instead. In order to compare percentages of participants taking supplementation versus those non-supplemented and their respective Vitamin D level categories (deficient versus nondeficient), Fisher's exact test or Pearson's chi-square for categorical variables or Wilcoxon rank sum were used for continuous variables.

Moreover, RQ2 used ANCOVA to test group difference post–pre in CD4 count/percent changes. Supplementation was considered the design factor, the pretest (baseline score) as the covariate (to eliminate between-subject variability from supplementation effect), while the posttest measurement was the response variable. In other words, to control the effect of baseline pretest Vitamin D on response to supplementation, baseline measurement or pre-test was treated as a potential confounding variable. Generally, ANCOVA has the potential of producing a significant treatment effect, especially if there is some kind of uncontrolled pre-existing differences between groups to start with. Comparing the difference between Vitamin D levels pre–post in the supplemented group was done using paired *t* test. In order to display variations and differences within the supplemented group, the participants' pre and post 25(OH)D levels, and the immune function parameters across time (T0, T1, & T2) were compared using repeated measures ANOVA.

The between-subjects comparison between supplemented group versus non-supplemented on the same variables was done through ANCOVA model. In looking for confounding variables for the ANCOVA model, univariate and successive multivariate analysis (when applicable) checked factors that were associated with 25(OH)D level or with Vitamin D status (group allocation as fixed effect). The confounding variables that were hypothesized to affect Vitamin D were: baseline 25(OH)D level, age, season of measurement, ART, smoking, diabetes, history of AIDS, BMI, and other variables provided that they showed significant correlation with Vitamin D levels in univariate analysis. ANCOVA would allow to examine if the change in 25(OH)D level induced by supplementation would translate into significant impact on CD4 count, percent, and viral load- after adjusting for the aforementioned variables. Box plot was used to show the increase in Vitamin D level at different time frames compared with baseline. Descriptive analysis was run to compare proportions of subjects in the deficiency group at baseline and after supplementation, to see how many subjects achieved Vitamin D sufficiency.

Consideration for the Rights of Human Subjects

The data that was used in this analysis were retrieved from medical charts at ACCESS clinic in Dearborn, Michigan. Upon receipt of special permission from the department of community health and research at the clinic, and upon signing HIPAA patient safety and privacy rule forms, the researcher was allowed to access the medical charts and to retrieve de-identified data and to make sure that no personal identifying information was collected or reported, and that patient confidentiality was honored. Data collection process took place at the clinic with the use of the researcher's personal

computer and SPSS database. All charts were given numbers and data was coded to avoid retaining personal identifiers. Upon analysis, all data was presented in statistical forms. Since this study was retrospective in nature and only dealt with medical charts, there was no contact with participants. Therefore, informed consent was not requested from any patient included in the sample and in the analysis. In accordance with an earlier contact with the Institutional Review Board at Walden University, the IRB member expressed the lack of necessity to obtain consent forms from study participants.

Conclusion

This chapter described in detail the proposed methodology, the research variables, and the corresponding statistical plan for the study. This study aimed to expand our understanding of the controversial relationship between Vitamin D and CD4 in the context of impact on immune function of HIV patients with Vitamin D deficiency. After data collection and analysis, the results and the conclusions were presented and discussed in Chapters 4 and 5, respectively.

Chapter 4: Results

Introduction

This chapter presented the results of the study about the relationship between Vitamin D level and the immune function of HIV patients as depicted by CD4 count/percent, CD8 percent, CD4/CD8 ratio, and viral load. It also reported the findings about the effect of Vitamin D supplementation on the aforementioned immune function parameters in Vitamin D deficient HIV patients. The chapter began with a presentation of major descriptive statistics, including sample demographics and variables studied. The chapter discussed in details the research questions and hypotheses, as well as illustrated the assumptions for the selected statistical tests used in the analyses. The chapter concluded with a summary of the data analysis procedures and a general assessment of the findings.

Data Collection

The data for this analysis is based on medical chart abstraction that took place after IRB approval between January 8, 2015 and February 20, 2015. The sample consisted of 70 African American, HIV-infected male patients who presented between 2010 and 2014 to an HIV specialty clinic (ACCESS) located in the city of Dearborn in southeast Michigan. This sample size is smaller than the sample size estimated originally in the principal proposal (100 participants). Despite the difference in sample size to what was originally proposed ($N = 70$ versus $N = 100$), a retesting of power analysis for the correlation analysis would still demonstrate at least 80% chance of finding a potential medium effect (Cohen's medium effect of 0.5). Despite the abundance in the number of

HIV-infected, African American patients that seek medical services at the clinic and that meet the basic inclusion criteria for the study based on an initial sample frame ($N = 125$), the thorough review during the data abstraction process resulted in disqualification of a sizable number of medical charts. To a great extent, the retrospective and the longitudinal nature of collected data displayed some inconsistencies in measuring the main variables under study with every routine visit or at follow-up visits whenever Vitamin D supplementation was administered.

Descriptive Statistics

All data were entered electronically into SPSS (version 21.0) and the statistical analyses were completed through SPSS software version 21.0 as well (SPSS, Chicago, IL, USA). The sample included 70 male African American, HIV-infected patients who attended an underserved HIV clinic in southeast Michigan between 2010 and 2014. They range in age from 21 to 57 years (*Mean* age = 37.6 years, standard deviation *[SD]* = 11.3). The general characteristics of the total study sample are presented in Table 4. Of the 70 participants, the majority (80%) were MSM (men who have sex with men), 57% were smokers, 8.6% had type II diabetes, 1.4% had history of injection drug use, 3% had hepatitis C, almost 49% had normal body mass index (BMI) and 11% were considered obese. Moreover, only one patient (1%) had a history of contracting HIV through injection drug use (IDU), about 90% were on ART at admission, and 18.6% had experienced AIDS (defined as a CD4 count below 200 ng/mL or prior AIDS defining event). Approximately, 26% of the participants had undetectable HIV plasma RNA or viral load in their blood at baseline, reflecting a good adherence to ART. A viral load

value of “0” denoted undetectable level < 48 copies/mL. Table 4 portrayed the descriptive characteristics of the study population.

Table 4

Baseline Descriptive and Demographic Characteristics of Study Population

Variables	**No. (*N* = 70)**	**%**
Transmission mode		
MSM	56	80
Heterosexual contact	14	20
Injection Drug Use	1	1.4
Smoking	40	57.1
Diabetes type II	6	8.6
Hepatitis C	2	2.9
BMI		
Underweight	1	1.4
Normal	34	48.6
Overweight	27	38.6
Obese	8	11.4
ART on Admission	63	90
History of AIDS	13	18.6

At baseline, of the 70 participants, 40 percent (n = 28) had sufficient or normal Vitamin D level (25(OH)D ≥ 30 ng/μL), while 60% (n = 42) had Vitamin D insufficiency and deficiency (25(OH)D < 30 ng/μL) – of which 14.3% (n = 10) have severe Vitamin D deficiency (25(OH)D < 10 ng/μL). The study sample was split into two main groups: (1) Vitamin D deficient group and (2) Vitamin D sufficient group (internal comparison). Only patients in the deficient group received supplementation and were followed for at least one year with repeated measurements of Vitamin D levels and corresponding HIV-related laboratory parameters, i.e., CD4 count/percent, CD8, CD4/CD8 ratio, and viral load.

Moreover, an independent *t* test was run to compare the baseline age and BMI characteristics (both normally distributed variables) between the Vitamin D deficient group (n = 42) and the Vitamin D sufficient group (n = 28). As per Levene's test for equality of variances for the two variables in both groups, the assumption for the *t* test was met in these continuous scaled variables. The mean age of participants in the deficient group (M = 37.40, SD = 11.13) was comparable to that in the sufficient group (M = 38.00, SD = 11.80), $t(68) = -0.214$, $p = 0.831$. The distribution of HIV duration since diagnosis was found skewed, therefore, Mann-Whitney *U* test was used to compare the mean ranks HIV duration in both deficient and sufficient groups. As a result, both groups did not differ on HIV duration since diagnosis (ranged from one year to 26 years); the deficient participants had a higher mean rank of 38.3 (median = 7, *IQR* = 8, range = 1 – 22) as compared to 31.3 (median = 4.3, *IQR* = 7, range = 1–26) for the participants in the sufficient group ($U(68) = 470.0$, $Z = -1.419$, $p = 0.156$). The means for HIV duration in years were also comparable M = 8.10, SD = 5.70 versus M = 7.11, SD = 7.13, in deficient and sufficient group, respectively. Similarly, BMI in the deficient group (M = 25.70, SD = 4.71) and in the sufficient group (M = 25.54, SD = 4.57) did not differ significantly at baseline, $t(68) = 0.138$, $p = 0.890$.

Baseline categorically scaled variables were compared between both groups using Chi-square test to determine differences in proportions or nonparametric Fisher's exact test (whenever appropriate, i.e., for expected cell sizes with less than 5 cases). These results are depicted in Table 5. Both groups were comparable on all these variables except for diabetes. Diabetes was only present in six patients in the deficient group and

none have diabetes in the sufficient group. Fisher's exact test has showed that the two groups differed significantly in having diabetes at baseline (p = 0.040).

Table 5

Group Comparison on Baseline Categorical Variables

	Vitamin D Deficient (n = 42) n (%)	Vitamin D Sufficient (n = 28) n (%)	p value
Smoking			
Yes	26 (62)	14 (50)	χ^2 (1, N = 70) = 0.972
No	16 (38)	14 (50)	p = 0.324
Diabetes			
Yes	6 (14.3)	0 (0)	Fisher's exact test
No	36 (85.7)	28 (100)	p = 0.040*
History of AIDS			
Yes	8 (19)	5 (18)	χ^2 (1, N = 70) = 0.016
No	34 (81)	23 (82)	p = 0.900
Transmission mode			
MSM	33 (78.6)	23 (82)	χ^2 (1, N = 70) = 0.134
Heterosexual	9 (21.4)	5 (18)	p = 0.714
Chronic Hepatitis C			
Yes	1 (2.4)	1 (3.6)	Fisher's exact test
No	41 (97.6)	27 (96.4)	p = 0.643
Measurement Season			
Winter months	14 (33.3)	9 (32.1)	χ^2 (1, N = 70) = 0.011
Sunlight months	28 (66.7)	19 (67.9)	p = 0.917
ART on admission			
Yes	36 (86)	27 (96.4)	Fisher's exact test
No	6 (14)	1 (3.6)	p = 0.145

*Significance = $p \leq 0.05$

Prior to running inferential analysis, a bivariate logistic regression model using SPSS was conducted to assess which independent variables at baseline can be useful in predicting the probability or likelihood of having a Vitamin D deficiency status (deficiency versus sufficiency) in the sample. The candidate risk factors for the logistic model were selected based on the literature review that showed the major predictors of deficient Vitamin D levels. For this analysis, the goal was to test whether the proportion

of the variance in Vitamin D deficiency status (deficiency status being the indicator variable) can be explained by the following independent variables: Age ≥ 40 years old, smoking, having AIDS, being MSM, high BMI, being on ART, and Vitamin D measured in winter months. All independent variables were dummy coded (using 0 and 1) before running the analysis. Before running the logistic model, a preliminary bivariate correlation analysis was done separately for each independent factor with the categorical dependent variable, that is, with Vitamin D status (deficiency versus sufficiency) before inputting the significant correlations into the binary logistic regression model.

The results from bivariate correlation analyses and later from the binary logistic regression model run on SPSS for all the variables combined showed that none of the aforementioned variables have demonstrated a significant relationship with Vitamin D status (deficiency status as an indicator) or Vitamin D levels. Neither the logistic regression model has produced a significant prediction of the outcome of Vitamin D deficiency status at baseline (Omnibus test showed a $p = 0.555$, that means that the model is not a good predictor; the Hosmer & Lemeshow test has a $p = 0.744$, which could have indicated a good model but all the B coefficients and Wald chi-square statistics have p values > 0.05, χ^2 (8) = 5.13, $p = 0.744$ ($p > 0.05$), $R^2 = 0.042$ (Cox and Snell) and 0.057 (Nagelkerke); in other words, in best case scenario, a significant model could have predicted 4.2% to 5.7% of the outcome. As no significant correlations have presented in the bivariate and the binary logistic regression, the multivariate analysis of predictors of Vitamin D deficiency was deemed unnecessary.

A preliminary evaluation of Vitamin D level and the HIV immune function

laboratory data at baseline included running a total sample analysis and then a comparative analysis between Vitamin D deficient and sufficient groups on the following parameters: 25(OH)D level, CD4 count, CD4 percent, CD8 percent, CD4/CD8 ratio, and viral load. Before proceeding with analysis and fitting data into statistical models and tests, it was essential to explore these main outcome variables and examine the spread and dispersion of their values and frequency in the distribution. For the sake of avoiding inaccuracies in results and flaws in hypothesis testing, normality of distribution check through plotting histograms and using Shapiro-Wilk tests of normality was imperative before deciding on the use of parametric or non-parametric tests. Consequently, descriptive statistics (kurtosis and skewness) and histograms (plus Q-Q and P-P plots), in addition to normality tests were performed on all data at baseline (using SPSS) for the aforementioned variables.

The findings indicated that Vitamin D levels, CD4 count, CD4/CD8 ratio, and viral load data were not normally distributed (as per histograms and the Shapiro-Wilk test significant p values < 0.05) in the study population. On the other hand, CD4%, and CD8% data are normally distributed (Shapiro-Wilk test p values > 0.05). For non-normally distributed variables, median and IQR were used instead of mean and standard deviation, and the latter statistics were considered for normally distributed variables. Table 6 describes a summary of the baseline Vitamin D and immune parameters characteristics for the total study population.

Table 6

Baseline Vitamin D Level and HIV Immune Function Markers of Study Population

	Mean (*SD*)	Median (*IQR*)	Range
CD4 count	542.93 (286.34)	539.5 (370.0)	108 - 1503
CD4%	26.93 (10.90)	28.00 (14.0)	5 – 54
CD8%	45.80 (12.64)	45.86 (13.2)	21 – 74
CD4/CD8 ratio	0.70 (0.48)	0.61 (0.47)	0.07 – 2.14
Viral load	36266 (87846.4)	321.0 (26917)	0 – 506000
25(OH)D level	25.47 (14.47)	25.5 (22)	5 – 70

Prior to comparative analysis, separate descriptive statistics were explored in a split file for each group (deficient versus sufficient) in order to check for normal distribution. The findings indicated that Vitamin D levels, CD4/CD8 ratio, and viral load data were not normally distributed (as per histograms and the Shapiro-Wilk test significant p values < 0.05) in either deficient or sufficient group. On the other hand, CD4 count, CD4%, and CD8% data were found normally distributed in both groups (Shapiro-Wilk test p values > 0.05) and they also met the homogeneity of variance assumption for t test (as per Levene's test p values > 0.05). The baseline comparative analysis findings are presented in Table 7.

The mean difference in serum 25(OH)D levels between Vitamin D deficient group ($M = 15.83$, $SD = 7.3$) and Vitamin D sufficient group ($M = 39.93$, $SD = 9.7$) as calculated using independent t test showed a statistically significant difference at baseline with $t(68) = -11.88$, $p = 0.000$. However, since Vitamin D level variable did not meet normal distribution assumption based on the shape of the histograms and the Shapiro-

Wilk normality tests (deficient group $p = 0.010$ and sufficient group $p = 0.000$), the non-parametric Mann–Whitney *U* test was used instead of student *t* test to compare the medians and mean ranks of the two independent groups. The median levels of 25(OH)D was 15.5 ng/µL (*IQR* = 12, range 5 – 28) and 36 ng/µL (*IQR* = 11, range 30 - 70) for deficient and sufficient groups, respectively; there was an evidence that the groups differed significantly with higher mean rank of 56.5 in the sufficient group as compared to a mean rank of 21.5 in the deficient group ($U = 0.000$, $Z = -7.055$, $p = 0.000$). Mann-Whitney *U* test was also used to compare CD4/CD8 ratio in both groups.

Table 7

Baseline HIV Immune Function Markers by Vitamin D Group

	Vitamin D Deficient ($n = 42$)	Vitamin D Sufficient ($n = 28$)	Sig. *p* value
CD4 count (cells/µL)[Ŧ]	529.14 (266.16)	563.61 (318.151)	0.625
CD4%[Ŧ]	27.43 (10.13)	26.18 (12.12)	0.642
CD8%[Ŧ]	45.83 (12.42)	45.75 (13.20)	0.979
CD4/CD8 ratio[§]	0.61 (0.46)	0.51 (0.55)	0.513
Viral load (< 5000)[†]	22 (52.4%)	22 (78.6%)	0.026*

[Ŧ] Mean ± standard deviation (SD). Independent *t*-test.
[§] Median (IQR). Mann-Whitney test.
[†] *n* (%). Chi-square utilized to compare proportions by Vitamin D status group.
*Significance = $p \leq 0.05$

It should be noted that based on the frequency distribution analysis for viral load level and the corresponding wide dispersion of data, the scaled variable was transformed into a dichotomous nominal variable with two main categories: (1) HIV plasma RNA level below 5000 copies/mL and (2) HIV plasma RNA level above 5000 copies/mL. Chi-

square test was used to compare proportions between both groups with the characteristic of interest viral load level < 5000 copies/mL. The results of Table 7 revealed that the immune function parameters between deficient and sufficient groups did not differ significantly at baseline, except for viral load ($\chi^2(1, N = 70) = 4.936, p = 0.026$). Univariate analysis indicated that 76.9% of those with viral load > 5000 copies/mL were more likely to be Vitamin D deficient as compared to only 23.1% in the sufficient people.

Research Questions and Hypotheses

The first research question was developed based on conflicting results in the existing literature about the association between Vitamin D level and CD4 count/percent.

Research Question 1: Do Vitamin D levels significantly correlate with CD4 count and CD4 percent in Vitamin D deficient group versus sufficient group?

H_0: There is no statistically significant correlation between Vitamin D levels and CD4 count or percent in Vitamin D deficient group versus sufficient group.

H_1: There is statistically significant correlation between Vitamin D levels and CD4 count, or CD4 percent in Vitamin D deficient group versus sufficient group.

In order to answer this research question and to test the hypothesis, a bivariate linear correlation was carried out with both parametric and non-parametric correlation coefficients, Pearson's *r* and Spearman rho (r_s), respectively. However, since Pearson's correlation was sensitive to normal distribution, and based on lack of meeting normality assumptions for Vitamin D and CD4/CD8 ratio, Spearman rho (r_s) was used to rank the data and to provide better correlation coefficient estimates.

At baseline, there was no significant correlation between Vitamin D levels and CD4 count in all participants ($N = 70$), $r_s = 0.090$, $p = .0.458$. Moreover, CD4 count was not significantly related to Vitamin D levels in either Vitamin D groups, deficient and sufficient ($r_s = 0.107$ versus $r_s = 0.132$, $p > 0.05$, respectively). The negative correlation between Vitamin D levels and CD4 percent for the whole sample did not reach statistical significance as well on Spearman rho correlation ($r_s = -0.035$, $p = 0.776$). The bivariate correlation analysis failed to show sufficient evidence of significant negative correlation between baseline Vitamin D level and each of the following: CD8 percent, CD4/CD8 ratio and viral load for all study participants at baseline. Table 8 summarizes the bivariate correlations analyses between Vitamin D level and each of the immune parameters.

A binary logistic regression analysis was not needed as not all immune variables had statistically significant correlations with Vitamin D levels. A linear regression analysis was performed to examine any association between the aforementioned variables and Vitamin D levels at baseline, especially to define whether CD4 count or CD4 percent were associated with Vitamin D levels. The model failed to show significant relationship for the entire sample ($R = 0.271$, $p = 0.285$) whereby all variables accounted for 7.3% of the variation in Vitamin D levels ($R^2 = 0.073$). However, only CD4 percent had a weak but significant negative relationship with Vitamin D level ($\beta = -0.852$, $p = 0.044$). That meant that every one-unit increase in CD4% corresponded with 8.5 units decrease in Vitamin D levels.

In order to test the hypothesis whether Vitamin D levels and CD4 count and CD4 percent were equally correlated or the same in deficient versus sufficient group (Vitamin

D status), bivariate correlations for each group were performed and later a Fisher's *Z*-transformation test was calculated to test the significance of the difference between the correlations in both groups (see Table 8). Fisher's *Z*-transformation calculator was used to reduce skewness and convert the correlation coefficients into a *Z* score, that is, a normally distributed *Z* statistic.

Table 8

Baseline Comparison of Bivariate Correlations Between 25(OH)D Levels and Immune Function Variables

	Vitamin D Deficient ($n = 42$) Spearman r_s (sig.)	Vitamin D Sufficient ($n = 28$) Spearman r_s (sig.)	Fisher *Z*-Transformation *Z* (sig.)
CD4 count	.107 (0.502)	.132 (0.504)	-0.1 (0.920)
CD4 percent	-.084 (0.598)	.204 (0.298)	-1.14 (0.254)
CD8 percent	.028 (0.861)	-.336 (0.081)	1.47 (0.141)
CD4/CD8 ratio	-.094 (0.555)	.279 (0.151)	-1.49 (0.136)
Viral load	.286 (0.067)	-.194 (0.321)	1.92 (0.055)

Overall, the difference between correlations for both groups did not reach statistical significance ($p > 0.05$) in any of the immune parameters with Vitamin D levels (the sample sizes were too small to detect a significant difference). At the same time, multivariate analysis (MANOVA) failed to provide evidence of a statistical difference in the means of the combination of dependent variables (CD4 count, CD4%, CD4/CD8 ratio, & CD8%) between both Vitamin D groups; in other words, there was no effect of Vitamin D status on all the immune variables as a group in the model (all *p*s > 0.05). In conclusion, correlation testing for the first research question has resulted in accepting the

null hypothesis for CD4 count and CD4 percent. Moreover, both Vitamin D groups did not differ in their correlations with the immune parameters when Fisher Z-transformation was applied. Only viral load and CD4 % at baseline were significantly predictive of Vitamin D deficiency in logistic regression.

Research Question 2: Does Vitamin D supplementation have a statistically significant effect on CD4 count/percent in HIV-infected African American adult men in this study?

H_0: There is no statistically significant effect of Vitamin D supplementation on CD4 count/percent in HIV-infected African American men in this study.

H_1: There is a statistically significant effect of Vitamin D supplementation on CD4 count/percent in HIV-infected African American men in this study.

Vitamin D. After evaluating baseline Vitamin D levels, all HIV-infected patients in the deficiency group (n = 42) were prescribed Vitamin D supplementation (oral 50000 IU cholecalciferol once per week) and were followed-up for a time frame period that stretched up to 14 months from date of entry. A T0 denotes baseline visit, while T1 (up to 6 months from baseline) and T2 (up to 8 months from T1) denote follow-up visits, respectively. Of these 42 patients, only 28.6% (n = 12) normalized their Vitamin D levels by 6 months (i.e., 25(OH)D ≥ 30 ng/µL) and 71.4% remained deficient. The proportion of patients achieving Vitamin D sufficiency increased considerably by T2, whereby 21 patients (50%) have become Vitamin D sufficient and 21 (50%) remained deficient. (Figure 7).

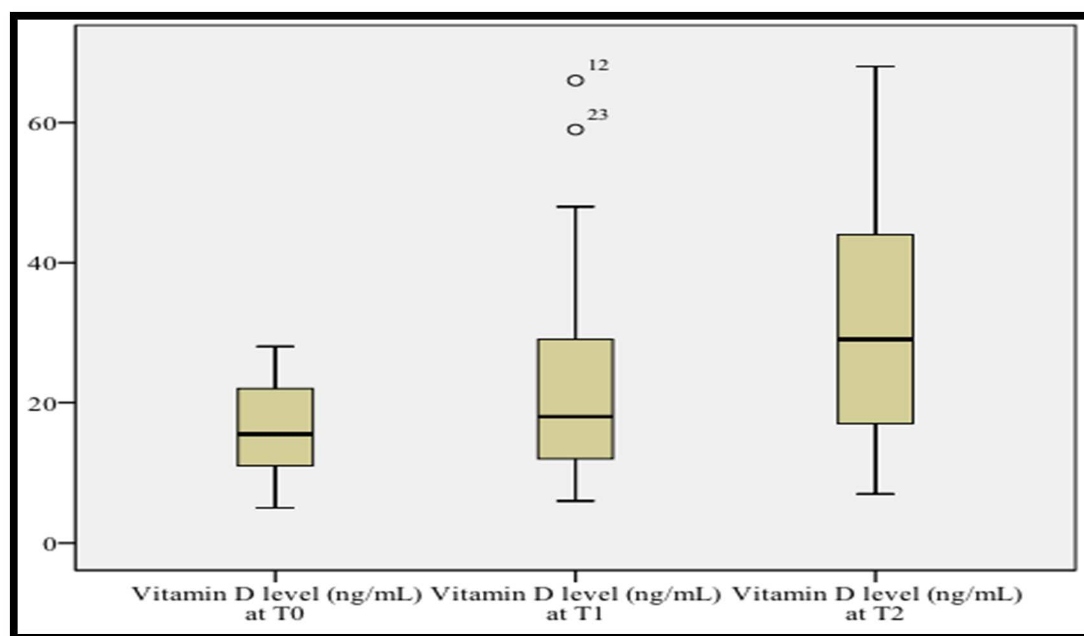

Figure 7. Change in 25(OH)D level from pre- to post-supplementation in deficient group.

Since Vitamin D levels were not normally distributed at baseline and follow-up visits (as per the histograms, Q-Q plots, P-P plots, and the Shapiro-Wilk tests of normality, $p < 0.05$), nonparametric Wilcoxon signed rank test are carried out to analyze presupplementation and postsupplementation Vitamin D levels. The Wilcoxon signed rank test showed that Vitamin D supplementation produced statistically significant changes in Vitamin D levels in the deficient patients at T1 ($Z = -2.256$, $p = 0.024$) and T2 ($Z = -4.916$, $p = 0.000$). The median 25(OH)D level at baseline is 15.50 ($IQR = 12$, range = 5–28) as compared to 18 ($IQR = 28$, range = 7–68) and 29 ($IQR = 17$, range = 6–66) in T1 and T2 visits, respectively. Moreover, Wilcoxon Rank's table had shown that from baseline T0 to T1, 14 participants out of 42 had lower Vitamin D levels at T1 than at presupplementation T0, while 25 participants had improved and normalized their Vitamin D levels at T1 post-supplementation (only 3 participants have no change in their levels).

From baseline to T2, 6 participants failed to normalize their Vitamin D levels as compared to 36 participants who significantly increased their levels postsupplementation (of which 21 achieved sufficiency by T2).

Overall, in comparing the Vitamin D level measurements on the same participants at three different time variables (T0, T1, and T2), the nonparametric Friedman test was used as an alternative to one-way ANOVA with repeated measures; the test compared the mean ranks to examine how the groups differed on their medians too. There was a statistically significant difference in Vitamin D levels after Vitamin D supplementation, $\chi^2(2) = 23.263$, $p = 0.000$. For post hoc analysis aiming to check where the differences really occurred among the related groups, the Wilcoxon signed-rank tests were used on multiple comparisons (T0 versus T1; T0 versus T2; and T1 versus T2) using Bonferroni adjustment on the *p* values obtained in the results. Consequently, a Bonferroni-adjusted significance level (known as Bonferroni correction) was set at 0.017, whereby the original significant *p* value of 0.05 was divided by number of comparisons, or by 3 in this case. Based on the results (presented in Table 9), the groups differed in their median Vitamin D levels (see values above) most significantly between baseline T0 and T2 ($p = 0.000$, < Bonferroni *p* of 0.017) and between T1 and T2 ($p = 0.004$, also less than 0.017). In other words, the increase in Vitamin D level was most significant between T0 to T2 and between T1 to T2. Statistically, the Wilcoxon median posttest ranks were significantly higher than the median pretest ranks (see Table 9).

Table 9

Vitamin D Levels Pre-Post Supplementation Changes: Wilcoxon Signed-Rank Test

	Vitamin D level at T1 - Vitamin D level at T0	Vitamin D level at T2 - Vitamin D level at T0	Vitamin D level at T2 - Vitamin D level at T1
Z	-2.256*	-4.916*	-2.905*
Asymp. Sig. (2-tailed)	.024	.000	.004

* Based on negative ranks.

HIV immune parameters. In order to check whether there was any effect of Vitamin D supplementation on CD4 count and percent, and the rest of the immune variables, a univariate analysis was run to determine the changes in levels at T1 and T2 before conducting comparative analysis. Moreover, a correlation analysis was performed between postsupplementation Vitamin D levels and postsupplementation CD4 count/percent. Univariate results show that CD4 count, CD4 percent, and CD8 percent were normally distributed (Shapiro-Wilk p values > 0.05) at baseline, T1, and T2, and accordingly, changes in their levels were examined through parametric tests such as paired t test and repeated measures ANOVA. CD4/CD8 ratio data lacked normal distribution, therefore, nonparametric tests such as Wilcoxon signed test and Friedman test were used.

In the normally distributed immune variables, the data were checked for outliers prior to comparing their means in order to reduce any effect of these values on the significance results (although they did not skew the distribution, means would remain sensitive to extreme values). For CD4 count, there were three outliers (one case at T0, and 2 cases at T1). Transformations of these outlier scores did not guarantee more robust

results nor did case removal due to small sample size. However, as per good practice guidelines suggested by Field (2007), the outlier scores have been changed and recalculated based on the mean value plus two times the standard deviation. Paired *t*–test before transformed outliers showed non-significant results ($p > 0.05$), and after adjusting the outliers, the paired *t*–test results remained non-significant. CD4 count, CD4 percent, and CD8 percent values with repeated measures remained normally distributed. Table 10 and Table 11 depict the immune parameters changes from pre- to post- supplementation at T1 and T2.

Table 10

HIV Immune Parameters Changes: Pre–and Post–Supplementation (T0 –T1)

	T0 (Presupplementation) *n* = 42 Mean (*SD*)	T1 (Postsupplementation) *n* = 42 Mean (*SD*)	*P* value
CD4 count	529.14 (266.16)	550.02 (257.52)	0.438
CD4 percent	27.26 (9.73)	28.10 (10.36)	0.449
CD8 percent	45.83 (12.42)	42.69 (12.11)	0.013*
CD4/CD8 ratio†	0.614 (0.47)	0.680 (0.55)	0.276

† Median (IQR), Wilcoxon signed rank test.
* Significant $p < 0.05$, based on paired *t* test.

The findings of the paired t–test for the within-group analysis of pre–post changes in Tables 10 and 11 suggested that after Vitamin D supplementation and up to the first follow-up period (T1), there was a significant change in the CD8 percent ($p = 0.013$) only. More importantly, these findings did not confirm any significant influence of Vitamin D supplementation on the main HIV immune parameters presented in the

hypothesis, which are CD4 count and CD4 percent. Despite a statistically significant increase in Vitamin D levels from baseline to follow-up visits, there was no corresponding influence of Vitamin D supplementation on the immune markers - which failed to exert any significant change in either direction, positive or negative.

Table 11

HIV Immune Parameters Changes: Pre–and Post–Supplementation (T0 –T2)

	T0 (Presupplementation) *n* = 42 Mean (*SD*)	T2 (Postsupplementation) *n* = 42 Mean (*SD*)	*P* value
CD4 count	529.14 (266.16)	586.48 (280.40)	0.084
CD4 percent	27.26 (9.73)	29.02 (10.79)	0.101
CD8 percent	45.83 (12.42)	44.26 (12.25)	0.380
CD4/CD8 ratio†	0.614 (0.47)	0.659 (0.56)	0.107

† Median (IQR), Wilcoxon signed rank test.

Repeated measures ANOVA were performed to examine the effect of Vitamin D supplementation on the immune variables, that is, to test the hypothesis whether there was any overall significant change in the CD4 count, CD4 percent, and CD8 percent values within subjects and across the three different time periods (T0, T1, and T2). All assumptions for the test were met except for Mauchly's sphericity (this assumption was violated, $p = 0.009$); therefore, in order to avoid distorted results, the Greenhouse-Geisser correction in the SPSS for within-subjects output table was reported instead. As a result, the ANOVA with repeated measures with Greenhouse-Geisser correction indicated that the mean values for CD4 count were not statistically significant; there was no significant

effect of supplementation on CD4 count, $F(1.654, 67.808) = 2.278$, $p = 0.119$ (Wilk's Lambda = 0.915, $F(2, 40) = 1.867$, $p = 0.168$). The strength of the relationship between Vitamin D supplementation and the change in the CD4 count scores as assessed by partial eta squared (i.e., effect size), was weak, with supplementation accounting for only 5.3% of the variance of the dependent variable, CD4 count.

Similarly, Vitamin D supplementation had no effect on CD4 percent change across baseline, T1, and T2. However, Mauchly's sphericity assumption was met for CD4 percent ($p = 0.963$). The repeated measures ANOVA again failed to demonstrate a significant effect of Vitamin D supplementation on CD4 percent, Wilk's Lambda = 0.936, $F(2, 40) = 1.379$, $p = 0.264$. Partial eta squared or effect size was also weak, with supplementation accounting for only 6.4% of the variance in CD4 percent. Post-hoc pairwise differences among the means of CD4 percent with the use of Bonferroni correction coincided with the earlier results of paired *t*–tests, and accordingly, have failed to elicit significant changes postsupplementation.

As for CD8 percent, supplementation did not produce statistically significant changes in the mean levels from baseline to postsupplementation, but it had elicited a significant reduction in mean value from baseline to T1 ($p = 0.038$). On the other hand, the nonparametric Friedman test of differences in medians among repeated measures for CD4/CD8 ratio was conducted and rendered a $\chi^2(2, n = 42)$ of 1.280, which was not significant ($p = 0.527$). Beside repeated measures, bivariate Pearson's correlations between Vitamin D levels and postsupplementation CD4 count, CD4 percent, and CD8% at T1 and T2, failed to yield significant results (all p values > 0.05).

Supplemented versus nonsupplemented. Although the intention of this study was to follow-up only on Vitamin D deficient participants, as the abstractor, I did collect data on Vitamin D and the corresponding immune parameters from sufficient subjects with subsequent visits that took place within six months of baseline. Only 18 (64%) nonsupplemented Vitamin D sufficient participants out of 28 had complete data for T1. Bivariate Spearman correlations between T1 Vitamin D levels and CD4 count and percent after supplementation also remained non-significant. Paired *t*–test analysis for the nonsupplemented group revealed no statistical difference between T0 and T1 (all p values > 0.05) for the immune parameters. Only Vitamin D levels for the sufficient group have failed the normality assumption, and a comparison of their median Vitamin D levels at T0 and T1 showed a statistically significant difference with Wilcoxon signed rank test ($Z = -2.288$, $p = 0.022$). According to the negative ranks, fourteen participants had higher levels at baseline than at T1, and four had higher levels at T1 without supplementation. T1 median = 30 (*IQR* = 21, range = 18–72) compared to T0 median = 36 (*IQR* = 11, range = 30–70), $p = 0.022$.

Mann-Whitney *U* test was performed to examine group difference in Vitamin D levels between supplemented and nonsupplemented group at T0 and T1. The result of Mann-Whitney revealed that compared to the supplemented group, the nonsupplemented group still had higher medians and mean ranks at T0 and T1 (mean ranks: 50.5 versus 21.5, and 42.4 versus 25.4, at T0 and T1, respectively). Most importantly, the group difference in Vitamin D levels at T1 was highly significant, $U(60) = 163.5$, $Z = -3.463$, $p = 0.001$.

As for viral load, the proportion of participants in the supplemented group belonging to the undetectable to the very low viral load category (0–1000 copies/mL) had increased postsupplementation from 50% at T0 to 69% at T1, as compared to 83% for the nonsupplemented group at T0 to 89% at T1. Fisher's exact test showed that there was no difference postsupplementation at T1 ($p = 0.192$) between both groups, unlike at T0 ($p = 0.021$). Mann-Whitney median and mean ranks comparison between groups at T0 and T1, indicated a significant difference in viral load at baseline only ($U(60) = 252.000$, $Z = -2.395$, $p = 0.017$) with higher viral load mean ranks for the deficient group, but did not yield any significant difference in viral load between both groups postsupplementation ($U(60) = 303.000$, $Z = -1.613$, $p = 0.107$).

In order to examine the effect of supplementation on the HIV immune parameters, the last statistical analysis consisted of running ANCOVA to compare changes in post–pre mean scores for these parameters, while treating their presupplementation or baseline scores as time-varying covariate and as a confounding variable, and the postsupplementation scores as the response. Group allocation to supplementation was considered the fixed effect. All ANCOVA assumptions were considered and were met (Levene's test of sphericity $p = 0.145$; homogeneity of regression slopes $p = 0.302$). The findings (see Table 12) revealed that there was no statistically significant effect of Vitamin D supplementation on CD4 counts at postsupplementation after controlling for baseline Vitamin D levels ($F(1, 56) = 1.563$, $p = 0.216$). Even after adjusting the means for CD4 count following the removal of the effect of the covariate (the mean had increased for the nonsupplemented group to 714.5, and to 566.07 for the supplemented

group), the *F* ratio remained nonsignificant, $F(1, 56) = 1.039$, $p = 0.312$. If the covariance were significant, the increase in the adjusted means would have indicated a possible positive covariance between CD4 count and supplementation status (group allocation), especially in the nonsupplemented group. The results failed to reject the null hypothesis of significant effect of supplementation on CD4 count.

Table 12

Changes in Vitamin D Levels and Immune Parameters in Supplemented Group Versus Nonsupplemented Group: Baseline to T1.

Parameter	Supplemented Group (*n* = 42)		Nonsupplemented Group (*n* = 18)		Sig. † (*p* Value)
	Baseline	**T1**	**Baseline**	**T1**	
25(OH)D level	15.83 ± 7.3	21.88 ± 13.9	40.83 ± 10.7	35.61 ± 15.2	0.001*
CD4 count	529.14 ± 266.2	550.02 ± 257.5	566.9 ± 349.5	590.2 ± 335.2	0.216
CD4 percent	27.26 ± 9.7	28.10 ± 10.4	26.22 ± 13.1	27.22 ± 10.7	0.918
CD8 percent	45.83 ± 12.4	42.69 ± 12.1	43.83 ± 12.8	43.00 ± 11.7	0.367

† P value calculated based on Analysis of Covariance (ANCOVA).
* Significance $p < 0.05$. Based on Mann-Whitney U test.

ANCOVA results for CD4 percent showed a statistically non-significant relationship between baseline Vitamin D levels and CD4 percent within the groups, $F(1, 56) = 0.011$, $p = 0.918$). The covariate did not contribute at all to the variance of the CD4 percent. The adjusted means have shown a reduction in CD4 percent in the nonsupplemented group as compared to an increase in CD4 percent in the supplemented group; however, these adjustments remained non-significant, $F(1, 56) = 0.055$, $p = 0.816$. Again, this result failed to reject null hypothesis of no statistically significant effect of

Vitamin D supplementation on CD4 percent. Similar to the results for CD4 count and CD4 percent, supplementation has failed to exert any significant influence on CD8 percent, $F(1, 56) = 0.829$, $p = 0.367$.

Summary and Conclusion

This chapter has displayed the statistical analyses of this retrospective chart review study that ranged from descriptive to inferential and hypotheses testing. All statistical tests have been performed using SPSS 21. The chapter has provided detailed presentation of all the analyses and the results related to two main research questions and their corresponding hypotheses. The first research question examined the presence of correlation between Vitamin D level and CD4 count/percent levels. The results have failed to reject the null hypothesis of no correlation between Vitamin D levels and CD4 count/percent. The second research question evaluateed the effect of Vitamin D supplementation on CD4 count and CD4 percent, in addition to comparing the difference in Vitamin D levels between the deficient/supplemented group and the nonsupplemented group (like a placebo group). Despite a significant increase in Vitamin D levels postsupplementation, again, the effect of Vitamin D supplementation was not evident on the CD4 count and CD4 percent levels; the study has failed to reject the null hypothesis of no significant impact of supplementation on these main HIV immune markers. Chapter 5 presents a detailed interpretation of all findings in this chapter, along with an extrapolation to the findings in the existent literature on this topic.

Chapter 5: Discussion, Conclusions, and Recommendations

Overview

The purpose of this quantitative, observational retrospective chart review study was to examine if any association between Vitamin D deficiency and CD4 exists in HIV positive persons and to then evaluate the impact of Vitamin D supplementation. The literature has shown that Vitamin D is an immunomodulator that regulates immune target cell function, especially CD4 T cells that express Vitamin D receptors, the fact which might enable Vitamin D to influence their immune responses against the invading pathogens (Cantorna, 2011). The immune influence of Vitamin D deficiency remains understudied in HIV and is rather controversial, especially with regard to the conflicting results in the literature that support or reject such influence. Moreover, there is scarce knowledge about the impact of Vitamin D supplementation on immune function in the field of HIV.

The relationship between Vitamin D and CD4 T cells constituted the conceptual framework for this study. The proposed conceptual framework suggests that Vitamin D in its active form in the body binds to the Vitamin D receptors (VDR) on the immune cells, especially the CD4 T cells and B lymphocytes. This leads to an activation of the CD4 and to an increase in the number of VDR that is translated into enhanced modulating effects of Vitamin D on CD4 cells (Baeke et al., 2010c; Beard et al., 2011; Deluca & Cantorna, 2001; Kamen & Tangpricha, 2010; Mahon et al., 2003), followed by a regulation of immune responses to pathogens (e.g., increasing the production of antimicrobial peptides – cathelicidin) (Baeke et al., 2007; Heany, 2008; Kamen &

Tangpricha, 2010; Liu et al., 2006; Liu et al., 2007; Wang et al., 2004; Wolff et al., 2008) and of cytokines production (Kamen & Tangpricha, 2010; Lake & Adams, 2011). Since HIV is characterized by a general pro-inflammatory condition, restoration of Vitamin D enhances the cytokine production that favors an anti-inflammatory milieu at the immune level, and improves CD4 count, which eventually slows progression towards AIDS (Lake & Adams, 2011).

In the context of framework, this study speculated that restoring Vitamin D to normal levels after supplementation would ensue in a simultaneous increase in CD4 count/percent, which in turn would enhance the overall immune health of HIV patients. This study addressed the gap in the literature through framing two research questions that were also directly relevant to the framework: the first examined the correlation between Vitamin D level and CD4 count/percent, and the second question evaluated the impact of Vitamin D supplementation as depicted by improvements in Vitamin D levels and the corresponding changes in CD4 count/percent in a group of Vitamin D deficient HIV-infected African American patients.

In order to answer these research questions, data was collected from a convenience cohort of 70 African American HIV-infected male participants (aged 21 years and up) who attended a specialized underserved HIV clinic in Southeast Michigan. The cohort was split into two groups based on first Vitamin D level at study entry: 1- Vitamin D deficient group (n = 42), and 2- Vitamin D sufficient (n = 28). The deficient group was followed up for more than a year retrospectively, and the participants' clinical parameters pertaining to Vitamin D and immune function were assessed at three points in

time: baseline (T0), T1 (up to 6 months), and T2 (up to 14 months). The sufficient group was mainly assessed at T0, and only 18 participants had follow-up data at T1. The researcher used chart review to abstract data and entered it into an electronic database using SPSS 21.0 software. Chapter 4 discussed the data analyses processes in details and presented the results. This chapter scrutinizes and interprets the main findings of the study, upon which conclusions, social change implications, and recommendations are furnished.

Interpretation of the Findings

It is well documented that there is a high prevalence of Vitamin D deficiency among HIV-infected patients of Vitamin D deficiency that can range from 70 percent to more than 90 percent (Bang et al., 2010; Dao et al., 2011; Vescini et al., 2011; Viard et al., 2011). As the study population consisted solely of African Americans, who have an assumed innate susceptibility to Vitamin D deficiency, 60 percent (n = 42) of the total study population were Vitamin D deficient at study entry; however, this prevalence could have constituted a vast majority if most of the 125 charts reviewed by the researcher and pertaining to HIV African American patients seen at the clinic had complete data on Vitamin D at subsequent visits within one year time frame from first baseline Vitamin D level. It was interesting to find that 40 percent of the total sample (n = 28) had sufficient Vitamin D levels, despite the applicable factors that constitute some known predisposing factors to Vitamin D deficiency with residual confounding effect such as, black race/ethnicity, geographic location and northern latitude, inadequate sunlight exposure, and low Vitamin D diet. The number of patients who had history of AIDS in the study

population was low (13 patients total, 8 in the deficient group and 5 in the sufficient group), but this was not associated with Vitamin D deficiency in the bivariate correlation or in the logistic model, unlike the findings by Mueller et al. (2010).

The results from the present study have shown that deficient and sufficient groups differed on baseline Vitamin D levels. The sufficient group had higher Vitamin D level mean rank (56.5) and median (36 ng/µL) as compared to the mean rank (21.5) and median (15.5 ng/µL) of Vitamin D level in the deficient group. Both groups were comparable at baseline on almost all characteristics that are commonly considered significant predictors of Vitamin D deficiency and that are accounted for in this study, accordingly: Age, BMI, HIV duration, smoking, history of AIDS, transmission mode, having hepatitis C, being on ART, and Vitamin D level measurement season. Upon examining if these risk factors constituted predictors of Vitamin D deficiency levels and/or Vitamin D status (deficiency being an indicator) in this study population, the bivariate logistic regression model failed to show significant results with any of these factors, in contrast to most findings in the literature. Most likely, this could be due to the inability to infer significant and stable correlations from a small sample size (Schonbrodt & Perugini, 2013). It should be noted that both groups differed on diabetes only, whereby none of the participants in the sufficient group had diabetes as compared to six participants in the deficient group. Despite the study small sample size, this finding on diabetes is consistent with what the literature highlights about the inverse association between low Vitamin D levels and higher incidence of type II diabetes, secondary to a propensity to have an increase in insulin resistance in Vitamin D deficient people

(Forouhi et al., 2012); however, a causal relationship between Vitamin D deficiency and type II diabetes is still far from being confirmed and, thus, constitutes a fertile area to investigate (Baz-Hecht & Goldfine, 2010; Tai, Need, Horowitz, & Chapman, 2008).

At baseline, both groups did not differ significantly on CD4 count, CD4 percent, CD8 percent, CD4/CD8 ratio, and viral load. Although group comparability at baseline could provide an unbiased platform upon which it would be easier to assess changes in Vitamin D and the immune parameters over the follow-up period, this could influence the whole analysis, which is based on baseline Vitamin D and on presumed difference in the main immune parameters, specifically CD4 count and CD4 percent. Such comparability reflected some considerable degree of immune competence as depicted by high means of CD4 count in both groups ($M = 529 \pm 266.16$ versus $M = 563.61 \pm 318.15$, in deficient and sufficient groups, respectively). The fact that 90 percent of the total participants were on ART at time of entry into study could imply that these participants have some degree of preserved immunity. Another possible reason for this enhanced immunity could be explained by the closeness of follow-up visits, hence the seeking of medical care those participants from both groups initiated. Conversely, being on ART did not seem to have an influential effect on Vitamin D level in this study population because of lack of evident correlation between ART and Vitamin D deficiency status, unlike other studies that found a high correlation between Vitamin D deficiency and ART (Childs et al., 2012; Conesa-Botella et al., 2010; Dao et al., 2011; Giusti et al., 2011; Griffin & Arnold, 2012; Mueller et al., 2010; Rodriguez et al., 2009; Van Den Bout- Van Den Beukel et al., 2008; Viard et al., 2011). However, adherence to ART was partially reflected by low or

undetectable levels of viral load in both groups, but more pronounced in the sufficient group. This study did not consider the type of ART or the duration of receiving ART to avoid methodological complexity issues with data collection and analysis, especially in view of the small study sample size, and this not being one of the objectives of the study.

Research Question 1. This study was conducted to emphasize the role of Vitamin D as an immune-modulator and as an immune response enhancer based on the HIV literature that discussed the unique Vitamin D characteristic that enables it of regulating the immune cells functions, mainly the CD4 T cells. Therefore, the first research question in this study examined the correlation between Vitamin D level and CD4 count and CD4 percent in both deficient and sufficient groups. At baseline, Spearman rho correlation coefficient, for the total sample, did not show significant correlation between Vitamin D levels and CD4 count ($p = 0.458$) or with CD4 percent ($p = 0.776$). Similarly, there was no correlation between Vitamin D levels and either of the group's CD4 count/percent, at baseline or at T1. Results of bivariate correlations, MANOVA, and linear regression model analyses, could not find a significant relationship between Vitamin D levels and the immune parameters: CD4 count, CD4 percent, CD8 percent, CD4/CD8 ratio, and viral load. In concordance with previous studies (Bang et al., 2010; Bearden et al., 2013; Gedala et al., 2013; Kim et al., 2011; Mehta et al., 2009, 2010; Sudfeld et al., 2012; Turett et al., 2013; Van Den Bout- Van Den Beukel et al., 2008; Viard et al., 2011; Wasserman & Rubin, 2010), this study could not provide evidence of any significant relationship, correlation or association, between Vitamin D levels and CD4 count or CD4 percent, unlike the findings of the studies done by De Luis

et al. (2002), Egan et al., 2008; Giusti et al., 2011, Haug et al. (1994, 1998), Legeai et al. (2013), Ross et al. (2011), Stein et al. (2010), Theodorou et al. (2013), and Welz et al. (2010). However, in most of these studies, the observed correlation was mostly between Vitamin D deficiency status and low CD4 counts, as compared to this study that sought to find any correlation between Vitamin D levels and CD4 count/percent mostly irrespective of categorization for parameters (knowing that the study population has preserved immune function). This explanation is in agreement with Sudfeld et al. (2012) who viewed the results of these studies (Haug et al., 1998; Rustein et al., 2011; Stein et al., 2010; Welz et al., 2010) as proving a “biologically plausible” (p. 5) phenomenon that patients with low CD4 counts are more likely to be Vitamin D deficient secondary to increased risk for opportunistic infections that is accompanied by high immune cells activation and a corresponding high use of Vitamin D by these cells. This explanation is in agreement with the studies that correlated Vitamin D deficiency with greater risk for HIV disease progression and mortality (Mehta et al., 2010, 2011; Sudfeld et al., 2012; Viard et al., 2011) and yet could not find a correlation between Vitamin D levels and CD4 counts.

The study finding that there is no correlation could be indicative of methodological limitation; whereby, the analysis was based on the available data (for both groups) at baseline, thus, could be viewed as similar to using a cross-sectional approach that could not account for longitudinal data when testing for correlation. Even, when the correlation analysis was run at a consecutive follow-up visit (T1), still, the study failed to establish a correlation between Vitamin D levels and CD4 count/percent in

view of the limited or insignificant changes in the parameters at T1 under the context of Vitamin D supplementation. The small sample size of the deficient group and the baseline comparability between both deficient and sufficient groups in most characteristics, especially in the immune parameters, could partly explain the inability to find a plausible correlation.

Research Question 2. The second research question investigated the effect of Vitamin D supplementation (50,000 IU once weekly) on CD4 count and CD4 percent in Vitamin D deficient group at six months (T1) and at one year (T2), and in Vitamin D sufficient group at T1. Changes in Vitamin D levels, CD4 count, and CD4 percent were compared between deficient/supplemented group and sufficient/nonsupplemented group. The nonsupplemented group consisted of 18 participants out of 28 sufficient participants and who had complete follow-up data at T1. This group was treated as a placebo or control group against the supplemented group. Taking the deficient group alone (n =48), 28.6 percent normalized their Vitamin D levels by T1, and 50 percent by T2 on post-supplementation analysis. A total of 36 (86%) participants improved their Vitamin D levels post-supplementation. There was a statistically significant increase in Vitamin D levels from a median of 15.5 ng/µL at T0, to 18 and 29 ng/µL at T1 and T2, respectively. The results of the Friedman test that compared the changes in Vitamin D levels across time confirmed that median posttest ranks were significantly higher than median pretest ranks (p = 0.000), with most pronounced increase taking place between T0 and T2 and between T1 and T2, as compared to between T0 and T1, as shown in post-hoc analysis. The increase in serum Vitamin D postsupplementation is consistent with findings from

Arpadi et al. (2009), Giacomet et al. (2013), Kakalia et al. (2011), and Van Den Bout-Van Den Beukel et al. (2008).

Post-supplementation, paired *t* test was performed to assess changes in the immune parameters (CD4 count, CD4 percent, CD8 percent, and CD4/CD8 ratio) from baseline to T1, and from baseline to T2 (for the deficient/supplemented group only). The findings showed no statistically significant changes in the parameters at T1, except for a lower CD8 percent ($p = 0.013$). In general, a lower CD8 percent could be indicative of either an ongoing non-HIV related infection or to an increased risk for HIV progression or even for higher risk for mortality (Hellebergh et al., 2014). The mean CD4 count increased from 529.14 cells/µL at T0 to 550.02 cells/µL at T1 ($p = 0.438$). From baseline to T2, the increase in CD4 count was more evident by 57 units but failed to be statistically significant (CD4 count at T2: 586.48 cells/µL, $p = 0.084$). Similarly, the supplemented group did not show significant improvement in the CD4 percent, whereby the increase in CD4 percent from baseline was by one unit at each follow-up, T1 (28.10 ± 10.36) and T2 (29.02 ± 10.79) postsupplementation ($p > 0.05$). A repeated measure ANOVA was conducted to test the overall change in the parameters within the supplemented subjects and across time (T0, T1, and T2). The findings indicated that Vitamin D supplementation had no effect on the immune parameters in question, CD4 count ($p = 0.168$) and CD4 percent ($p = 0.264$), in the supplemented group.

Upon comparing the supplemented group versus the nonsupplemented group, they still differed significantly on their Vitamin D level at T0 and at T1. Surprisingly, the nonsupplemented group showed better results in their Vitamin D medians and mean

ranks than the supplemented group at T1 (T0 mean ranks: 50.5 versus 21.5, and T1 mean ranks: 42.4 versus 25.4; $p = 0.001$). As for viral load, there was an increase in the proportion of participants who lowered their viral load to undetectable level or less than 1000 copies/mL among the supplemented (from 50% at T0 to 69% at T1) as well as among the nonsupplemented (from 83% at T0 to 89% at T1); both groups differed at baseline, but did not differ statistically on viral load change at T1, $p = 0.107$. Lastly, ANCOVA test was performed to examine the effect of supplementation on HIV immune parameters through comparing changes in post-pre scores between supplemented and nonsupplemented groups, after controlling for baseline Vitamin D levels. The nonsupplemented group ($n = 18$) at T1 showed no statistically significant difference in their CD4 count and CD4 percent compared to baseline. Actually, 14 subjects (78%) of the nonsupplemented had a significant drop in their CD4 count at T1 from a median of 36 (*IQR* = 11, range = 30 -70) at baseline to a median of 30 (*IQR* = 21, range = 18 -72) at T1, $p = 0.022$. However, the nonsupplemented group had an increase in their mean scores of CD4 count from 566.9 cells/μL (*SD* = 349.5) at baseline to 590.2 (*SD* = 335.2), which was not statistically significant ($p = 0.216$). Likewise, the increase in CD4 percent scores in the nonsupplemented group from 26.22 (*SD* = 13.1) to 27.22 (*SD* =10.7) at T1 did not yield statistical significance ($p = 0.918$). Overall, the results of ANCOVA failed to reject the null hypothesis of no statistically significant effect of Vitamin D supplementation on the main parameters of immune function, CD4 count and CD4 percent, in both groups.

The study hypothesized an improvement in the immune function of Vitamin D deficient HIV- infected participants following supplementation. However, the reported

findings of no effect on immune parameters resonate with results from previous studies, despite some differences in the methodological approaches. Kakalia et al. (2011) evaluated the impact of Vitamin D supplementation on CD4 count in 54 children with HIV (3-18 years old, mean age = 10.3 ± 3.9) in a randomized, non-blinded, controlled clinical trial. About 85% were deficient and 15% were sufficient; the majority was on ART, and had some preserved immunity (baseline mean CD4 count = 927 cells/μL ± 468). The authors randomized the study population to three groups: placebo Group 1 (no supplementation), and two supplemented groups (800 IU/day for Group 2, and 1600 IU/day for Group 3). Similar to the findings from the present study, supplementation significantly improved Vitamin D levels in supplemented groups (Vitamin D normalization increased from 17% to 39% in Group 2 and from 6% to 67% in Group 3) but not in the placebo group. However, the mean change in vitamin level from baseline to six months was not statistically significant in Group 3. On the other hand, similar to this study finding, Kakalia et al. (2011) failed to provide evidence of a significant impact of Vitamin D supplementation on CD4 count, CD4 percent, or viral load. The authors argued that because the study population had some preserved immunity at baseline, it was less likely for Vitamin D supplementation would exert more significant changes in their immune parameters; besides, the findings could not be generalized to a population with advanced HIV disease and more immune-compromised status. This explanation is also applicable to the present study population who has relatively some degree of immune competence.

Arpadi et al. (2009) evaluated a 12-month Vitamin D supplementation intervention in a randomized controlled study that recruited 56 HIV-infected children and split them into two groups: placebo (Vitamin D sufficient $n = 27$) and supplemented (Vitamin D deficient $n = 9$). Although the objective of the study was to evaluate the effect of bimonthly supplementation of 100,000 IU of Vitamin D on bone mass, the authors assessed changes in CD4 count and viral load and found no impact of supplementation on these parameters. Giacomet et al. (2013) also could not find significant changes in CD4 counts after supplementing a group of Vitamin D deficient children ($n = 26$) with 100,000 IU D3 administered every three months against a placebo group ($n = 26$) with comparable immune function and Vitamin D profiles at baseline. Just in keeping with Kakalia et al. (2011) and the present study, the fact that the study populations had some degree of preserved immunity could have underpowered the impact of supplementation on CD4 count/percent.

Poowuttikul et al. (2013) study remains the closest to the present study in terms of design (retrospective chart review), population ($N = 160$ dark skinned HIV-infected patients with low Vitamin D), and geographic location (Detroit, Michigan). However, their study population was younger and consisted of children and young adults aged 2 to 26 years who were recruited as part of routine clinical care. The authors used a cutoff point for Vitamin D deficiency/insufficiency at ≤ 35 ng/μL, where 152 had Vitamin D deficiency and received Vitamin D3 supplementation 1000 IU/day. Similar to this study, Poowuttikul et al. (2013) aimed to evaluate whether Vitamin D supplementation in HIV patients would improve the disease immune markers such as, CD4 count, CD4 percent,

and viral load. The changes in Vitamin D levels post-supplementation were statistically significant in the supplemented groups, whereby 39.5 percent (n = 60) reached normalization. On the other hand, the changes in CD4 count, CD4 percent, or viral load failed to reflect any significant effect of Vitamin D supplementation. A plausible explanation for this lack of supplementation effect could be that the study population had high pre-supplementation CD4 counts (M = 734 cells/μL ± 469.9) that did not significantly change post-supplementation (M = 702.3 cells/μL ± 446.7), p = 0.26. This is applicable to CD4 percent pre-post absolute change (p = 0.60) and to viral load (p = 0.31). This study by Poowuttikul et al. (2013) provided similar insights concerning the effect of Vitamin D supplementation on the immune function of HIV patients. The following section discusses the limitations of the present study, some of which could be pertinent to the above-mentioned studies dealing with Vitamin D supplementation in HIV.

Limitations of the Study

There are some limitations that can explain why the study findings could not reach the intended statistical significance. First and foremost, the present study is observational and retrospective in nature; thus, it brings along some typical limitations that are applicable to this kind of design, such as: lacking control over the included variables; introducing selection bias (threat to internal validity) by including only patients with complete data; presenting threat to external validity through inability to generalize results to other subpopulations; failing to control for compliance issues; and lacking the ability to account for exposure variables (e.g., Vitamin D intake through diet or sunlight

exposure, or even using a multivitamin with Vitamin D). The sample consists of a convenience sample and it comes from a single-site clinic; the sample size could have been too small to have statistical power that can demonstrate the intended effect. Besides, this study, being retrospective and longitudinal, could not provide any guarantee with compliance to the supplementation regimen, the fact that could have influenced considerably the findings. Despite prescribing the same supplementation regimen (50,000 IU/weekly of cholecalciferol or D3) to all study participants, it is hard to assume that all participants dispensed the same Vitamin D type (i.e., D3), especially that D2 is more commonly used in prescriptions but is less potent and has higher degradation than D3. It is noteworthy to mention that Vitamin D testing is expensive and this can impede routine checking for Vitamin D in clinical settings with poor resources (similar to the clinic in this study). This was evident in the inability of the researcher to include more patients in the sample size due to Vitamin D testing limitations and despite the high prevalence of Vitamin D deficiency among the HIV patients attending the clinic. Last but not least, this study did not account for the type of ART, knowing that some ART types are negatively implicated in Vitamin D deficiency (e.g., Efavirenz). It remains unclear though whether accounting for ART could have influenced the findings related to immune parameters.

Recommendations

Given the findings of this study, there is a need for large prospective longitudinal studies that take into account all the above-mentioned limitations. Multicenter studies could provide even more insights concerning the different HIV populations with high prevalence of Vitamin D deficiency. Randomized controlled trials (RCTs) that use

Vitamin D supplementation with appropriate doses (controlled intervention) are the only designs that can provide unbiased, evidence-based, and generalizable results pertained to the potential effect of supplementation on Vitamin D levels or immune function parameters in HIV. The controversial issue concerning the correlation between Vitamin D levels and CD4 count remains unsolved. Therefore, more studies should be undertaken to clearly establish the relationship between Vitamin D deficiency and CD4 not only in the context of evaluating supplementation but also in the context of highlighting the immune impact of Vitamin D, the potent immune-modulator targeting the CD4 cells in HIV. There are many studies that have demonstrated the overall health benefits of normal Vitamin D levels for general health and in multitude of diseases (Bischoff-Ferrari et al., 2006; Holick, 2004, 2006, 2007; Holick et al., 2011; Holick & Chen, 2008; Hossein-Nezhad & Holick, 2013). However, the variety in the recommendations for Vitamin D supplementation regimen could be responsible for the lack of consensus on the clinical efficacy of correcting Vitamin D deficiency in some populations, including HIV, and therefore, present an ongoing gap in the literature that need to be addressed in future studies.

Implications

This study recognized the importance of acknowledging and treating Vitamin D deficiency, a very common issue that is highly prevalent in the general population worldwide, as well as in the HIV subpopulation. The negative implications of Vitamin D deficiency in various chronic diseases discussed in the vast medical literature, besides HIV, have pinpointed to the importance of addressing and managing this easily

preventable condition. A general purpose of this study is to provide an eye-opener to an often-overlooked problem in routine HIV care, that of high prevalence of Vitamin D deficiency in African American HIV-infected patients. African Americans, being dark-skinned, have an inherent biologic tendency to be Vitamin D deficient, the fact that puts them at a disadvantage and more prone for health problems as compared to other racial groups.

In the context of HIV, Vitamin D has proved to be an immune-modulator and an immune response booster, and therefore, public health efforts should seek to educate the patients, the clinicians, the health policy makers, and the public on the significance of screening for and correcting the deficiency status, especially in view of the studies that linked Vitamin D deficiency to greater risk for disease progression or mortality. Although the evidence about the benefits or efficacy of Vitamin D supplementation on the immune function of HIV patients is still controversial, the knowledge on the risk factors and the negatives outcomes associated with Vitamin D deficiency in HIV are known and provide a solid platform to embrace good practices to manage this epidemic. A social change implication secondary to the findings of the study (whether significant or not) would be to acknowledge the need to modify the clinical approach in caring for HIV patients, in a way that make clinicians more aware of factors that contribute to HIV disease process (i.e., Vitamin D deficiency), notably in highly-impacted and high-risk populations such as African American patients. Vitamin D deficiency supplementation is a both cost-effective and easy strategy that can dramatically impact the general health of the

population; needless to say, this overhaul encompasses healthy individuals to reach patients from all walks of life, including those with HIV.

Conclusion

Vitamin D deficiency remains a worldwide health problem that is often overlooked, and yet merits special acknowledgment in the health field because of its association with a variety of chronic diseases, including HIV. The literature has shown that, as compared to the general population, the HIV population seems to be more susceptible to Vitamin D deficiency and to its adverse effects. More specifically, the discovered immune-modulatory role of Vitamin D has kindled a series of studies that targeted HIV populations in an attempt to reduce HIV disease burden and halt disease progression towards AIDS. Since CD4 count presents an important immune function parameter in HIV, this retrospective observational study opted to examine the relationship between Vitamin D levels and CD4 count/percent and to examine the impact of Vitamin D supplementation (as part of routine care) on CD4 count/percent. The fact that this study could not find significant results should be viewed with some caution because it may imply that the study had inadequate power to test the relationship or to find an effect of Vitamin D supplementation on CD4. This remains an observational study with non-generalizable results secondary to selection bias of study population and to the inadequate sample size due to low eligibility of participants at study entrance; these limitations led to low statistical power that could have undermined the possibility of detecting any effect, besides potential increased type II error and inflated variance in the variables under study that could have influenced the results. However, while the data may

not be statistically significant in this current study, the theoretical framework should not be rejected altogether, rather should provide a valuable insight to be tested in large prospective longitudinal studies and RCTs that take into consideration feasibility and limitation issues to maximize their methodological rigor and extend an evidence-based knowledge in the field. The findings from this study could not establish enough bases for clinical relevance to market implementing Vitamin D testing and supplementation in routine HIV care; yet, this remains a challenge that is incumbent on future public health research endeavors to draw on all findings and limitations in order to tailor better suited studies capable of improving the health status of individuals with HIV infection.

References

Abuzeid, W. M., Akbar, N. A., & Zacharek, M. A. (2012). Vitamin D and chronic rhinitis. *Current Opinion in Allergy and Clinical Immunology*, *12*(1), 13–17. Retrieved from http://www.medscape.com/viewarticle/757649_4

Adams, J. S., & Hewison, M. (2010). Update in Vitamin D. *Journal of Clinical Endocrinology and Metabolism*, *95*(2), 471–478. doi:10.1210/jc.2009-1773

Adeyemi, O. M., Agniel, D., French, A. L., Tien, P., Weber, K., Glesby, M. J. … Cohen, M. (2011). Vitamin D deficiency in HIV-infected and un-infected women in the US. *Journal of Acquired Immune Deficiency Syndrome*, 57(3), 197–204. doi:10.1097/QAI.0b013e31821ae418

AIDS/HIV Program-Wisconsin Department of Health Services. (2012). *AIDS/HIV surveillance annual review: Cases reported through December 31, 2011*. Retrieved from http://www.dhs.wisconsin.gov/aidshiv/Stats/2011SurvAnnualReview.pdf

AIDS InfoNet. (2011). *Glossary of HIV/AIDS-Related Terms.* 7th Edition. Available at http://aidsinfo.nih.gov/contentfiles/glossaryhivrelatedterms_english.pdf

AIDS InfoNet. (2013, February 12). *Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents: CD4 T-cell count*. Retrieved from http://aidsinfo.nih.gov/contentfiles/lvguidelines/glchunk/glchunk_4.pdf

AIDS InfoNet. (2014, April 16). *CD4 cell tests* [Fact sheet number 124]. Retrieved from http://www.aidsinfonet.org/uploaded/factsheets/13_eng_124.pdf

Allison J. J., Wall T. C., Spettell, C. M., Calhoun, J., Fargason, C. A., Kobylinski, R. W. … Kiefe, C. (2000). The art and science of chart review. *Journal of Quality Improvement, 26*(3), 115–136.

American Heart Association. (2012, August 31). Vitamin D supplement fails to lower cholesterol in short term. *ScienceDaily*. Retrieved September 30, 2012, from http://www.sciencedaily.com /releases/2012/09/120904162124.htm

Antico, A., Tampoia, M., Tozzoli, R., & Bizzaro, N. (2012). Can supplementation with Vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. *Autoimmunity Reviews*, *12*, 127–136. doi:10.1016/j.autrev.2012.07.007

Aranow, C. (2011). Vitamin D and the immune system. *Journal of Investigative Medicine, 59*(6), 881–886. doi:10.231/JIM.0b013e31821b8755

Armas, L. A., Hollis, B. W., & Heaney, R. P. (2004). Vitamin D2 is much less effective than Vitamin D3 in humans. *Journal of Clinical Endocrinology and Metabolism*, *89,* 5387–5391. Retrieved from http://www.direct-ms.org/pdf/VitDGenScience/Hollis%20D3%20versus%20D2.pdf

Arpadi, S. M., McMahon, D., Abrams, E. J., Bamji, M., Purswani, M., Engelson, E. S. … Shane, E. (2009). Effect of bimonthly supplementation with oral cholecalciferol on serum 25-hydroxyVitamin D concentrations in HIV-infected children and adolescents. *Pediatrics, 123*(1), e121–e126. doi:10.1542/peds.2008-0176

Aziz, M., Livak, B., Burke-Miller, J., French, A. L., Glesby, M. J., Sharma, A. … Adeyemi, O. M. (2013). Vitamin D insufficiency may impair CD4 recovery

among women's Interagency HIV Study participants with advanced disease on HAART. *AIDS, 27*(4), 573–578. doi:10.1097/QAD.0b013e32835b9ba1

Baeke, F., Gysemans, C., Korf, H., & Mathieu, C. (2010a). Vitamin D insufficiency: Implications for the immune system. *Pediatric Nephrology, 25*, 1597–1606. doi:10.1007/s00467-010-1452-y

Baeke, F., Korf, H., Overbergh, L., Van Etten, E., Verstuyf, A., Gysemans, C., & Mathieu, C. (2010b). Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D3 in the immune system. *Journal of Steroid Biochemistry and Molecular Biology, 121*(1–2), 221–227. doi:10.1016/j.jsbmb.2010.03.037

Baeke, F., Takiishi, T., Korf, H., Gysemans, C., & Mathieu, C. (2010c). Vitamin D: Modulator of the immune system. *Current Opinion in Pharmacology, 10*(4), 482–496. doi:10.1016/j.coph.2010.04.001

Baeke, F., Van Etten, E., Overbergh, L., & Mathieu, C. (2007). Vitamin D3 and the immune system: Maintaining the balance in health and disease. *Nutrition Research Reviews, 20*(1), 106–118. doi:10.1017/S0954422407742713

Bang, U. C., Shakar, S. A., Hitz, M. F., Jespersen, M. S., Andersen, O., Nielsen, S. D., & Jensen, J. E. (2010). Deficiency of 25-hydroxyvitamin D in male HIV-positive patients: A descriptive cross-sectional study. *Scandinavian Journal of Infectious Diseases, 42*(4), 306–310. doi:10.3109/00365540903463981

Bang, U. C., Kolte, L., Hitz, M., Nielsen, S. D., Schierbeck, L. L., Andersen, O. … Jensen, J. E. B. (2012). Correlation of increase in 1,25-dihydroxyvitamin D

during vitamin therapy with activation of CD4+ T lymphocytes in HIV-1-infected males. *HIV Clinical Trials, 13*(3), 162–170. doi:10.1310/hct1303-162

Barbosa, N., Costa, L., Pinto, M., Rosinha, P., Rosinha, I., & Couto, M. (2014). Vitamin D and HIV infection: A systematic review. *Journal of Immunodeficiency & Disorders, 3*, 1–11. doi:10.4172/2324-853X.1000107

Barlett, J. G. (2010). The natural history and clinical features of HIV infection in adults and adolescents. Retrieved from HIV UpToDate at http://www.uptodate.com/contents/the-natural-history-and-clinical-features-of-hiv-infection-in-adults-and-adolescents?source=search_result&search=The+stages+and+the+natural+history+of+HIV+infection&selectedTitle=31~150#H3

Baz-Hecht, M., & Goldfine, A. B. (2010). The impact of Vitamin D deficiency on diabetes and cardiovascular risk. *Current Opinions in Endocrinology, Diabetes, and Obesity, 17*, 113–119. doi:10.1097/MED.0b013e3283372859

Beard, J. A., Bearden, A., & Striker, R. (2011). Vitamin D and the antiviral state. *Journal of Clinical Virology, 50*(3), 194–200. doi:10.1016/j.jcv.2010.12.006

Bearden, A., Abad, C., Gangnon, R., Sosman, J. M., Binkley, N., & Safdar, N. (2013). Cross-sectional study of Vitamin D levels, immunologic and virologic outcomes in HIV-infected adults. *Journal of Clinical Endocrinology and Metabolism, 98*(4), 1726–1733. doi:10.1210/jc.2012-4031

Bergman, P., Walter-Jallow, L., Broliden, K., Agerberth, B., & Soderlund, J. (2007). The antimicrobial peptide LL-37 inhibits HIV replication. *Current HIV Research, 5*(4), 410–415.

Beyrer, C., Baral, S. D., Van Griensven, F., Goodreau, S. M., Chariyalertsak, S., Wirtz, A. L., & Brookmeyer, R. (2012). Global epidemiology of HIV infection in men who have sex with men. *Lancet, 380*, 367–377. doi:10.1016/S0140-6736(12)60821-6

Bhavan, K. P., Kampalath, V. N., & Overton, E. T. (2008). The aging of the HIV epidemic. *Current HIV/AIDS Report, 5*(3), 150–158.

Biancuzzo, R. M., Clarke, N., Reitz, R. E., Travison, T. G., & Holick, M. F. (2013). Serum concentrations of 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3 in response to Vitamin D2 and Vitamin D3 supplementation. *Journal of Clinical Endocrinology and Metabolism, 98*(3), 973–979. doi:10.1210/jc.2012-2114

Bischoff-Ferrari, H. A., Giovannucci, E., Willett, W. C., Dietrich, T., & Dawson-Hughes, B. (2006). Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *American Journal of Clinical Nutrition, 84*(1), 18–28. Retrieved from http://ajcn.nutrition.org/content/84/1/18.long

Boonstra, A., Barrat, F. J., Crain, C., Heath, V. L., Savelkoul, H. F., & O'Garra, A. (2001). 25-Dihydroxyvitamin D3 has a direct effect on naïve CD4(+) T cells to enhance the development of Th2 cells. *Journal of Immunology, 167*(9), 4974–4980. doi:10.4049/jimmunol.167.9.4974

Berger, A. (2000). Th1 and Th2 responses: What are they? *321*(12), 424. doi:10.1136/bmj.321.7258.424

Brown, T. T., & McComsey, G. A. (2010). Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. *Antiviral Therapy*, *15*(3), 425–429. doi:10.3851/IMP1502

Buell, J.S., & Dawson-Hughes, B. (2008). Vitamin D and neurocognitive Dysfunction: Preventing "D"ecline? *Molecular Aspects of Medicine, 29*(6), 415–422. doi:10.1016/j.mam.2008.05.001

Campbell, G. R., & Spector, S. A. (2012). Vitamin D inhibits Human Immunodeficiency Virus Type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. *PLOs Pathogens, 8*(5), e1002689. doi:10.1371/journal.ppat.1002689

Campbell, Y., Fantacone, M. L., & Gombart, A. F. (2012). Regulation of antimicrobial peptide gene expression by nutrients and by-products of microbial metabolism. *European Journal of Nutrition, 51*(8), 899–907. doi:10.1007/s00394-012-0415-4

Cantorna, M.T., Zhu, Y., Froicu, M., & Wittke, A. (2004). Vitamin D status, 1,25-dihydroxyVitamin D_3 and the immune system. *American Journal of Clinical Nutrition, 80*(6), 1717s–1720s. Available at http://ajcn.nutrition.org/content/80/6/1717S.long

Cantorna, M. T. (2011). Why do T cells express the Vitamin D receptor? *Annals of the New York Academy of Sciences, 1217*, 77–82. doi:10.1111/j.1749-6632.2010.05823.x

CDC. (1982). A cluster of Kaposi's sarcoma and pneumocystis carinii pneumonia among homosexual male residents of Los Angeles and Orange Counties, California. *Morbidity and Mortality Weekly Report, 31*(23), 305-307. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/00001114.htm

CDC. (2008a). Trends in HIV/AIDS diagnoses among men who have sex with men---33 states, 2001-2006. *Morbidity and Mortality Weekly Report, 57*(25), 681-686. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5725a2.htm

CDC. (2008b). Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 months and for HIV infection and AIDS among children aged 18 months to <13 years—United States, 2008. *MMWR, 57*(RR10), 1-8. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5710a1.htm

CDC. (2009). Late HIV testing---34 States, 1996-2005. *Morbidity and Mortality Weekly Report, 58*(24), 661-665. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5824a2.htm

CDC. (2011a). HIV surveillance--- United States, 1981-2008. *Morbidity and Mortality Weekly Report, 60*(21), 689-693. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6021a2.htm

CDC. (2011b). Disparities in diagnoses of HIV infection between Blacks/African Americans and other racial/ethnic populations---37 states, 2005-2008. *Morbidity and Mortality Weekly Report, 60*(04), 93-98. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6004a2.htm.

CDC. (2011c). Reported CD4+ T-lymphocytes and viral load results for adults and adolescents with HIV infection- 37 states, 2005-2007. *HIV Surveillance Supplemental Report 2010, 16*(1). Retrieved from http://www.cdc.gov/hiv/pdf/statistics_2005_2008_HIV_Surveillance_Report_vol_16_no1.pdf

CDC. (2012a). HIV in the United States: At a glance. Retrieved from http://www.cdc.gov/hiv/resources/factsheets/us.htm#ref2.

CDC. (2012b). Diagnosis of HIV infection and AIDS in the United States, 2010. *HIV Surveillance Report, vol.22.* Retrieved from http://www.cdc.gov/hiv/surveillance/resources/reports/2010report/pdf/2010_HIV_Surveillance_Report_vol_22.pdf#Page=21

CDC. (2012c). Estimated HIV incidence in the United States, 2007-2010. *HIV Surveillance Supplemental Report, 17*(4). Retrieved from http://www.cdc.gov/hiv/surveillance/resources/reports/2010supp_vol17no4/index.htm

CDC. (2012d). Terms, definitions, and calculations used in CDC HIV surveillance publications. Retrieved from http://www.cdc.gov/hiv/topics/surveillance/resources/factsheets/surveillance-table.htm.

Cervero, M., Agud, J., Torres, R., Garcia-Lacalle, C., Alcazar, V., Jusdado, J., & Moreno, S. (2013). Higher Vitamin D levels in HIV-infected out-patients on treatment with

boosted protease inhibitor monotherapy. *HIV Medicine, 14*(9), 556–562. doi:10.1111/hiv.12049

Childs, K., Welz, T., Samarawickrama, A., & Post, F. A. (2012). Effects of Vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. *AIDS, 26*, 253–262. doi:10.1097/QAD.0b013e32834f324b

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences (2nd ed.).* Mahwah, NJ: Lawrence Erlbaum.

Conroy, R. (2004). Sample size calculation: A quick guide. Retrieved from http://www.rcsiethics.info/uploads/7/5/8/0/758034/samplesizeguideforprotocolsandethicsapplications.pdf

Conesa-Botella, A., Florence, E., Lynen, L., Colebunders, R., Menten, J., & Moreno-Reyes, R. (2010). Decrease of Vitamin D concentration in patients with HIV infection on a non-nucleoside reverse transcriptase inhibitor-containing regimen. *AIDS Research and Therapy, 7*, 40. doi:10.1186/1742-6405-7-40

Cozzolino, M., Vidal, M., Arcidiacono, M. V., Tebas, P., Yarasheski, K. E., & Dusso, A. S. (2003). HIV-protease inhibitors impair Vitamin D bioactivation to 1,25-dihydroxyVitamin D. *AIDS, 17*, 513–520.

Crutchley, R. D., Gathe, J. Jr., Mayberry, C., Trieu, A., Abughosh, S., & Garey, K. W. (2012). Risk factors for Vitamin D deficiency in HIV-infected patients in the South Central United States. *AIDS Research & Human Retroviruses, 28*(5), 454–9. PMID: 21878055.

Dao, C. N., Patel, P., Overton, E. T., Rhame, F., Pals, S. L., Johnson, C., … The Study to Understand the Natural History of HIV and AIDS in the Era of Effective Therapy (SUN) Investigators. (2011). Low Vitamin D among HIV-infected adults: Prevalence of and risk factors for low Vitamin D levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clinical Infectious Diseases, 52*, 396-405. doi:10.1093/cid/ciq158

Das, P. & Horton, R. (2012). The cultural challenge of HIV/AIDS. *Lancet, 380* (9839), 309–310. Available at http://www.thelancet.com/journals/lancet/article/PIIS0140-6736%2812%2961074-5/fulltext

Dawson-Hughes, B. (2004). Racial/ethnic considerations in making recommendations for Vitamin D for adult and elderly men and women. *American Journal of Clinical Nutrition, 80*, 1763S–1766S.

De La Torre, M. S., Torres, G., Nieto, S., Vergara, S., Carrero, A. S., Macia, J., … Fibla, J. (2008). Vitamin D receptor gene haplotypes and susceptibility to HIV-1 infection in injection drug users. *Journal of Infectious Diseases, 197*, 405–410. doi:10.1086/525043

De Luis, D. A., Bachiller, P., Aller, R., De Luis, J., Izaola, O., Terroba, M. C., Cuellar, L., & Gonzalez Sagrado, M. (2002). Relation among micronutrient intakes with CD4 count in HIV infected patients [Abstract]. *Nutricion Hospitalaria, 17*(6), 285–289.

Deluca, H. F. & Cantorna, M. T. (2001). Vitamin D: Its role and uses in immunology. *FASEB Journal, 15*(14), 2579–2585. doi:10.1096/fj.01-0433rev. Available at http://www.fasebj.org/content/15/14/2579.full.pdf+html

Department of Human and Health Services –Panel on Antiretroviral Guidelines for Adults and Adolescents. (2012). Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. http://aidsinfo.nih.gov/guidelines. (Accessed on March 2014).

Department of Human and Health Services–Panel on Antiretroviral Guidelines for Adults and Adolescents. (2014). Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents [Revised]. Retrieved from http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf?source=govdelivery&utm_medium=email&utm_source=govdelivery (Accessed on May 2014).

Douek, D. C., Picker, L. J., & Koup, R. A. (2003). T cell dynamics in HIV-1 infection. *Annual Review of Immunology, 21*, 265–304. doi:10.1146/annurev.immunol.21.120601.141053

Egan, K. M., Signorello, L. B., Munro, H. M., Hargreaves, M. K., Hollis, B. W., & Blot, W. J. (2008). Vitamin D insufficiency among African Americans in the Southeastern United States: Implications for cancer disparities (United States). *Cancer Causes Control, 19*(5), 527–535.

Egger, M., May, M., Chene, G., Phillips, A. N., Ledergerber, B., Dabis, F., … ART Cohort Collaboration. (2002). Prognosis of HIV-1 infected patients starting highly

active antiretroviral therapy: A collaborative analysis of prospective studies. *Lancet*, 360(9327), 119–129.

Elhed, A., & Unutmaz, D. (2010). Th17 cells and HIV infection. *Current Opinion in HIV AIDS, 5*(2), 146–150. doi:10.1097/COH.0b013e32833647a8

Etminani-Esfahani, M., Khalili, H., Soleimani, N., Jafari, S., Abdollahi, A., Khazaeipour, Z., & Gholami, K. (2012). Serum Vitamin D concentration and potential risk factors for its deficiency in HIV positive individuals. *Current HIV Research, 10*(2), 165–70. doi:10.2174/157016212799937272

Fawzi, W. W., Msamanga, G. I., Spiegelman, D., Wei, R., Kapiga, S., Villamor, E., … Hunter, D. J. (2004). A randomized trial of multivitamin supplements and HIV disease progression and mortality. *New England Journal of Medicine, 351*, 23–32. doi:10.1056/NEJMoa040541

Fevrier, M., Dorgham, K., & Rebollo, A. (2011). CD4+ T cell depletion in human immunodeficiency virus (HIV) infection: Role of apoptosis. *Viruses, 3*(5), 586–612. doi:10.3390/v3050586

Findley, T. W., & Daum, M. C. (1989). Research in physical Medicine and rehabilitation III. The chart review or how to use clinical data for exploratory retrospective studies. *American Journal of Physical Medicine & Rehabilitation, 68*(3), 150–7.

Fisher Z-Transformation Test. (n.d.). Retrieved March 19, 2015, from Vassarstats website, http://vassarstats.net/rdiff.html.

Forouhi, N. G., Ye, Z., Rickard, A. P., Khaw, K. T., Luben, R., Langenberg, C., & Wareham, N. J. (2012). Circulating 25-hydroxyVitamin D concentration and the

risk of type 2 diabetes: Results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. *Diabetologia, 55*(8), 2173–2182. doi:10.1007/s00125-012-2544-y

Fox, J., Peters, B., Pakrash, M., Arribas, J., Hill, A., & Moecklinghoff, C. (2011). Improvement in Vitamin D deficiency following antiretroviral regime change: Results from the MONET trial. *AIDS Research and Human Retroviruses, 27*(1), 29–34. doi:10.1089/aid.2010.0081

Ganji, V., Milone, C., Cody, M. M., McCarthy, F., & Wang, Y. T. (2010). Serum Vitamin D concentrations are related to depression in young adult US population: The Third National Health and Nutrition Examination Survey. *Internal Archives of Medicine, 3*, 29. doi:10.1186/1755-7682-3-29

Garrett-Mayer, E., Wagner, C. L., Hollis, B. W., Kindy, M. S., & Gattoni-Celli, S. (2012). Vitamin D3 supplementation (4000 IU/d for 1 y) eliminates differences in circulating 25-hydroxyVitamin D between African American and white men. *American Journal of Clinical Nutrition, 96*(2), 332–336. doi:10.3945/ajcn.112.034256

Gearing, R. E., Mian, I. A., Barber, J., & Ickowicz, A. (2006). A methodology for conducting retrospective chart review research in child and adolescent psychiatry. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, 15(3), 126–134.

Gedela, K., Edwards, S. G., Benn, P., & Grant, A. D. (2013). Prevalence of Vitamin D deficiency in HIV-positive, antiretroviral treatment-naive patients in a single

centre study. *International Journal of STD & AIDS*, ISSN 0956–4624. doi:10.1177/0956462413515194

Giacomet, V., Vigano, A., Manfredini, V., Cerini, C., Bedogni, G., Mora, S., … Zuccotti, G. V. (2013). Cholecalciferol supplementation in HIV-infected youth with Vitamin D insufficiency: Effects on Vitamin D status and T-cell phenotype. A randomized controlled trial. *HIV Clinical Trials, 14*(2), 51–60. doi:10.1310/hct1402-51

Ginde, A. A., Liu, M. C., & Camargo, C. A. Jr. (2009). Demographic differences and trends of Vitamin D insufficiency in the US population, 1988-2004. *Archives of Internal Medicine, 169*, 626–632. doi:10.1001/archinternmed.2008.604

Giorgi, J. V., Lyles, R. H., Matud, J. L., Yamashita, T. E., Mellors, J. W., Hultin, L. E., … Multicenter AIDS Cohort Study. (2002). Predictive value of immunological and virologic markers after long or short duration of HIV-1 infection [Abstract]. *Journal of Acquired Immune Deficiency Syndrome, 29*(4), 346–355.

Giusti, A., Penco, G., & Pioli, G. (2011). Vitamin D deficiency in HIV-infected patients: A systematic review. *Nutrition and Dietary Supplements, 3*, 101–111. doi:http://dx.doi.org/10.2147/NDS.S6921

Glade, M. J. (2012). A 21st century evaluation of the safety of oral Vitamin D. *Nutrition, 28*, 344–356. doi:10.1016/j.nut.2011.11.006

Gombart, A. F., O'Kelly, J., Saito, T., & Koeffler, H. P. (2007). Regulation of the CAMP gene by 1,25(OH)2D3 in various tissues. *Journal of Steroid Biochemistry and Molecular Biology, 103*(3–5), 552–557. doi:10.1016/j.jsbmb.2006.12.095

Griffin, A. T., & Arnold, F. W. (2012). Review of metabolic, immunologic, and virologic consequences of suboptimal Vitamin D levels in HIV infection. *AIDS Patient Care STDS*, 26(9), 516–525. doi:10.1089/apc.2012.0145

Grigoryan, A., Hall, I., Durant, T., & Wei, X. (2009). Late HIV diagnosis and determinants of progression to AIS or death after HIV diagnosis among injection drug users, 33 US States, 1996-2004. *PLoS ONE, 4*(2), e4445. doi:10.1371/journal.pone.0004445

Grossman, Z., Meier-Schellersheim, M., Sousa, A. F., Victorino, R. M. M., & Paul, W. E. (2002). CD4+ T-cell depletion in HIV infection: Are we closer to understanding the cause? *Nature Medicine, 8*, 319–323. doi:10.1038/nm0402-319

Guiguet. M., Kendjo, E., Carcelain, G., Abgrall, S., Mary-Krause, M., Tattevin, P., … FHDH-ANRS CO4 Epidemiology Group. (2009). CD4+ T-cell percentage is an independent predictor of clinical progression in AIDS-free antiretroviral-naive patients with CD4+ T-cell counts >200 cells/mm3. *Antiviral Therapy, 14*(3), 451–457.

Gunville, C. F., Mourani, P. M, & Ginde, A. A. (2013). The role of Vitamin D in prevention and treatment of infection. *Inflammation & Allergy Drug Targets, 12*(4), 239–245. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3756814/pdf/nihms503787.pdf

Hall, I., Byers, R. H., Ling, Q., & Espinoza, L. (2007). Racial/ethnic and age disparities in HIV prevalence and disease progression among men who have sex with men in

the United States. *American Journal of Public Health, 97*, 1060–1066. doi:10.2105/AJPH.2006.087551

Hall, L. M., Kimlin, M. G., Aronov, P. A., Hammock, B. D., Slusser, J. R., Woodhouse, L. R., & Stephensen, C. B. (2010). Vitamin D intake needed to maintain target serum 25-hydroxyVitamin D concentrations in participants with low sun exposure and dark skin pigmentation is substantially higher than current recommendations. *Journal of Nutrition, 140*(3), 542–550. doi:10.3945/jn.109.115253

Hannan, M. T., Litman, H. J., Araujo, A. B., McLennan, C. E., McLean, R. R., McKinlay, J. B., Chen, T. C., & Holick, M. F. (2008). Serum 25-hydroxyVitamin D and bone mineral density in a racially and ethnically diverse group of men. *Journal of Clinical Endocrinology and Metabolism, 93*, 40–46. doi:10.1210/jc.2007-1217

Harris, S. S. (2006). Vitamin D and African Americans. *Journal of Nutrition, 136*, 1126–1129. Available at http://jn.nutrition.org/content/136/4/1126.full.pdf+html

Harrison, K. M., Song, R., & Zhang, X. (2010). Life expectancy after HIV diagnosis based on National HIV Surveillance data from 25 states, United States. *Journal of Acquired Immune Deficiency Syndrome*, 53, 124–130. Available at http://natap.org/2010/HIV/Life.pdf

Hart, P. H., Gorman, S., & Finlay-Jones, J. (2011). Modulation of the immune system by UV radiation: More than just the effects of Vitamin D? *Nature, 11*, 584–596. doi:10.1038/nri3045

Haug, C., Müller, F., Aukrust, P., & Frøland, S. S. (1994). Subnormal serum concentration of 1,25-Vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. *Journal of Infectious Diseases, 169*(4), 889–893. doi:10.1093/infdis/169.4.889

Haug, C. J., Aukrust, P., Haug, E., Morkrid, L., Muller, F., & Froland, S. S. (1998). Severe deficiency of 1,25-DihydroxyVitamin D3 in human immunodeficiency virus infection: Association with immunological hyperactivity and only minor changes in calcium homeostasis. *Journal of Clinical Endocrinology and Metabolism, 83*(11), 3832–3838.

Havens, P. L., Mulligan, K., Hazra, R., Flynn, P., Rutledge, B., Van Loan, M. D., … Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 063 Study Team. (2012). Serum 25-hydroxyVitamin D response to Vitamin D3 supplementation 50,000 IU monthly in youth with HIV-1 infection. *Journal of Clinical Endocrinology and Metabolism, 97*(11), 4004–4013. doi:10.1210/jc.2012-2600

Hazenberg, M., Otto, S. A., Van Benthem, B., Roos, M., Coutinho, R., Lange, J., … Miedema, F. (2003). Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS, 17*(13), 1881–1888. doi:10.1097/01.aids.0000076311.76477.6e

Heany, R. P. (2008). Vitamin D in health and disease. *Clinical Journal of American Society of Nephrology, 3*(5), 1535–1541. doi:10.2215/CJN.01160308

Heaney, R. P., Davies, K. M., Chen, T. C., Holick, M. F., & Barger-Lux, M. J. (2003). Human serum 25-hydroxycholecalciferol response to extended oral closing with cholecalciferol. *American Journal of Clinical Nutrition, 88*, 1535–1542. Retrieved from http://ajcn.nutrition.org/content/77/1/204.full.pdf+html

Heaney, R. P., Recker, R. R., Grote, J., Horst, R. L., & Armas, L. A. G. (2011). Vitamin D3 is more potent than Vitamin D2 in Humans. *Journal of Clinical Endocrinology and Metabolism, 96*(3), E447–E452. doi:10.1210/jc.2010-2230

Hellebergh, M., Kronborg, G., Ullum, H., Ryder, L. P., Obel, N., & Gerstoft, J. (2014). Course and clinical significance of CD8 counts in a large HIV cohort [Abstract]. *Journal of Infectious Diseases*, pii: jiu669. doi:10.1093/infdis/jiu669

Hellerstein, M., Hanley, M. B., Cesar, D., Siler, S., Papageorgopoulos, C., Wieder, E., … McCune, J. M. (1999). Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nature Medicine, 5*(1), 83–89. doi:10.1038/4772

Herbein, G., & Varin, A. (2010). The macrophage in HIV-1 infection: From activation to deactivation. *Retrovirology, 7*, 33. doi:10.1186/1742-4690-7-33

Hewison, M. (2010). Vitamin D and the immune system: New perspectives on an old theme. *Endocrinology Metabolism Clinics of North America, 39*(2), 365–379. doi:10.1016/j.ecl.2010.02.010

Hewison, M. (2012). An update on Vitamin D and human immunity. *Clinical Endocrinology, 76*(3), 315–325. doi:10.1111/j.1365-2265.2011.04261.x

HICDEP-HIV Cohorts Data Exchange Protocol. (2008). Version 1.30. Available at http://www.hicdep.org/

Ho, D. D. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature, 373*, 123–126. doi:10.1038/373123a0

Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M., & Markowitz, M. (1999). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature, 373*(6510), 123–126. doi:10.1038/373123a0

Hoffman, J., Griensven, J. V., Colebunders, R., & McKellar, M. (2010). Role of the CD4 count in HIV management. *HIV Therapy, 4*(1), 27–39. Available at http://www.futuremedicine.com/doi/pdf/10.2217/hiv.09.58

Hogg, R. S., Yip, B., Chan, K. J., Wood, E., Craib, K. J. P., O'Shausghnessy, M. V., & Montaner, J. S. G. (2001). Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. *JAMA, 286*(20), 2568–2578. doi:10.1001/jama.286.20.2568

Hogg, R. S., Althoff, K. N., Samji, H., Cescon, A., Modur, S., Buchacz, K., … Gange, S. J. (2013). Increases in life expectancy among treated HIV-positive individuals in the United States and Canada, 2000-2007. 7th IAS Conference on HIV Pathogenesis, Treatment and Prevention, June 30-July 3, 2013, Kuala Lumpur. Abstract TUPE260.

Holick, M. F. (1996). Photobiology of Vitamin D. In Feldman, D., Glorieux, F. H., & Pike, J. W. (Eds.), *Vitamin D* (pp. 33–39). Academic Press, San Diego, CA.

Holick, M. F. (2003). Vitamin D: A millennium perspective. *Journal of Cellular Biochemistry, 88*, 296–307. doi:10.1002/jcb.10338

Holick, M. F. (2004). Sunlight and Vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *American Journal of Clinical Nutrition, 80*, 1678S–1688S. Retrieved from http://ajcn.nutrition.org/content/80/6/1678S.full.pdf+html

Holick, M. F. (2006). High prevalence of Vitamin D inadequacy and implications for health. *Mayo Clinic Proceedings, 81*, 353–373. Retrieved from http://www.mayoclinicproceedings.org/article/S0025-6196%2811%2961465-1/pdf

Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine, 357*(3), 266–281. doi:10.1056/NEJMra070553

Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: A worldwide problem with health consequences. *American Journal of Clinical Nutrition*, 87(suppl.), 1080S–1086S.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., & Weaver, C. M. (2011). Evaluation, treatment, and prevention of Vitamin D deficiency: An endocrine society clinical practice guideline. *Journal of Endocrinology and Metabolism, 96*, 1911–1930. doi:10.1210/jc.2011-0385

Hollis, B. C. (2005). Circulating 25-hydroxyVitamin D levels indicative of Vitamin D sufficiency: Implications for establishing a new effective dietary intake

recommendation for Vitamin D. *Journal of Nutrition, 135*(2), 317–322. Retrieved from http://jn.nutrition.org/content/135/2/317.long

Hossein-Nezhad, A., & Holick, M. F. (2013). Vitamin D for health: A global perspective. *Mayo Clinic Proceedings, 88*(7), 720–755. Retrieved from http://www.mayoclinicproceedings.org/article/S0025-6196%2813%2900404-7/pdf

Houghton, L. A., & Vieth, R. (2006). The case against ergocalciferol (Vitamin D2) as a vitamin supplement. *American Journal of Clinical Nutrition, 84*(4), 694–697.

Hulgan, T., Shepherd, B. E., Raffanti, S. P., Fusco, J. S., Beckerman, R., Barkanic, G., & Sterling, T. R. (2007). Absolute count and percentage of CD4+ lymphocytes are independent predictors of disease progression in HIV-infected persons initiating highly active antiretroviral therapy. *Journal of Infectious Diseases, 195*(3), 425–431. doi:10.1086/510536

Institute of Medicine (2011). Dietary Reference intakes for Calcium and Vitamin D. Washington DC, National Academies Press. Retrieved from http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-calcium-and-vitamin-D.aspx

Jeng, L., Yamshchikov, A. V., Judd, S. E., Blumberg, H. M., Martin, G. S., Ziegler, T. R., & Tangpricha, V. (2009). Alterations in Vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. *Journal of Translational Medicine, 7*, 28–36. doi:10.1186/1479-5876-7-28

Johnson, L. F. Mossong, J., Dorrington, R. E., Schomaker, M., Hoffmann, C. J., Keiser, O., … The International Epidemiologic Databases to Evaluate AIDS Southern Africa (IeDEA-SA) Collaboration. (2013). Life expectancies of South African adults starting antiretroviral treatment: Collaborative analysis of cohort studies. *PLoS Med*, doi:10.1371/journal.pmed.1001418.s003

Jones, G., (2008). Pharmacokinetics of Vitamin D toxicity. *American Journal of Clinical Nutrition, 88*(2), 582S–586S. Retrieved from http://ajcn.nutrition.org/content/88/2/582S.long

Judd, S. E., Nanes, M. S., Ziegler, T. R., Wilson, P. W., & Tangpricha, V. (2008). Optimal Vitamin D status attenuates the age-associated increase in systolic blood pressures in White Americans: Results from the Third National Health and Nutrition Examination Survey. *American Journal of Clinical Nutrition, 87*, 136–141. Retrieved from http://ajcn.nutrition.org/content/87/1/136.full.pdf+html

Kamen, D. L., & Tangpricha, V. (2010). Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *Journal of Molecular Medicine (Berl), 88*(5), 441–450. doi:10.1007/s00109-010-0590-9

Kestens, L. (2005). *Virologic and immunologic basis of HIV infection + HIV pathogenesis* [PDF document]. Retrieved from http://www.itg.be/internet/e-learning/written_lecture_eng/Ref_Virology_and_immunology.pdf

Khoo, A. L., Chai, L., Koenen, H., Joosten, I., Netea, M., & Van der Ven, A. (2012). Translating the role of Vitamin D_3 in infectious diseases. *Critical Review in Microbiology, 38*(2), 122–135. doi:10.3109/1040841X.2011.622716

Kibirige, D., Mutebi, E., Ssekitoleko, R., Worodria, W., & Mayanja-Kizza, H. (2013). Vitamin D deficiency among adult patients with tuberculosis: A cross-sectional study from a national referral hospital in Uganda. *BMC Research Notes, 6*(1), 293. Available at http://www.biomedcentral.com/1756-0500/6/293

Kim, J. H., Gandhi, V., Psevdos, G. Jr., Espinoza, F., Park, J., & Sharp, V. (2012). Evaluation of Vitamin D levels among HIV-infected patients in New York City. *AIDS Res Hum Retroviruses, 28*(3), 235–41.

Kimlin, M. G. (2004). The climatology of Vitamin D producing ultraviolet radiation over the United States [Abstract]. *Journal of Steroid Biochemistry and Molecular Biology, 89–90*, 479–483. doi:10.1016/j.jsbmb.2004.03.111

Kosciuczuk, E. M., Lisowski, P., Jarczak, J., Strzalkowska, N., Jozwik, A., Horbanczuk, J., … Bagnicka, E. (2012). Cathelicidins: Family of antimicrobial peptides. A review. *Molecular Biology Reports*, doi:10.1007/s11033-012-1997-x

Kovacs, J. A., Lempicki, R. A., Sidorov, I. A., Adelsberger, J. W., Herpin, B., Metcalf, J. A., … Lane, H. C. (2001). Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV. *Journal of Experimental Medicine, 194*(12), 1731–1741. Available at http://jem.rupress.org/content/194/12/1731.long

Kulie, T., Groff, A., Redmer, J., Hounshell, J., & Schrager, S. (2009). Vitamin D: An evidence-based review. *Journal of the American Board of Family Medicine, 22*(6), 698–706. doi:10.3122/jabfm.2009.06.09.0037

Kurts, C. (2008). Th17 cells: A third subset of CD4+ T effector cells involved in organ-specific autoimmunity. *Nephrology Dialysis Transplantation, 23*(3), 816–819. doi:10.1093/ndt/gfm800

Lagunova, Z., Porojnicu, A. C., Lindberg, F., Hexeberg, S., & Moan, J. (2009). The dependency of Vitamin D status on body mass index, gender, age and season. *Anticancer Research, 29*, 3713–3720. Available at http://ar.iiarjournals.org/content/29/9/3713.full.pdf

Lake, J. E., & Adams, J. S. (2011). Vitamin D in HIV-infected patients. *Current HIV/AIDS Reports, 8*(3), 133–141. doi:10.1007/s11904-011-0082-8

Lang, P. O., Samaras, N., Samaras, D., & Aspinall, R. (2013). How important is Vitamin D in preventing infections? *Osteoporosis International, 24*(5), 1537–1553. doi:10.1007/s00198-012-2204-6

Langford, S. E., Ananworanich, J., & Cooper, D. A. (2007). Predictors of disease progression in HIV infection: A review. *AIDS Research and Therapy, 4*, 11–24. doi:10.1186/1742-6405-4-11

Lansky, A., Brooks, J. T., DiNenno, E., Heffelfinger, J., Hall, H. I., & Mermin, J. (2010). Epidemiology of HIV in the United States. *Journal of Acquired Immune Deficiency Syndrome, 55*(Suppl. 2), S64–8. doi:10.1097/QAl.0b013e3181fbbe15

Lappe, J. M. (2011). The role of Vitamin D in human health: A paradigm shift. *Journal of Evidence-Based Complementary & Alternative Medicine, 16*(1), 58–72. doi:10.1177/1533210110392952

Laureate, Education, Inc. (Producer). (2010). *The absolute essentials of sample size analysis: You too can be a statistical power guru* [PowerPoint]. Baltimore, MD: Burkholder, G. J., Walden University, College of Health Sciences.

Lavie, C. J., Lee, J. H., & Milani, R. V. (2011). Vitamin D and cardiovascular disease will it live up to its hype? *Journal of American College of Cardiology, 58*(15), 1547–1556. doi:10.1016/j.jacc.2011.07.008

Lawrie, D., Coetzee, L. M., Becker, P., Mahlangu, J., Stevens, W., & Glencross, D. K. (2009). Local reference ranges for full blood count and CD4 lymphocyte count testing. *South African Medical Journal, 99*(4), 243–248.

Legeai, C., Vigouroux, C., Souberbielle, J. C., Bouchaud, O., Boufassa, F., Bastard, J. P., … the ANRS-COPANA Cohort Study Group. (2013). Associations between 25-HydroxyVitamin D and immunologic, metabolic, inflammatory markers in treatment-naïve HIV-infected persons: The ANRS CO9 “COPANA” Cohort Study. *PLoS ONE, 8*(9), e74868. doi:10.1371/journal-pone.0074868

Lin, R. & White, J. H. (2004). The pleiotropic actions of Vitamin D. *BioEssays, 26*(1), 21–28. doi:10.1002/bies.10368

Liu, P. T., Stenger, S., Li, H., Wenzel, L., Tan, B. H., Krutzik, S. R., … Modlin, R. L. (2006). Toll-like receptor triggering of a Vitamin D-mediated human antimicrobial response. *Science*, *311*(5768), 1770–1773. doi:10.1126/science.1123933

Liu, P. T., Krutzik, S. R., & Modlin, R. L. (2007). Therapeutic implications of the TLR and VDR partnership. *Trends in Molecular Medicine*, *13*(3), 117–124. doi:10.1016/j.molmed.2007.01.006

Liu, P. T., Stenger, S., Tang, D. H., & Modlin, R. L. (2007). Cutting edge: Vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *Journal of Immunology*, *179*(4), 2060–2063. doi:10.4049/jimmunol.179.4.2060

Lodi, S., Fisher, M., Phillips, A., De Luca, A., Ghosn, J., Malyuta, R., Zangerie, R., … Porter, K., [CASCADE Collaboration in EuroCoord]. (2013). Symptomatic illness and low CD4 cell count at HIV seroconversion as markers of severe primary HIV infection. *PLoS ONE, 8*(11), e78642. doi:10.1371/journal.pone.0078642

Logan, V. F., Gray, A. R., Peddie, M. C., Harper, M. J., & Houghton, L. A. (2013). Long-term Vitamin D3 supplementation is more effective than Vitamin D2 in maintaining serum 25-hydroxyVitamin D status over the winter months. *British Journal of Nutrition*, *109*(6), 1082–1088. doi:10.1017/S0007114512002851

Mahon, B. D., Wittke, A., Weaver, V., & Cantorna, M. T. (2003). The target of Vitamin D depends on the differentiation and activation status of CD4- positive T cells. *Journal of Cellular Biochemistry*, *89*(5), 922–932. doi:10.1002/jcb.10580

Maki, K. C., Rubin, M. R., Wong, L. G., McManus, J. F., Jensen, C. D., Marshall, J. W., & Lawless, A. (2009). Serum 25-hydroxyVitamin D is independently associated

with high-density cholesterol and the metabolic syndrome in men and women. *Journal of Clinical Lipidology, 3*, 289–296. doi:10.3109/09637486.2010.536146

Mastaglia, S. R., Mautalen, C. A., Parisi, M. S., & Oliveri, B. (2006). Vitamin D2 dose required to rapidly increase 25OHD levels in osteoporotic women. *European Journal of Clinical Nutrition, 60*, 681–687. doi:10.1038/sj.ejcn.1602369

Mathieu, C., & Adorini, L. (2002). The coming age of 1,25-dihydroxyVitamin D_3 analogs as immunomodulatory agents. *Trends in Molecular Medicine, 8*(4), 174–179. doi:10.1016/S1471-4914(02)02294-3

Mathieu, C., & Van Der Schueren, B. J. (2011). Vitamin D deficiency is not good for you. *Diabetes Care, 34*, 1245–1246. doi:10.2337/dc11-0307

Matsuoka, L. Y., Ide, L., Wortsman, J., MacLaughlin, J. A., & Holick, M. F. (1987). Sunscreens suppress cutaneous Vitamin D3 synthesis. *Journal of Clinical Endocrinology & Metabolism, 64*(6), 1165–1168. doi: 10.1210/jcem-64-6-1165

May, M., Sterne, J. A., Sabin, C., Costagliola, D., Justice, A. C., Thiebaut, R., … Egger, M. (2007). Prognosis of HIV-1 infected patients up to 5 years after initiation of HAART: Collaborative analysis of prospective studies. *AIDS*, 21(9), 1185–1197. doi:10.1097/QAD.0b013e328133f285

May, M., Gompels, M., Delpech, V., Porter, K., Post, F., Johnson, M. … Sabin, C. (2011). Impact of late diagnosis and treatment on life expectancy in people with HIV-1: UK Collaborative HIV Cohort (UK CHIC) study. *BMJ*, 343–353. doi:10.1136/bmj.d6016

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature, 449*(7164), 819–826. doi:10.1038/nature06246

Mehta, S., Giovannucci, E., Mugusi, F., Spiegelman, D., Aboud, S., Hertzmark, E., … Fawzi, W. W. (2010). Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. *PLoS One, 5*(1), e8770.

Mehta, S., Mugusi, F. M., Spiegelman, D., Villamor, E., Finkelstein, J. L., Hertzmark, E., … Fawzi, W. W. (2011). Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. *AIDS Patient Care STDS, 25*(10), 579–585.

Meyaard, L., & Miedema, F. (1997). Immune dysregulation and CD4+ T cell loss in HIV-1 infection. *Springer Seminars in Immunopathology, 18*, 285–303. Available from http://link.springer.com/article/10.1007%2FBF00813499#close

Miedema, F., Hazenberg, M. D., Tesselaar, K., Van Baarle, D., de Boer, R. J., & Borghans, J. A. M. (2013). Immune activation and collateral damage in AIDS pathogenesis. *Frontiers in Immunology, 4*, 298. doi:10.3389/fimmu.2013.00298

Miller, J., & Gallo, R. L. (2010). Vitamin D and innate immunity. *Dermatologic Therapy, 23*, 13–22. doi:10.1111/j.1529-8019.2009.01287.x

Millet, G. A., Peterson, J. L., Flores, S. A., Hart, T. A., Jeffries, W. L. Wilson, P. A., … Remis, R. S. (2012). Comparisons of disparities and risks of HIV infection in black and other men who have sex with men in Canada, UK, and USA: A meta-

analysis. *Lancet, 380*, 341–48. Available at http://dx.doi.org/10.1016/S0140-6736(12)60899-X

Mistretta, V. I., Delanaye, P., Chapelle, J. P., Souberbielle, J. C., & Cavalier, E. (2008). Vitamin D2 or Vitamin D3? *Revue de Medecin Intern, 29*(10), 815–820. doi:10.1016/j.revmed.2008.03.003

Mohri, H., Perelson, A. S., Tung, K., Ribeiro, R. M., Ramratnam, B., Markowitz, M., … Ho, D. D. (2001). Increased turnover of T lymphocyte in HIV-1 infection and its reduction by antiretroviral therapy. *Journal of Experimental Medicine, 194*(9), 1277–1288. Available from http://jem.rupress.org/content/194/9/1277.full

Moller, K. I., Kongshoj, B., Philipsen, P. A., Thomsen, V. O., & Wulf, H. C. (2005). How Finsen's light cured lupus vulgaris [Abstract]. *Photodermatology Photoimmunology Photomedicine, 21*(3), 118–124.

Monk, R. D., & Bushinsky, D. A. (2011). Making sense of the latest advice on Vitamin D therapy. *Journal of the American Society of Nephrology, 22*, 994–998. doi:10.1681/ASN.2011030251

Moore, D. M., Hogg, R. S., Yip, B., Craib, K., Wood, E., & Montaner, J. S. (2006). CD4 percentage is an independent predictor of survival in patients starting antiretroviral therapy with absolute CD4 cell counts between 200 and 350 cells/µL. *HIV Medicine, 7*(6), 383–388. doi:10.1111/j.1468-1293.2006.00397.x

Morgan, G. A., & Harmon, R. J. (2000). Research questions and hypotheses. *Journal of American Academy of Child and Adolescence Psychiatry, 39*(2), 261–263.

Retrieved from http://cinbiocli.files.wordpress.com/2013/01/art1_research-questions-and-hypo-copy.pdf

Mueller, N. J., Fux, C. A., Ledergerber, B., Elzi, L., Schmid, P., Dang, T., … Swiss HIV Cohort Study. (2010). High prevalence of severe Vitamin D deficiency in combined antiretroviral therapy-naïve and successfully treated Swiss HIV patients. *AIDS, 24*(8), 1127–34. doi:10.1097/QAD.0b013e328337b161

Murphy, A. B., Kelley, B., Nyame, Y. A., Martin, I. K., Smith, D. J., Castaneda, L., … Kittles, R. A. (2012). Predictors of serum Vitamin D levels in African American and European American men in Chicago. *American Journal of Men's Health, 6*(5), 420–426. doi:10.1177/1557988312437240

National Academy of Sciences. (2000). Beyond discovery: Unraveling the enigma of Vitamin D. Available at http://www.beyonddiscovery.org/content/view.article.asp?a=414

National Institute of Health/National Institute of Allergy and Infectious Diseases. (2012). HIV/AIDS. Available at http://www.niaid.nih.gov/topics/hivaids/understanding/Pages/Default.aspx

Neri, D., Miller, T., & Potter, J. E. (2012). Vitamin D deficiency and HIV. *HIV CareLink*, *13*(3). Available at http://fcaetc.org/newsletters/2012/CareLinkNewsletter_Vol13Iss3.pdf

Nevado, J., Tenbaum, S. P., Castillo, A. I., Sanchez-Pacheco, A., & Aranda, A. (2007). Activation of the human immunodeficiency virus type 1 long terminal repeat by

1α,25-dihydroxyVitamin D_3. *Journal of Molecular Endocrinology, 38*(6), 587–601. doi:10.1677/JME-06-0065

Nimitphong, H., & Holick, M. F. (2011). Vitamin D, neurocognitive functioning and immunocompetence. *Current Opinion in Clinical Nutrition and Metabolic Care, 14*(1), 7–14. doi:10.1097/MCO.0b013e3283414c38

Norman, A. W. (1998). Sunlight, season, skin pigmentation, Vitamin D, and 25-hydroxyVitamin D: Integral components of the Vitamin D endocrine system. *American Journal of Clinical Nutrition, 67*, 1108–1110. Retrieved from http://ajcn.nutrition.org/content/67/6/1108.long

Norman, A. W. (2006). Minireview: Vitamin D receptor: New assignments for an already busy receptor. *Endocrinology, 147*(12), 5542–5548. doi:http://dx.doi.org/10.1210/en.2006-0946

Norman, A. W. (2012). The history of the discovery of Vitamin D and its daughter steroid hormone. *Annals of Nutrition and Metabolism, 61*(3), 199–206. doi:10.1159/000343104

Ormesher, B., Dhaliwal, S., Nylen, E., Gibert, C., Go , C., Amdur, R., & Benator, D. (2011). Vitamin D deficiency is less common among HIV-infected African American men than in a matched cohort [ABSTRACT]. *AIDS, 25*(9), 1237–1239.

Overton, E. T., & Yin, M. T. (2011). The rapidly evolving research on Vitamin D among HIV-infected populations. *Current Infectious Diseases Report, 13*(1), 83–93. doi:10.1007/s11908-010-0144-x

Pasquet, A., Viget, N., Ajana, F., De La Tribonniere, X., Dubus, S., Paccou, J., Legroux-Gerot, I., … Yazdanpanah, Y. (2011). Vitamin D deficiency in HIV-infected patients: Associated with non-nucleoside reverse transcriptase inhibitor or Efavirenz use? *AIDS, 25*(6), 873. doi:10.1097/QAD.0b013e32834542fa

Paul, T. V., Asha, H. S., Thomas, N., Seshadri, M. S., Rupali, P., Abraham, O. C., Pulimood, S. A., & Jose, A. (2010). Hypovitaminosis D and bone mineral density in human immunodeficiency virus-infected men from India, with or without antiretroviral therapy. *Endocrinology Practice, 16*(4), 547–553. doi:10.4158/EP09197.OR

Penna, G., Roncari, A., Amuchastegui, S., Daniel, K. C., Berti, E., Colonna, M., & Adorini, L. (2005). Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+regulatory T cells by 1, 25-dihydroxyVitamin D3. *Blood, 106*(10), 3490–3497. doi: 10.1182/blood-2005-05-2044

Phillips, A. N., & Lundgren, J. D. (2006). The CD4 lymphocyte count and risk of clinical progression. *Current Opinion in HIV and AIDS*, 1(1), 43–49. doi:10.1097/01.COH.0000194106.12816.b1

Pinzone, M. R., Di Rosa, M., Malaguarnera, M., Madeddu, G., Foca, E., Ceccarelli, G., … Nunnari, G. (2013). Vitamin D deficiency in HIV infection: An underestimated and undertreated epidemic. *European Review for Medical and Pharmacological Sciences, 17*, 1218–1232.

Pirotte, B. F., Rassenfosse, M., Collin, R., Devoeght, A., Moutschen, M., & Cavalier, E. (2013). Role of Vitamin D in HIV infection [French]. Revue Medicale de Liege, *68*(1), 25–31.

Pirzada,Y., Khuder, S., & Donabedian, H. (2006). Predicting AIDS-related events using CD4 percentage or CD4 absolute counts. *AIDS Research and Therapy, 3*, 20. doi:10.1186/1742-6405-3-20

Poowuttikul, P., Thomas, R., Hart, B., & Secord, E. (2013). Vitamin D insufficiency/deficiency in HIV-infected inner city youth. *Journal of the International Association of Providers of AIDS Care*, 1–5. July 23. doi:10.1177/2325957413495566

Prejean, J., Song, R., Hernandez, A., Ziebell, R., Green, T., Walker, F., … Hall, I. (2011). Estimated HIV incidence in the United States, 2006-2009. *PLoS ONE*, 6(8): e17502. doi:10.1371/journal.pone.0017502

Prentice, A., Goldberg, G. R., & Schoenmakers, I. (2008). Vitamin D across the lifecycle: Physiology and biomarkers. *American Journal of Clinical Nutrition, 88*(2), 500S–506S. Available at http://ajcn.nutrition.org/content/88/2/500S.long

Prietl, B., Treiber, G., Pieber, T. R., & Amrein, K. (2013). Vitamin D and immune function. *Nutrients, 5*, 2502–2521. doi:10.3390/nu50725502

Reichrath, J. (2009). Skin cancer prevention and UV-protection: how to avoid Vitamin D deficiency? *British Journal of Dermatology, 161*(suppl. 3), 54–60. doi:10.1111/j.1365-2133.2009.09450.x

Reitzel, L. R., Regan, S. D., Nguyen, N., Cromley, E. K., Strong, L. L., Wetter, D. W., & McNeill, L. H. (2014). Density and proximity of fast food restaurants and body mass index among African Americans. *American Journal of Public Health, 104*(1), 110–116. doi:10.2105/AJPH.2012.301140

Rodriguez, M., Daniels, B., Gunawardene, S., & Robbins, G. K. (2009). High frequency of Vitamin D deficiency in ambulatory HIV-positive patients [ABSTRACT]. *AIDS Research and Human Retroviruses, 25*(1), 9–14. doi:10.1089/aid.2008.0183

Rosen, C. J. (2011). Vitamin D insufficiency. *New England Journal of Medicine, 364*, 248–254. doi:10.1056/NEJMcp1009570

Ross, A. C., & McComsey, G. A. (2012). The role of Vitamin D deficiency in the pathogenesis of osteoporosis and in the modulation of the immune system in HIV-infected patients. *Clinical Reviews in Bone and Mineral Metabolism, 10*(4), 277–287. doi:10.1007/s12018-012-9131-0

Ross, A. C., Judd, S., Kumari, M., Hileman, C., Storer, N., Labbato, D., … McComsey, G. A. (2011). Vitamin D is linked to carotid intima-media thickness and immune reconstitution in HIV-positive individuals. *Antiviral Therapy, 16*(4), 555–563. doi:10.3851/lMP1784

Rustein, R., Downes, A., Zemel, B., Schall, J., & Stallings, V. (2011). Vitamin D status in children and young adults with perinatally acquired HIV infection. *Clinical Nutrition, 30*, 624–628. doi:10.1016/j.clnu.2011.02.05

Sant, A. J. & McMichael, A. (2012). Revealing the role of CD4+ T cells in viral immunity. *The Journal of Experimental Medicine, 209*(8), 1391–1395. doi:10.1084/jem.20121517

Shintani, A. (2008). *Sample size estimation and power analysis* [PDF document]. Retrieved from Vanderbilt University-Department of Biostatistics website: https://medschool.vanderbilt.edu/cqs/files/cqs/media/2008Mar2008Shintani.pdf

Schonbrodt, F. D., & Perugini, M. (2013). At what sample size do correlations stabilize? *Journal of Research in Personality, 47*(5), 609–612. Retrieved from http://www.sciencedirect.com/science/article/pii/S0092656613000858

Schwalfenberg, G. K. (2010). A review of the critical role of Vitamin D in the functioning of the immune system and the clinical implications of Vitamin D deficiency. *Molecular Nutrition and Food Research, 54*, 1–13. doi:10.1002/mnfr.201000174

Simon, V., Ho, D. D., & Abdool Karim, Q. (2006). HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *Lancet, 368*(9534), 489–504. doi:10.1016/S0140-6736(06)69157-5

Smolders, J., Thewissen, M., Peelen, E., Menheere, P., Tervaert, J. W. C., Damoiseaux, & Hupperts, R. (2009). Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. *PLoS One, 4*(8), e6635. doi:10.1371/journal.pone.0006635

Spector, S. A. (2009). Vitamin D earns more than a passing grade. *Journal of Infectious Diseases, 200*(October), 1015–1017. doi:10.1086/605723

Spector, S. A. (2011). Vitamin D and HIV: letting the sun shine in. *Topics in Antiviral Medicine, 19*(1), 6–10.

Spina, C. S., Tangpricha, V., Uskokovic, M., Adorinic, L., Maehr, H., & Holick, M. F. (2006). Vitamin D and cancer. *Anticancer Research, 26*, 2515–2524. Retrieved from http://ar.iiarjournals.org/content/26/4A/2515.long

Stein, E. M., Yin, M. T., McMahon, D. J., Shu, A., Zhang, C. A., Ferris, D. C., … Shane, E. (2011). Vitamin D deficiency in HIV-infected postmenopausal Hispanic and African-American women. *Osteoporosis International, 22*(2), 477–487. doi:10.1007/s00198-010-1299-x

Stephensen, C. B., Marquis, G. S., Kruzich, L. A., Douglas, S. D., Aldrovandi, G. M., Wilson, C. M. (2006). Vitamin D status in adolescents and young adults with HIV infection. *American Journal of Clinical Nutrition, 83*(5), 1135–1141. Retrieved from http://ajcn.nutrition.org/content/83/5/1135.full

Sudfeld, C. R., Wang, M., Aboud, S., Giovannucci, E. L., Mugusi, F. M., & Fawzi, W. W. (2012). Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. *PLoSOne, 7*(6), e40036. doi:10.1371/journal.pone.0040036

Suresh, K., Thomas, S. V., & Suresh, G. (2011). Design, data analysis and sampling techniques for clinical research. *Annals of Indian Academy of Neurology, 14*(4), 287–290. doi:10.4103/0972-2327.91951

Tafazoli, A., & Khalili, H. (2013). Vitamin D and HIV infection: A review of the clinical evidence. *Future Virology, 8*(6), 589–606. doi:10.2217/fvl.13.40

Tai, K., Need, A. G., Horowitz, M., Chapman, I. M. (2008). Vitamin D, glucose, insulin, and insulin sensitivity. *Nutrition, 24*(3), 279–85. doi:10.1016/j.nut.2007.11.006

Teichmann, J., Stephan, E., Lange, U., Discher, T., Friese, G., Lohmeyer, J., … Bretzel, R. G. (2003). Osteopenia in HIV-infected women prior to highly active antiretroviral therapy. *Journal of Infection, 46*(4), 221–227. doi:10.1053/jinf.2002.1109

The White House- Office of National AIDS Policy. (2010). National HIV/AIDS strategy. Retrieved from http://www.whitehouse.gov/administration/eop/onap/nhas

Theodorou, M., Serste, T., Van Gossum, M., & Dewit, S. (2014). Factors associated with Vitamin D deficiency in a population of 2044 HIV-infected patients. *Clinical Nutrition, 33*(2), 274–279. doi:10.1016/j.clnu.2013.04.018

Trang, H., Cole, D. E., Rubin, L. A., Pierratos, A., Siu, S., & Vieth, R. (1998). Evidence that Vitamin D3 increases serum 25-hydroxyVitamin D more efficiently than does Vitamin D2. *American Journal of Clinical Nutrition, 68*, 854–48. Retrieved from http://ajcn.nutrition.org/content/68/4/854.long

Trinchieri, G., & Sher, A. (2007). Cooperation of toll-like receptor signals in innate immune defence. *Nature Reviews Immunology, 7*, 179–190. doi:10.1038/nri2038

Tripkovic, L., Lambert, H., Hart, K., Smith, C. P., Bucca, G., Penson, S., … Lanham-News, S. (2012). Comparison of Vitamin D2 and Vitamin D3 supplementation in raising serum 25-hydroxyVitamin D status: A systematic review and meta-analysis. *American Journal of Clinical Nutrition, 95*(6), 1357–1364. doi:10.3945/ajcn.111.031070

Tseng, M., Giri, V., Bruner, D. W., & Giovannucci, E. (2009). Prevalence and correlates of Vitamin D status in African American men. *BMC Public Health, 9*, 191–197. doi:10.1186/1471-2458-9-191

Turett, G. S., Pollack, S., Seligman, J. B., & Kaufman, D. L. (2013). Vitamin D levels in an HIV-infected and uninfected cohort in New York City. *Journal of Virology and Anti-viral Research, 2*, 2. Retrieved from http://scitechnol.com/vitamin-d-levels-in-an-hivinfected-and-uninfected-cohort-in-new-york-city-IxwB.pdf

United Nations Programme on HIV/AIDS. (2012). Global report: UNAIDS report on the global AIDS epidemic 2012. Retrieved from http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_with_annexes_en.pdf

Van Den Bout-Van Den Beukle, C. J., Fievez, L., Michels, M., Sweep, F. C., Hermus, A. R., Bosch, M. E., … Van Der Ven, A. J. (2008). Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: Effects of antiretroviral therapy [Abstract]. *AIDS Research and Human Retroviruses, 24*(11), 1375–1382.

Vassar, M., & Holzmann, M. (2013). The retrospective chart review: Important methodological considerations. *Journal of Educational Evaluation for Health Professions., 10*, 12–18. Retrieved from http://jeehp.org/DOIx.php?id=10.3352/jeehp.2013.10.12

Vescini, F., Cozzi-Lepri, A., Borderi, M., Re, M. C., Maggiolo, F., De Luca, A., … Icona Foundation Study Group. (2011). Prevalence of hypovitaminosis D and factors associated with Vitamin D deficiency and morbidity among HIV-infected patients

enrolled in a large Italian cohort. *Journal of Acquired Immune Deficiency Syndrome*, 58(2), 163–172. doi:10.1097/QAI.0b013e31822e57e9

Viard, J. P., Souberbielle, J. C., Kirk, O., Reekie, J., Knysz, B., Losso, M., … Mocroft, A. (2011). Vitamin D and clinical disease progression in HIV infection: Results from the EuroSIDA Study. *AIDS, 25*, 1305–1315. doi:10.1097/QAD.0b013e328347f6f7

Vieth, R., Bischoff-Ferrari, H., Boucher, B., Dawson-Hughes, B., Garland, C. F., Heaney, R. P., … Zittermann, A. (2007). The urgent need to recommend an intake of Vitamin D that is effective. *The American Journal of Clinical Nutrition, 85*(3), 649–650. Available at http://ajcn.nutrition.org/content/85/3/649.long

Villamor, E. (2006). A potential role for Vitamin D on HIV infection? *Nutrition Reviews, 64* (5), 226–233. doi:10.1111/j.1753-4887.2006.tb00205.x

Wacker, M., & Holick, M. F. (2013). Vitamin D-Effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients, 5*, 111–148. doi:10.3390/nu5010111

Walker, V. P., & Modlin, R. L. (2009). The Vitamin D connection to pediatric infections and immune function. *Pediatric Research, 65*(5), 106R–113R.

Wang, T. T., Nestel, F. P., Bourdeau, V., Nagai, Y., Wang, Q., Lia, J., … White, J. H. (2004). Cutting edge: 1,25-dihydroxyVitamin D3 is a direct inducer of antimicrobial peptide gene expression. *The Journal of Immunology, 173*(5), 2909–2912. Available at http://www.jimmunol.org/content/173/5/2909.long#ref-8

Wang, T. T., Tavera-Mendoza, L. E., Laperriere, D., Libby, E., MacLeod, N. B., Nagai, Y., … White, J. H. (2005). Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyVitamin D3 target genes. *Molecular Endocrinology, 19*(11), 2685–2695. Available at http://mend.endojournals.org/content/19/11/2685.long

Wasserman, P., & Rubin, D. S. (2010). Highly prevalent Vitamin D deficiency and insufficiency in an urban cohort of HIV-infected men under care. AIDS Patient Care STDS*, 24*(4), 223–227. doi:10.1089/apc.2009.0241

Wejse, C., Gomes, V. F., Rabna, P., Gustafson, P., Aaby, P., Lisse, I. M., … Sodemann, M. (2009). Vitamin D as supplementary treatment for tuberculosis: A double blind, randomized, placebo-controlled trial. *American Journal of Respiratory & Critical Care Medicine, 79*(9), 843–850. doi:10.1164/rccm.200804-567OC

Welz, T., Childs, K., Ibrahim, F., Poulton, M., Taylor, C. B., Moniz, C. F., & Post, F. A. (2010). Efavirenz is associated with severe Vitamin D deficiency and increased alkaline phosphatase. *AIDS, 24*(12), 1923–1928. doi:10.1097/QAD.0b013e32833c3281

White, J. H. (2008). Vitamin D signaling, infectious diseases, and regulation of innate immunity. *Infection and Immunity, 76*(9), 3837–3843. doi: 10.1128/IAI.00353-08

Wiboonchutikul, S., Sungkanuparph, S., Klertiburanakul, S., Chailurkit, L. O., Charoenyingwattana, A., Wangsomboonsiri, W., Chantratita, W., & Ongphiphadhanakul, B. (2012). Vitamin D insufficiency and deficiency among

HIV-1 infected patients in a tropical setting. *Journal of International Association of Physicians AIDS Care (Chic), 11*(5), 305–10.

Williams, B., Lima, V., & Gouws, E. (2011). Modelling the impact of antiretroviral therapy on the epidemic of HIV. *Current HIV Research, 9*(6), 367–382. doi:10.2174/157016211798038533

Williams, S., Malatesta, K., & Norris, K. (2009). Vitamin D and chronic kidney disease. *Ethnicity and Disease, 19*(Suppl.5), S5-8–S5-11. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2878736/

Wolff, A. E., Jones, A. N., & Hansen, K. E. (2008). Vitamin D and musculoskeletal health. *Nature Clinical Practice Rheumatology, 4*(11), 580–588. Available at http://www.nature.com/nrrheum/journal/v4/n11/pdf/ncprheum0921.pdf

WHO. (2006). WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV related disease in adults and children. Retrieved from *www.who.int/**hiv**/pub/guidelines/WHO%20**HIV**%20**Staging**.pdf*

WHO. (2007). WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Retrieved from www.who.int/**hiv**/pub/guidelines/WHO%20**HIV**%20**Staging**.pdf

Worster, A., & Haines, T. (2004). Advanced statistics: Understanding medical record review (MRR) studies. *Academic Emergency Medicine, 11*(2), 187–192. Retrieved from http://www.emergencymedicine.utoronto.ca/Assets/EmergeMed+Digital+Assets/event/Medical+Records+Review.pdf

Worster, A., Bledsoe, R. D., Cleve, P., Fernandes, C. M., Upadhye, S., & Eva, K. (2005). Reassessing the methods of medical record review studies in emergency medicine research. *Annals of Emergency Medicine, 45*(5), 448–451. doi:10.1016/j.annemergmed.2004.11.021

Yan, H., Yang, H., Li, J., Liu, X., & McFarland, W. (2012). *Disparities in progression from HIV to AIDS and mortality among HIV-positive men who have sex with men, Jiangsu province, China.* Poster Abstract Session: AIDS 2012 - XIX International AIDS Conference: Abstract no. MOPE105.

Yin, M. (2012). Vitamin D, bone, and HIV infection. *Topics in Antiviral Medicine, 20*(5), 168-172. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4019218/

Youssef, D. A., Miller, C. W. T., El-Abbassi, A. M., Cutchins, D. C., Cutchins, C., Grant, W. E., & Peiris, A. N. (2011). Antimicrobial implications of Vitamin D. *Dermato-Endocrinology, 3*(4), 220–229. doi:10.4161/derm.3.4.15027

Yuk, J. M., Shin, D. M., Lee, H. M., Yang, C. S., Jin, H. S., Kim, K. K., Lee, Z. W., … Jo, E. K. (2009). Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin [ABSTRACT]. *Cell Host Microbe, 6*(3), 231–243. doi:10.1016/j.chom.2009.08.004