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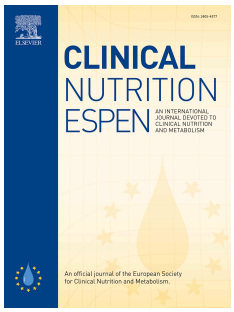

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Raiha Qurban, Shamreen Saeed, Wajiha Kanwal, Kashaf Junaid, Abdul Rehman

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Conflict of interest

The authors have declared that no competing interests exist. All authors have contributed in the design, write up and final editing of the manuscript.

Potential immune modulatory effect of vitamin D in HIV infection: A review

Raiha Qurban[a], Shamreen Saeed[a], Wajiha Kanwal[a], Kashaf Junaid[b], Abdul Rehman[a]*

[a]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore-54590, Pakistan

[b]College of Applied Medical Sciences, Jouf University, Sakaka, Al Jouf, Saudi Arabia

*Correspondence author
Dr. Abdul Rehman
Associate Professor
Department of Microbiology & Molecular Genetics
University of the Punjab, New Campus,
Lahore 54590, Pakistan
Tel: 92-42-9231249
Email: rehman.mmg@pu.edu.pk

Abstract

Vitamin D is a fat soluble hormone that is majorly involved in the classical function of calcium and phosphorus hemostasis and bone mineralization as well non classical functions of immune modulation in various viral and autoimmune diseases. Both innate and adaptive immunity is aided by vitamin D. Deficiency of vitamin D is not only linked with bone and muscle disorders but it has a critical role in many infectious and noninfectious diseases. A growing body of literature suggests vitamin D deficiency in human immunodeficiency virus (HIV) infected patients. HIV affects 36.7 million people worldwide. Currently a valuation of 0.13 million people are infected with acquired immunodeficiency syndrome (AIDS) in Pakistan. Various studies showed that hypovitaminosis D may aggravate the disease severity in HIV patients by compromising the immune system. Calcidiol supplementation is credibly a promising adjuvant of combination anti-retroviral therapy (cART) for the treatment of HIV by increasing CD4 T-cells and lowering the viral load. This review accentuates vitamin D's functions as an immune modulator in HIV, the effect of hypovitaminosis D in diseases severity, and its supplementation impact on the treatment of HIV infected patients.

Key words: Hypovitaminosis D; immune modulation; calcitriol; HIV infection; cART.

1. Introduction

Vitamin D is essential for bone health and has been associated with bone mineralization issues over the years. Vitamin D can potentially influence various extra-skeletal health outcomes, such as muscular function, immunological function, cardiovascular disease, diabetes, and cancer [1,2]. This, together with evidence of widespread vitamin D insufficiency worldwide [3], has

sparked a surge in interest in vitamin D and the potential benefits of supplementation in many infectious and chronic diseases [4].

According to various studies, vitamin D deficiency is common in HIV patients, just as it is in the general population [5,6]. Chronic HIV infection is linked to an increased risk of comorbidities such as heart disease, osteoarthritis and osteoporosis, malignancy, and other age-related diseases. Considering the similar risk of chronic disease outcomes as observed during vitamin D deficiency, and given the consequences of immunological function in HIV disease progression and susceptibility to an opportunistic pathogen, there has been a lot of interest in determining the clinical significance of vitamin D in HIV infected patients [7].

The current review focuses on vitamin D's role in developing and effective functioning of the immune system, primarily the defense system against HIV. An online search in PubMed and Google Search was performed for the present review using the keywords; vitamin D, 25(OH)D, hypovitaminosis D, and HIV infection for the period from September 2020 to April 2021. We included both the original and review articles related to the subject, focusing more on the most recent epidemiological and experimental data. An overall 88 studies have been included for this review article, and we concentrate on the most recent epidemiological and clinical data. Figure 1 represents the study selection and selection of studies included in this review article. The current study describes vitamin D metabolism and its deficiency, vitamin D as an immune modulator, and vitamin D's role against viral diseases, emphasizing HIV disease.

2. Vitamin D

In the early 20th century, vitamin D was first identified as a vitamin, known as ergo-calciferol and sunshine vitamin [8]. Different studies on rickets are linked with the discovery of vitamin D.

vitamin D has been proven to be a rickets-prevention agent in one study from 1920s [9]. In 1921, Hess and Unger manifested the significance of sunlight in curing rickets leading to the discovery of vitamin D [10]. Vitamin D is a fat-soluble hormone and ingested through skin [11]; primarily responsible for the absorption of calcium and phosphorus hemodynamics to maintain bone mineral density and credibility [12]. A vast body of work and epidemiological studies have exemplify that vitamin D plays a crucial role in various cellular processes such as immune tolerance, cellular differentiation and proliferation, neurological functions, cardiorespiratory endurance, oxidative stress reduction, anticancer, and anti-aging effects [13-15]. Besides its classical functions of body mineral homeostasis; it also has a critical impact on novel functions like providing immunity against pathogenic infections [16]. Many studies showed the role of vitamin D in autoimmune and metabolic ailments [17]. Recent epidemiological studies have expanded our knowledge regarding the currency of vitamin D deficiency among HIV infected individuals [17,18].

3. Chemical reactions-vitamin D

Vitamin D activation needs two steps hydroxylation; one in the liver and the other in the kidney, regardless of its source, either from diet or sunlight [19]. Vitamin D binding proteins (VDBP) also known as Gc-globulins as belonging to the Gc protein family; binds with both active and inactive forms of vitamin D and promote their transmission into the whole body [20]. The inactive form of vitamin D (Pre-vitamin D3) travels to the liver via DBP protein, where it undergoes very first hydroxylation. By cytochrome P450 and enzyme 25 hydroxylase (CYP2R1/CYP27A1) D3 is converted to active 25-hydroxyvitamin D3 or calcidol [21]. Some

extrahepatic hydroxylation of D3 has also been mentioned. Serum 25(OH)D or calcidol is the prime circulating and proffer form to measure in blood having a shelf life of 2 weeks [22].

The 25(OH)D is transported to the kidney, where it is hydroxylated again aided by an enzyme 25-hydroxyvitamin D 1-alpha-hydroxylase (25(OH)D-1αOHase or CYP27B1). Calcitriol or 1,25(OH)D is the active metabolite, produced here with a half-life of just 10-12 hours which is even lesser than 25(OH)D [21,23]. The hydroxylation of 25(OH)D in the kidney to its active form is highly synchronized by parathyroid hormone, calcium, phosphorus and 1,25(OH)D (Fig. 2).

Parathyroid hormone increases vitamin D synthesis, enhancing the calcium absorption from the intestine to regulate calcium homeostasis [24]. Vitamin D stimulation in the extra-renal system is also evident because of enzyme 25(OH)D-1αOHase (CYP27B1) in extra renal tissues like skin, prostate, pancreas, brain and macrophages. The substantial build of circulating vitamin D is calcidiol or 25(OH)D and higher by 500-1000 times than 1,25(OH)D. Both calcidiol and calcitriol are protein bound once entered into the circulatory passage. The 25(OH)D is chiefly bound to VDB, a small amount to albumin, and very little is free in the circulation. According to studies, the free form of 25(OH)D is less than 1% [25-27].

4. Vitamin D deficiency

When people have inadequate exposure to sunlight, then they suffer from vitamin D deficiency. There is no agreed-upon concept of hypovitaminosis D, but numerous polls employ various threshold values of serum 25(OH)D below called Vitamin D deficiency or insufficiency. Literature from other researches orthodox that level below 50nM/L or 70nM/L is considered vitamin D deficiency, which causes alteration in bone metabolism [22,28,29]. Recent ecological

guidelines regarding vitamin D supplementation suggested that a healthy life serum 25(OH)D level must be maintained between 75 to 125nmol/l. For vitamin D's pleiotropic and extraskeletal roles, a dose of 400- 2000IU/day is recommended [30].

5. Non classical functions of vitamin D

From the past few years, comprehensive studies are done to estimate the non-classical functions of vitamin D. Aside from the predominant skeletal roles of vitamin D of bone mineralization; many extra skeletal functions have also been discovered [31]. In the last two decades, the emergence of the pleiotropic outcomes of vitamin D makes it helpful to find that receptors of vitamin D (VDR) and vitamin D activating enzymes CYP27B1 are present on various cells other than bone, kidney and liver [23,32]. Recently discovered tissues and cells having receptors of vitamin D are; small intestine, prostate, colon, β- islet of pancreas, keratinocytes, osteoblast, active lymphocytes, and any other organs of the body like brain, skin, hair and breast [33,34].

Vitamin D as an immune-modulator showed that many immune cells like macrophages and hematopoietic cells also generate 25(OH)D at the site of infection increasing their phagocytic ability [35,36]. The occurrence of chronic diseases may decrease the serum vitamin D ratio, which designates the inverse association of vitamin D with chronic diseases. Different epidemiological studies have suggested that low serum vitamin D status is responsible for different chronic autoimmune infections like congestive heart failure, diabetes, tuberculosis, hypertension, chronic liver diseases, progression of acquired immune deficiency syndrome and metabolic disorders [37].

6. Autoimmune disorders and vitamin D

The reluctance of an organism to acknowledge its own cells, directing to demolishment of body's own healthy cells by auto-reactivity of its immune cells is termed as autoimmunity [38]. The etiology of more than 100 autoimmune diseases (ADs) has been recognized and principally associated with the genetic factors, epigenetic factors, infectious diseases, hormonal and low exposure to sunlight [39,40]. There is a clear cut gender difference in prevalence of autoimmune diseases as females are more susceptible to Ads [41]. Various studies have illustrated that vitamin D insufficiency is affiliated with immune intolerance, playing a major part in innate and acquired immune responses [42,43].

All immune cells possess VDR which make them prone to calcitriol- mediated articulation. The engagement of calcitriol with VDR of dendritic cells (DCs) cease the maturation and migration of DCs, decreases the production of pro-inflammatory cytokines (IL-6, IL-12, IL-23), TNF-α, enhances the formation of anti-inflammatory cytokines (IL-8, IL-10), slumping the MHC I, II expression and surface co-stimulatory factors (CD-40, CD-80,CD-83,CD-86) and thus enhances the tolerogenic and immune-regulatory effects of DCs [44].

The 25(OH)D halts the antibodies production by inhibiting the maturation of B cells into plasma cells. It also interacts with T cells, inhibiting the production of Th1 and Th17 subpopulations and increasing the differentiation of Th2 to CD4+, inducing IL-4. Regulatory cells also suppress the immune responses by the stimulation of calcitriol [45].

7. HIV genome and structure

HIV was first insinuated in the human population between 1920 to 1940 years. HIV is a retrovirus, consisting of two identical positive sense single stranded RNA strands encoded in viral capsid and enzymes reverse transcriptase, integrase and protease, grouped to genus Lentivirus within the family of Retroviridae [46]. The viral envelope consists of external spikes

of glycoproteins gp120 and transmembrane gp41 glycoproteins encoded by virus. The RNA compartment of HIV contains approximately 9749 nucleotides long having 5'cap (Gppp) and 3' poly A tail and various open reading frames (ORFs). CD4 cells are the focal target of HIV. The interaction between chemokine receptors CXCR4 and CCR5 with the envelope glycoprotein gp120 instigate the penetration of the HIV genome into the host cell [47] (Fig. 3).

7.1. HIV as a life threat and its impact on Pakistan

Cosmo-politically, there are approximately 36.7 million people who are living with HIV. Deadly disease like AIDS is caused by HIV infection and becomes a recrudescence throughout the word [48]. In 2016, almost 1 million lives were lost of AIDS while 1.8 million new cases of HIV/AIDS were discovered in the same time span. In Pakistan, a valuation of 0.13 million people are currently infected with AIDS, out of which 17% are enrolled with the AIDS control programme and 54% of total people living with HIV infection and getting treatment from anti-retroviral therapy (ART) centers. The pervasiveness rate of HIV is 0.1% in Pakistan. Over the decades, there was a concentrated epidemic of HIV in key population of Pakistan. Still, now the epidemic shifted from key population to female sex workers (FSWs), among people who inject drugs (PWIDs), transgender (TG), male sex workers (MSWs), is increased in Pakistan. As reported by Integrated Biological and Behavioral Survey (IBBS), the incidence of HIV in people who inject drugs (PWIDs) was 38.4%, in Transgender (TG) 7.1%, among men who had sex with other men (MSWs) was 3.5% and among female sex workers (FSWs) was 2.2% [45,49]. Evidence from different studies suggested that HIV to SWs is interlinked with the ethical and sociological aspects such as cultural disgrace, illiteracy, poor knowledge about prevention of HIV and lack of access to services [50,51].

7.2. HIV and vitamin D

In recent decades, vitamin D has been a counter stone for the investigator stalks as HIV medicaments. A considerable number of studies are available that showed the influence of vitamin D on cardiovascular diseases [52,53] under the infection of HIV, co-infection of HIV/HCV [54,55], and literature suggested the detection, mitigation and cure of vitamin D insufficiency in HIV suffering patients [56-59] (Table 1). Clinical specifications commended that, ideally, the daily dose of vitamin D must be between 1500 and 2000IU/d. For the HIV positive adults, 6000-10,000IU/d vitamin D intake is advocated. A serum concentration of 25(OH)D3 below 20ng/mL(50nm/L) is considered as a sign for the cure of hypovitaminosis D [60].

7.3. Universality of vitamin D insufficiency in HIV

From various studies it is evident that hypovitaminosis D causes the very late or compromised immune response over the course of HIV medication. A study from 2005-2007 scrutinized the AIDS patients from different countries: Haiti, India, Brazil, Peru, Malawi, South Africa, USA, Thailand and Zimbabwe. 49% of these patients have vitamin D levels lower than baseline. The occurrence of vitamin D insufficiency differed remarkably by country varying from 27% in Brazil to 78% in Thailand [61,62]. A study conducted by Havers at el. hat calculated the role of vitamin D as a factor affecting the virologic reactions during anti-retroviral therapy (ART) [63].

8. Vitamin D as immune-modulator against HIV

8.1. Vitamin D's participation in innate immune response

The immunomodulation of vitamin D is provided by its autocrine passage in human monocytes and macrophages which in turn stimulates the toll like receptors (TLRs1/2, TLR4) and interferon receptors (IFN- γ and CD40) [64,65] (Fig. 4). These receptors commences the signal cascade that causes the conversion of 25(OH)D to 1,25(OH)D by up-regulating VDR and CYP27B1. Functions of monocytes and macrophages modulated during infection by binding of 1,25(OH)D with the VDR leading to multitarget gene expression. Vitamin D halts the maturation of dendritic cells (DCs) thus inhibiting the excessive inflammatory response against infectious diseases[66,67]. High levels of vitamin D and VDR are linked with the in-build resistance against HIV. This may be owing to the overexpression of IL-10 and excitation of anti-HIV defensins in the mucosa of individuals who have been unprotected with HIV-1 [68].

The VDR expression is directly associated with the up-regulation of quite few anti HIV molecules such as cathelicidin microbial peptide (CAMP) and RNase7 which elicit natural resistance of HIV [69]. I,25(OH)D in monocytes minimizes the chances to HIV by preventing the entry of virus, limiting CD4 expression and reducing the monocytes proliferation [70,71]. TLR8 agonists are thought to impede the HIV malady by the way of CAMP and vitamin D mediated autophagy mechanism in macrophages. Moreover, vitamin D activates the autophagy in macrophages inhibiting the HIV infection [72]. Vitamin D deficiency stimulates the over secretion of (CXCL10, IL-6, TNF -α, D-dimer) and energized monocytes subpopulations (CCR2+ and CX3CR1) in HIV positive individuals and which may causes tissue disruption, co-infection development and AIDS progression and ultimately leading to death in HIV patients [73,74].

8.2. Vitamin D's participation in adaptive immune response:

Over expression of CYP27B1 VDR and by T-cells activation increases the conversion of 25(OH)D to 1,25(OH)D, which modify the effector function of vitamin D. vitamin D changes the T-cells phenotype and function by down regulation of Th1,Th17 and Th2 cytokine profile production [75] (Fig. 5). In case of B-cells expression of surface VDR and CYP27B1 up-regulated, decreasing memory B-cells differentiation into plasma cells, reducing the plasma cell count leading to low immunoglobulin production [76].

8.3. Vitamin D as a forestallment and cure of HIV

Vitamin D insufficiency in general populace of all ages is a global issue. Different studies have announced that more than 75% of the US population has vitamin insufficiency. The cohort studies worldwide have reported about 100% hypovitaminosis D in the HIV suffering people [77]. In spite of the certitude that HIV infected patients are suffering from hypovitaminosis D, no safe and clear dose additive protocol has been published. Even so, recently, the Institute of Medicine suggested a daily dose of 600IU to keep up the necessity of 97.5% of the population, with 4000IU/d deemed the highest allowable dose [78]. The North American Endocrine Society(NAES) anticipated triple the recommended dose for HIV infected persons adhering to cART [29].

For the vitamin D supplementation trials were conducted in HIV living individuals with a greater contribution of men 60%, predominantly African-American followed by the Caucasians. The number of participants for each trial is about 17 to 365, all of which are given oral supplementation of vitamin D, apart from in Falasca et al's report in which individuals are also supplemented through intramuscular route [79]. Before supplementation vitamin D were noted which were <20ng/mL, suggesting that HIV infected individuals suffer from vitamin D

deficiency. In most of the studies viral load, co-morbidities and CD4+ counts are also noted. The duration of each group is different from 4 to 104 weeks. Even though most of the supplementation increases the vitamin D level, it was a bit challenging because of severe hypovitaminosis D in HIV individuals. The most potent dose was use of 7000IU/d [78,80-83], maintaining the sufficient levels of about >30ng/mL in 80% of supplemented individuals attaining the higher vitamin level following 12 months of treatment [84].

8.4. Vitamin D supplementation affects CD4+ cells and viral load

The CD4+ count and viral burden are the vital index to measure the progression of HIV infection. Recent research has concluded that hypovitaminosis D is linked to low CD4+ count in HIV infected individuals [18]. According to Coelho et al. [85] about 88% of the individuals having CD4+ count <50 cells/mm^3 were deficient in vitamin D, only 6% of the participating individuals have normal levels of vitamin D. In the same study, the supplementation of 1ng/mL of 1,25(OH) up-grade the CD4+ cells count by 3,3 cells per cubic millimeter, showing a favorable role of calcidiol on immune response retrieval [85] (Fig. 4). Furthermore, Stallings et al. showed a decrease in viral count after vitamin D dosage [86]. Another study observed that CD4+ and CD8+ activation and monocytes decreased considerably in 51 HIV-infected patients who received 18,000IU, 60,000IU, or 120,000IU vitamin D3 monthly [87]. According to one most recent review article, they reviewed 29 clinical studies and suggested when Vitamin D levels were increased to normal ranges in HIV-infected patients, regardless of cART, inflammatory markers associated with bone turnover and the risk of secondary hyperparathyroidism were reduced; however, the anti-bacterial response was enhanced [88].

9. Conclusion

In conclusion, vitamin D plays a beneficial role in HIV suffering individuals. Calcidiol supplementation in HIV positive individuals boosts the serum vitamin D levels, nonetheless of cART, race, and regions of the individuals being dosed. High serum vitamin D level has a positive impact on immune response. The most salient effects of increased cacidiol levels included prevention of secondary hyperthyroidism, comorbidities like tuberculosis, increasing CD4+ cells count, decreasing viral load and the biomarkers which ultimately increase bone turnover and chronic inflammation. On the whole, studies suggested that supplementation of vitamin D is a promising adjuvant of cART. Additionally, effects of supplementation are mediated by the dosage and span of supplementation. As a general rule the dosage of 4000 to 7000 IU/d for at least 12 weeks showed potent and successful effects in HIV infected individuals.

Ethical Approval Statement

Ethical approval was not required.

Conflict of interest

The authors declare that they have no competing interests.

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Figure Legends

Figure 1: Figure 1 represents the study selection and selection of studies included in the current review.

Figure 2: Representation of Vitamin D synthesis and metabolism [23].

Figure 3: HIV's viral genome and its structure [47].

Figure 4: Vitamin D as immune-modulator [65].

Figure 5: Vitamin D supplementation has an impact on clinical and immunological features of HIV infection. In HIV infected individuals supplementation lowers the PTH levels leading to secondary hyperparathyroidism which induces the antimicrobial peptides (AMP) including CAMP and HBD which improves bone formation by lowering bone turnover biomarkers. The supplementation of this hormone may have little on CVD and effectiveness of vit D replacement is independent of cART regimen. It increases the CD4 cell count and engorging the differentiation toward Th2 and Treg profile and decreasing the Th2, Th17 and CD8 cells profile [75].

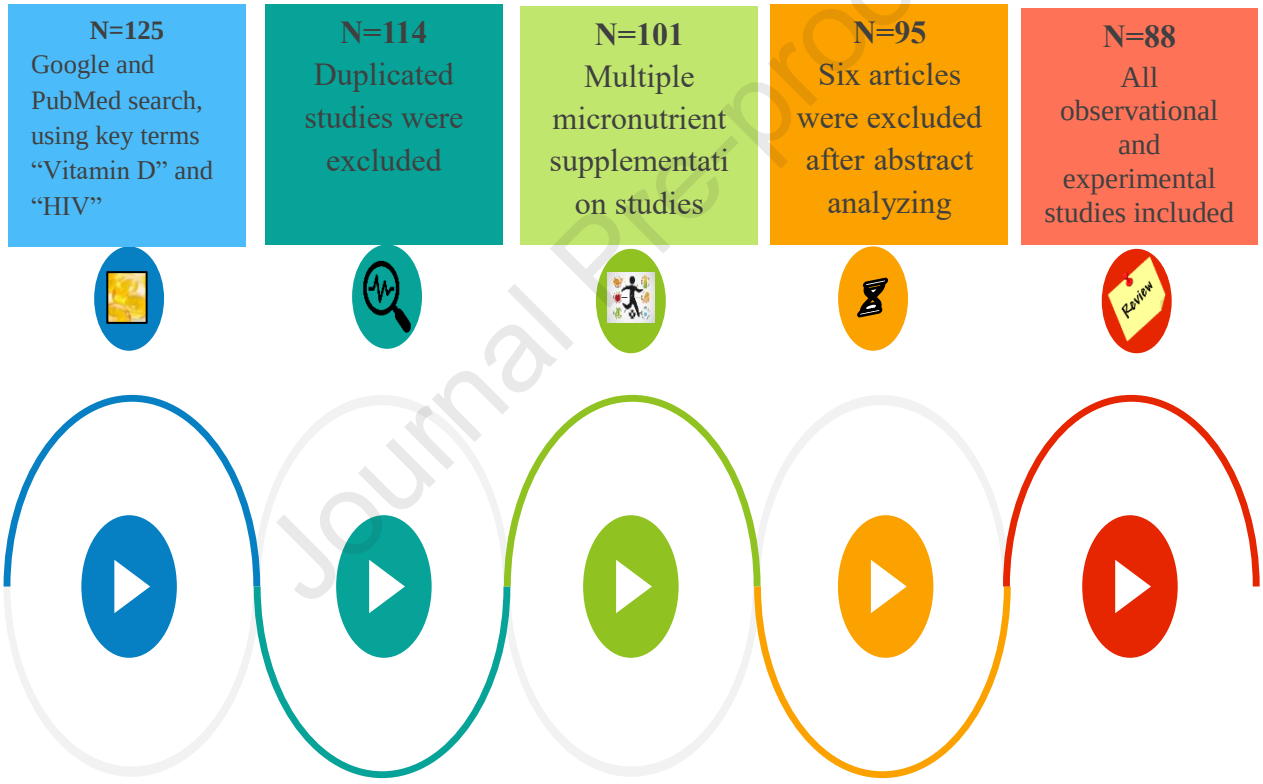


Figure 1

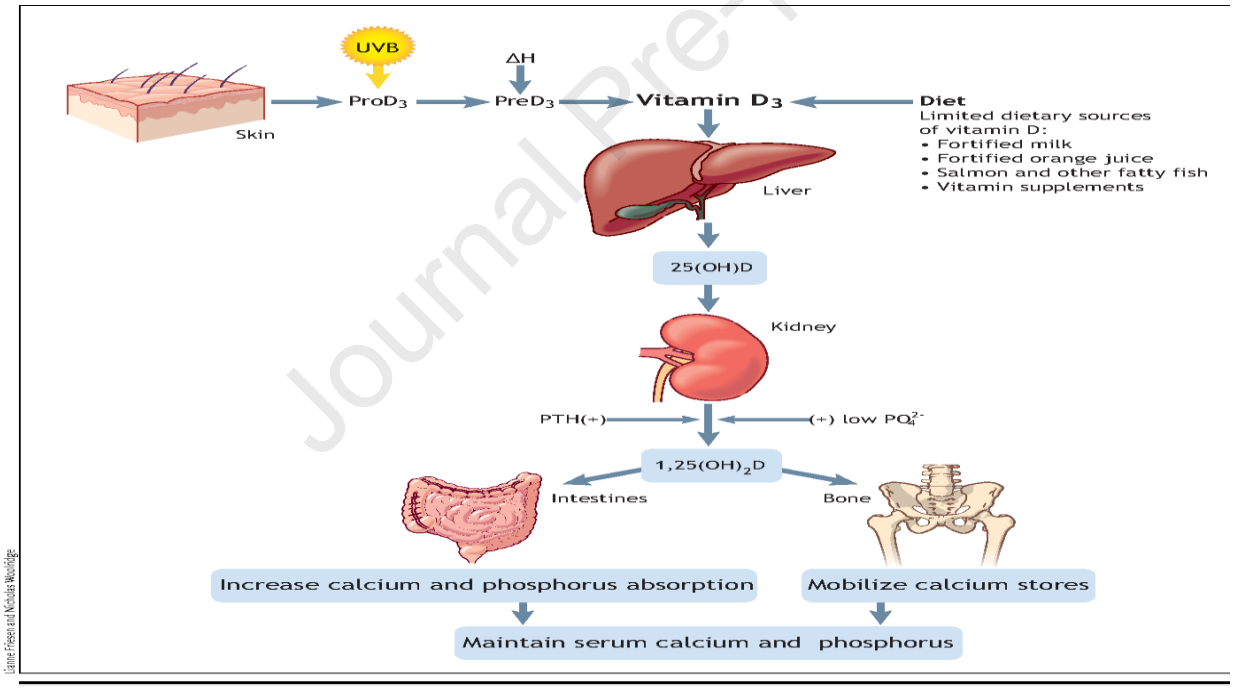
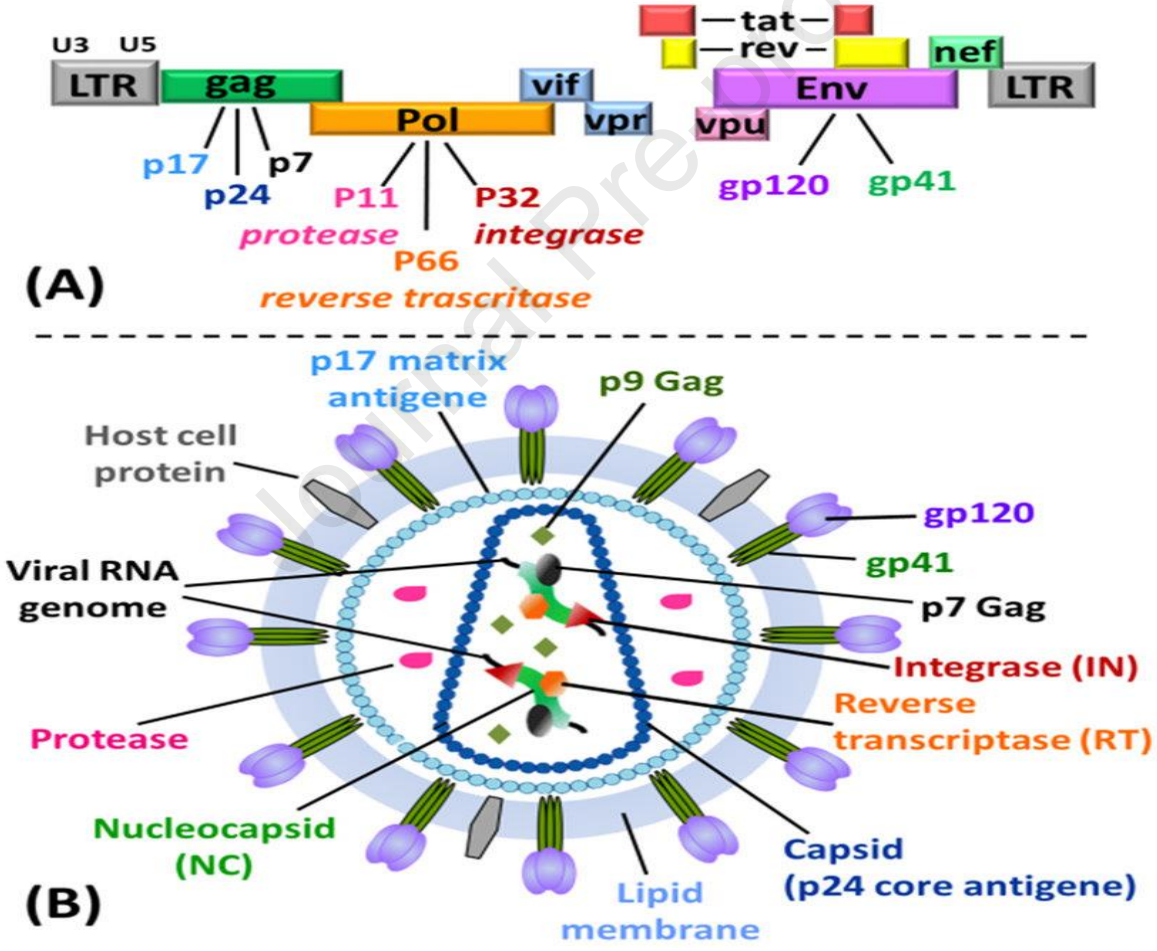


Figure 2


U3 U5
LTR
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p24
p7
P11
protease
P66
reverse trascritase
P32
integrase
gp120
gp41
(A)
p17 matrix antigene
p9 Gag
Host cell protein
gp120
gp41
Viral RNA genome
p7 Gag
Integrase (IN)
Reverse transcriptase (RT)
Protease
Nucleocapsid (NC)
Capsid (p24 core antigene)
Lipid membrane
(B)

Figure 3

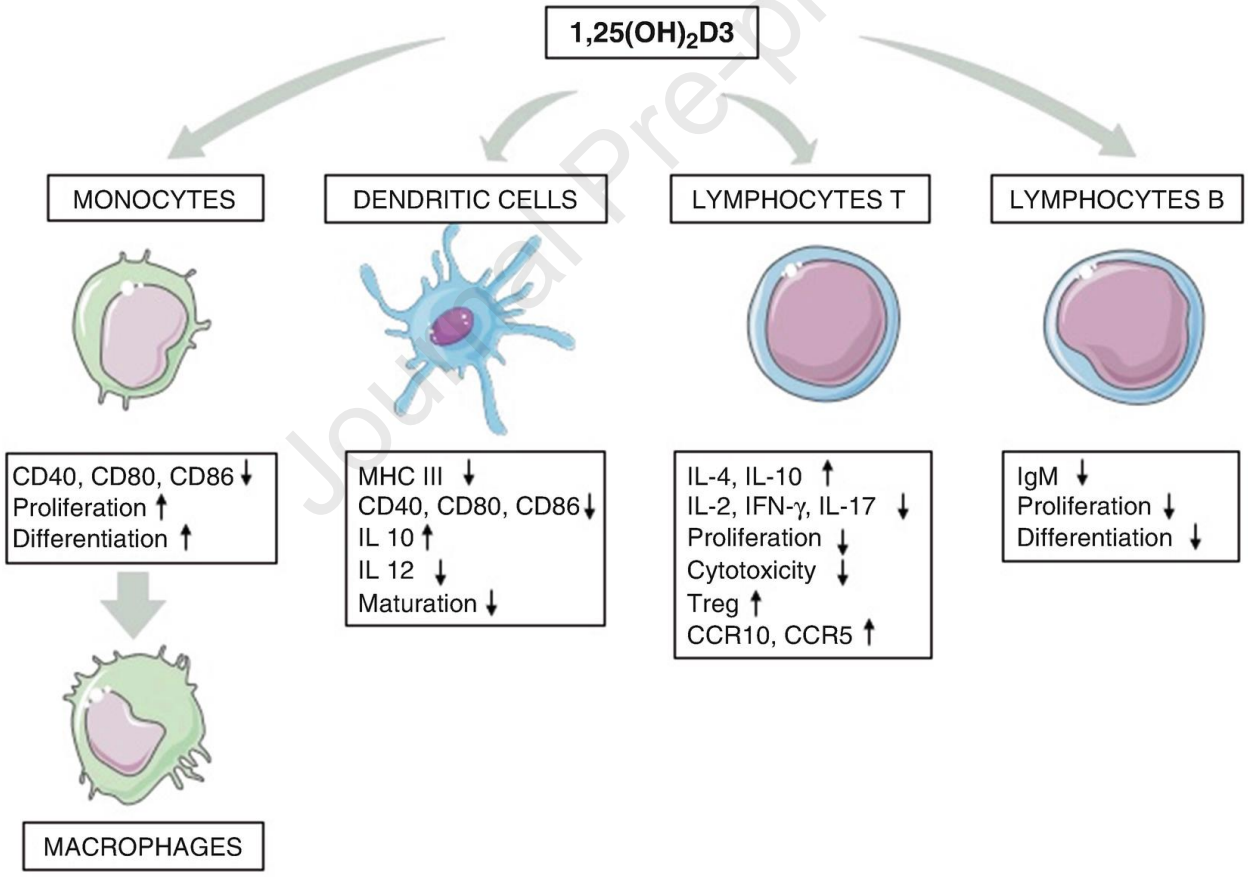

Figure 4

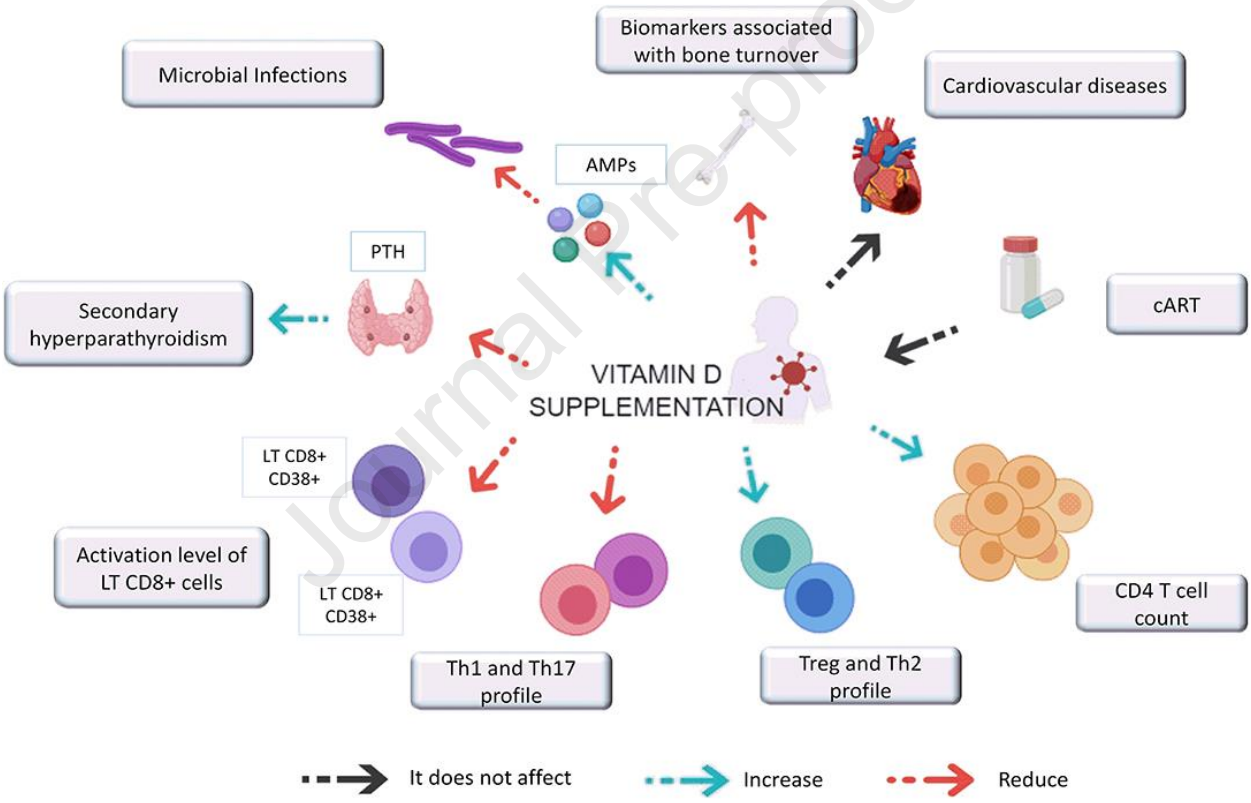
Microbial Infections
Biomarkers associated with bone turnover
Cardiovascular diseases
AMPs
PTH
Secondary hyperparathyroidism
cART
VITAMIN D SUPPLEMENTATION
LT CD8+ CD38+
Activation level of LT CD8+ cells
LT CD8+ CD38+
Th1 and Th17 profile
Treg and Th2 profile
CD4 T cell count
It does not affect
Increase
Reduce

Figure 5

Table 1: Different vitamin D dose supplementation decreases viral load and increase CD4 T-cells by immunomodulation and a good adjuvant to cART against HIV treatment.

Sr. no	Mean age	Dosage (IU)	Patients on cART (%)	Efficacy of dose to restore levels	Main outcome	Topic of interest	Reference
1	9-25	7000 daily for 52 weeks	>76	High	Supplementation was efficient regardless of cART	supplementation	Schall et al. [78]
2	<25	4000 or 7000 daily for 12 weeks	50-75	High	CAMP expression increased after 52 weeks of follow-up	Antibacterial response	Chun et al. [80]
3	35	200,000 once for 4 weeks	65%	High	↑ CAMP and MP-β linked with anti HIV-1 effect ↓ CD38+ T-cells post induction	Immune modulation, Antibacterial response	Lashmann et al. [56]
4	46	20,000 weekly for 52 weeks	100	42-78%	42-78% patient restore vitamin D levels after supplementation No effect on CD4 T-cells count	Immune modulation	Noe et al. [57]
5	34-56	300,000 IM every 10 months Or 25000 orally once for 40 week	100	30-50%	Oral supplementation is more effective than IM No change in CD4 cell count	Immune modulation	Falasca et al. [79]
6	41-45	100,000 every 14 day for 48 weeks	100	High	↓activation levels ↑ CD4/CD8 cells count	Immune modulation	Fabree-Massreman et al. [81]
7	45	4000 daily for 12 weeks	78	Low	Severe deficient did not reach sufficient levels ↓PTH levels	cardiovascular	Longeneker et al. [58]
8	5-25	7000 daily for 48	76	33-40%	↓viral load ↑ vitamin D and CD4 cell count	Immune modulation	Stallings et al. [86]

		weeks					
9	38-50	100,000 weekly for 5 weeks then 16,000 weekly for 12 weeks	100	83%	Association between CD4 cells recovery and vitamin D levels	Immune modulation and induction in cART	Coelho et al. [85]
10	35-57	32000 daily for 96 weeks	100	100%	Supplementation not affect bone mass but ↓ PTH levels	PTH levels and bone turnover	Mela et al. [59]