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Cholecalciferol in Cirrhotic Patients

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Abstract:

Vitamin D deficiency is a common finding in patients with chronic liver disease, particularly cirrhosis. The liver plays a central role in the hydroxylation of vitamin D, and progressive hepatic dysfunction impairs this metabolism. Additionally, patients with cirrhosis often have reduced sunlight exposure, malnutrition, impaired bile acid secretion leading to fat malabsorption, and altered vitamin D–binding protein levels, all contributing to deficiency. Deficiency has been associated with increased risk of infections, impaired bone mineralization, sarcopenia, hepatic encephalopathy, and poorer overall prognosis. Recent studies have also linked low vitamin D levels to higher mortality in cirrhosis, suggesting a role not only as a biomarker of severity but potentially as a therapeutic target.

Keywords: Vitamin D; Cirrhosis; Chronic liver disease; Vitamin D deficiency; Bone metabolism; Prognosis; Mortality; Hepatic dysfunction.

Introduction:

Vitamin D deficiency is highly prevalent among patients with cirrhosis, with studies reporting up to 90% of cases showing suboptimal serum 25(OH)D levels. This is due to impaired hepatic hydroxylation, malnutrition, and decreased sun exposure in this population (1).

The liver is central to vitamin D metabolism, as it converts vitamin D into 25-hydroxyvitamin D [25(OH)D], the major circulating form. In cirrhosis, reduced hepatocellular function and cholestasis further limit vitamin D bioavailability (2).

Clinically, vitamin D deficiency in cirrhosis is associated with osteoporosis, increased fracture risk, muscle weakness, and higher rates of infections. Importantly, low vitamin D has been linked with poor prognosis and increased mortality in advanced liver disease (3).

Vitamin D supplementation has been suggested as a potential adjunct therapy in cirrhotic patients, although optimal dosing and its impact on long-term outcomes remain under investigation (4).

Vitamin D, also known as calciferol, comprises a group of fat-soluble seco-sterols. The two major forms are vitamin D_2 and vitamin D_3. Vitamin D_2 (ergocalciferol) is largely human-made and added to foods, whereas vitamin D_3 (cholecalciferol) is synthesized in the skin of humans from 7-dehydrocholesterol and is also consumed in the diet via the intake of animal-based foods. Table 1 summarizes the various sources of this fat-soluble vitamin(5).

Table 1 Sources of vitamin D(6)

Food (naturally present)	Vitamin D_3: oily fish (e.g. salmon, mackerel, tuna, sardines), egg yolk Vitamin D_2: mushrooms
Food (fortified)	e.g. margarine, breakfast cereals, milk (global variation in fortified foods) May contain vitamin D_2 or vitamin D_3
Oral supplements	May contain vitamin D_2 or vitamin D_3
Sun exposure[a]	Photochemical conversion of 7-dehydrocholesterol to previtamin D_3

[a] : Daily sunlight exposure for 5–15 minutes (between 10 am and 3 pm) at latitudes above 37° during spring, summer and autumn is suggested as adequate for individuals with lighter skin 86.

Vitamin D *Synthesis and metabolism*

- **The synthesis of vitamin D3 from the skin and the factors affecting this synthesis**

Formation of vitamin D3, which is the first step of vitamin D synthesis, takes place in the epidermis by a non-enzymatic process (Figure 1). Vitamin D3 is the most important source of vitamin D in the body. 90–95% of vitamin D3 in the human body is produced from the skin with the effect of sunlight. Therefore, sunlight is the main source of vitamin D synthesis, and if there is sufficient exposure to sunlight, there is no need to take additional vitamin D. The mechanism of non-enzymatic photolysis of vitamin D by ultraviolet B (UVB) rays with wavelengths in the range of 290–315 nm involves the breaking of a bond in the B ring of 7-dehydrocholesterol (pro-vitamin D3), resulting in pre-vitamin D3 formation in the epidermis. Subsequently, two different double bonds are formed between the broken carbon atoms in the B ring by thermo-sensitive non-enzymatic process, and the formation of vitamin D3 from pre-vitamin D3 is completed (Figure 1) (7)

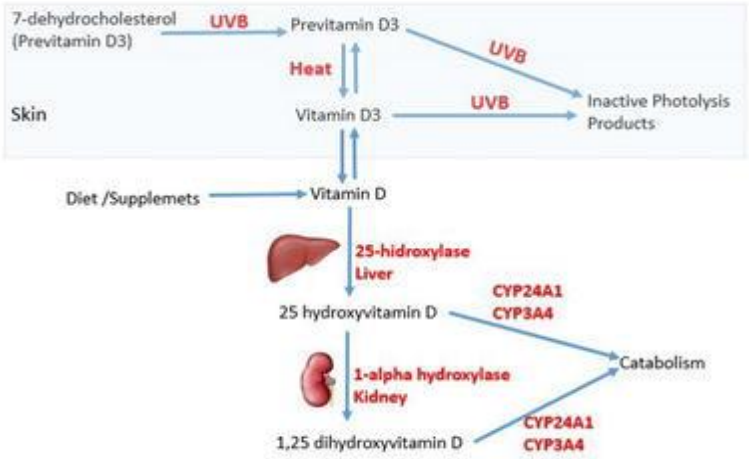

Figure 1. Vitamin D metabolism(8).

The synthesis of vitamin D3 from pro-vitamin D3 in the skin is adjusted according to the needs of the organism. In a period of just fifteen minutes, pre-vitamin D3 is synthesized from pro-vitamin D3 with the effect of ultraviolet light. Conversion from pre-vitamin D3 to vitamin D3 occurs by isomerization in a rather slow and thermo-sensitive manner. In the case of exposure to UV rays or solar radiation for a long period, pre-vitamin D3 converts to a number of photolyzed inactive by-products, such as lumisterol (irreversible) or tachysterol (which can be converted back to pre-vitamin D3). These by-products have no biological effects (Figure 2).(8)

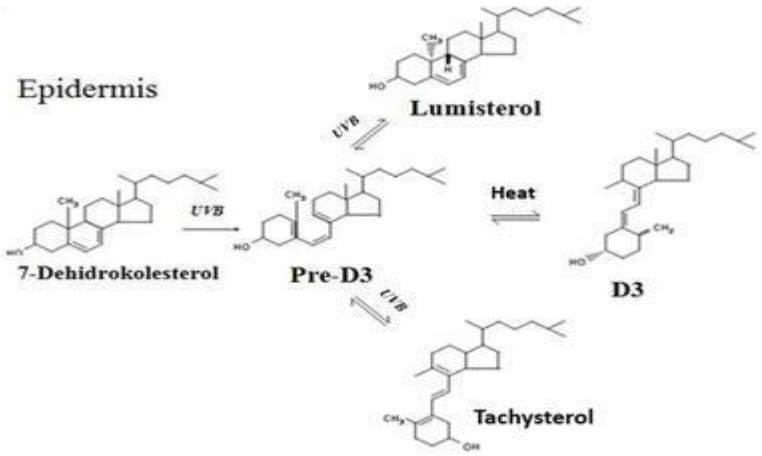

Figure 2. Vitamin D3 synthesis from 7-dehydrocholesterol in the epidermis.(8)

In other words, once pre-vitamin D3 is formed in the skin, it turns into either vitamin D3 or inactive metabolites. This is a physiological control mechanism that protects the body from vitamin D intoxication by preventing unnecessary vitamin D synthesis Some conditions that prevent UVB rays from reaching the skin cause a decrease in vitamin D production. One of these reasons is the ozone (O3) layer surrounding the atmosphere, which reflects some of the sun's rays, preventing them from reaching the Earth and their harmful carcinogenic effects on the skin. The peak UVB wavelength required for optimal vitamin D synthesis from the skin is 297 (290–315) nm (9)

In addition, air pollution, aerosols, water vapors, and increased nitrogens in the air also play a role in preventing sunlight reaching the Earth, and consequently result in a potential reduced synthesis of vitamin D. Melanin is a large, opaque polymer synthesized by melanocytes in the skin through the stimulus of exposure to UVB rays. Melanin competes with dehydrocholesterol 7 in the skin to absorb UVB photons and thus inhibits vitamin D synthesis (10)

- **Biosynthesis of 25OHD3 (25-hydroxylase) in liver**

Vitamin D3 synthesized in the skin is released into the systemic circulation and all forms are transported by binding to VDBP in serum. A portion of vitamin D, a fat-soluble vitamin, is stored in adipose tissue for use when necessary. The ability of vitamin D to be stored in adipose tissue extends its total half-life in the body up to approximately 2 months. When vitamin D3 is transported to the liver, it is first converted into 25OHD3 by the cytochrome P450 25-hydroxylase enzyme. 25OHD3 is the main circulating form of vitamin D, and it is the parameter that provides the best estimation about the body's vitamin D pool (9)

Various enzymes that show 25-hydroxylase properties have been described in the body. Among these, the first one is CYP27A1 located in mitochondria, and the second is microsomally located CYP2R1. CYP27A1 also exerts 27-hydroxylase effect and is involved in bile acid synthesis. Although CYP27A1 is expressed in different tissues of the body, the tissues where it is most commonly found are liver and skeletal muscle tissues (11)

In addition, individuals with a CYP27A1-inactivating mutation develop a cerebrotendinous xanthomatosis disease with bile and cholesterol synthesis disorders, but without rickets manifestation . Besides CYP27A1, different CYP-450 enzymes with 25-hydroxylase activity (CYP2D25, CYP2J2, CYP2J3, and CYP2C11) have been identified in humans and animals, with the most important one in human being CYP2R1. It is assumed that enzymes other than CYP2R1 have effects only on serum 25OHD3 levels (12)

- **Formation of active vitamin D [1,25 (OH) 2D3] by 1-alpha hydroxylase (CYP27B1) in the kidney**

The final step of active vitamin D formation takes place in the proximal tubules of the kidney, led by the enzyme 1-alpha hydroxylase. 25OHD3, which is bound to VDBP, is taken into tubule cells and metabolized (1-alpha hydroxylation) through megalin and cubilin, which are transmembrane proteins located in renal tubules and act as surface receptors for VDBP in tubules. 25OHD3, which then undergoes 1-alpha hydroxylation (13)

The 1-alpha hydroxylase enzyme hydroxylates the first carbon atom in the A ring of 25OHD3, resulting in the formation of 1,25 (OH) 2D3. CYP27B1 is the only enzyme that has 1-alpha hydroxylase activity. This enzyme, which belongs to the cytochrome P-450 enzyme system, is located in the inner mitochondrial membrane and carries out electron transport to NADPH via ferrodoxin-ferrodoxin reductase . The gene for the enzyme consists of nine exons and is located 12q14.1 chromosomal region. Four different groups reported the cloning and sequencing of the gene from rats, mice and humans (14)

In biallelic inactivating mutations of this enzyme, which is highly homologous to some mitochondria located cytochrome P-450 enzymes (CYP27A1 and CYP24A1), 25OHD3 cannot be converted to 1.25 (OH) 2D3, which is the active vitamin D form. In this case, the clinical picture of vitamin D-dependent rickets type 1A (also called pseudo-vitamin D deficiency rickets) occurs (8)

This disease is typically characterized by rickets, with clinically observed very low 1.25 (OH) 2D3, low serum calcium/phosphorus, and high parathyroid hormone (PTH) levels. CYP27B1 is expressed mainly in the renal proximal tubules and in the placenta during pregnancy . While the expression of the gene encoding this enzyme increases with the effect of PTH, it decreases with FGF23 (fibroblast growth factor 23) and $_{1.25}$ (OH) 2D3. CYP27B1 gene is also expressed in lung, brain, breast and intestinal system epithelial cells, immune system cells (macrophage, T/B lymphocytes and dendritic cells), osteoblasts, chondrocytes, and some tumor cell types (9)

The regulation of the extra-renal localized 1-alpha hydroxylase enzyme differs. In some granulomatous diseases where monocyte/macrophage cells play an important role (sarcoidosis, tuberculosis, Chron's disease, etc.), with the effect of IL-1, TNF-α, IFN-γ, 1-alpha hydroxylase enzyme activity increases and 1,25 (OH) 2D3 is synthesized in greater quantities than normal, and consequently, hypercalcemia and hypercalciuria emerge(8)

Additionally, since cells in these tissues do not have PTH receptors, it is not yet understood how PTH exerts its enhancing effect on the 1-alpha hydroxylase enzyme activity in these cells. In one study, it has been suggested that this enhancing effect of PTH may have occurred through post-transcriptional effects . Moreover, 1-alpha hydroxylase enzyme in these cells is not inhibited by 1,25 (OH) 2D3 or hypercalcemia, unlike the renal tubules.(10)

- **Inactivation of vitamin D by 24-hydroxylase (CYP24A1)**

The 24-hydroxylase enzyme is located in the mitochondrial inner membrane of the cells located in the proximal kidney and, like CYP27B1, uses the electron transport system that enables electron transport to NADPH via ferrodoxine-ferrodoxin reductase. It is known that CYP24A1, which is the only enzyme showing 24-hydroxylase enzyme activity in humans, can also exhibit 23-hydroxylase enzyme activity. Which enzyme will be more prominent varies according to the species. The 23-hydroxylase, another enzyme that degrades vitamin D, is the first step activity in the conversion of 1,25 (OH) 2D3 to 1,25 (OH) 2D3-23,26-lactone.(15)

The CYP24A1 enzyme, encoded in 20q13 chromosomal region and having 24-hydroxylase enzyme activity, initiates catabolic processes that lead to the inactivation of vitamin D by hydroxylating the 24th carbon atom. This enzyme can use both 25OHD3 and 1.25 (OH) 2D3 as substrates, but has a higher affinity for 1.25 (OH) 2D3. As a result of a series of enzymatic reactions, calcitroic acid is formed, which becomes biologically inactive. On the other hand, it has been suggested that the 1,25 (OH) 2D3-23,26-lactone, which is formed in the 23-hydroxylase pathway, lowers serum calcium level, inhibits bone resorption induced by 1.25 (OH) 2D3, and stimulates the formation of collagen tissue in bone tissue. In addition, it has been suggested that 24,25 (OH) 2D3 is not only a degradation product, but has an important role in bone metabolism, especially in endochondral bone formation (16)

There are two vitamin D response elements (VDRE) in the promoter region of the CYP24A1 gene . When active vitamin D is bound to the these one of VDRE after heterodimerization with various molecules, thus initiates the inactivation process of vitamin D. In addition, it has been shown that CYP24A1 gene expression decreases with the effect of PTH, whereas it increases with increased FGF23 concentrations. Inactivating mutations in CYP24A1 lead to an idiopathic infantile hypercalcemia clinic characterized by hypercalcemia, hypercalciuria,

nephrocalcinosis, low PTH, low 24.25 (OH) 2D3 and high 1.25 (OH) 2D3 levels. As a result, CYP24A1 is a critical enzyme that protects the body from excessive accumulation and possible intoxication of vitamin D.(17)

- **3-epimerization of Vitamin D**

3-epimerase activity was first demonstrated in 2001, with the detection of the 3-epi form of 1,25 (OH) 2D3 in keratinocytes . In the following years, epimer forms of 25OHD3 and other vitamin D metabolites were discovered. However, the enzyme or enzymes involved in epimerization has not yet been identifiedpurified or cloned. This enzyme changes the hydroxyl group in the 3rd carbon of the A ring from the alpha orientation to the beta orientation, causing the three-dimensional structure to change and consequently alter the activity of CYP27B1 and CYP24A1 enzymes on vitamin D metabolism. These epimers can be detected by special liquid chromatography-mass spectroscopy (LC-MC) measurement methods. C-3 epimer forms of 25OHD3 and 1,25 (OH) 2D3 have been shown to have lower affinity for VDR and VDBP compared to non-epimer forms (18)

The C-3 epimer form of 1,25 (OH) 2D3 has been shown to cause PTH suppression similar to the non-epimer form, but its effects on bone tissue are not clear. In addition, epimer forms have also been shown to have non-calcium effects (anti-proliferative effect, surfactant synthesis). It has been shown that the serum levels of vitamin C-3 epimer forms are found to be 60% higher in the period between the neonatal period and one year old, and decrease after one year of age and decrease to very low levels in adulthood (13)

The reason why epimer forms with limited biological activity are important is that they cause interference and false high results in serum 25OHD3 and 25OHD2 measurement. Therefore, it is important to prefer the method (especially LC–MS / MS) that can exclude this effect of epimer forms that cause serum vitamin D measurement interference. However, the use of LC–MS/MS method in the measurement of vitamin D has not become widespread in the world, and the use of this method is only recommended in selected cases.(19)

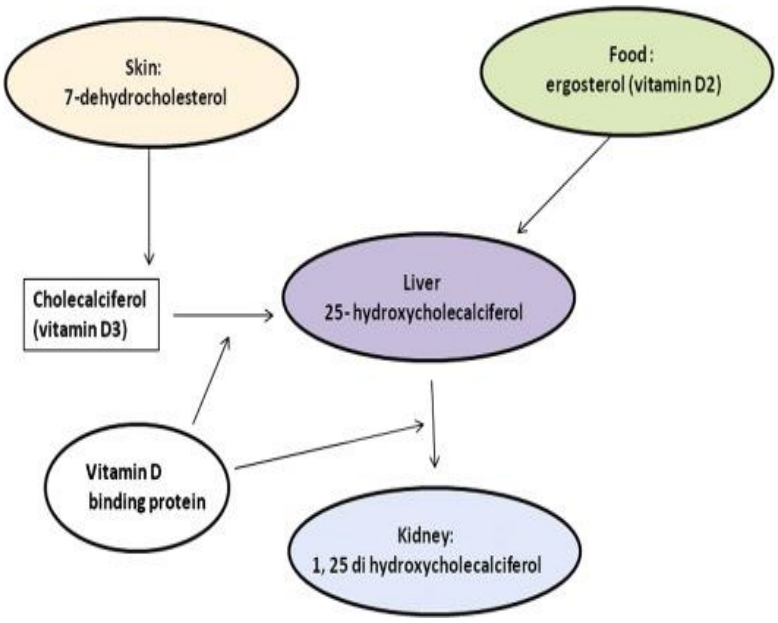

Figure3: Vitamin D metabolism is illustrated (8)

Transport of Vitamin D

The largest part of the circulating vitamin D is in the form of 25OHD3, and its serum concentration is in equilibrium with the level of vitamin D stored in muscle and adipose tissues. The parameter that gives the best information about the whole vitamin D pool in the body is 25OHD3 and its known half-life of 15–20 days. Most of all forms of vitamin D in circulation (85–88%) are transported by binding to VDBP and the remaining part (12–15%) to albumin.(13)

The serum concentration of VDBP is 4–8 nM and only 2% of it is bound with vitamin D metabolites. Moreover, the affinity of VDBP to 25OHD3 is 20 times higher than 1.25 (OH) 2D3. 0.03% of 25OHD3 and 0.4% of 1.25 (OH) 2D3 are in free form . In chronic liver disease or nephrotic syndrome, VDBP and albumin levels and thus total serum 25OHD3 and 1.25 (OH) 2D3 levels decrease, but the levels of free forms are not affected. Likewise, since the VDBP level may decrease during the acute disease period, evaluating the body's vitamin D pool by measuring the serum 25OHD3 level with standard immunoassays may lead to misinterpretations (20)

Biological actions of vitamin D:

- **Classical Vitamin D Actions**

1. **Vitamin D and Bone**

Mechanisms Vitamin D exerts both direct and indirect actions on bone. Vitamin D is a major determinant of mineral homeostasis, promoting intestinal calcium and phosphorus absorption, which are required for optimal mineralization of bone. Vitamin D also exerts direct actions on bone.(21)

The direct actions of vitamin D on bone are more complex to demonstrate, and studies on VDR or *CYP27B1* knockout animal models treated with a rescue high-calcium, high-phosphorus, and high-lactose diet have shown that even though severe bone abnormalities such as rickets (i.e., defective mineralization of the growth plate and adjacent metaphysis in the growing skeleton) and osteomalacia (i.e., the accumulation of unmineralized osteoid at sites other than the growing metaphysis) are prevented , changes in osteoblast number, mineral apposition rate, and bone volume remain (22)

Even though it is well established that acquired or genetic alterations in the vitamin D endocrine system can lead to rickets and osteomalacia and that, vice versa, treatment with an adequate quantity of vitamin D prevents rickets, osteomalacia, and renal osteodystrophy, the role of vitamin D in the skeleton of adults and older adults is often disputed. (23)

2. **Vitamin D, Muscle Strength, Muscle Mass, Muscle Power, and Risk of Falls**

Mechanisms VDD has been associated with musculoskeletal dysfunction, a reduction in muscle strength and size, and increased intramuscular noncontractile tissue(24)

An improvement in lower limb muscle strength could be a promising mechanism through which vitamin D supplementation could reduce the risk of falls, since, on one hand, quadriceps strength is a significant predictor of falls and, on the other hand, VDD has also been linked to an increased risk of falls. Thus, whether vitamin D supplementation confers protection from falls has received a lot of interest, but meta-analyses on this topic have yielded conflicting results(25).

Non-Classical Vitamin D Actions

1. **Vitamin D and Hypertension**

Mechanisms Preclinical studies have shown that VDD may predispose to hypertension through upregulation of the renin–angiotensin–aldosterone system (RAAS) and increased vascular resistance and vasoconstriction. On the other hand, VDR activation has been shown to inhibit intrarenal mRNA levels and protein expression of key components of the RAAS .Evidence shows that vitamin D supplementation is effective in reducing blood pressure in patients with hypertension and VDD (26)

Once again, the modality of vitamin D supplementation impacts the outcome, with daily or weekly administrations of vitamin D improving hypertension outcomes, whereas large bolus vitamin D dosing (e.g., 100,000 IU VD every 2 months) failed to reduce blood pressure in vitamin D deficient subjects (27)

Large doses of vitamin D might also have detrimental vascular effects, since they can result in vascular calcification . On the contrary, vitamin D supplementation in vitamin D replete subjects has null effects on lowering blood pressure. Antihypertensive medications may also affect whether vitamin D supplementation will affect blood pressure. (28)

Low serum 25(OH)D levels have also been associated with an increased risk of developing hypertension, which raises the question of whether vitamin D supplementation can impact the incidence of hypertension, and this is

of great clinical interest. It is important to note that to evaluate the effects of vitamin D supplementation on the incidence of chronic diseases, such as hypertension, the intervention period should be long enough (> 5 years) to record a sufficient number of events (29)

2. Cardiovascular Events

Mechanisms VDR is expressed in endothelial cells, vascular smooth muscle cells, and cardiac myocytes. Vitamin D preserves endothelial function through inhibition of the proliferation of vascular smooth muscle cells, and also reduces oxidative stress, inflammation, and thrombogenesis. It has also been suggested that it can modify lipid metabolism by increasing the activity of lipoprotein lipase in adipose tissue and by reducing fatty acid absorption. (30)

3. Acute Respiratory Tract Infection and Influenza

Mechanisms Vitamin D is involved in the control of both the innate and adaptive immune response. Virtually all immune cells express VDR and CYP27B1, and it has been shown that macrophages, activated T and B cells, dendritic cells, and endothelial cells lining the upper and lower respiratory tracts can hydroxylate 25(OH)D into the active form(31)

Neutrophils express VDR, but it seems that they do not possess CYP27B1. Evidence suggests that 1,25(OH)2D controls the innate immune response through a negative feedback loop on macrophages and other immune cells. More specifically, IFNγ-activated macrophages induce 1,25(OH)2D release, which in turn activates VDR on macrophages, suppressing the expression of key genes producing proinflammatory proteins (31)

Regarding regulation of adaptive immune responses, 1,25(OH)2D has been shown both to inhibit proliferation and differentiation of activated human B cells, to inhibit T helper cells, and also to promote Treg cells ; the net outcome of these effects would be to limit inflammatory processes. In the specific case of influenza virus, it has been shown that incubation of human lung A549 epithelial cells with 1,25(OH)2D before or after exposure to influenza A virus led to decreased production of TNF-α, IFN-β, and IFN-stimulated gene-15, and downregulated interleukin (IL-8 and IL-6 RNA levels (32)

4. Tuberculosis

Vitamin D was used in the pre-antibiotic era for the treatment of patients with tuberculosis (TB), when the ancient Greeks had first introduced “heliotherapy” (i.e., sunlight exposure) to treat TB (33)

5. COVID-19

Considering the previous implications of vitamin D in acute respiratory tract infections, soon after the outbreak of the COVID-19 pandemic the research community started investigating whether vitamin D supplementation may have an impact in preventing infection with Severe acute respiratory syndrome coronavirus (SARS-COV2), or on the severity of COVID-19. This was especially important at the beginning of the pandemic when the medical community had almost no treatments in the fight against COVID-19.(34)

6. Type 2 Diabetes (T2D)

Mechanisms Preclinical studies have shown that vitamin D may modulate β-cell growth and differentiation, enhance insulin secretion , increase the expression of the insulin receptor , and enhance insulin-mediated glucose transport (35)

7. Diabetic Neuropathy and Diabetic Foot Ulcers (DFU)

Mechanisms The role of vitamin D in the function of peripheral nervous system has not been extensively studied . Vitamin D has been shown to induce production of antimicrobial peptides in keratinocyte cells from DFU . Preclinical studies have shown that topical application of vitamin D promotes wound healing in a dose-dependent manner, and activates the expression of angiogenic molecules in keratinocytes and the migration of endothelial and keratinocyte cells in a diabetic foot ulceration model (36)

8. Neuroprotection

Mechanisms VDR and 1α-hydroxylase are expressed throughout the brain, and they are particularly highly expressed in the substantia nigra and in the hippocampus, two important regions for Parkinson’s disease and cognition, respectively. It has been suggested that vitamin D may confer neuroprotection through several

mechanisms, including regulation of neurotrophic factors and of nerve growth, protection against cytotoxicity, and reduced oxidative stress. Vitamin D has also been implicated in the regulation of acetylcholine and clearing of amyloid beta (37).

9. Cancer

Mechanisms Early studies have shown that $1,25(OH)_2D$ analogs have potent antiproliferative and pro-differentiating effects on cancer cells in vitro. Also, vitamin D decreases tumor invasiveness, angiogenesis, and metastatic propensity Systematic reviews and meta-analyses on the levels of vitamin D and mortality outcomes in cancer patients have shown that higher vitamin D levels are protective in a series of cancers such as breast cancer , colorectal cancer , prostate cancer, and hematological malignancies. However, these promising data, based on observational studies, may be biased by a generally better health status and/or a healthier lifestyle (e.g., exercising with greater sunlight exposure) in the subjects who had higher levels of 25(OH)D.(38)

10. Inflammatory Bowel Disease (IBD)

Mechanisms IL-10 knockout mice is an animal model used for the study of IBD; these animals spontaneously develop enterocolitis within 5–8 weeks of birth due to an uncontrolled immune response to resident intestinal flora. People who have an *IL-10* gene polymorphism also have an increased risk of developing colitis (39)

11. Autoimmune Disorders

Mechanisms Activation of the VDR by $1,25(OH)_2D$ has been shown to inhibit the differentiation and proliferation of B and T helper lymphocytes, promoting a shift from an inflammatory to a more tolerant immune status . Also, $1,25(OH)_2D$ inhibits the production of proinflammatory Th1 cytokines while stimulating Th2 and regulatory T-cell activity . Independent of VDR activation, $1,25(OH)_2D$ and other vitamin D hydroxyl-metabolites can bind to RORa and RORg, and result in IL17 inhibition. Both these pathways have been implicated in the protective role of vitamin D from autoimmune disorders. An acquired form of vitamin D resistance has also been hypothesized to play a role in the development of autoimmune disorders (40)

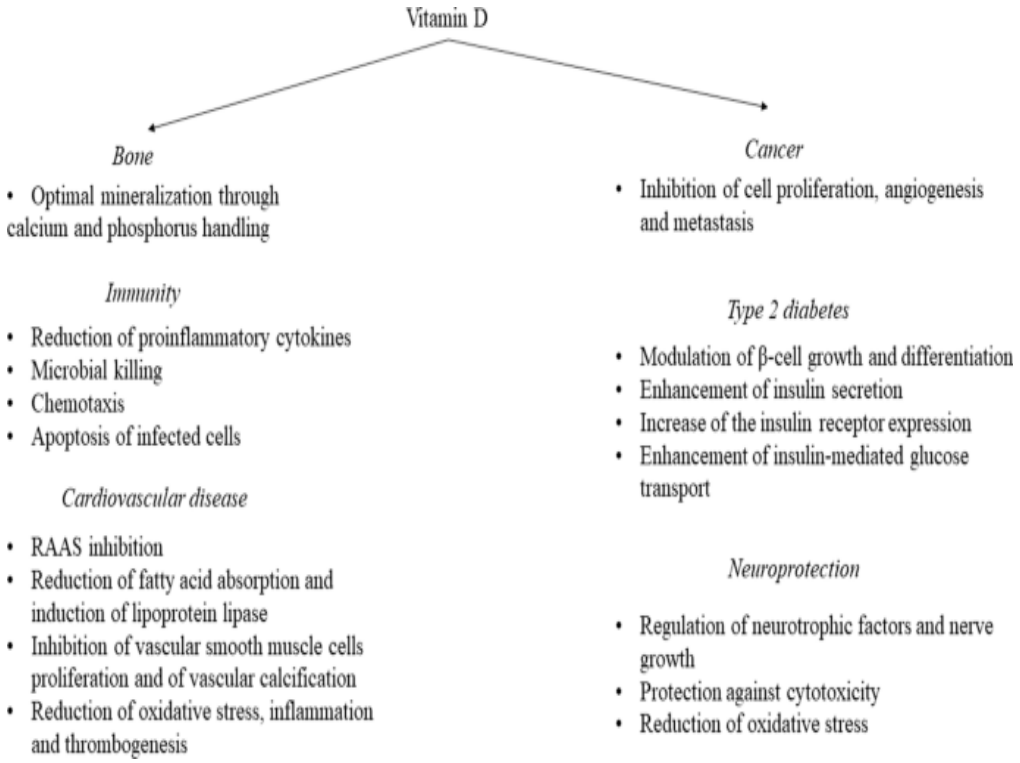

Figure4: Mechanisms through which vitamin D may impact on bone health, immunity, cancer, cardiovascular disease, and neuroprotection(32)

Deficiency of vitamin D

- **Definition**

Vitamin D deficiency is characterized by a lack of the active vitamin D metabolite calcitriol (1,25-dihydroxyvitamin D) in its target cells leading to hypocalcemic rickets in infants and to osteomalacia in adults. The defect in vitamin D action can occur secondarily as a consequence of an inadequate vitamin D supply or primarily as the result of two hereditary forms of rickets that lead to impaired vitamin D action: The two forms are vitamin D-dependent rickets type I (VDDR I) and VDDR II (41).

- **Mechanisms for Deficiency of vitamin D**

The etiology of vitamin D deficiency in liver disease is generally multifactorial, including decreased oral absorption (eg, cholestatic liver disease or portal hypertensive enteropathy) and decreased exposure to ultraviolet light. Patients with severe cholestasis have decreased absorption of vitamin D compared to patients with milder disease. Patients with severe parenchymal disease or obstructive hepatic disease may have reduced synthesis of 25(OH)D. However, the majority of the liver must be dysfunctional before synthesis is reduced.(42)

Other risk factors for vitamin D deficiency include high latitudes, seasonal variation with decreased sun exposure, obesity, medications increasing vitamin D metabolism, and chronic medical conditions, such as chronic kidney disease, leading to decreased synthesis of 1,25(OH)D. Cirrhosis, non-white race, acute decompensation of cirrhosis, and triceps skin fold thickness (a measurement for estimating body fat) are associated with lower vitamin D levels . (42)

Vitamin D deficiency in cirrhosis:

Vitamin D deficiency correlates with the severity of underlying chronic liver disease and is associated with worse outcomes. In patients with cirrhosis and vitamin D deficiency, levels improve with oral vitamin D supplementation and fall without supplementation.(43)

In a study of 75 cirrhotic patients in an outpatient liver clinic, vitamin D deficiency correlated with the Child-Pugh score and Model for End-Stage Liver Disease scores and was associated with hepatic decompensation and mortality.(44)

In a study of 88 hospitalized patients with cirrhosis, a severe deficiency (vitamin D < 10 ng/mL) occurred in 56.8% of patients, with low levels of 25(OH)D being independently associated with bacterial infections, including bacteremia, urinary tract infections, and spontaneous bacterial peritonitis.Vitamin D deficiency occurrs in up to 1 billion people worldwide and approximately 25% to 50% of the adult population in the United States. (45)

Vitamin D is an important secosteroid most widely known for its role in calcium homeostasis and bone mineralization, but has gained recognition for its extraskeletal effects, including the pathophysiology and treatment of chronic diseases, the immune system, and cellular proliferation and differentiation.(46)

Vitamin D deficiency has important implications in chronic liver disease, including associations with the degree of fibrosis and outcomes, such as infections, hepatocellular carcinoma, and mortality.(42)

Serum levels of vitamin D in patients with liver cirrhosis

Vitamin D deficiency in cirrhosis is related to liver dysfunction rather than etiology and it is no longer considered prevalent only in cholestatic disorders . **Malham *et al*** (47) compared vitamin D status between patients with alcoholic cirrhosis (ALC) and PBC. ALC patients hadlower vitamin D levels compared to PBC patients.

A number of studies have supported the prevalence of hypovitaminosis D in chronic liver disease and cirrhosis with one study eporting a low prevalence of 25(OH)D deficiency in a cohort of patients with genotype 1 chronic HCV infection and compensated liver disease (15% cirrhotic patients): 48% and 16% of the cohort had vitamin levels of <75 nmol/L and <50 nmol/L, respectively (Table 2). (46)

Table 2: Serum levels of vitamin D in patients with liver cirrhosis(48)

Author [ref]	Year (publication)	Etiology of liver disease	Study population (n)	Proportion of cirrhotics/ALF patients included	Study in favor of hypovitaminosis	Vitamin D levels
Terrier *et al* [67]	2011	HIV-HCV	189	25% F3-F4 0% F0	Yes	F3/F4: 16.2±10.0 ng/mL; F2: 18.9±8.5 ng/mL; F1: 20.9±11.1 ng/mL
Putz-Bankuti *et al* [68]	2011	Various, mainly alcohol (61%)	75	100% cirrhotic (33 CP-A, 32 CP-B, 10 CP-C)	Yes	Baseline: 16.0±9.2 ng/mL
Bitetto *et al* [69]	2011	HCV	211	Decompensated disease excluded, baseline staging: 2 (0-6 Ishak stage)	Yes	Median: 20.7 ng/mL (2.1-59.6); 46.4% <20 ng/mL; 16.1% <10 ng/mL
El-Maouche *et al* [70]	2013	HIV-HCV	116 African-American	13 cirrhotics	Yes	41% <15 ng/mL
Venu *et al* [71]	2013	Various, mainly alcohol and HCV	63	Liver transplant candidates	Yes	75% <20 ng/mL; 6.3% <10 ng/mL
Stokes *et al* [72]	2014	Various, mainly alcohol (66%)	65	100% cirrhosis, 82% with advanced disease (CP: B,C stage)	Yes	Median: 8.2 ng/mL (4.0–95.8)
Anty *et al* [73]	2014	Various, mainly alcohol (71%)	88	100% cirrhosis, with active infection	Yes	Median: 8.8 (5.3–14.1) ng/mL
Savic *et al* [74]	2014	Alcohol	30	100% cirrhosis	Yes	66.7% <50 ng/mL
Finkelmeier *et al* [75]	2014	Various, mainly alcohol and HCV	200	HCC	Yes	Mean: 17±13 ng/mL (1–72)
Kitson *et al* [77]	2013	HCV genotype 1	274	15% cirrhotics with compensated disease	No	Mean: 79.6 nmol/L; 48% <75 nmol/L; 16% <50 nmol/L

ALF, advanced liver fibrosis; CP, Child-Pugh stage; HCC, hepatocellular carcinoma

Clinical implications of vitamin D in the cirrhotic setting

Several clinical applications of 25(OH)D levels have been suggested, including its use as a non-invasive marker of liver fibrosis in chronic hepatitis C , as a prognostic predictive factor for mortality and infections in patients with liver cirrhosis and as a marker of unfavorable outcome and advanced disease stage in patients with hepatocellular carcinoma

Malham *et al* (47) emphasized the importance of monitoring vitamin D in all cirrhotic populations, especially those with alcoholic liver cirrhosis, and commented on the efficacy of treatment in liver insufficiency-associated bone disease, on the possible extraskeletal benefits (muscle function, cancer risk, and immune impairment) and on the probable benefit of higher than standard doses of vitamin D supplementation for repletion. Garcia-Alvarez *et al* (49) also recommended vitamin D screening in HCV patients.

The possible benefit of vitamin D substitution, as a preventive measure for the development of liver fibrosis in patients with chronic hepatitis C, has also been suggested (Baur et al., 2012) . The same authors comment on expanding the indications for vitamin D supplementation to all patients with chronic liver diseases irrespective of the presence of bone disease. A recent meta-analysis suggested the cutoff of 30 ng/mL as an appropriate threshold to prevent both fibrosis and treatment failure in patients with chronic hepatitis C (49)

The Endocrine Society Clinical Practice Guideline (ESCPG) (50) recommend screening for vitamin D deficiency (cutoff <20 ng/mL) in individuals at high risk for deficiency, including those with hepatic failure, and recommends vitamin D supplementation in cases of deficiency. They also suggest that vitamin D requirements may be greater for sick patients than for healthy individuals and that serum vitamin D levels above 30 ng/mL may have additional benefits in reducing the risk for various disease conditions. They recommend that all adults

who are vitamin D deficient to be treated with 50000 IU of vitamin D2 or vitamin D3 once a week for 8 weeks or its equivalent of 6000 IU of vitamin D2 or vitamin D3 daily to achieve a serum level of 25(OH)D above 30 ng/mL, followed by maintenance therapy of 1500–2000 IU/day. Furthermore, they have set minimal daily dietary recommendations of vitamin D intake for patients at high risk for vitamin D deficiency, depending on their age group. The extent of the recommended screening indications is limited to hepatic failure and does not cover the full spectrum of liver diseases.

International liver study associations (both EASL and AASLD) recommend fat-soluble vitamin substitution for the management of all patients with cholestatic liver diseases. These recommendations focus mostly on the prevention of osteoporosis. More specifically, they propose clinical assessment of the risk of osteoporosis for all cholestatic patients and emphasize the importance of both identifying reversible risk factors and applying appropriate lifestyle changes. It is highlighted that the risk of osteoporosis is increased in decompensated disease and in high degree of cholestasis and it is suggested at least annual screening intervals following diagnosis. Finally, it is recommended that calcium and vitamin D supplementation should be considered in all cholestatic disease patients (48)

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