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An Overview Of Vitamin D And Ll-37 And Their Correlation With Sepsis

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Abstract

Background: Vitamin D is a pro-hormone important for serum calcium and phosphorus homeostasis for proper neuromuscular function and optimal skeletal health. Vitamin D can be obtained from diet or made in the skin after exposure to ultraviolet B radiation from the sun, it is then converted to its major circulating form, 25-hydroxyvitamin D (25(OH)D), by the liver and to its hormonally active form, 1,25-dihydroxyvitamin D (1,25(OH)2D), by the kidney to increase the efficiency of intestinal absorption of calcium as its classic function. Recent studies suggest that Vitamin D exhibits a plethora of effects on the innate and adaptive immune responses, endothelial function and the mucosal barrier. It has been demonstrated that Vitamin D also has a role in the regulation of inflammatory responses against infection. Available evidence suggests that vitamin D deficiency may be a predictor of sepsis or increased mortality rate in critically ill patients.

Keywords: Vitamin D, Sepsis

Background

Sepsis is defined as a “life threatening organ dysfunction caused by a dysregulated host response to infection”. Organ dysfunction is characterized by the acute increase of at least two points in the SOFA score **(1).** If untreated, sepsis may progress to septic shock and lead to further complications such as multiple organ failure (MOF)

Sepsis exists on an escalating level of severity ranging from infection to septic shock, which can lead to multiple organ dysfunction syndrome (MODS) and death. The definitions of sepsis and septic shock have been rapidly evolving since the early 1990s **(2)**.

Recently, the European Society of Intensive Care Medicine (ESICM) and the Society of Critical Care Medicine (SCCM) convened a task force of 19 critical care, infectious disease, surgical and pulmonary specialists with the aim to update the definitions and clinical criteria identifying the “septic patient.” This became known as the sepsis-3 task force **(3).**

The sepsis-3 task force recognized sepsis as more complex than infection and inflammation and defined sepsis as a life-threatening organ dysfunction due to a dysregulated host response to infection. While septic shock was defined as a subset of sepsis in which underlying circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than sepsis alone **(3).**

Epidemiology:

The global epidemiological burden of sepsis is difficult to ascertain. An estimated 48·9 million incident cases of sepsis were recorded worldwide, and 11 million sepsis-related deaths were reported, representing 19·7% of all global deaths. Age-standardized sepsis incidence fell by 37% and mortality decreased by 52·8% from 1990 to 2017 **(4).**

Despite declining age-standardized incidence and mortality, sepsis remains a major cause of health loss worldwide and has an especially high health-related burden in low- and middle-income countries (LMICs) **(4).**

The burden of sepsis is most likely highest in LMICs. Factors increasing the risk for sepsis in LMICs are poverty and overcrowding, inadequate basic healthcare, inadequate hygiene and public health programs, and in some countries, a very high prevalence of HIV infection. The increased risk of sepsis in LMICs is illustrated by the 5 to 16 fold increase in risk for nosocomial infections in studies conducted primarily in those countries when compared with nosocomial infection rates seen in the United States **(5)**

25 hydroxy vitamin D

The classical functions of vitamin D are to regulate calcium-phosphorus homeostasis and control bone metabolism. However, vitamin D deficiency has been reported in several chronic conditions associated with increased inflammation and deregulation of the immune system, such as diabetes, asthma and rheumatoid arthritis. These observations, together with experimental studies, suggest a critical role for vitamin D in the modulation of immune function. This leads to the hypothesis of a disease-specific alteration of vitamin D metabolism and reinforces the role of vitamin D in maintaining a healthy immune system **(6).**

Epidemiology of 25(OH) D deficiency:

Vitamin D deficiency is a global public health issue; about 1 billion people worldwide have vitamin D deficiency, while 50% of the population has vitamin D insufficiency. The prevalence of patients with vitamin D deficiency is highest in the elderly, obese patients, nursing home residents and hospitalized patients. The prevalence of vitamin D deficiency was 35% higher in obese subjects irrespective of latitude and age **(7).**

Chemical Structure of 25(OH) D:

The two major forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_3 is synthesized in the skin of humans and is consumed in the diet via the intake of animal-based foods, mainly fish oils, whereas vitamin D_2 is derived from plant sources and added to foods. Vitamins D_2 and D_3 forms differ only in their side chain structure. This structural difference does not affect metabolism (i.e., activation) and both forms have the prohormone function **(8).**

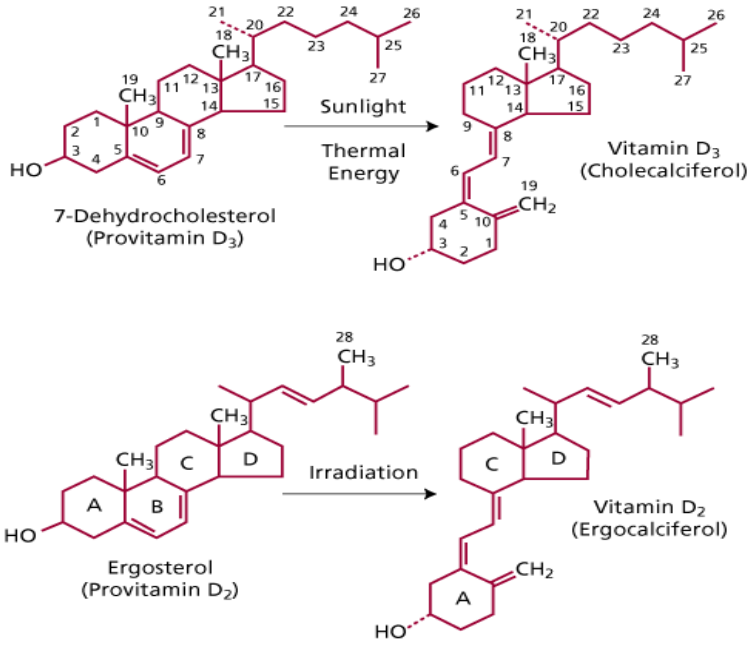

Fig. (1): Chemical structure of vitamin D (9)

Physiological function of vitamin D:

A) Calcium homeostasis:

Vitamin D is a principal factor involved in the control of mineral balance. Vitamin D deficiency during bone development causes growth retardation and rickets, while in adults, vitamin D deficiency can cause secondary hyperparathyroidism resulting in osteoporosis and/or osteomalacia and increased risk of fracture **(10,11).**

Plasma calcium concentrations are maintained at a very constant level, and this level is supersaturating with respect to bone mineral density. If the plasma becomes less than saturated with respect to calcium and phosphate, then mineralization fails, which results in rickets among children and osteomalacia among adults **(12).**

B) immune-modulatory functions:

1,25(OH) 2D (calcitriol) has important immunomodulatory actions, namely, the enhancement of the innate immune system and inhibition of the adaptive immune responses, associated with an increased synthesis of IL-4 by T helper (Th2) lymphocytes and the upregulation of regulatory T lymphocytes (Treg) **(8).**

1) Innate immunity:

Vitamin D increases the defense capacity of macrophages inducing their differentiation, phagocytic capacity and antimicrobial activity via increasing the expression of cathelicidins**(8).**

A previous study investigating the underlying mechanisms in humans identified the CYP27B1 (cytochrome P 27B1) or 1α-hydroxylase and VDR genes as two genes that are uniquely upregulated in monocytes/macrophages in response to TLR activation by Mycobacterium tuberculosis. In the presence of sufficient 25(OH) D, this expression leads to a 1, 25(OH) 2D dependent induction of the antimicrobial peptide cathelicidin and enhanced killing of intracellular M. tuberculosis. In the same study, sera from African American subjects with low 25(OH) D concentrations were inefficient in supporting cathelicidin mRNA induction **(13)**.

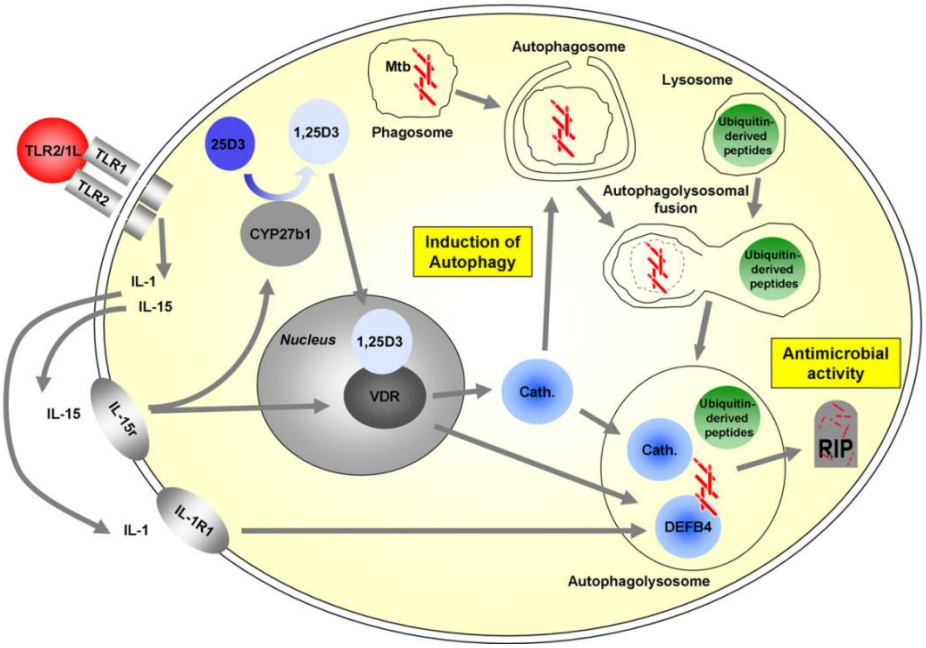

Fig. (2): Vitamin D and the Immune Response to Mycobacterium Tuberculosis (14).

Activation of the TLR 2/1 heterodimer by M. tuberculosis upregulates the expression of CYP27B1 and VDR genes in monocytes and macrophages. In the presence of sufficient 25 (OH) D, this upregulation leads to a 1,25 (OH) 2D–dependent induction of the antimicrobial peptide cathelicidin and enhanced killing of intracellular M. tuberculosis. In a granuloma, surrounding (Th1) cells produce INF-γ which further enhances CYP27B1 expression. Excess 1,25 (OH) 2D inhibits the differentiation of undifferentiated T-helper cells (Th0) to Th1 cells in a paracrine fashion, providing a mechanism to prevent excessive 1,25 (OH) 2D production **(14).**

2) Adaptive immunity:

Besides promoting innate immunity, another major effect of 1, 25(OH) 2D on the immune system is the suppression of the adaptive immune system and generation of tolerance and anergy. At the cellular level, dendritic cells exhibit reduced expression of major histocompatibility complex (MHC) class II molecules and adhesion molecules necessary for full T-cell stimulation in the presence of 1, 25(OH) 2D. In contrast to its stimulatory effects on monocyte-macrophages, 1, 25(OH) 2D3 acts as an immunosuppressive agent in lymphocytes. The treatment of systemic lupus erythematosus (SLE) patients with 1,25 (OH) 2D has been shown to activate and increase the number of T regulatory cells **(15)**, suppress IL- 17 and decrease production of IFN-α **(16).**

C) Control of differentiation and function in the skin:

Previous observational studies have related lower vitamin D status to increased markers of atopic diseases such aseczema, anaphylaxis, food allergy and chronic urticaria**.** The efficacy of thesterol in treating psoriasis, melanoma, and scleroderma could be mediated by theimmunosuppressive properties of vitamin D on Langerhans cells, theantigen-presenting cells of the epidermis **(17).**

D) Control of the nervous system:

Expression of functional VDRs within both neurons and glia of the adult hippocampus provide further evidence for vitamin D's importance in the adult central nervous system. In the human brain, both VDR and 1α-hydroxylase, the enzymes required for 1, 25 (OH) 2D production, have been observed to be in high levels in the substantia nigra, suggesting a potential link between this vitamin and the dopamine neuron population linked with Parkinson's disease **(18, 8).**

E) Anticancer effect:

Calcitriol and VDR have been shown to control the expression of genes associated with cellular proliferation and differentiation, suggesting a key role in cancer prevention. There is some evidence that vitamin D levels provide a protective status to lower the risk of cancer. Some analyses on publications of colon, breast, prostate, and ovarian cancer revealed that in numerous cases, vitamin D3 levels correlated with reduced incidence of cancer. Conversely, other studies suggest no or only weak evidence for a link between vitamin D levels and cancer protection, and there are examples where high vitamin D levels may actually increase risk of pancreatic cancer **(8).**

Preclinical studies show that calcitriol and its analogs have antitumor effects in vitro and in vivo through multiple mechanisms including the induction of cell cycle arrest, apoptosis, differentiation and the suppression of inflammation, angiogenesis, invasion and metastasis **(19)**.

F) Control of the renin-angiotensin system:

The renin-angiotensin system plays a central role in the regulation of blood pressure, electrolytes and volume homeostasis. As 1,25 (OH) 2D3 acts as a negative endocrine regulator of the renin-angiotensin system, several epidemiological and clinical studies have suggested an association between inadequate sunlight exposure or low serum 1,25(OH) 2D3 with high blood pressure and/or high plasma renin activity **(20)**.

G) Control of insulin secretion:

The association between vitamin D and diabetes mellitus (DM) is explained by the discovery of VDR and 1α-hydroxylase enzyme inside beta cells and the presence of calcium-linking protein, DBP, in the pancreatic tissue **(21).**

Clinical significance of Vitamin D in diseases:

1) Vitamin D in infection and sepsis:

One of the most promising extra-skeletal roles of vitamin D for patients with sepsis is its role in the immune system. This was initially indicated by the discovery of VDRs in nearly all types of immune cells **(22)** spanning the body's innate and adaptive immune responses to pathogens. Further studies have revealed that vitamin D modulates immune responses to the pro-inflammatory bacterial endotoxin LPS in vitro and in rodent models of sepsis **(23).**

In addition to affecting the humoral response to sepsis, vitamin D acts in the local tissue response to infection **(24)** and is integral to the production of AMPs **(25).**

2) Vitamin D in autoimmune disorders

Vitamin D regulates the differentiation and activity of $CD4^+$ T cells, resulting in a more balanced Th1/Th2 response that limits development of self-reactive T cells, thereby preventing inflammation and autoimmunity **(26).** Therefore, a role for vitamin D deficiency in the pathogenesis of autoimmune diseases has been proposed.

3) Vitamin D and cardiovascular diseases

Experimental studies have established that calcitriol and VDR are critical regulators of the structure and function of the heart. In addition, clinical studies have associated vitamin D deficiency with chronic vascular disease. Emerging evidence demonstrates that calcitriol is highly involved in chronic vascular disease related signaling pathways **(27).**

4) Vitamin D and diabetes

25 hydroxy vitamin D deficiency has been shown to be related to the development of diabetes **(28).** Available studies have shown that 25 (OH) D levels are negatively correlated to prevalence of diabetes

mellitus type 2 (T2DM), islet beta cell function, insulin resistance, body fat and body mass index levels (BMI) **(28).**

Conversely, 25(OH) D levels were positively correlated with insulin sensitivity. 25 (OH) D deficient individuals have a higher insulin resistance and type 2 diabetes risk **(29).**

5) Vitamin D and neuropsychiatric disorders

There are a variety of similar studies relating 25 (OH) D deficiency with increased risk for depression, Alzheimer disease, epilepsy and neurocognitive decline **(30).** 1, 25(OH) 2D3 could also act by increasing serotonin levels in the brain. Furthermore 1, 25(OH) 2D3 has also been demonstrated to stimulate amyloid-β phagocytosis and clearance by macrophages in Alzheimer patients **(31).**

This may help explain the association between neurocognitive decline, dementia, depression and Alzheimer diseases and a high prevalence of 25 (OH) D deficiency **(31).**

6) Vitamin D and bronchial asthma

Vitamin D, by binding and activating VDR, has been found to alleviate inflammation associated with allergic asthma. In airway smooth muscle cells, vitamin D reduced proliferation and production of pro-inflammatory cytokines, matrix metalloproteinases (MMP) and mucus secretion, which decreased airway hyper-responsiveness, inflammation, and remodeling in asthma **(32).**

7) Vitamin D and viral infection (Influenza virus and COVID-19)

Several reviews consider the mechanisms in which vitamin D reduces the risk of viral infections **(33).** The role of vitamin D in reducing the risk of viral infection grouped those mechanisms into three categories: physical barrier, cellular natural immunity and adaptive immunity **(34)**. Several articles discussed how viruses disturb cellular junction integrity of the respiratory tract, increasing infection by the virus and other microorganisms.Vitamin D enhances cellular innate immunity partly through the induction of AMPS, including human cathelicidin, LL-37 **(35).** A previous study demonstrated that LL-37 reduced influenza A virus replication **(36).**

In another laboratory study, 1,25 (OH) 2D reduced the replication of rotavirus both in vitro and in vivo**(37).** A clinical trial reported that supplementation with 4000 IU/day of vitamin D decreased dengue virus infection **(38)**

Vitamin D also enhances cellular immunity, in part by reducing the cytokine storm induced by the innate immune system. The innate immune system generates both pro-inflammatory and anti-inflammatory cytokines in response to viral and bacterial infections, as observed in COVID-19 patients **(39)** Vitamin D can reduce the production of pro-inflammatory Th1 cytokines, such as TNF and IFN **(40).**

Administering vitamin D reduces the expression of pro-inflammatory cytokines and increases the expression of anti-inflammatory cytokines by macrophages**.** Vitamin D supplementation also enhances the expression of genes related to antioxidation (glutathione reductase and glutamate–cysteine ligase modifier subunit. The increased glutathione production spares the use of ascorbic acid (vitamin C), which has antimicrobial activities. and has been proposed to prevent and treat COVID-19 **(41).**

Serum 25(OH) D concentrations tend to decrease with age, which may be important in COVID-19 because case-fatality rates (CFRs) increase with age. A recent review suggested using vitamin D loading doses of 200,000–300,000 IU in 50,000-IU capsules to reduce the risk and severity of COVID-19 **(41).**

A) Plasma/serum concentration of 25(OH) D:

25 hydroxy vitamin D is the principal criterion by which vitamin D sufficiency is determined **(11)** Plasma or serum concentration of 25(OH) D represents total vitamin D from both exposure to UV-irradiation (cutaneous synthesis) and dietary sources. It can also be used as a biomarker of vitamin D intake in people with low exposure to UV-B irradiation from sunlight. Serum 25(OH) D has a long half-life of approximately 13–15 days and is considered a useful marker of vitamin D status (both D2 and D3) **(42)**

It is evident that circulating levels of 25(OH) D of at least 80 nmol (32 µg/L) are required to optimize bone mineral density**.** However, the reference range for 25 (OH) D in most laboratories is 25 - 80 ng/mL. Though to date there is no globally accepted definition regarding vitamin D deficiency, but in many countries, it is assumed that a serum level of vitamin D <20 ng/mL denotes vitamin D deficiency, 20–30 ng/mL denotes insufficiency and >30 ng/mL is regarded as normal **(12).**

B) Free serum 25(OH) D concentration:

The fraction of serum 25(OH) D that circulates without being bound to DBP and albumin is free serum 25(OH) D. This free form accounts for less than 1% of total 25(OH)D in the body but has been hypothesized to be a potential marker of vitamin D status, because this free fraction is readily available to target cells **(43).**

C) Plasma/serum 1, 25(OH) 2D concentration:

The biologically active 1, 25(OH) 2D has a half-life measured in hours (approximately 4 hours) and is closely linked with blood calcium, parathyroid hormone (PTH) and phosphate concentrations. Hence its very short half-life, it cannot be used to assess vitamin D status **(43).**

D) Serum parathyroid hormone (PTH) concentration:

Serum PTH concentration and its relationship with 25(OH) D concentration has been suggested as a possible biomarker or functional endpoint of vitamin D status**.** The principal function of the biologically active metabolite 1, 25(OH) 2D is to maintain calcium and phosphorus homeostasis in the circulation, together with PTH and fibroblast growth factor (FGF) **(42).**

Assay of 25(OH) D:

Serum 25 (OH) D represents the best biomarker of vitamin D status, but there is much debate surrounding the performance of some of the assay methods. Programs such as the Vitamin D Standardization Program and Vitamin D External Quality Assessment Scheme (DEQAS) in the United Kingdom allowed the assessment of the accuracy and reproducibility of the available methods **(42).**

There are multiple methods for the measurement of 25(OH) D in serum including high-performance liquid chromatography with UV detection (HPLC/UV), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and immunoassays (radio-immunoassays [RIA], competitive protein binding assays [CPBA], enzyme-linked immunosorbent assays [ELISA]) that are either manual or automated. LC-MS/MS and HPLC methods are considered the gold standard methods, since these methods have the advantage of measuring 25(OH) D3 and 25(OH) D2 separately, which is needed in specific situations **(44).**

Serum 25 (OH) D assays fall into two main categories: (44).

1) Those based on a separation step of chromatography, the most popular of which is liquid chromatography–tandem mass spectrometry (LC-MS/MS).
2) Non-chromatographic methods based on antibody or protein binding, such as radioimmunoassays and chemilumenescent technique.

Vitamin D and the Sepsis Cascade

Monocytes play important roles in the innate immune system as antigen presenting cells as well as in phagocytosis. Human monocytes recognize some PAMPs by a family of transmembrane molecules, the Toll-Like Receptors (TLRs). TLR4 specifically recognizes and binds to lipopolysaccharide (LPS), a substance produced by gram-negative bacteria and a potent stimulator of the sepsis inflammatory cascade. **Sadeghi et al. (23)** demonstrated that human monocytes stimulated with LPS and treated with 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), showed dose-dependent decreases in TLR2 and TLR4 synthesis, with an increase in CD14, a TLR co-stimulatory molecule. They further found that $1,25(OH)_2D$ decreased TNFα and tissue factor, both end products of LPS activation and important inflammatory molecules in sepsis. These

effects were reversed with the introduction of a VDR antagonist, reinforcing a key role of vitamin D in this signaling mechanism. **(23)**

Further studies have revealed a role for vitamin D in the endothelial response to LPS. In sepsis, LPS activates endothelial cells to produce transcription factor NFκB, the pro-inflammatory cytokines IL-6 and IL-8, and the chemokine, RANTES. In a study by Equils et al., human endothelial cells treated with $1,25(OH)_2D$ then stimulated with LPS, showed significant inhibition of these molecules when compared with cells only exposed to LPS. These findings may suggest that vitamin D acts to modulate the pro-inflammatory endothelial response to LPS **(45).**

Over the past two decades, these intriguing vitamin D-dependent cellular responses to LPS have also been studied in rat and mouse models of sepsis. Horiuchi et al. exposed mice simultaneously to intraperitoneal LPS and oral $1,25(OH)_2D$. Compared with controls, mice that received vitamin D had less expression of the inflammatory molecule, $iTXB_2$, and a decrease in mortality. In 2001, Asakura et al. demonstrated that compared with low-molecular weight heparin, treatment with oral $1,25(OH)_2D$ had equal or improved effects on hemostatic parameters and markers of organ dysfunction in rats infused with LPS. In 2007, Moller et al. performed placebo-controlled trials of treatment with $1,25(OH)_2D$ in three different rat models of sepsis showing varied results. While the different models of sepsis and vitamin D treatments in these experiments make them difficult to compare, when combined with the in vitro data they suggest that vitamin D has important modulatory effects on the innate immune response to LPS-induced sepsis.

While LPS is an important molecule in gram-negative sepsis, vitamin D may also have a role in the sepsis cascade induced by fungal organisms. A study by **Khoo et al (46).** treated peripheral blood mononuclear cells (PBMC) with $1,25(OH)_2D$ and exposed them to C. albicans. The PBMCs demonstrated significant dose-dependent decreases in production of pro-inflammatory cytokines with a decrease in expression of the PRRs that recognize C. albicans**. (46)**

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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