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Review Article

Deciphering Controversies About the Effect of Vitamin D in Hepatitis B

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Abstract

Chronic infection with hepatitis B virus has a major public health impact. Current antiviral therapy in chronic hepatitis B keeps the infection under control, but does not cure the disease; therefore, different areas of research aiming to reduce the risk of progressive disease are open. Despite numerous experimental data showing a benefit from vitamin D supplementation, clinical studies do not consistently support this finding. This review covers the possible explanations for these controversies, highlighting the complexity of vitamin D metabolism and of hepatitis B virus biology that should be considered for proper comparison of the results of the clinical studies.

Introduction

The global prevalence of hepatitis B virus (HBV) infection is estimated to almost 3 million cases [1] and the lack of access to suitable treatment or to prevention programs in certain areas contributes to the maintenance of these high figures. The consequences of this infection are enormous, as the cumulative 5-year incidence of cirrhosis is 8-17% [2] and the 5-year risk of developing hepatocellular carcinoma is estimated to be between 10-15% [2]. Even in the absence of significant cirrhosis, HBV is capable of leading to hepatocellular carcinoma at any time during disease evolution. Current antiviral therapy in chronic hepatitis B keeps the infection under control, but does not cure the disease, with rare exceptions; therefore, different areas of research aiming to reduce the risk of progressive disease are open. One of these directions explores the positive reported effects of vitamin D in the immune response against HBV infection [3,4]. The bone metabolism is frequently modified in hepatitis B. The hepatic osteodystrophy is found in 20% of patients with chronic hepatitis and reaches up to 50% in viral-induced cirrhosis [5]. The excessive chronic production of inflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-alpha) increases the expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates osteoclastogenesis along with bone resorption. Low vitamin K, low level of insulin growth factor 1, hypogonadism, malabsorption and low body mass index are contributing factors. The significance of the vitamin D insufficiency should not be ignored but the relation between vitamin D and liver pathology is far more complex, exceeding the domain of the bone metabolism.

Role of Vitamin D in Hepatitis B Virus Infection

Experimental data show that vitamin D is active in the inflammatory process, in the innate and in the adaptative response. In the hepatic stellate cells, vitamin D has an inhibitory effect on transforming growth factor (TGF) β [6] and reduces the matrix deposition [7], subsequently delaying the fibrosis evolution. The anti-proliferative effects of vitamin D rely on the prevention of the initiation process: the nuclear binding of vitamin D receptors (VDR)-vitamin D increases the expression of tumor suppressor genes, promoting the DNA repair process [8] stabilizing the cells and preventing oncogenesis.

A recent systematic review and meta-analysis showed that low vitamin D levels were inversely correlated to viral load [9]. Before further considerations, it is important to underline that current practice of vitamin D status evaluation is plasma or serum measurement of the total 25-hydroxyvitamin D2/D3 (25(OH)D) by immunoassay tests or by liquid chromatography tandem mass spectrometry (LC/MS). The arguments for considering 25(OH)D as representative for the vitamin D status are the short plasma half time (limited to a couple of hours) of the active metabolite, 1,25-dihydroxyvitamin D2/D3 (1,25(OH)2D), and the response of the parathormone (PTH) axis that rapidly increases the 1,25(OH)2D after a deficiency level is attained [10].

Several prospective studies confirmed that serum levels of vitamin D are inversely associated with the incidence [11] and prognosis of HBV-related hepatocellular carcinoma [12], but a Cochrane review did not find a benefit from vitamin D supplementation [13]. Some promising results concerning the vitamin D immunomodulation of hepatitis B evolution were found in a large multicentric study [14], but were not confirmed in another [15]. Other studies suggest that polymorphisms of the genes involved in vitamin D metabolism might even predict the efficacy of the current treatment for HBV infection [16,17]. Hence, although experimental data are rather consistent on vitamin D role, clinical data on supplementation are not, at least not to the same extent.

Possible Explanations for the Controversy of the Clinical Results

This inconsistency might be explained by various factors: the virus genotype, the variations in vitamin D metabolism, and the biases related to technique of the vitamin D determination.

a) In what concerns the **virus genotype,** the genotype D and E of the HBV were associated with lower vitamin D levels [15]. The major hydrophilic region of the HBsAg contains sequences of amino acids that are targets for the neutralizing antibodies and mutations in this area, called "escape mutations", and have critical role in the infection reactivation and in chronic evolution of the hepatitis [18]. It is significant to notice that mutations of these epitopes were found in vitamin D deficient or insufficient patients infected with HBV [19] and they might explain, on one side, the severity of the evolution, and on the other side, the lack of benefit from standard treatment, as these mutations are frequently associated with drug resistance [20]. As there is no proof of immune-escape mutations compensation by vitamin D supplementation, there are no clear benefits of the adjuvant therapy with respect to immunologic response or viral load.

b) **The vitamin D metabolism.** Hepatic cells convert both vitamin D2 and D3 in 25-hydroxyvitamin D2/D3 (25(OH)D). The process is catalyzed mainly by CYP2R1 in the endoplasmic reticulum and, in less significant quantity, in the mitochondria by CYP27A1. Variation in CYP2R1 polymorphisms influences the level of the circulating vitamin D [21]. Some polymorphisms also influence the results of vitamin D supplementation [22], but others do not [21]. In the liver, 25(OH)D3 can be further metabolized to 23,25$(OH)_2$D3 or to 25,26(OH)D3 by CYP3A4 [23] or sent into the circulation. Under physiological conditions, about 85% of the 25(OH)D in plasma is bound to vitamin D binding protein (DBP), about 15% is bound to albumin and only 0.03% is free (bioavailable form) [24]. The expression of the DBP, including the response to supplementation with vitamin D and the affinity of vitamin D binding seem to be genetically determined [24]. These variations of DBP have consequences on the vitamin D distribution and on the active form availability, affecting the clinical significance of the total vitamin D level currently measurement. They also influence the efficacy of the vitamin D treatment, in general. In hepatitis, this influence is even more significant, as the most important source of DPB is the liver. The diminished synthesis of the DBP and the reduced conversion of D2 and D3 in D2/D3 25(OH)D, which can occur in chronic hepatitis or hepatic cirrhosis, would lead to lower levels of total 25(OH)D, but will not necessarily reduce the level of the active form 1,25 $(OH)_2$D3 (calcitriol), produced by the kidney and regulated by specific mechanisms (described bellow). It is, however, reasonable to presume that DBP synthesis is not equally affected in all patients, and therefore, the interpretation of 25(OH)D values should take into account the DBP level. According to the free hormone theory, stipulating that the biological functions of the vitamin D (as any other hormone) are ensured by the free level of 25(OH)D and 1,25$(OH)_2$D, a lower level of DBP should be considered biologically beneficial. In fact, a multicentric study published in 2018 found higher levels of free but also of total 25(OH)D in patients with cirrhosis than in healthy subjects, medically-stable outpatients, prediabetes patients or pregnant women [25]. In a graphical representation, the relationship between free and total 25(OH)D has the steepest slope among the studied groups, suggesting higher bioavailability of Vitamin D in patients with cirrhosis. As the biologically active 1,25$(OH)_2$D is generally not measured in clinical studies, the clinical significance of the results based on the total 25(OH)D determination could be biased.

The kidney is the main source of the major vitamin D active form, by converting 25(OH)D3 to 1,25$(OH)_2$D3. The reaction is mediated in mitochondria by 1α-hydroxylase, the product of CYP27B1, highly regulated by PTH and the fibroblast growth factor 23 (FGF-23). Genetic variations of CYP27B1 could create an apparently normal level of serum vitamin D, with an abnormal, low level of the active form and could also lead to misinterpretation of the results [26].

Expression of CYP27B1 is not limited to the renal cells. A significant circulating level of $1,25(OH)_2D3$ through non-renal cells output reflects a pathophysiological status, being reached only from activated tissue macrophages and placenta [27]. Inflammatory cytokines upregulate CYP27B1 expression in macrophages [28] and other immune cells in an autocrine or paracrine manner with apparently neither PTH nor FGF-23 feedback. Due to this lack of a systemic control, at least in theory, this mechanism of conversion to calcitriol is unlimited in inflammation [29], a reason why 25(OH)D might not be reliable enough for the estimation of the treatment results.

Vitamin D receptors are present in a variety of tissues. Evolutionarily, the VDR are closely related to receptors involved in xenobiotic detoxification and elimination, such as the pregnane X receptor, or the farnesoid X receptor [28]. The VDR recognizes a specific DNA sequence, the vitamin D response element (VDRE). The VDRE is composed by 2 hexameric nucleotide half-sites separated by 3 base pairs that are recognized by the heterodimer formed by one molecule of VDR and one of retinoid X receptor (RXR). It is estimated that as much as 5% of the human genome is regulated by calcitriol, via VDR-RXR link to the VDRE or by the ability of the VDR to facilitate the recruitment, in a gene-specific manner, of large and diverse co-regulatory complexes. Epigenetic modifications enlarge the influence of the vitamin D on the gene expression [30]. VDR is not present in the hepatocytes from normal human liver but is strongly expressed in hepatic stellate cells, sinusoidal endothelial cells and Kupffer cells [31]. In inflammation, the VDR expression increases in hepatic stellate cells and Kupffer cells, but also in hepatocytes [32]. The literature regarding the impact of the genetic variation of VDR in hepatic disorders is rather consistent, showing an increase in the susceptibility of progression to liver cirrhosis and portal hypertension for some polymorphisms [33], or to hepatic carcinoma associated to chronic hepatitis B virus infection [34,35]. Other polymorphisms led to some protective effects [36].

c) **The vitamin D determination in plasma.** As previously mentioned, in order to assess the current practice is to determine the total 25(OH)D concentration in plasma by immunoassay or LC/techniques. The 25(OH) D3 is the major circulating form of vitamin D3, but numerous other metabolites of vitamin D3 were measured in serum [37]. The most significant in terms of quantity are 3-epi-25(OH)D, the $24R,25(OH)_2D3$, the $1,25(OH)_2D3$ and $1,25(OH)_2D2$, the 25-hydroxyvitamin D3-3-sulfate and the D-hydroxyderivatives (20S(OH)D3 and 22(OH)D3 and their metabolites). The active forms of vitamin D are $1,25(OH)_2D3$ and 1,25(OH)2D2, which bind to the VDR and produce both the calcemic and the non-calcemic effects. The functional role of the other compounds is not enough characterized and its clinical significance is not yet well understood. An exception are the D-hydroxyderivatives; they bind to the VDR, without inducing the calcemic effects; instead, they are almost as potent as $1,25(OH)_2D3$ in the extra-osseous effects, including the antifibrogenic one [38].

The total level of 25(OH)D is also the only therapeutic target of the vitamin D substitution, although there are reports showing that free 25(OH)D would better reflect the achievement of the normal vitamin D status [39,40]. The liver produces both DBP and albumin, the carriers of vitamin D in the blood stream; depending on the severity of the liver disease, both proteins might be significantly reduced. The free form of vitamin D is less dependent on the hepatic function and therefore the measurement of the total vitamin D is not a good reflection of the real status of this hormone in hepatic disorders. Even more, results could be biased by an increased percentage of free serum 25(OH)D in patients with vitamin D deficiency [41]. Due to difficulties in the measurement of the free 25(OH)D level, the latter is rarely communicated in clinical studies. However, a recent extensive review of the published studies related to free vitamin D measurement strongly recommends the measurement of the free 25(OH)D in liver diseases [42].

Conclusions

There are no large studies addressing all these issues in a systematic manner and therefore the results are difficult to compare. Without considering the complex biology of the vitamin D metabolism and the biology of the HBV it is impossible to obtain reliable results and to conclude on the vitamin D supplementation in chronic hepatitis.

Due to this diversity, it is not yet well established what specific profiles of patients are suitable for treatment, and a patient-centered approach still waits to be defined.

Conflicts of Interests

Nothing to declare.

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