Research Article

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Association of baseline vitamin D levels with clinical parameters and treatment outcomes in chronic hepatitis B

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Background & Aims: The relationship between vitamin D levels and chronic hepatitis B (CHB) infection and treatment outcomes are poorly elucidated. We measured pre-treatment serum vitamin D (25-hydroxyvitamin D_3; 25[OH]D_3) levels and determined their association with clinical parameters and treatment outcomes in active CHB patients without advanced liver disease enrolled in a global clinical trial.

Methods: Patients were randomly assigned to either 48 weeks of tenofovir disoproxil fumarate (TDF) plus peginterferon alfa-2a (PegIFN), TDF plus PegIFN for 16 weeks followed by TDF for 32 weeks, PegIFN for 48 weeks, or TDF for 120 weeks. Univariate and multivariate analyses were conducted to determine associations between vitamin D, baseline factors, and week 48 clinical outcome.

Results: Of 737 patients, 35% had insufficient ($\geqslant$20 but <31 ng/ml) and 58% had deficient (<20 ng/ml) vitamin D levels. In univariate analysis, lower vitamin D levels were significantly associated with the following baseline parameters: younger age, lower uric acid levels, HBeAg-positive status, lower calcium levels, blood draw in winter or autumn, and HBV genotype D. On multivariate analysis, only HBV genotype, season of blood draw, calcium level, and age retained their association. High baseline level of vitamin D was associated with low HBV DNA, normal ALT and HBsAg at week 48 independent of treatment groups, but the association, with the exception of ALT, became statistically insignificant after adjusting for age, gender, HBeAg and HBV genotype.

Conclusions: Abnormally low vitamin D levels are highly prevalent among untreated, active CHB patients. Baseline vitamin D levels are not associated with treatment outcomes, but were associated with normal ALT.

Keywords: Hepatitis B; Seasonal variation; Tenofovir disoproxil fumarate; Vitamin D deficiency.

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Abbreviations: CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; TDF, tenofovir disoproxil fumarate; HBV, hepatitis B virus; 25[OH]D, 25-hydroxyvitamin D_3; HCV, hepatitis C virus; ISG, interferon-stimulating gene; PegIFN, pegylated interferon-α 2a; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TSH, thyroid-stimulating hormone; HBsAg, hepatitis B surface antigen; anti-HBs, hepatitis B surface antibody.

Introduction

Worldwide, an estimated two billion people have been infected with hepatitis B virus (HBV), and more than 240 million of them have developed chronic infection [1]. The mechanisms underlying the chronicity of infection are unclear but are likely a function of inadequate activation of immune responses for clearing infection. Identifying mediators of the immune response, particularly modifiable factors, may have significant implication on disease management.

Vitamin D, through its active form (1,25-dihydroxyvitamin D_3, calcitriol), enhances intestinal calcium absorption, plays a central role in maintaining calcium homeostasis and skeletal integrity [2], and has immunomodulatory activities, with regulatory roles impacting both innate and adaptive immune systems [3]. Vitamin D from the skin and diet is hydroxylated in the liver into 25-hydroxyvitamin D_3 (25[OH]D_3). 25(OH)D_3 is the major circulating form of vitamin D and is the form measured to determine an individual's vitamin D status [4]. From the liver, 25(OH)D_3 is transported to the kidney, where it undergoes a second hydroxylation and is converted into 1,25-dihydroxyvitamin D_3.

Recent observations have implicated vitamin D in the clinical course of infectious diseases. Vitamin D enhances host protective immune responses to *Mycobacterium tuberculosis*, and vitamin D supplementation can accelerate clinical improvement in patients with pulmonary tuberculosis [5]. In three small studies of patients with chronic hepatitis C virus (HCV) infection, adding vitamin D to therapy with pegylated interferon alfa (PegIFN) and ribavirin improved rates of sustained virological response [6–8]. Furthermore, *in vitro* analyses revealed that calcitriol

augments intrahepatic interferon-α signaling by removing constitutive vitamin D receptor inhibition of STAT1, resulting in nuclear localization of phosphorylated STAT1 and increased interferon-stimulated gene (ISG) expression [9]. In a population of CHB patients predominantly ineligible to receive antiviral therapy, low vitamin D levels were associated with high viral load [10].

Given the paucity of information on the role of vitamin D in CHB, along with recent data suggesting vitamin D may contribute to ISG expression, we evaluated the baseline serum vitamin D levels and their association with baseline clinical parameters and clinical outcomes among a large population of patients with treatment eligible CHB infection. The population included patients enrolled in a large global clinical trial designed to investigate the efficacy of tenofovir disoproxil fumarate (TDF) plus PegIFN alfa-2a compared with either TDF or PegIFN alone.

Methods

Study design

In this global, randomized, open-label, active-controlled clinical trial (NCT01277601), we evaluated the efficacy and safety of 48 weeks of TDF plus PegIFN (Arm A), TDF plus PegIFN for 16 weeks followed by TDF for 32 weeks (Arm B), TDF for 120 weeks (Arm C), and PegIFN for 48 weeks (Arm D). Eligible patients were aged 18–75 years with CHB, either hepatitis B e antigen (HBeAg)-positive or -negative, without advanced liver disease and naïve to interferon and anti-HBV nucleotide treatment (oral anti-HBV nucleoside therapy was acceptable if the last dose was ⩾24 weeks prior to screening). After completing 48 weeks of treatment, patients underwent follow-up for 72 weeks.

For inclusion in the study, patients had to have HBV DNA ⩾20,000 IU/ml if HBeAg-positive and ⩾2,000 IU/ml if HBeAg-negative; alanine aminotransferase (ALT) >54 and ⩽400 U/L for men and >36 and ⩽300 U/L for women; and creatinine clearance rates of ⩾70 ml/min as calculated by the Cockcroft-Gault equation.

Patients were excluded from participating in the study if they had any of the following conditions or disease characteristics: bridging fibrosis or cirrhosis as confirmed by liver biopsy or Fibroscan (⩾8 kPa) within 12 months prior to screening; history of clinical hepatic decompensation; evidence of hepatocellular carcinoma; significant renal, autoimmune, or bone disease; thyroid dysfunction; or co-infection with HIV, HCV, or hepatitis D virus. Serum levels of calcium, phosphate, or vitamin D were not among criteria for inclusion or exclusion.

The study protocol was approved by each institution's review board or ethics committee prior to study initiation. The study was performed in accordance with either Good Clinical Practice guidelines outlined by the International Conference on Harmonization and the principles of the Declaration of Helsinki. All patients provided written informed consent before undertaking any study-related procedures.

Data collection

At baseline, data were collected regarding patient demographics (sex, age at baseline, height, weight, race); date of first dose; laboratory values for ALT, aspartate aminotransferase (AST), calcium, phosphorous, alkaline phosphatase, albumin, thyroid-stimulating hormone (TSH), alpha-fetoprotein, uric acid; and HBV virology, such as hepatitis B surface antigen (HBsAg) level, hepatitis B surface antibody (anti-HBs), HBV DNA level, HBV genotype, and HBeAg status. The season of enrollment was determined by the date of enrollment and the location of the study site. The three locations were equatorial, northern hemisphere, or southern hemisphere. All sites in the equatorial zone, defined as within 24° of the equator, were considered to be in spring-summer year-round. For northern and southern sites, the season was delimited by the respective equinox dates (e.g., spring-summer if the northern site was between Mar 21 and Sept 21). All laboratory data were measured by a central laboratory (Bio Analytical Research Corporation N.V.) with three separate sites globally. Concomitant medication log was tracked on all patients throughout the study.

Vitamin D was also assessed at baseline by measuring serum concentration of $25(OH)D_3$. The test was performed using either a chemiluminescence immunoassay (LIAISON 25 OH Vitamin D TOTAL assay, DiaSorin, Inc., Stillwater, MN) with a measuring range of 4.0 to 150.0 ng/ml or a radioimmunoassay (25-Hydroxyvitamin D ^{125}I RIA kit, DiaSorin, Inc.) with a measuring range of 5.0 to 100.0 ng/ml. Levels of vitamin D were categorized as follows: <20 ng/ml = deficient; ⩾20 but <31 ng/ml = insufficient; ⩾31 ng/ml = normal.

Statistical analyses

Standard descriptive statistics (e.g., mean, standard deviation) were used to summarize continuous variables. For categorical variables, frequencies and proportions were used. Comparisons between groups were done via Fisher exact test for categorical data and Wilcoxon Rank-Sum test for continuous data. Associations between vitamin D and each of the baseline demographics and lab values were assessed in univariate analyses using General Linear Models. Each of the following was treated as a class variable: sex, race (white *vs.* non-white), randomized treatment assignment, season of blood draw (spring/summer *vs.* autumn/winter), HBV genotype, HBeAg status, dichotomous anti-HBs (mIU/ml), and dichotomous ALT. Quantitative anti-HBs was dichotomized at ⩽3 mIU/ml *vs.* >3 mIU/ml. ALT was dichotomized at >54 U/L *vs.* ⩽54 U/L for males and >36 U/L *vs.* ⩽36 U/L for females. Each of the following was treated as a continuous variable: baseline age, height, weight, BMI, calcium, AST, phosphorous, alkaline phosphatase, albumin, TSH, alpha-fetoprotein, uric acid, HBV DNA level (log_{10} IU/ml), and HBsAg level (log_{10} IU/ml). In addition, significant variables in univariate analyses at a 0.15 level were fitted simultaneously into a multivariate General Linear Model using a backward selection method until each remaining variable had an independent significance of 5% controlling for all of the remaining variables.

As a supplementary analysis, vitamin D was dichotomized into normal/insufficient *vs.* deficient (⩾20 ng/ml *vs.* <20 ng/ml). Univariate logistic regression analyses were employed to explore the association of vitamin D deficiency with various baseline characteristics mentioned above. A multivariate logistic regression model using a backward selection method was employed to explore the associations of vitamin D deficiency with all the variables simultaneously.

Also examined were associations between baseline vitamin D and the following outcome variables at week 48: HBsAg (log_{10} IU/ml) decline from baseline, HBsAg <100 IU/ml, HBV DNA <15 IU/ml, ALT normalization, HBeAg loss and seroconversion, and HBsAg loss.

Results

Study population

Between March 2010 and March 2013, 1597 patients were screened and 740 were randomized and treated at 139 study sites (45 in the European Union, 37 in Asia, 31 in North American, 17 in Australia, and nine in India). More than half of patients (66%) were male, 22% were white, and 75% were of Asian race, including Indian (Table 1). The mean age of patients was 37.1 years; the mean ALT was 110.2 U/L; the mean HBV DNA level was 7.0 log_{10} IU/ml; and the mean HBsAg level was 3.8 log_{10} IU/ml. Additionally, 58% of patients were HBeAg-positive. Of the 737 patients with baseline vitamin D level and included in this analysis, 73 (10%) started taking vitamin D or a multivitamin supplement (Arm A, 18 [10%]; Arm B, 15 [8%]; Arm C, 18 [10%]; Arm D, 22 [12%]) between baseline and week 48.

Associations of vitamin D level with baseline characteristics

The mean baseline vitamin D level in the study was 18.4 ng/ml. Among all patients, 7% had normal vitamin D levels, 35% had insufficient levels, and 58% were deficient for vitamin D (Table 2). Vitamin D deficiency was significantly more common among HBeAg-positive patients (62%) than HBeAg-negative patients (53%) ($p = 0.0214$). Mean vitamin D levels were lower among