

Assessment of Interferon INF- γ and Vitamin D levels among Chronic Hepatitis B Virus Patients in Khartoum -Sudan

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Received: 10 June 2021; Accepted: 06 July 2021

Citation: Ahmed DM, Elhadad IO, Al Dawi AF. Assessment of Interferon INF- γ and Vitamin D levels among Chronic Hepatitis B Virus Patients in Khartoum -Sudan. Clin Rev Cases. 2021; 3(2): 1-7.

ABSTRACT

Introduction: In Sudan Hepatitis B virus, (HBV) infection is the most common cause of acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) worldwide, Interferon INF- γ is significantly associated with chronic hepatitis B virus (HBV) infection. However, the relationship between low serum vitamin D levels and HBV chronicity is unknown.

Objective: The aim of this study was to assess (INF γ) levels and the pattern of vitamin D levels, in patients with chronic HBV infection compared with healthy individuals.

Methodology: 90 chronically HBV-infected, were randomly selected in the present study. A serum vitamin D level measured by using Electro-chemiluminescence binding assay (ECLIA). The INF γ and HBsAg serum level were measured by using an in vitro diagnostic (ELISA).

Results: A total of 180 individuals were selected in the present study, 90 of them were case study of chronic hepatitis B virus infection, and 90 were case control healthy individual. The mean serum level of 25(OH) D was for case was 11.63 ng/mL. Out of the 180 participant, 93 (53.8%) were female and 87(46.2%) were male. Vitamin D and INF γ were significantly decreased in case group in comparison with the control (p value 0.002).

Conclusions: Vitamin D deficiency and interferon γ are significantly associated with chronic HBV infection.

Keywords

Hepatitis B virus, Vitamin D, Interferon γ .

Introduction

Hepatitis B virus (HBV) infection is the most common cause of acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) worldwide. Up to date, more than two billion individuals have been infected, of which 350 million are chronic carriers of the virus [1,2].

The genome of HBV is a circular, partially double-stranded DNA of approximately 3.2 kb. According to genetic heterogeneity, HBV

can be classified into eight genotypes (A–H), and recently, two additional genotypes (I and J) were proposed [3].

High genetic variability is a characteristic feature of HBV because virus polymerase lacks proofreading activity and very high virion production per day (> 1,000 viruses) [4].

In addition to natural evolutionary changes that contribute to genetic variations, increased genome mutations might also be caused by the host immune system in order for the virus to evade immune clearance [5,6].

Vitamin D is not only a steroid hormone with classical actions

related to mineral metabolism and skeletal health, but also acts as an important modulator involved in cell proliferation and differentiation, immune modulation, and inflammation [7,8].

Recently they found that there is a significant association between low serum concentrations of 25(OH) D3 and higher levels of HBV replications in chronically infected patients [9].

In order to further understand the effects of vitamin D metabolism on chronic hepatitis B virus infection (CHB), we performed this study to analyze the relationships between low serum vitamin D levels and the high level of HBSAg in CHB patients.

According to recent studies, the prevalence of vitamin D insufficiency and deficiency is higher in patients with chronic liver disease than in general population ranging between 64 and 92% [10,11]. It has been also reported that the incidence of vitamin D deficiency increases as the liver disease progresses [12,13].

Interactions between host immune responses and HBV determine the clinical manifestations of HBV infection. Innate immune mechanisms have important roles in the clearance of HBV through production of inflammatory cytokines such as interferons γ (IFN- α/β and IFN- γ) [14].

Besides the classical function in regulating calcium and bone homeostasis, vitamin D (cholecalciferol, 25-(OH) D3) is also relevant in modulating both innate and adaptive immune responses [15,16].

Deficiency of vitamin D has been shown to be involved in carcinogenesis and the course of several infectious diseases [17,18], among them distinct liver infections [19,20]. Vitamin D is also involved in aspects of innate immunity and immune-related disease. In the face of infection, 1 α ,25(OH) $_2$ D is able to induce the production of cathelicidins and other antimicrobial peptides (AMPs) through binding with VDR on numerous immune cells, such as T-cells, B-cells, NK cells, and monocytes [21].

Vitamin D deficiency is associated with several adverse health outcomes, and vitamin D appears to have systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses [22].

HBV is a DNA virus classified in the virus family Hepadnaviridae. Humans are the only known natural host. HBV enters the liver via the bloodstream, and replication occurs only in liver tissue. The intact, infectious virus is 42–47 nm in diameter and circulates in the blood in concentrations as high as 108 virions per ml. The inner core of the virus contains hepatitis B core antigen, hepatitis B e antigen (HBeAg), a partially double-stranded 3,200-nucleotide DNA molecule, and DNA polymerase with reverse transcriptase activity. Hepatitis B surface antigen (HBsAg) is found both on the surface of the virus and as self-assembling, noninfectious spherical or tubular particles [23].

Vitamin D deficiency is associated with several adverse health outcomes, and vitamin D appears to have systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illnesses.

Vitamin D may also improve survival in acute illness by boosting innate immunity, and appears to exhibit systemic antimicrobial effects that may be crucial in a variety of both acute and chronic illness [24].

Vitamin D is not only a secosteroid hormone with classical actions related to mineral metabolism and skeletal health, but also acts as an important modulator involved in cell proliferation and differentiation, immunomodulation, and inflammation [25].

According to World Health Organization (WHO) recommendations, 25(OH) D (the main circulation form of vitamin D) levels of (≥ 14 and < 30 ng/mL) and < 14 ng/mL are defined as vitamin D insufficiency and vitamin D deficiency, respectively [26].

Recently, they found that there is a significant association between low serum concentrations of 25(OH) D3 and higher levels of HBV replications in chronically infected patients [27].

In order to further understand the effects of vitamin D metabolism on chronic hepatitis B virus infection (CHB), we performed this study to analyze the relationships between low serum vitamin D levels and the mutations of HBV determinant in CHB patients [28].

Vitamin D has an important role in various chronic diseases, such as infectious and cardiovascular diseases, diabetes mellitus and some types of cancer [29,30].

In addition, vitamin D has been associated with chronic liver diseases and it has been reported that low vitamin D status is a common feature in different types of liver diseases [31].

On the other hand, interferon- γ is a cytokine involved in the activation of cellular immunity, particularly cytotoxic CD8+T cells, during an acute self-limited HBV infection, high level of IFN- γ is secreted by T-lymphocytes, which has significant role in HBV clearance. HBV or viral proteins also have been clearly reported to inhibit IFN production. Major vault protein (MVP) is a novel virus-induced cellular protein that upregulates IFN production [32].

Rationale

Incidence of liver diseases (viral hepatitis) increasing all over the world with significant amount of mortality.

Recent studies reported a significant association between vitamin D level and hepatitis B virus infection. That is due to the important role of vitamin D and maintenance of immunity.

Objectives

General Objective

To determine serum vitamin D and interferon γ (INF γ) levels among Sudanese patients with chronic hepatitis B virus in Khartoum state.

Specific Objectives

- To measure serum Vitamin D and INF γ in patient with hepatitis B and compare with healthy individuals.
- To find out the correlation between vitamin D and INF γ levels with age of patients.
- To compare between vitamin D and INF γ levels according to gender of patients.
- To find the association of vitamin D and INF γ levels and the duration of the disease.

Material and Method

Study Design

This is a prospective hospital-based cross-sectional study.

Study Area and Duration

The study was conducted at Khartoum state during Feb 2016- Feb 2017.

Study population and sample size

90 Sudanese patients with HBV as case and 90 Healthy individuals as control group.

Patients and Criteria of Selection and Rejection

90 chronically HBV-infected patients were randomly selected from hospitals and clinic of the infection diseases department, and 90 healthy individuals (HBsAg negative) Booth female and male in case study and control.

Inclusion Criteria

For the present study were chronic infection with BV, defined as a positive result of HBsAg (HBsAg +ve) and treatment-naive status.

Rejection Criteria

Patients were excluded if they were co-infected with HCV, human immunodeficiency virus (HIV), or hepatitis delta virus (HDV), if excessive alcohol consumption had been reported, if they had received a liver allograft, or if a malignant disease (including HCC) or diabetes mellitus, and renal problem had been diagnosed.

Samples

Hundred venues blood samples (approx. 2-5 ml each) were obtained from each selected patient and healthy individuals (for control) on a blood container(plan and heparinized).

Blood samples were centrifuged (2to 4minutes at 3000 RPM) and the serum were processed for measuring of Serum 25-hydroxyvitamin D (25-OHD) and HBSAg and diagnosis by autoanalyzer and ELISA respectively all samples were preserve at 4°C in laboratory refrigerator.

Laboratory Method of Investigation For HBV, Vitamin D and INF γ

HBV HbsAg (HS)

Fortress HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

Electro-chemiluminescence binding assay (ECLIA)

For the in-vitro determination of total 25-hydroxyvitamin D.

INF γ

The RayBio® Human IFN-gamma ELISA kit is an in-vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IFN-gamma in serum, plasma.

Statistical Analysis

All data will be performed using the Statistical Package for the Social Sciences software package (SPSS) v21.

Results

A total of 180 individuals were selected in the present study according to the above-described criteria, 90 of them were case study of chronic hepatitis B virus infection, and 90 were case control healthy individuals.

The mean \pm SD serum level of 25(OH)D for both of them (case study & control) was showed significant relation between the low level of vitamin D and high rate of HBSAg (11.633 ± 2.277 ng/ml 35.125 ± 7.871 ng/ml respectively) (p. value 0.000).

INF γ level was significantly decreased in case group in comparison with the control (mean, $11.63 \pm$ SD 2.27 case, $35.12 \pm$ SD 7.87 control respectively (p value 0.002). (Mean $7.6 \pm$ SD 3.5 pg/mL case, $11.3 \pm$ SD 2.4 pg/mL).

Out of the 180 participant, 99 (53.8%) were female and 83(46.2%) were male their insignificant relation between the vitamin D concentration and the gender of our group study (case and control) (p. value 0.099).

The age of all the study members was ranged from 27 to 52 years old with mean \pm SD of 37.68 ± 6.12 years for case study & 39.67 ± 5.59 years for control group respectively and also showed significant relation between them with vitamin D concentrations with (p. value 0.027).

There was significant relation between the decrees of vitamin D level and increase of disease duration with (mean \pm SD 2.874 ± 1.808) (p value 0.000), the duration of disease in case study which range between one to 7 years.

Table 1: Relation of vitamin D, IFN- γ & HBSAg in case and control group.

Parameters	Case (Mean \pm SD) 90	Control (Mean \pm SD) 90	P-value
HbsAg rate	2.874 ± 1.808	0.008 ± 0.015	0.000
vitamin D ng/ml	11.633 ± 2.277	35.125 ± 7.871	0.000
IFN- γ pg/mL	$7.6 \pm$ SD 3.5	$11.3 \pm$ SD 2.4	0.002

T-test was used to calculate P value

- P value less than 0.05 considered significant
- Mean \pm Std. Deviation

Tables 1 shows significant relationship between HBSAg rate and vitamin D concentration in Case group (mean \pm SD 11.633 ± 2.277 ng/ml, (P-value 0.000).

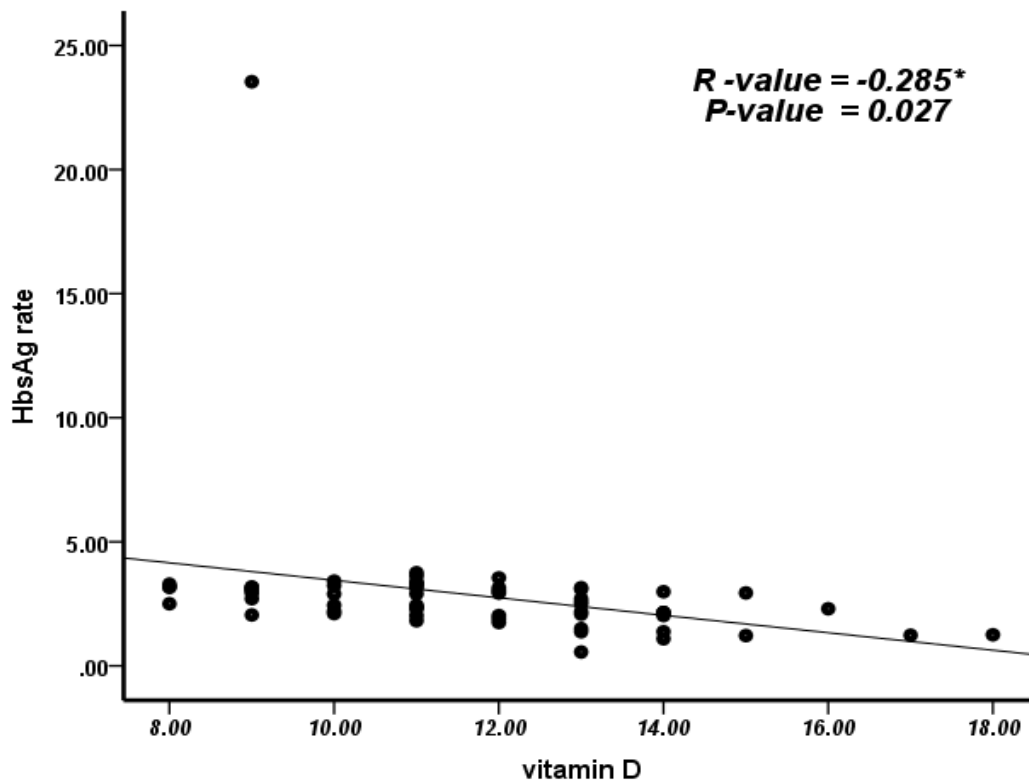


Figure 1: Relation between HBSAg rate and vitamin D concentration.

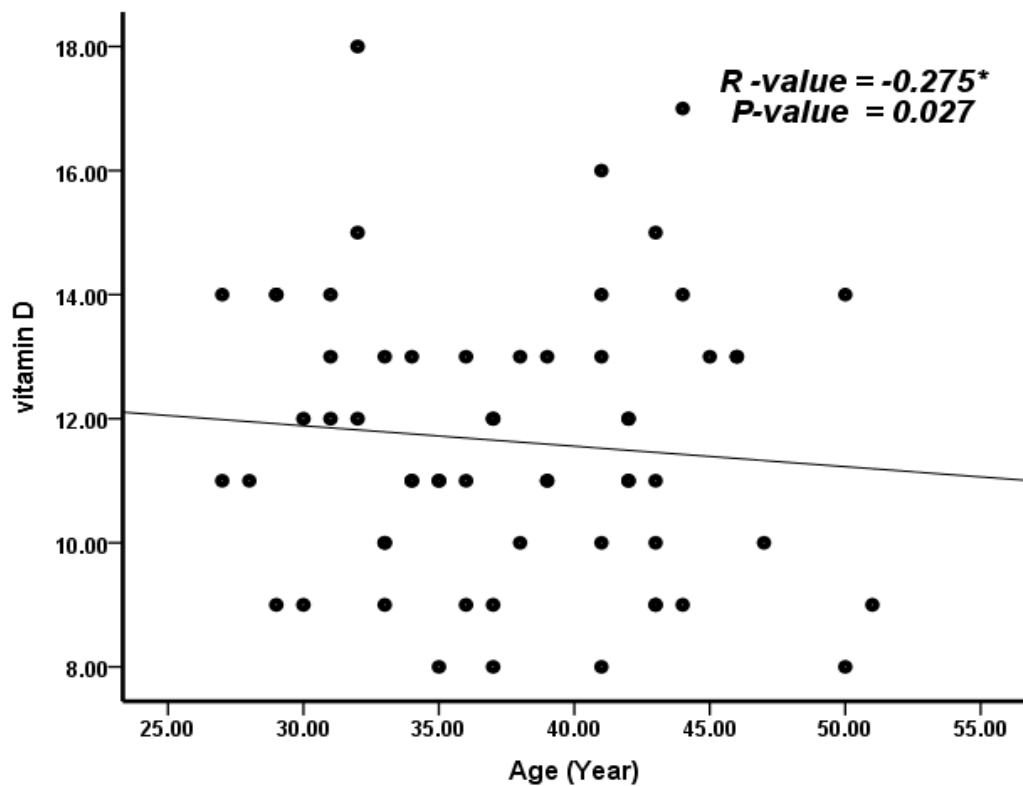


Figure 2: The relation between vitamin D concentration and the age of study group.

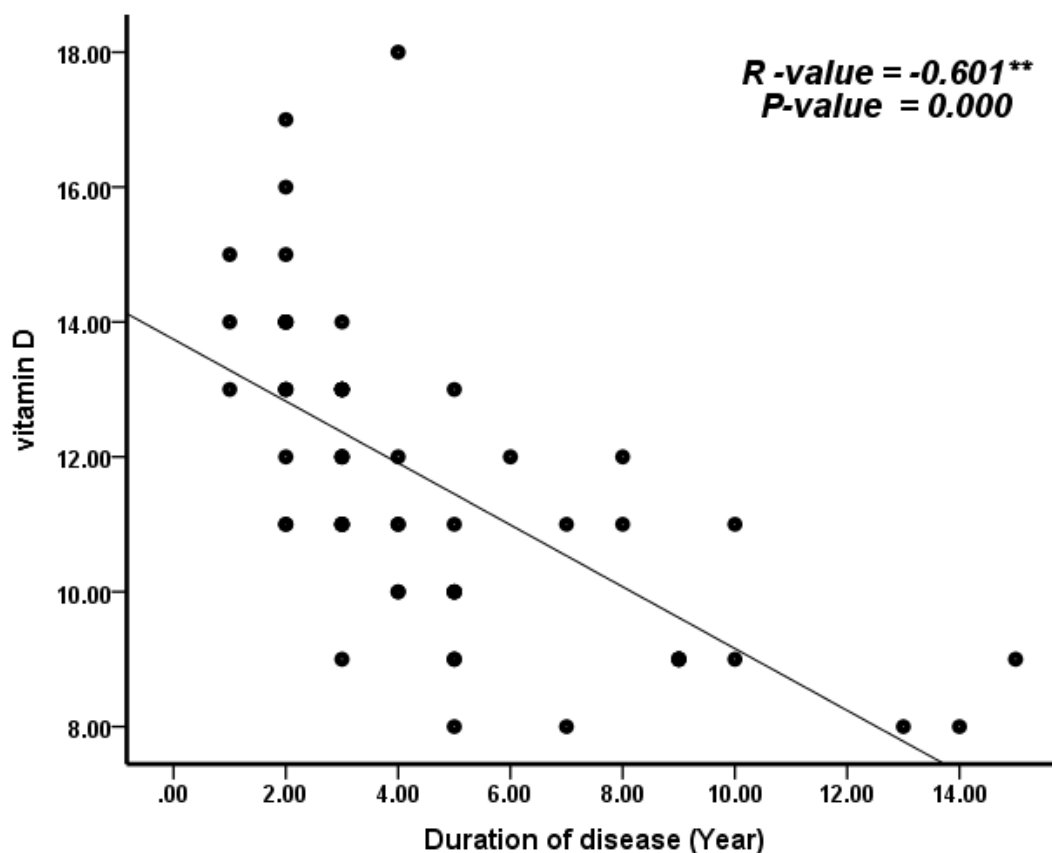


Figure 3: The relation between vitamin D concentration and disease duration.

Table 2: The rate of HBSAg in male & female of case group.

Variable	Male (Mean ± SD)	Female (Mean ± SD)	P-value
HbsAg rate	2.57 ± 0.766	3.69 ± 1.33	0.415

t-test was used to calculate P value

- P value less than 0.05 considered significant
- Mean ± Std. Deviation

Tables 2 shows insignificant relationship between sex and HBSAg rate in both male and female (mean ± SD 2.57 ± 0.766, 3.69 ± 1.33 P-value 0.415) respectively.

Table 3: Vitamin D concentration in male and female of case group.

Variable	Male (Mean ± SD)	Female (Mean ± SD)	P-value
vitamin D/ ng/ml	11.77 ± 2.24	11.25 ± 2.41	0.439

t-test was used to calculate P value

- P value less than 0.05 considered significant
- Mean ± Std. Deviation

Tables 3 Shows insignificant relationship between sex and vitamin D concentration in both male and female (mean ± SD 11.77 ± 2.24 ng/ml, 11.25 ± 2.41 ng/ml P-value 0.439) respectively.

Discussion

This study was carried out to comparing, the relation between vitamin D level, and INF level in chronic hepatitis B virus infection, by measuring the concentration of vitamin D, HBSAg

rate and assess the relation of both test with disease duration, age and sex of patients and compare the results of patients with control group, so vitamin D levels were examined in patients with chronic HBV infection and healthy individuals.

Vitamin D levels were found to be lower in the chronic hepatitis B patients compared with control individuals. There was a strong correlation between the low level of vitamin D (p- value 0.000) in case study and high rate of HBSAg (p- value 0.000).

There was significant relation between the level of vitamin D level, and the disease duration, the duration of disease in our case study was range between one to seven years (p- value 0.000).

INF γ level was significantly decreased in the chronic hepatitis B patients compared with control individuals. there was a significant correlation between the low level of INF γ (p- value 0.002) in case study and high rate of HBSAg (p- value 0.000).

The age of case, study group show also significant relationship the oldest one the lowest vitamin D level (p value 0.027). Sabetta *et al.*, demonstrated that maintenance of a vitamin D serum concentration of 38 ng/mL or higher could significantly reduce the incidence of acute viral [33].

But their insignificant relation between the vitamin D concentration and the gender of our study group (p. value 0.439).

Recent study showed that the activity of cytokines such as IFN γ is an important indicator of the clinical severity and progression of HBV-related infections. The chronicity of hepatitis B infection is also influenced by mutations in the vitamin D receptor gene, with polymorphisms being associated with higher viral load and increased disease progression and severity [34].

Conclusion

The results report that vitamin D level and INF γ levels were significantly associated with HBV chronic infection, disease, duration and age but there is insignificant relation between the vitamin D and the gender of study group.

Recommendations

In future studies we recommended that, large sample size must be taken in more than state (to reliable data for Sudanese population).

Molecular test should be study to increase understanding of this correlation between vitamin D, INF γ , and chronic HBV infection.

Finally, the introducing of vitamin D supplement must take place in hepatic clinics, to improve the immunity of patients and delay the mortality of the disease.

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