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The Role of Vitamin D In The Susceptibility To Hepatitis B Virus Infection

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Abstract

Background: The Hepatitis B virus (HBV) infection is a major contributor to liver damage. The initial phase of infection is frequently asymptomatic; nonetheless, this virus has a notable propensity to persist and advance. **Objectives**: This study attempts to evaluate the concentrations of Vitamin D3 and IFN-α in the serum of individuals infected with Hepatitis B virus. **Materials and Methods:** This study employed a case-control design involving forty patients. Serum levels of Vitamin D3 were assessed using the immunofluorescence technique with Cobas-e, in accordance with the manufacturer's instructions (Roche, Switzerland). Melsin's enzyme-linked immunosorbent assay (ELISA) methodology was employed to quantify IFN-α levels. Melsin originates from Jiling, China. **Results:** The findings indicated a significant reduction in Vitamin D3 levels (14.28 ± 3.11 ng/ml) among HBV patients relative to healthy individuals (39.72 ± 6.21 ng/ml). The INF-α levels exhibited significant differences among the tested subjects ($P < 0.05$).The mean perforin level was decreased in HBV patients to (0.12 ± 0.084 ng/ml), whereas it increased to (1.81± 0.14 ng/ml) in healthy individuals. Significant differences in vitamin D3 concentrations were observed between males and females. The mean INF-α levels for males surpassed those of females; however, no statistically significant differences were observed between the two groups. **Conclusion:** Vitamin D3 insufficiency is common and frequently observed in chronic liver diseases associated with HBV. This study demonstrates that sufficient levels of vitamin D3 are essential during antiviral treatment for HBV infections. Interferon alpha plays a crucial role in HBV infection through the regulation of immune responses and the exhibition of antiviral properties.

Keywords: Vitamin D, IFN-α, Hepatitis B virus

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دور فيتامين D في قابلية الاصابة بعدوى فيروس التهاب الكبد B ،

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المستخلص

يُعدّ التهاب الكبد الوبائي نوع (HBV) أحد الأسباب الرئيسية لتلف الكبد. غالبًا ما تكون المرحلة الأولى من العدوى بدون أعراض؛ ومع ذلك، يميل هذا الفيروس إلى الاستمرار والتطور. تهدف هذه الدراسة تقييم تركيزات فيتامين (D3) و (INF-α) في مصل الأفراد المصابين بفيروس التهاب الكبد B.اذ اعتمدت الدراسة على اخذ عينات (40) مريضًا. قُيّمت مستويات فيتامين (D3) في مصل الدم باستخدام تقنية الفلورسنت المناعي باستخدام Cobas-e، واستُخدمت منهجية اختبار الممتز المناعي المرتبط بالإنزيم (ELISA) لتحديد مستويات (INF-α).

حيث أشارت النتائج إلى انخفاض كبير في مستويات فيتامين (D3) (14.28 ± 3.11 نانوغرام/مل) بين مرضى التهاب الكبد ب مقارنة بالأفراد الأصحاء (39.72 ± 6.21 نانوغرام/مل). أظهرت مستويات INF-α اختلافات كبيرة بين الأشخاص الذين تم اختبارهم ($P < 0.05$). انخفض متوسط مستوى البيرفورين لدى مرضى التهاب الكبد ب إلى (0.12 ± 0.084 نانوغرام/مل)، بينما ارتفع إلى (1.81 ± 0.14 نانوغرام/مل) لدى الأفراد الأصحاء. لوحظت اختلافات كبيرة في تركيزات فيتامين (D3) بين الذكور والإناث. تجاوز متوسط مستويات INF-α لدى الذكور تلك الموجودة لدى الإناث؛ ومع ذلك، لم تُلاحظ أي فروق ذات دلالة إحصائية بين المجموعتين. نقص فيتامين (D3) شائع ويُلاحظ بشكل متكرر في أمراض الكبد المزمنة

معلومات البحث
تأريخ النشر: تشرين الاول 2025

المرتبطة بفيروس التهاب الكبد B. توضح هذه الدراسة أن المستويات الكافية من فيتامين (D3) ضرورية أثناء العلاج المضاد لعدوى فيروس التهاب الكبد B. يلعب INF-α دورًا حاسمًا في عدوى فيروس التهاب الكبد ب من خلال تنظيم الاستجابات المناعية وإظهار خصائص مضادة للفيروسات.

الكلمات المفتاحية: فيتامين د، إنترفيرون ألفا، فيروس التهاب الكبد ب

Introduction

Hepadnaviridae is a family. HBV comprises a circular, partly double-stranded DNA genome that is 3.2 kb in length. HBV is a 40-42 nanometer enclosed [1].HBV infection weakly induces the innate immune response, but co-infection with Hepatitis D Virus (HDV) activates a stronger interferon response, enhancing immune cell recruitment and activation [2]. HBV impairs macrophage function by inducing hyperacetylation of metabolic enzymes, leading to reduced TCA cycle activity and M2-like polarization, which diminishes their antiviral capabilities [3]. Chronic HBV infection leads to T cell and NK cell exhaustion due to persistent viral antigens, resulting in ineffective immune responses and viral persistence [4]. Sunlight stimulates the skin to manufacture cholecalciferol, generally known as vitamin D3[5]. The liver initially transforms cholecalciferol into 25-hydroxyvitamin D (25-OHD) via a process termed 25-hydroxylation [6]. Vitamin D shortage causes calcium and phosphorous metabolism to be distorted [7]. Furthermore hypothesised to have a major impact include autoimmune illnesses, Type 2 Diabetes Mellitus, cancer, and the development of numerous viral infections [8].People with vitamin D deficiency with Hepatitis B virus show clearly hepatic necrosis [9]. Advanced fibrosis speeds up the development [10]. The 25-hydroxylase enzymes CYP27A1 are downregulated at the cellular level in liver tissue deprived of sufficient vitamin D. The negative connection between CYP27A1 expression and the degree of necro-inflammatory activity indicates the toxicity of it[10][11].Interferon-α (IFN-α) is a fundamental component of antiviral immune responses used in treatment of Hepatitis B virus (HBV) infections. Even if IFN-α has therapeutic value, long-term use of it usually results in significant side effects including severe depressive episodes and paralyzing tiredness[12]. Many studies have been conducted on the reasons of depression, which typically manifests one-third of patients between 4 to 8 weeks after beginning IFN-α treatment and affects [13]. Still, little study has focused on weariness, which affects almost all patients undergoing therapy and shows considerably more quickly usually within hours following the initial injection.[14]. Furthermore, one of the most functionally incapacitating side effects of IFN-α-based treatment is generally weariness, which could last even after treatment is stopped [15].Still, not much is known about the mechanics of cytolytic effector activities especially those mediated by perforin. Cytotoxic T cells are believed to carry enzymes that induce cell death, including granzymes and perforin, a protein creating holes in secretory granules already developed. Released, it starts polymerization, creating a transmembrane channel. This allows additional proteases and granzymes penetrate target cells and induce death there[16]. Many studies have examined the pre-formed perforin levels in CD8+ T cells from individuals recently infected with HBV. Their findings imply quite little of it exists. This holds

true even with a long-term illness [17]. Many data points, nevertheless, hint to a relationship between viral control and perforin expression. According to a recent study, chimpanzees that lost HBV also showed perforin in their liver T cells during the late acute phase of infection [18]. Furthermore, those who effectively clear HBV during antiviral therapy have more perforin than those who fail to eradicate the virus [19]. The aim of this work is to investigate the levels of IFN-α, perforin, and vitamin D3 in blood of Hepatitis B carriers.

Materials and Methods

Study design: Admitted to private labs in Babylon province, 45 individuals verified clinically identified as HBV patients. The patients span is 21 to 68 years; the men were 19 and the women were 26. Furthermore, 45 seemingly healthy individuals match age and sex to patients.

Blood samples: Every person had three milliliters taken by venous punctures. To separate serum, place it in throwaway tubes with separating gel. The peripheral blood samples let to clot at room temperature for thirty minutes. They then spent around three minutes under 3000 g in centrifugation. The serum was collected and kept for study at -20°C.

Determining immunological factors: Each research participant turned in whole blood samples. Blood samples processed using an enzyme-linked immunosorbent test (ELISA) kit from Demeditec Diagnostics, Kiel, Germany, revealed immunological markers including Perforin and IFN-α in line with the given instructions. Following manufacturer recommendations, the vitamin D3 was measured using the immunofluorescence technique with co base (Roche, Switzerland).

Statistical analysis

We used the statistical package for the Social Sciences (Version 24.0, IBM Corp., Chicago, IL, USA) for the analysis. The group means were compared using Fisher's Exact Test, Mann-Whitney U test, independent samples t-test, and Chi-square (X^2).The possibilities for data displays were number, percentage, average, and standard deviation. A P-value below 0.05 denotes statistical relevance.

Ethical approval

The present work according to the ethical standards set in the Declaration of Helsinki. Patients verbal permission for the surgery to be carried out before sample collecting. Reference number 34 (dated September 12, 2024) is the study protocol and patient informed consent supplied by the Babylonian Health Authority and the Publication Ethics Committee of the University of Babylon in Iraq.

Results

Comparatively to healthy controls, serum vitamin D3 levels were much lower in HBV patients ($p < 0.005$). as shown in Table (1)

Table (1): HBV patient and control vitamin D3 levels (ng/ml)

Vitamin D_3	Study groups		P value
	Case (45)	Control (45)	
	Mean ± SD	Mean ± SD	

	14.28 ± 3.11	**39.72 ± 6.21**	**≤ 0.005***

*** p value ≤ 0.05 was significant.**

ELISA allowed one to measure INF-α levels. The mean values showed a notable variation among the several study groups. As table (2) shows, the average value of patients diagnosed with HBV was lower than that of the healthy control group (P < 0.005).

Table (2): INF-α concentration in HBV patients' and control's (ng/ml)

	Study groups		**P value**
INF-α	**Case (45)**	**Control (45)**	
	Mean ± SD	Mean ± SD	
	0.58 ± 0.89	**1.78 ± 0.33**	**≤ 0.005***

***p value ≤ 0.05 was significant.**

The results showed a clear variation in the average perforin levels among the several study groups. Table (3) shows that compared to control, those with HCV showed notable lower average values (P < 0.005).

Table (3): Perforin concentration (ng/ml) in HBV patients and control

	Study groups		**P value**
Perforin	**Case (45)**	**Control (45)**	
	Mean ± SD	Mean ± SD	
	0.12 ± 0.084	**1.81 ± 0.14**	**≤ 0.005***

*** p value ≤ 0.05 was significant.**

Table (4) shows the very different concentrations of vitamin D3 between men and women. Though the mean for men surpassed that of women, there were no appreciable variations between the two groups in terms of Perforin and INF-α levels.

Table (4) : Concentration of vitamin D_3 , Perforin and INF-α according to sex distribution in HBV patients

Sex	**vitamin D_3 (ng/ml)**	**INF-α (ng/ml)**	**Perforin (ng/ml)**	**P value**
male	**18.31± 3.43**	**0.87± 0.081**	**0.48± 0.21**	**≤ 0.005***
female	**9.36± 1.87**	**1.29± 0.63**	**0.71± 0.31**	

*** Significant difference regarding Vitamin D_3 sex (male more than female).**

Discussion

According to the present study, compared to men, a considerable number of women have inadequate or deficient levels of vitamin D. Vitamin D is converted by the liver into 1,25-dihydroxyvitamin D3, the active varient of the vitamin [20]. Those diagnosed with chronic liver illness may show poor transformation of vitamin D3 and its physiologically active metabolites [21]. Vitamin D insufficiency and fibrosis might be linked, so chronic liver disease increases the likelihood of vitamin D insufficiency. Putz-Bankuti et al.[22] Since low levels of 25(OH)D have been connected to fibrosis, low levels of this vitamin may serve as indicators for hepatic decompensation and risk death in individuals with chronic liver disease. Gal-Tanamy with companions [23]. shown in cell culture that vitamin D3 reduced viral proliferation and improved VDR expression. numerous disorders, including bone abnormalities, numerous autoimmune and infectious diseases, asthma, malignancies, and psychiatric problems, are associated with vitamin D inadequacy[24]. Vitamin D increases the efficacy of interferon-based therapy, therefore contributing to the HBV infection.Their metabolites, 25-(OH)D3 and 1α,25-(OH)2D3,reduce apolipoprotein expression and change interferon signaling, therefore hindering the growth of HBV[25]. Vitamin D helps the immune system work and could influence the infection with chronic liver disease (HBV) [26]. Although direct involvement requires more research, vitamin D, via the vitamin D receptor (VDR), has a function in immunoregulation and anti-inflammatory processes, therefore perhaps influencing the progression of ongoing hepatitis B infection and related liver disease[27]. Affecting treatment efficacy, oxidative stress, and hepatic fibrosis, vitamin D deficiency corresponds with chronic hepatitis B (HBV) infection. It is linked with genetic variations that affect vitamin D metabolism and may function as an autonomous predictor of sustained virological response (SVR)[28]. In chronic hepatitis B virus (HBV) infection, vitamin D functions as a powerful immunomodulator, therefore possibly enhancing viral response and treatment efficacy. Still, blood vitamin D levels by themselves might not be a consistent indicator of therapy efficacy[29]. Since it increases the cytotoxic action of cytotoxic T lymphocytes (CTLs), perforin is crucial in the infection with the hepatitis C virus (HBV). Together with granzyme B, cytotoxic T cells generate this pore-forming molecule to start death in infected hepatocytes. The study found increased perforin mRNA levels in patient liver tissues with chronic hepatitis B, indicating that activated CTLs employ the perforin-mediated route to induce liver cell death during HBV infection [30]. HBV specific CTL cytotoxicity requires perforin. It mostly acts at a low effector to target ratio by destroying antigen presenting, sensitive cells. Perforin helps lysing both antigen presenting and bystander cells; yet, its effectiveness depends on a higher effector to target ratio for bystander cell killing. In the context of HBV infection, this approach is crucial particularly in terms of targeting contaminated hepatocytes [31]. It emphasizes the crucial function of perforin in controlling viral infections generally and its correlation with pathological diseases including the breakdown of the blood-brain barrier. Although immune responses to many viruses, including HBV, depend on perforin, the exact mechanisms and effects of perforin in HBV infection are yet unknown [32].

The loss of perforin highlights its crucial function in viral defense as it causes a fatal immunodeficiency in those affected with HBV. This emphasizes the need of the perforin/granzyme pathway in controlling HBV and preserving strong antiviral response [33]. Approved originally in 1991, IFN-α was the main treatment used for persistent HBV infection. When given ribavirin, it improved sustained virologic response rates; yet, side effects reduced its efficacy, therefore reducing its relevance with the development of direct-acting antivirals [34]. Regulating immune responses and proving antiviral effectiveness makes interferon alpha crucial in HBV infection. Still, its practical application is limited by negative effects and a short serum half-life, hence latent forms must be created for maximum safety[35]. For decades, IFN-α has been used to treat chronic hepatitis B virus (HBV), mostly by encouraging the production of interferon-stimulated genes (ISGs), which are important antiviral agents that stop HBV replication and boost the immune response in the host[36]. By always increasing antiviral responses in human hepatocytes, IFN-α is crucial in HBV infection. The constant production improves the expression of IFN-stimulated genes, thereby controlling the HBV distribution and reducing early viral replication[37]. Essential for natural antiviral immunity and a basic component of treatment regimens for chronic hepatitis B virus (HBV), alpha interferon (IFN-α) helps control viral replication and influences the effectiveness of antiviral drugs[38]. Important new perspectives for enhancing antiviral responses come from the interplay among vitamin D3, perforin, and IFN-α in hepatitis B virus (HBV) infection. Particularly its active form 1α,25-dihydroxyvitamin D3, vitamin D3 has shown the capacity to increase the efficacy of IFN-α therapy, thereby demonstrating a synergistic relationship strengthening the immune response against HBV[39].

Conclusions

Patients with HBV-related chronic liver disease often have D3 vitamin shortage. Several studies indicate that vitamin D3 levels should be kept under HBV antiviral treatment. Although perforin helps CD8+ T cells lyse HBV-replicating human liver hepatoma cells, prolonged viral infections may prevent this process because of reduced perforin expression in HCV-specific CD8+ T cells during early and intermediate development stages. Hepatitis B virus (HBV) infection is treated with IFN-α, a crucial control of antiviral immune responses agent.

Recommendations

Essential for natural antiviral immunity and a basic component of treatment regimens for chronic hepatitis B virus (HBV), alpha interferon (IFN-α) helps control viral replication and influences the effectiveness of antiviral drugs[38]. Important new perspectives for enhancing antiviral responses come from the interplay among vitamin D3, perforin, and IFN-α in hepatitis B virus (HBV) infection.

Suggestions

Particularly its active form 1α,25-dihydroxyvitamin D3, vitamin D3 has shown the capacity to increase the efficacy of IFN-α therapy, thereby demonstrating a synergistic relationship strengthening the immune response against HBV[39].

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