

Normal vitamin D levels are associated with spontaneous hepatitis B surface antigen seroclearance

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Abstract

AIM: To investigate a possible association between serum vitamin D levels and spontaneous hepatitis B surface antigen (HBsAg) seroclearance.

METHODS: Fifty-three patients diagnosed with chronic inactive hepatitis B and spontaneous HBsAg seroclearance were followed up in two Israeli liver units between 2007 and 2012. This retrospective study reviewed medical charts of all the patients, extracting demographic, serological and vitamin D rates in the serum, as well as medical conditions and current medical therapy. Spontaneous HBsAg seroclearance was defined as the loss of serum HBsAg indefinitely. Vitamin D levels were compared to all patients who underwent spontaneous

HBsAg seroclearance.

RESULTS: Out of the 53 patients who underwent hepatitis B antigen seroclearance, 44 patients (83%) had normal levels of 25-hydroxyvitamin D compared to 9 patients (17%) who had below normal levels. Multivariate analysis showed that age (> 35 years) OR = 1.7 (95%CI: 1.25-2.8, $P = 0.05$), serum vitamin D levels (> 20 ng/mL) OR = 2.6 (95%CI: 2.4-3.2, $P = 0.02$), hepatitis B e antigen negativity OR = 2.1 (95%CI: 2.2-3.1, $P = 0.02$), low viral load (hepatitis B virus DNA < 100 IU/mL) OR = 3 (95%CI: 2.6-4.2, $P = 0.01$) and duration of HBsAg seropositivity (> 8 years) OR = 1.6 (95%CI: 1.15-2.6, $P = 0.04$) were also associated with spontaneous HBsAg seroclearance.

CONCLUSION: We found a strong correlation between normal vitamin D levels and spontaneous HBsAg seroclearance.

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Key words: Hepatitis B; Vitamin D; Immune disease; Seroclearance; Viral load

Core tip: Vitamin D has recently been linked to many autoimmune diseases. Hepatitis B is a viral disease but shows many autoimmune characteristics. Spontaneous hepatitis B seroclearance is an unexplained phenomenon. The hypothesis of this paper was that normal vitamin D levels may be linked to a positive effect on hepatitis B. We showed that normal vitamin D levels correlate positively with spontaneous hepatitis B seroclearance. This finding may help to expand the therapeutic options for this disease.

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INTRODUCTION

The natural history of chronic hepatitis B virus (HBV) infection involves three sequential phases. The initial immune tolerant phase occurs when patients are positive for hepatitis B e antigen (HBeAg) and express normal levels of alanine aminotransferase (ALT). The immune clearance phase occurs when HBeAg-positive patients encounter an abnormal ALT elevation. The final phase takes place when HBeAg undergoes seroconversion to its antibody and ALT levels normalize, proceeding the transition to the inactive residual phase^[1]. Although one third of these so-called inactive hepatitis B surface antigen (HBsAg) carriers might come across HBV reactivation and develop HBeAg-negative chronic hepatitis, most inactive carriers remain permanently inactive over a lifetime and some may ultimately clear HBsAg from the serum^[2]. Spontaneous HBsAg seroclearance is defined as the loss of serum HBsAg remaining consistent on multiple examinations^[3]. Spontaneous HBsAg seroclearance is a rare event in the natural history of chronic HBV infection and this phenomenon is more common in Caucasians than in Asians. The annual rate of spontaneous HBsAg seroclearance varied between 0.12%-2.38% in cohorts from Asian countries and 1.54%-1.98% in cohorts from Western countries^[4,5]. Older age, male gender, normal ALT levels, steatosis, cirrhosis, HBeAg negative at baseline, HBV DNA negative at baseline, genotype and hepatitis C virus (HCV) superinfection have all been shown to have a significant correlation with spontaneous HBsAg seroclearance^[3]. Infection by HBV is accompanied by a number of immunopathological manifestations^[6]. A link between infection and autoimmunity is well documented for HCV infection but HBV infection has also been shown to provoke immunological reactions. These manifestations range from production of autoantibodies to overt autoimmune diseases, including thyroiditis, autoimmune hepatitis, cryoglobulinemia, glomerulonephritis and vasculitis^[7-9]. Vitamin D deficiency has been reported in more than one billion people worldwide, including in sun-rich countries like Israel^[10,11]. The key role played by vitamin D combined with calcium in bone health is well known but other non classical effects of vitamin D are recognized. Interaction with the immune system is one of the most well established non classical effects of vitamin D^[12,13]. Vitamin D deficiency has also been associated with increased risk of respiratory disease, including infections (such as influenza and mycobacterium tuberculosis) and chronic respiratory diseases such as cystic fibrosis^[14,15]. Considerable data about the connection between vitamin D deficiency and development of immune mediated diseases have been published. Studies suggest a link between vitamin D deficiency and autoimmune dis-

eases, such as rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus (SLE)^[16,17]. In this study, we aimed to look for a possible association between serum 25-hydroxyvitamin D [25 (OH) D] levels and the occurrence of spontaneous HBsAg seroclearance. Clinical, anthropometric and laboratory factors were also checked to find a correlation with HBsAg seroclearance.

MATERIALS AND METHODS

A retrospective study carried out between 2007 to 2012 included adult patients with spontaneous HBsAg seroclearance who were followed up at the liver unit of the Shaare Zedek Medical Center (SZMC), Jerusalem, Israel and the liver unit of the Holy Family Hospital, Nazareth, Israel. The study was reviewed and approved by the local ethics committee of each hospital. All patients aged 18-60 years with spontaneous HBsAg seroclearance between 2007 and 2012 were included. Exclusion criteria included patients with liver disease due to acute or chronic hepatitis C, hepatitis A and human immunodeficiency virus (HIV). All patients with another metabolic, infectious, autoimmune or inflammatory liver disease other than steatosis were excluded. Additionally, patients with an alcohol intake > 10 mg/d, receiving chronic immunosuppressive therapy, history of antiviral treatment, prior liver transplantation or the absence of 25 (OH) vitamin D levels in serum were all excluded. The medical charts of the patients were reviewed and multiple data were collected, including age, gender, body mass index (BMI), serum 25 (OH) vitamin D, year of HBsAg appearance, the status of HBeAg, antibodies for HBeAg and viral load (HBV DNA polymerase chain reaction). Information concerning medical conditions, drug therapy and results of laboratory tests were extracted from the medical charts of each subject. Spontaneous HBsAg seroclearance was defined as the loss of serum HBsAg on two occasions at least 6 mo apart and still absent at the last visit.

The normal range of 25 (OH) vitamin D levels was considered to be > 30 ng/mL. Levels of 25 (OH) vitamin D of 20-30 ng/mL were considered an insufficiency and levels < 20 ng/mL were considered a deficiency.

Statistical analysis

Data was analyzed using SPSS version 19 (IBM SPSS, Chicago, IL, United States). Continuous variables are expressed as the mean \pm SD. The χ^2 test was used to test differences in categorical variables between the cases and analysis of variance or the Student's *t* test was used for comparisons of continuous variables. Spearman rank correlation and univariate regression analysis was used to determine the strength of the relationship between the factors for spontaneous HBsAg seroclearance, namely age, gender, BMI, serum 25 (OH) vitamin D, duration of HBsAg positivity, the status of HBeAg, antibodies for HBeAg and hepatitis B viral load. A multiple logistic regression analysis was done to determine the association between the different factors and spontaneous HBsAg

Table 1 Laboratory, demographic and clinical data of the two groups *n* (%) or (mean ± SD)

Characteristic	Normal levels of 25 (OH) vitamin D (<i>n</i> = 44)	Below normal levels of 25 (OH) vitamin D (<i>n</i> = 9)	<i>P</i> value
Sex (male)	30 (68)	5 (55)	NS
Age (yr)	37 ± 12.3	33 ± 15.1	NS
BMI	25 ± 5.4	27 ± 3.2	NS
C-reactive protein (mg/L)	0.9 ± 2.57	0.7 ± 1.23	NS
Duration of HBsAg positivity (yr)	7.2 ± 3.4	8.4 ± 4.8	NS
HBeAg-positive	7 (16)	2 (22)	NS
Mean levels of 25 (OH) vitamin D (ng/mL)	31 ± 4	13.5 ± 7.2	< 0.001

Comparison of demographic and laboratory characteristics between patients with normal and below normal levels of serum vitamin D. Normal levels of 25-hydroxyvitamin [25 (OH)] vitamin D > 30 ng/mL. Below normal levels of 25 (OH) vitamin D ≤ 30 ng/mL. NS: Not significant; BMI: Body mass index; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen.

seroclearance. A significance level of *P* < 0.05 was used in this test.

RESULTS

The medical charts of 68 adult patients with spontaneous HBsAg seroclearance were reviewed during the years 2007-2012. Fifteen patients were excluded due to evidence of HCV antibodies (*n* = 2), alcohol intake > 10 mg/d (*n* = 2), chronic steroid use (*n* = 1), suspected autoimmune hepatitis (*n* = 1) and an unknown vitamin D status (*n* = 9). Altogether, 53 patients with spontaneous HBsAg seroclearance were included in the study. The patients were separated into 2 groups, one group with normal 25 (OH) vitamin D levels and the other with 25 (OH) vitamin D levels below normal, including insufficiency and deficiency. Age, gender, BMI and C-reactive protein (CRP) levels were similar between the two groups (Table 1). When comparing the two groups according to vitamin D levels, the normal vitamin D group had 44 (83%) patients, compared to 9 patients (17%) with below normal vitamin D levels. The duration of HBsAg and positivity of HBeAg did not show a significant difference between the two groups.

When performing a multiple logistic regression analysis, adjusted by age, gender, BMI, serum 25 (OH) vitamin D, duration of HBsAg positivity, the status of HBeAg, antibodies for HBeAg and the viral load, statistically significant findings were associated with spontaneous seroclearance of HBsAg. Age over 35 years, absence of HBeAg, low viral load (< 100 IU/mL) and duration of HBsAg (> 8 years) were all associated with spontaneous HBsAg seroclearance (Table 2).

DISCUSSION

Vitamin D deficiency has been associated with several adverse health consequences that include autoim-

Table 2 Results of multiple logistic regression analysis of spontaneous hepatitis B surface antigen seroclearance

Variables	OR (95%CI)	<i>P</i> value
Age > 35 (yr)	1.7 (1.25-2.8)	0.05
HBV DNA < 100 IU/mL	3 (2.6-4.2)	0.01
Serum 25 (OH) vitamin D > 20 ng/mL	2.6 (2.4-3.2)	0.02
HBeAg-negative	2.1 (1.2-3.1)	0.02
Duration of HBsAg-positivity > 8 years	1.6 (1.15-2.6)	0.04

Multiple logistic variables found to add a risk for spontaneous hepatitis B antigen clearance. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; 25 (OH): 25-hydroxyvitamin.

mune diseases, cardiovascular diseases and infections^[16]. 1,25-dihydroxyvitamin D3 acts as an immunomodulator targeting various immune cells, including monocytes, macrophages and dendritic cells, as well as T-lymphocytes and B-lymphocytes, hence modulating both innate and adaptive immune responses^[18]. Prospective studies in the involvement of vitamin D in autoimmune disorders are conceptually limited but most cross-sectional studies have shown an inverse relationship between vitamin D levels and disease activity^[19]. A study performed on patients with rheumatoid arthritis concluded that the serum concentrations of vitamin D were inversely related to disease activity^[19,20]. An *in vitro* study concluded that when vitamin D was added, many immunological abnormal characteristics of SLE were resolved, thus suggesting that vitamin D deficiency shifts the immunological response towards the loss of tolerance^[20]. Our study supports this possible link between normal vitamin D levels and the likelihood of a positive clinical and serological response. To the best of our knowledge, this is the first study that has investigated the association between vitamin D levels and spontaneous HBsAg seroclearance. Our findings suggest a link between normal vitamin D levels and the occurrence of spontaneous HBsAg seroclearance. Normal levels of vitamin D had a statistically significant association with spontaneous HBsAg seroclearance. The mechanisms that link vitamin D normal levels with spontaneous HBsAg seroclearance are unknown. HBV infection has also been associated with a variety of immunological manifestations, including non-organ-specific autoantibodies, membranous and membranous proliferative glomerulonephritis, mixed cryoglobulinemia and polyarteritis nodosa^[6]. Moreover, about one third of patients with polyarteritis nodosa are infected by HBV, the vasculitic lesions usually appear during primary HBV infection and are related to the presence of HBeAg. Anti-HBe seroconversion, either spontaneous or induced by antiviral treatment, may lead to a resolution of the vasculitic process^[21]. Another finding of our study was the importance of host and virological factors in spontaneous HBsAg seroclearance, similar to previously published data that indicate that older age, male gender, low viral load and HBeAg-seronegativity are associated with spontaneous HBsAg seroclearance^[3]. Our data supported these findings.

Our study contains some limitations. The link be-

tween vitamin D levels and HBsAg seroclearance was not shown to be causal but associative. The retrospective pattern of this study was unable to determine the cause effect of vitamin D levels to HBsAg seroclearance. More studies with a larger number of patients and with a prospective and controlled design are needed to confirm this hypothesis. Furthermore, this study did show that a very high percentage of spontaneous converters do have high levels of vitamin D but this percentage was not compared to a similar group of patients with hepatitis B without a spontaneous seroclearance. Other limitations are that the study had a small number of participants and did not exclude obese or overweight patients. These patients may have low levels of vitamin D. Patients with hepatic steatosis were also included in our study, although it is known that steatosis is an important predictor host factor for spontaneous HBsAg seroclearance.

In summary, we found a strong correlation between normal vitamin D levels and spontaneous HBsAg seroclearance. Vitamin D deficiency may be a significant risk factor for the lack of HBsAg seroconversion.

COMMENTS

Background

Vitamin D has recently been linked to many autoimmune diseases. Hepatitis B is a viral disease but shows many autoimmune characteristics. Spontaneous hepatitis B seroclearance is an unexplained phenomenon.

Research frontiers

A possible association between serum vitamin D levels and spontaneous hepatitis B surface antigen seroclearance should be investigated.

Innovations and breakthroughs

The authors showed that normal vitamin D levels correlate positively with spontaneous hepatitis B seroclearance.

Applications

This finding may help to expand the therapeutic options for this disease.

Peer review

The authors have performed a cross-sectional study examining the association between serum 25-hydroxyvitamin D levels and the severity of fatty liver disease in overweight and obese children. They report that vitamin D deficiency was significantly greater in obese vs overweight children, and that low vitamin D levels were independently associated with severity of fatty liver disease. This is an interesting finding, which is consistent with other reports of vitamin D deficiency in liver disease, and this paper provides a useful basis for further study.

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