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Original Article

Effect of vitamin D deficiency on the hepatitis-B-vaccine immune response in healthy population

Xiaojia Xiong[1], Min Li[2], Xindeng Tong[1], Yongliang Jiang[1], Xifei Yang[3], Guoxin Hu[1], Yanzhong Peng[1]

[1]Peking University of Shenzhen Hospital, Shenzhen, Guangdong Province, China; [2]The Sixth Affiliated Hospital Guangzhou Medical University, Qingyuan Peoples Hospital, Qingyuan, Guangdong Province, China; [3]Key Laboratory of Modern Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong Province, China

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Abstract: To determine the potential associations among vitamin D, metabolic enzymes, vitamin D receptors (VDR) and hepatitis-Bvaccine and whether vitamin D processing is involved in human hepatitis-B-vaccine immune response mechanisms in a healthy population, 100 healthy subjects who underwent active hepatitis B immunization were included in this study. These subjects included seroprotection (n=30, titer >100 U/L), non-responders (n=40, titer 1-9 U/L) and low-responders (n=30, titer 10-100 U/L). The responders contained low-responders and seroprotection (n=60, titer ≥10 U/L). 25-hydroxyvitamin D (25(OH)D) levels were measured by an chemiluminiscent immunoassay. The expression of vitamin D metabolic enzymes (*CYP24A1* and *CYP27B1*) and *VDR* in peripheral blood mononuclear cells (PBMC) were measured using quantitative RT-PCR. The non-responders showed lower 25(OH)D levels than the responders, but the difference was not significant (*P*>0.05). The gene expression of VDR, CYP27B1 and CYP24A1 in PBMCs was not significantly different among the various groups. Our data suggested thatvitamin D levels were not significantly different between the responders and the non-responders and vitamin D processing by PBMCs maybe not impaired in non-responders.

Keywords: Hepatitis B vaccination, vitamin D, vitamin D related genes, PBMC

Introduction

Hepatitis B virus (HBV) infection is one of the most common problems worldwide. Treatment with antiviral agents has been effective for most patients and vaccination is the most effective approach to prevent HBV infection. However, drug resistance is still serious and there are still 5%-10% of the subjects who fail to produce protective anti-HBsAgtitier after three times of vaccination irrespective of the source of the antigen [1-3]. Factors associated with vaccination responses are age, obesity, virus infection (such as hepatitis C virus) and genetic factors [4, 5]. The underlying mechanisms of the poor immune responses have been proposed, but not elucidated [4, 6, 7].

Vitamin D metabolic enzymes and vitamin D receptors (VDR) are expressed in PBMCs, including antigen-presenting-cells, T cells, B cells and monocytes [8, 9], suggesting a role of vitamin D for human health, especially in the field of human immunology. Metabolite 25-hydroxyvitamin D (25(OH)D) as the biologically inactive metabolite is the most abundant in the circulation. 1,25-dihydroxyvitamin D($1,25(OH)_2D$) is a biologically active hormone from 25(OH)D which is hydroxylized by the enzyme cytochrome *P27B1 (CYP27B1)* and is believed to influence both innate and adaptive immunity [10]. $1,25(OH)_2D$ can bind to the vitamin D receptor (VDR) of immune cells to mediate the functions of vitamin D [11]. The extra-renal synthesis of the active metabolite calcitriol-1,25(OH)2D-by immune cells has been proposed to have immunomodulation properties [12, 13]. In immune cells, a lack of feedback mechanisms compared to kidney cells allows the production of high local concentrations of 1,25(OH)D required for immunomodulation [14].

Vitamin D deficiency may be involved in chronic viral infection, including chronic hepatitis B (HBV) infection [15]. Vitamin D may exert an immune-regulatory effect following BCG vacci-

nation [16]. In patients with chronic kidney disease, vitamin D deficiency is associated with poor antibody formation upon hepatitis B vaccination [17]. But the other study showed that vitamin D levels did not differ between responding and non-responding dialysis patients [18]. Vitamin D deficiency is highly prevalent in many countries [19-21]. Not only the patients with chronic kidney diseases, but also the athletes who live in a sunny country and receive training outdoors are exposed to a high risk of vitamin D deficiency [22].

The objective of this study was to evaluate whether vitamin D deficiency is associated with poor response to hepatitis B vaccination in a healthy population.

Subjects and methods

Subjects

The healthy subjects who underwent active hepatitis B immunization at our Department in China between 2013 and 2015 were included in this study. All the subjects received 20 μg of recombinant HBs antigen vaccine (HBVAXPRO, Sanofi Pasteur, Lyon, France) by deltoid muscle at month 0, 1 and 6 in accordance with CDC guidelines for vaccination. Seroprotection (n= 30, titer >100 IU/L), Non-responder (n=40, titer <10 IU/L) and low-responders (n=30, titer 10-100 IU/L). Responders included low-responders and seroprotection (n=60, titer ≥10 IU/L). All the subjects were seronegative for anti-hepatitis B surface (anti-HBsAb), anti-hepatitis B core (anti-HBcAb), hepatitis B surface antigen (HBsAg), hepatitis C virus antibody, and human immunodeficiency virus (HIV). None of these subjects received immune-suppressive therapy, used vitamin D supplements and suffered the chronic diseases. The body mass index (BMI) of all the subjects is at the scope of 18.5-22.9.

Serum 25-hydroxynitain D measurement

Blood samples were collected from each subject prior to vaccination, and the concentration of 25(OH)D was measured by a chemiluminiscent immunoassay system (CLIA) (Liaison 25OH Vitamin D Total, Diasorin, Saluggia, Italy). According to the international guidelines, vitamin D deficiency could be diagnosed with plasma 25(OH)D<20 ng/ml which was further classified as mild deficiency (10-20 ng/ml) and severe deficiency (<10 ng/ml) [23].

Definition of immune response

Anti-HBs antibody titers were quantified using a commercial colorimetric ELISA kit (Diasorin ETI-AB-AUK3™). Non-responder was defined as anti-HBs titer <10 mIU/ml. Responder was defined as the subjects with anti-HBs antibody ≥10 mIU/ml, and seroprotection as a titer ≥100 IU/L.

Isolation of PBMCs

Peripheral (whole) blood was drawn just before the experiment. The PBMCs were separated via Ficoll-Hypaque density gradient centrifugation (Sigma-Aldrich, St. Louis, MO, USA) and washed twice with Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY, USA). The PBMCs were counted using trypan blue staining.

Gene expression

The expression of CYP27B1, CYP24A1, VDR and GAPDH genes were measured in groups. Total RNA was extracted using the TRIZOL method. The integrity and the purity of the RNA was verified by visualization of rRNA on agarose gels. Equal amounts of RNA (2 μg) were converted to cDNA using TaqMan High Capacity Reverse Transcriptase (Applied Biosystems) in a total reaction volume of 20 μl. For real-time quantitative PCR analysis, the cDNA was diluted in an equal volume of nuclease-free water, and 1 μl of the diluted cDNA was amplified using TaqMan Master Mix and predetermined TaqMan Gene Eexpression Assay primer and probe sets (Applied Biosystems) in a reaction volume of 50 μl in triplicate wells. These intron-spanning primers have been validated by the manufacturer to possess amplification efficiencies of 100%±10% under the assay conditions. The real-time PCR reaction was performed using an ABI 7300 instrument. Primer-Blast for CYP24A1 (fw: 5'-CATTCTTCTGGAGAAGCCCA-3', rv: 5'-CGTTGAAGACTTGTACACGC-3'), CYP27B1 (fw: 5'-GAGCTTGGCAGACATCCCAGGC-3', rv: 5'-CCCTGCACCTGCAGCTCGTGTAG-3'), and VDR (fw: 5'-ATCTGCATCGTCTCCCCAGAT-3', rv: 5'-AGCGGATGTACGTCTGCAGTG-3'). The levels of CYP24A1, VDR and CYP27B1 were normalized to GAPDH within each subject.

Table 1. Subject characteristics, vitamin D levels

	Total subjects (n=100)	Non-responders (n=40)	Responders (n=60)	Seroprotection (n=30)
Age (years)	28.6±4.3	29.5±3.9	27.9±4.5	28.2±4.4
Male:female	51:49	21:19	30:30	15:15
BMI (kg/m^2)	20.5±1.3	20.0±1.2	20.5±1.3	19.9±1.1
25(OH)D (ng/ml)	26.7±9.0	24.6±8.6	28.0±9.1	28.8±8.6
25(OH)D levels, n (%)				
<10 ng/ml	7 (7.0)	4 (10.0)	3 (5.0)	1 (3.3)
<20 ng/ml	24 (24.0)	10 (25.0)	14 (23.3)	6 (20.0)
20-30 ng/ml	29 (29.0)	17 (42.5)	19 (31.7)	10 (33.3)
>30 ng/ml	40 (40.0)	13 (32.5)	27 (45.0)	14 (46.7)

BMI: body mass index.

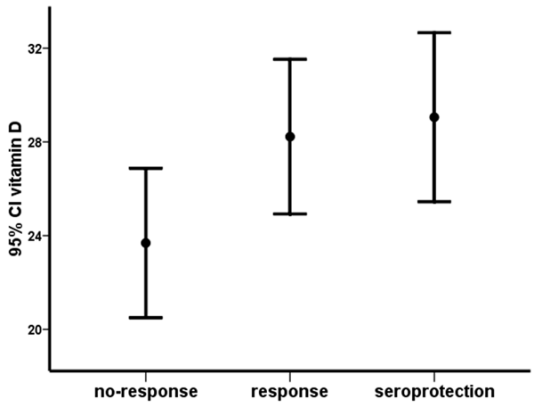

Figure 1. 25(OH)D levels and immune response.

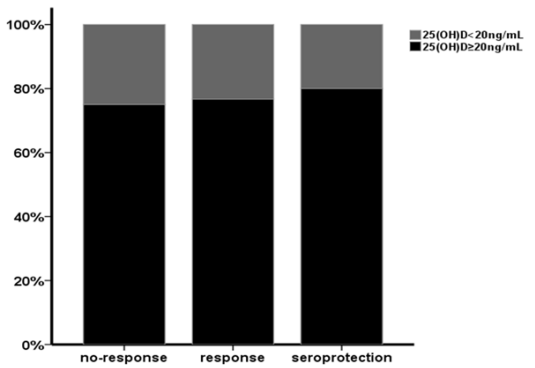

Figure 2. Proportion of patients with vitamin D deficiency and immune response.

Statistical analysis

The statistical analysis was performed using SPSS16.0 statistic software (SPSS Inc, USA). Independent samples-Ttest analysis was used to compare the difference of Mean ± SD of age, BMI, 25(OH), the levels of vitamin D related genes in three groups. One-way ANOVA was used for the difference of 25(OH)D levels and immune response. The difference of proportion of patients with vitamin D deficiency and immune response was using Chi-square test. *P*-values ≤0.05 were considered statistically different.

Results

Subject characteristics

A total number of 100 subjects (51 males and 49 females) with a mean age of 28.6 years were included in the study. The subject characteristics, mean 25(OH)D levels were shown in **Table 1**. Age, sex and BMI were not statistically different between groups (P>0.05).

Vitamin D levels

In non-responders: 13 subjects had a 25(OH)D level within the normal range (>30 ng/ml). Vitamin D deficiency defined as a level <20 ng/ml was observed in 24% of the subjects (**Table 1**). Non-responders showed lower 25(OH)D levels than the subject with responder and seroprotection (**Figure 1**). In the subjects with 25(OH)D concentrations below 20 ng/ml the number of subjects showing no response was greater and the number of responder or seroprotection lower compared to those with a level ≥20 ng/ml, but there were no statistically different (P>0.05) (**Figure 2**). The proportion of subjects with vitamin D deficiency (<20 ng/mL; n=24) and the subjects with sufficient levels (>30 ng/mL; n=40) were no statistically different in the groups (P=0.88 and P=0.37, respectively).

The expression of vitamin D metabolism-related genes

The expression levels of CYP27B1, CYP24A1 and VDR in PBMC were not significantly different between non responders and responders (**Table 2**).

Discussion

A lot of studies suggested that vitamin D may have an immune-regulatory effect in human

Table 2. Mean ± SD of expression levels of vitamin D related genes to GAPDH in non responders and responders

Parameter	Non-responders (n=40)	Responders (n=60)	*p*-value
VDR	0.038±0.010	0.032±0.19	0.50
CYP27B1	0.0098±0.003	0.0095±0.002	0.62
CYP24A1	0.00042±0.00017	0.00044±0.00016	0.46

body. A study suggested that vitamin D deficiency was indeed a predictor of failure to yield anti-HBsAg antibody in patients with CKD [17]. Jhorawat et al. had reported that both at 4 and 7 months after vaccination the difference of vitamin D levels between responders and non-responders did not reach statistic significance in their dialysis patients [18]. We observed that non-responders showed no significantly lower 25(OH)D levels than the responders at baseline before vaccination in the healthy population. So the data of this study did not support the direct effect of vitamin D level on the hepatitis B seroconversion in the healthly population. In our study, the prevalence of vitamin D deficiency (<20 ng/ml) in our subjects was 26%, and the mean of vitamin level was 26.7 ng/ml. According to the studies, vitamin D deficiency was not so highly prevalent in the healthy population in our city (the south of China). We measured the level of vitamin D at baseline before vaccination but the situation after vaccination was unknown. In these healthy participation did not have very low 25(OH)D levels might be the reason of lack statistical significance. Whether vitamin D deficiency is a causal factor for the poor vaccination response is still need more studies.

The contribution of immune cells to serum 1,25(OH)D levels is modest, but these cells can product high local concentrations of 1,25(OH)D in the tissues for the immunomodulation through vitamin D metabolic enzymes. This study showed that the expression profile of CYP27B1, CYP24A1, and VDR was not significantly different in PBMCs between non-response and response at the baseline before vaccination. These results suggested that vitamin D processing may be not impaired in PBMCs of non-responders at baseline before vaccination. Some other studies reported dramatic profound differences in vitamin D metabolism-related-related gene expression profile between CD4+ T cells and PBMCs [24]. Salazar. reported that non-resporders showed a defect in HBsAg reactive CD4+ helper T cells [25]. Thus, whether there is differential expression of vitamin D metabolism-related genes in subgroup immune cell needs to be further analyzed. In the future, we will examine vitamin D metabolism-related gene expression in T cells or other subgroup immune cells.

Conclusion

Present studies demonstrated that in patients with chronic kidney disease, seriousvitamin D deficiency is associated with poor antibody formation upon hepatitis B vaccination. Our study showed that in the healthy population, non-responders showed lower 25(OH)D levels than the responders, but the difference was not significant (P>0.05). And we further detected the vitamin D related genes. The gene expression of VDR, CYP27B1 and CYP24A1 in PBMCs was not significantly different among the various groups. Our data suggested that vitamin D deficiency was not significantly different between the responders and non-responders in the healthy population and that vitamin D processing by PBMCs maybe not impaired in non-responders.

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Disclosure of conflict of interest

None.

Address correspondence to: Yanzhong Peng, Department of Infectious, Perking University of Shenzhen Hospital, Shenzhen 518000, Guangdong Province, China. Tel: +86 13510331758; Fax: +86 755 2550-8584; E-mail: pyz9888@126.com

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