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Impact of Serum 25(OH) D Levels on Immune Responses to Hepatitis B Virus Vaccination in Healthy Adults: A Cross-Sectional Study in Mane and Samalghan City, Iran

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ABSTRACT

Backgrounds: Several studies have elucidated vitamin D as an important immunomodulatory factor regulating immune responses to different viral infections and vaccines. This study aimed to evaluate the impact of 25(OH) D serum levels on immune responses to hepatitis B virus (HBV) vaccine.

Materials & Methods: This study was conducted on 134 healthy individuals aged 18-35 years, referring to health centers for HBV vaccination in Mane and Samalghan city in North Khorasan, Iran from June to September 2021. Demographic data were collected through a questionnaire. Serum 25(OH) D levels were analyzed using commercial sandwich ELISA kits. Anti-hepatitis B surface antibody (anti-HBsAb) levels were determined in blood samples 4-6 weeks post-vaccination.

Findings: The prevalence of vitamin D deficiency and insufficiency among the participants was 46.3 was 34.3%, respectively. The level of 25(OH) D was insignificantly higher in women than in men. There was no significant association between serum 25(OH) D levels and participants' ethnicities and BMI ranges. Anti-HBsAb titer was significantly higher in participants with sufficient vitamin D levels compared to those with insufficient and deficient levels (1835 ± 252.55 vs. 1129 ± 120.7 and 1363 ± 0.125 ng/ml). Serum anti-HBsAb levels post HBV vaccination were significantly higher in women and younger individuals than in men and older individuals, respectively.

Conclusion: This study findings suggest that participants with different serum vitamin D levels produce seroprotective antibody titers post HBV vaccination, while those with sufficient vitamin D levels may produce higher titers against HBV vaccine.

Keywords: Hepatitis B surface antigen, Hepatitis B, Hepatitis B Vaccines, Vitamin D.

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Introduction

Hepatitis B is one of the most common viral diseases in humans, and one million people die every year due to its complications [1]. The main causes of death from this disease are liver cirrhosis and liver cancer, which are the final clinical outcomes of hepatitis [2]. Due to the severe complications as well as the difficult and expensive treatment of hepatitis B, the most important way to control and avoid hepatitis B infection is prevention through the administration of a vaccine containing hepatitis B surface antigen (HBsAg), obtained by recombinant DNA technology [3]. Since 1993, the national vaccination program against hepatitis B has been implemented for newborns in Iran [4]. At present, the vaccine is given to infants at the ages of zero, two, and six months and to high-risk groups at the ages of zero, one, and six months in health centers [4, 5]. The protective threshold of antibody response to hepatitis B virus (HBV) vaccine has been established by clinical studies as an antibody titer of 10 international units (IU) or higher [6]. Although previous studies have indicated that HBV vaccine is highly effective against hepatitis B infection, it is far from perfect in some vaccinated individuals [7]. Several studies have shown that 1-10% of healthy individuals are unable to produce protective antibodies after the end of the vaccination period, these people are considered as non-responders to HBV vaccine [6]. On the other hand, studies on different population groups at different ages have shown that the duration of protective immune responses induced by HBV vaccine varies from 5 to 10 years [5, 6]. In a systematic review and meta-analysis, Tazhibi et al. (2014) demonstrated that the prevalence of non-responders to HBV vaccine among Iranian adult population was approximately 14% [8].

Individual genetics, especially certain haplotypes of HLA, old age, sex, obesity, high smoking, immunodeficiency, low or very high dose of antigen in the vaccine, and inappropriate location and method of injection have been introduced as factors influencing protective immune responses induced by HBV vaccine [9]. Although several investigations have been conducted during the past 20 years, the impact of immunogenic factors on both the efficacy of HBV vaccine and the duration of post-vaccination immunity is not completely understood [9]. Moreover, during the last two decades, several studies have been carried out to increase the immunogenicity of HBV vaccine, especially in non-responders, to increase the duration of protective immune responses induced by HBV vaccine and to reduce the dosage and frequency of booster injections [9, 10]. Furthermore, in recent years, the use of adjuvants has been suggested as a strategy to improve immune responses to viral vaccines such as HBV vaccine in the elderly population and people with comorbidities [11]. Notably, alterations in the host's nutritional status and nutritional interventions are among the potential strategies to enhance immune responses to vaccination [12-14]. Vitamin D is one of the micronutrients whose impacts on immune responses to different viral infections and vaccines have been widely evaluated [15-17].

Moreover, various research studies have extensively demonstrated that vitamin D possesses anti-infective, anti-inflammatory, and immunomodulatory attributes, and its sufficient serum levels play a vital role in promoting the optimal performance of the immune system [18].

In a study conducted by Farnik et al. (2013), patients suffering from chronic hepatitis B and low serum levels of vitamin D exhibited increased replication of hepatitis B virus [19]. Also, in another study by Zitt et al. (2012), the results indicated that vitamin D deficiency in patients with chronic kidney

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disease was associated with poor antibody responses to HBV vaccine [20]. In their research, Mahamid et al. (2013) revealed a significant association between serum levels of vitamin D and spontaneous occurrence of hepatitis B surface antigen seroclearance [21]. Although few studies have investigated the effect of serum levels of vitamin D on immune responses to HBV in healthy adult populations, some studies have evaluated the association between serum levels of vitamin D and hepatitis B virus replication, severity of hepatitis B infection, disease progression, and response to treatment [19-22]. According to Sadarangani and colleagues (2015), the importance and effect of vitamin D on immune responses to HBV vaccine should be studied not only in populations with high incidence of vitamin D deficiency such as hemodialysis patients but also in other healthy populations [15]. Previous research has suggested that serum vitamin D levels may serve as a primary indicator of the body's response to HBV vaccine [17].

Habibesadat et al. (2014) reported a high prevalence of vitamin D deficiency and insufficiency in North Khorasan province, Iran [23]. However, the relationship between serum vitamin D levels and immune responses to HBV vaccine has not yet been fully elucidated.

Objectives: The present study aimed to investigate the effect of serum levels of vitamin D on immune responses to HBV vaccine in healthy adult population in Mane and Samalghan city in North Khorasan, Iran.

Materials and Methods

Subjects: This descriptive cross-sectional study was conducted on 134 healthy individuals aged 18 to 35 years, who referred to health centers for HBV vaccination in Mane and Samalghan city in North Khorasan, Iran from June to September 2021. Exclusion criteria included: taking vitamin D supplementations and immunosuppressive drugs within six months prior to the study, history of hepatitis B infection, BMI (body mass index) lower than 16 and upper than 40, having underlying diseases such as hyperparathyroidism, chronic kidney diseases, liver diseases, diabetes, autoimmune disease, etc. The participants were selected completely voluntarily based on the cluster sampling method. Demographic data (age, race, ethnicity, gender, BMI, etc.) of the participants were collected through a questionnaire.

Vaccination, blood collection, and measurement and assessment of serum 25(OH) D levels: Peripheral venous blood samples were collected from all the participants. Following blood collection, sera were isolated from the blood samples via centrifugation at 2000× g and stored at −80 ◦ C for subsequent analysis. The participants were administered with three doses of HBV vaccine: the initial dose at the time of blood sampling and subsequent doses one and six months later.

Then serum levels of 25(OH) D were measured using commercial sandwich ELISA (enzyme-linked immunosorbent assay) kits (Padtangostar, Iran). The intra-assay and inter-assay precision of the ELISA kit for 25(OH) D was less than 10 and 12%, respectively. According to previous studies [16, 23, 24], 30 and 20 ng/mL were considered as the thresholds of vitamin D insufficiency and deficiency, respectively. Accordingly, the participants with sufficient (30-100 ng/mL), insufficient (20-29 ng/mL), and deficient (> 20 ng/mL) serum 25(OH) D levels were evaluated in terms of anti-hepatitis B surface antibody (anti-HBsAb) titer.

Blood collection and measurement of anti-HBsAb titer: Blood samples were collected from the participants four to six weeks after the third dose of HBV vaccine. Anti-hepatitis B surface antibody (anti-HBsAb) titer was determined by analyzing isolated serum samples using the

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sandwich ELISA method (Padtangostar, Iran). The participants were subsequently classified into three groups based on their serum anti-HBsAb levels: (i) non-responders or sero-negatives (< 10 IU/L), (ii) low responders (10-100 IU/L), and (iii) good responders (> 100 IU/L) [10]. The ELISA kit demonstrated intra-assay and inter-assay precision of 4.9 and 5.1% for the mean HBsAb titers, respectively.

Statistical analysis: GraphPad Prism software Version 5.0 (GraphPad software, USA) was used for statistical analysis of data. Data distribution was analyzed by Kolmogorov–Smirnov test. According to the normality test results, comparisons between groups were performed using one-way ANOVA for parametric variables and Mann-Whitney U test for nonparametric variables. Chi-square test was used to examine the relationship between independent and dependent variables. Data were shown as mean ± standard deviation (SD), and *p* values less than 0.05 were considered as statistically significant.

Findings

Characteristics of sample: In this study, informed consent was obtained from a total of 134 participants. Also, 65.7% (n=88) of the participants were male, and 34.3% (n=34) were female. The average age of the study participants was 21.33 ± 2.34 years. The results showed that most of the study participants had insufficient (34.3%) and deficient (46.3%) serum 25 (OH) D levels. Table 1 summarizes the demographic characteristics of the study participants.

The results indicated that the level of 25 (OH) D was higher in women than in men (23.30 ± 1.81 and 20.85 ± 0.88, respectively); however, this difference was not statistically significant (*p*= .17). There was also no statistically significant association between serum levels of 25(OH) D and participants' various ethnicities and BMI ranges (*p*< .05).

Anti-hepatitis B surface antibody titer in participants with different serum vitamin D levels: The mean anti-HBsAb titer in the participants before vaccination and 4-6 weeks post vaccination was 3.74 ± 0.29 and 1340.44 ± 85.11 ng/mL, respectively. Of 134 individuals vaccinated with HBV vaccine, 130 (97%) were good responders (> 100 IU/L), whereas 2 (1.5%) were low responders (10-100 IU/L), and 2 (1.5%) were non-responders (< 10 IU/L).

This study results showed that the mean anti-HBsAb titer post HBV vaccination was significantly higher in women than in men (*p*= .004, Table 2). It was also evident that the mean anti-HBsAb titer post HBV vaccination was significantly higher in participants aged <21 years (*p*= .007) than in individuals aged ≥21 years. Table 2 compares the mean anti-HBsAb titer in individuals with different age, gender, and serum 25(OH) levels.

The comparative analysis results indicated that the difference in anti-HBsAb titer between participants with sufficient, insufficient, and deficient serum 25(OH) D levels was significant (*p*= .018). The mean anti-HBsAb titers in participants with sufficient, insufficient, and deficient serum 25(OH) D levels were 1835 ± 252.55, 1129 ± 120.7, and 1363 ± 0.125 ng/mL, respectively (Figure 1).

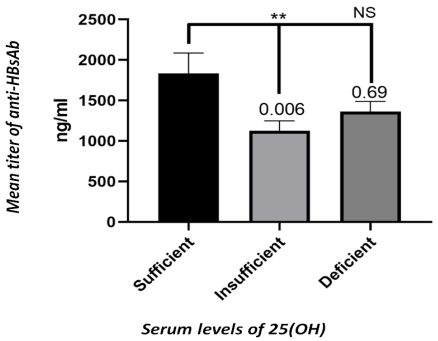

Figure 1) Comparison of the mean anti-HBsAb titer in individuals with different serum levels of 25(OH). The serum levels of anti-HBsAb were significantly higher in individuals who had sufficient levels of vitamin D compared to those who had insufficient levels. * *P* values less than 0.05 are significant. NS: Non significant

Discussion

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Table 1) Demographic characteristics of the study participants and their classification based on serum total 25(OH)D

Characteristics	N=134
Age (years)	21.33 ± 2.34
Gender, n (%)	
Male	88 (65.7)
Female	46 (34.3)
Marital status, n (%)	
Single	129 (96.3)
Married	5 (3.7)
Education levels, n (%)	
Low (elementary school)	24 (17.9)
Middle (high school)	43 (32.1)
High (college or university)	67 (50)
BMI	22.63 ± 2.96
Ethnicity, n (%)	
Persians	89 (66.4)
Khorasani Turks	15 (11.2)
Khorasani Kurds	14 (10.4)
Turkomen	11(8.2)
Other (Tats, Arabs, Baloch)	5 (3.7)
Serum total 25(OH)D (ng/L)	21.70 ± 9.93
Adequate (30-100 ng/mL)	26 (19.40)
Insufficient (20-29 ng/mL)	46 (34.32)
Deficient (> 20 ng/mL)	62 (46.26)

* BMI: Body Mass Index

Table 2) Comparison of the mean anti-HBsAb titer in individuals with different age, gender, and serum levels of 25(OH). It was noticed that males displaying Insufficient 25(OH) levels or a deficiency of 25(OH) tend to have significantly higher anti-HBsAb titer compared to females with corresponding 25(OH) levels. Furthermore, individuals under the age of 21 years with deficiency of 25(OH) had significantly higher serum levels of anti-HBsAb titer compared to those aged ≥21 years

Variable		Gender		Age (years)	
		Male	Female	21 >	21≤
Sufficient	Mean ± SD	2105 ± 520.0	2228 ± 454.4	2070 ± 607.3	2237.0 ± 401.2
	P-value	.866		.818	
Insufficient	Mean ± SD	856.2 ± 140.3	1382.0 ± 183.0	1311 ± 181.3	1019 ± 158.7
	P-value	.028 *		.244	
Deficient	Mean ± SD	1135 ± 170.7	1784 ± 121.7	1682 ± 145.0	982.3 ± 189.8
	P-value	.011 *		.004 *	

* *P* values less than 0.05 are significant.

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Since the introduction and approval of the recombinant HBV vaccine by the FDA (Food and Drug Administration) in 1986, there have been argues about the factors that could affect human immune responses to this vaccine [25]. Vitamin D is one of the factors suspected to be effective in improving immune responses to HBV vaccine. In this research, the influence of serum concentration of 25-(OH) D at the time of HBV vaccination on anti-HBsAb titer 4-6 weeks post vaccination was investigated in healthy adults.

The results showed that the prevalence of vitamin D deficiency and insufficiency among the participants was 46.3 and 34.3%, respectively. This finding indicates that most of the participants had insufficient and deficient levels of vitamin D, which is a concern, given that previous research has suggested that low vitamin D levels may be associated with an increased risk of developing infectious diseases [26]. However, this finding is consistent with previous research results suggesting that vitamin D insufficiency or deficiency is a common problem, particularly among younger populations [27]. Shakeri et al. (2017) and Moradzadeh et al. (2006) have also reported similar results [24, 28].

The results showed no significant difference between men and women in the serum level of 25(OH) D, which in inconsistent with the results of another study by Shakeri et al. (2017), indicating a higher prevalence of vitamin D deficiency and insufficiency in women [23, 24]. This difference may be due to differences in participants' age and geographical regions studied.

There are different reports on the percentage of vaccinated individuals who may develop effective immune responses following complete HBV vaccination. Studies conducted in Iran and other parts of the world have reported that 90-95% of people vaccinated against hepatitis B virus successfully respond to vaccination [7, 8], which is consistent with the present study results indicating 97% effective response to HBV vaccine. In this study, only a small proportion of vaccinated individuals did not respond to HBV vaccine and were classified as low or non-responders. Various factors including age, obesity, smoking, immune status, and genetic factors are involved in not responding to the standard dose of hepatitis B virus vaccine [9]. Among these factors, genetic factors play an important role in not responding to HBV vaccine. For example, certain human leukocyte antigen (HLA) gene polymorphisms are associated with poor vaccine response [29].

Interestingly, it was found that individuals with different serum 25(OH) D levels produced different anti-HBsAbs titers following vaccination. In other words, anti-HBsAb titer was significantly higher in individuals with sufficient 25 (OH) D levels than in individuals with insufficient or deficient 25 (OH) D levels (Figure 1). In line with the present study results, Kashi et al. (2021) conducted a study on 447 healthy adults and proved that low vitamin D level at the time of initial vaccination was associated with a poorer response to HBV vaccine [30]. Additionally, Jafarzadeh et al. (2017) conducted a study to evaluate the relationship between vitamin D status and persistence of anti-HBsAb and immune response to booster immunization 20 years after receiving the primary HBV vaccination. Their study findings suggested that individuals who maintained seroprotective levels of anti-HBsAb (≥10 IU/L) exhibited elevated concentrations of vitamin D. Furthermore, individuals who exhibited an anamnestic response to booster vaccination revealed considerably greater concentrations of vitamin D than those who did not display such a response [31]. In addition to conducting

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several studies on healthy subjects, Zitt et al. (2012) demonstrated that in patients with chronic kidney disease, vitamin D deficiency was associated with poor antibody response to HBV vaccine [20]. However. Viard et al. (2016) claimed that there was no significant association between 25(OH) D levels and immune responses elicited against HBV vaccine [32], which is different from the current study results. This inconsistency may be explained by the fact that they conducted their study on HIV patients but not on healthy populations.

It was also found that serum levels of anti-HBsAb post HBV vaccination were significantly higher in women and individuals under 21 years of age than in men and individuals over 21 years of age, respectively (Table 2). In line with the present study results, previous studies have demonstrated that women have higher anti-HBsAb titers than men, and anti-HBsAb titers decrease with age [33, 34].

Conclusion

Taken together, these results suggest that healthy individuals with different serum vitamin D levels produce seroprotective antibody titers post HBV vaccination, while subjects with sufficient vitamin D levels may produce higher titers against HBV vaccine. These findings may be partially influenced by the age range of the study participants, the season and geographical area studied, the small sample size, and the method used to measure 25 (OH) D levels.

An in-depth analysis of the correlation between vitamin D status and permanence of anti-HBsAb titer, as well as the relationship between anti-HBsAb titer following HBV vaccination and vitamin D-binding protein concentration and vitamin D receptor gene polymorphism, may promote a comprehensive comprehension of the effects of serum vitamin D status on immune responses to HBV vaccine in healthy adult populations.

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Ethical permissions: The study was approved by the Ethics Committee of North Khorasan University of Medical Sciences, Bojnourd, Iran (Ethic approval Code: IR.NKUMS.REC.1397.065).

Authors' contributions: M.R conceived the study, M.R and H.N.A designed the study, Z.A and S.K completed the questionnaire, S.M.J and H.N performed the experiments, M.R.S and Z.A performed statistical analyses, M.R, H.N.A, and S.M.J prepared the manuscript, and S.M.J and M.R.S edited the manuscript. All authors read and approved the final manuscript.

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References

1. Lavanchy D, Kane M. Global epidemiology of hepatitis B virus infection. Hepatitis B virus in human diseases: Springer; 2016. p. 187-203.
2. Tan Y-J. Hepatitis B virus infection and the risk of hepatocellular carcinoma. World journal of gastroenterology: WJG. 2011;17(44):4853.
3. Degli Esposti S, Shah D. Hepatitis B in pregnancy:

[DOI: 10.61186/iem.9.2.157]

challenges and treatment. Gastroenterology Clinics. 2011;40(2):355-72.
4. Moghadami M, Dadashpour N, Mokhtari AM, Ebrahimi M, Mirahmadizadeh A. The effectiveness of the national hepatitis B vaccination program 25 years after its introduction in Iran: a historical cohort study. Brazilian Journal of Infectious Diseases. 2020;23:419-26.
5. Alavian SM, Zamiri N, Gooya MM, Tehrani A, Heydari ST, Lankarani KB. Hepatitis B vaccination of adolescents: a report on the national program in Iran. Journal of public health policy. 2010;31(4):478-93.
6. Van Damme P. Long-term protection after hepatitis B vaccine. Oxford University Press; 2016. p. 1-3.
7. Chen D-S. Hepatitis B vaccination: the key towards elimination and eradication of hepatitis B. Journal of hepatology. 2009;50(4):805-16.
8. Tazhibi M, Hajivandi A, Tafti AD, Fallahzadeh H. The efficacy of hepatitis B vaccine in Iranian population: A systematic review and meta-analysis. Journal of education and health promotion. 2014;3.
9. Yang S, Tian G, Cui Y, Ding C, Deng M, Yu C, et al. Factors influencing immunologic response to hepatitis B vaccine in adults. Scientific reports. 2016;6(1):1-12.
10. Said ZNA, Abdelwahab KS. Induced immunity against hepatitis B virus. World journal of hepatology. 2015;7(12):1660.
11. Haq K, McElhaney JE. Immunosenescence: influenza vaccination and the elderly. Current opinion in immunology. 2014;29:38-42.
12. Calder PC, Berger MM, Gombart AF, McComsey GA, Martineau AR, Eggersdorfer M. Micronutrients to Support Vaccine Immunogenicity and Efficacy. Vaccines. 2022;10(4):568.
13. Inserra F, Tajer C, Antonietti L, Mariani J, Ferder L, Manucha W. Vitamin D supplementation: An alternative to enhance the effectiveness of vaccines against SARS-CoV-2? Vaccine. 2021;39(35):4930.
14. Fabrizi F, Dixit V, Martin P, Jadoul M, Messa P. Meta-analysis: the impact of nutritional status on the immune response to hepatitis B virus vaccine in chronic kidney disease. Digestive diseases and sciences. 2012;57(5):1366-72.
15. Sadarangani SP, Whitaker JA, Poland GA. "Let there be light": the role of vitamin D in the immune response to vaccines. Expert review of vaccines. 2015;14(11):1427-40.
16. Shakeri H, Azimian A, Ghasemzadeh-Moghaddam H, Safdari M, Haresabadi M, Daneshmand T, et al. Evaluation of the relationship between serum levels of zinc, vitamin B12, vitamin D, and clinical outcomes in patients with COVID-19. Journal of medical virology. 2022;94(1):141-6.
17. Hasanzadeh E, Moludi J, Kheirouri S, Naeimi AF, Alizadeh M. The predictive roles of obesity and serum vitamin D levels in body response to hepatitis B vaccine. PROGRESS IN NUTRITION. 2018;20:210-7.
18. Caprio M, Infante M, Calanchini M, Mammi C, Fabbri A. Vitamin D: not just the bone. Evidence for beneficial pleiotropic extraskeletal effects. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2017;22(1):27-41.
19. Farnik H, Bojunga J, Berger A, Allwinn R, Waidmann O, Kronenberger B, et al. Low vitamin D serum concentration is associated with high levels of hepatitis B virus replication in chronically infected patients. Hepatology. 2013;58(4):1270-6.
20. Zitt E, Sprenger-Mähr H, Knoll F, Neyer U, Lhotta K. Vitamin D deficiency is associated with poor response to active hepatitis B immunisation in patients with chronic kidney disease. Vaccine. 2012;30(5):931-5.
21. Mahamid M, Nseir W, Elhija OA, Shteingart S, Mahamid A, Smamra M, et al. Normal vitamin D levels are associated with spontaneous hepatitis B surface antigen seroclearance. World Journal of Hepatology. 2013;5(6):328.
22. Ko W-S, Yang Y-P, Shen F-P, Wu M-C, Shih C-J, Lu M-C, et al. The Study of Correlation between Serum Vitamin D3 Concentrations and HBV DNA Levels and Immune Response in Chronic Hepatitis Patients. Nutrients. 2020;12(4):1114.
23. Habibesadat S, Ali K, Shabnam JM, Arash A. Prevalence of vitamin D deficiency and its related factors in children and adolescents living in North Khorasan, Iran. Journal of Pediatric Endocrinology and Metabolism. 2014;27(5-6):431-6.
24. Shakeri H, Pournaghi S-J, Hashemi J, Mohammad-Zadeh M, Akaberi A. Do sufficient vitamin D levels at the end of summer in children and adolescents provide an assurance of vitamin D sufficiency at the end of winter? A cohort study. Journal of Pediatric Endocrinology and Metabolism. 2017;30(10):1041-6.
25. Pattyn J, Hendrickx G, Vorsters A, Van Damme P. Hepatitis B Vaccines. The Journal of infectious diseases. 2021;224(Supplement_4):S343-S51.
26. Kearns MD, Alvarez JA, Seidel N, Tangpricha V. Impact of vitamin D on infectious disease. The American journal of the medical sciences. 2015;349(3):245-62.
27. Haimi M, Kremer R. Vitamin D deficiency/insufficiency from childhood to adulthood: Insights from a sunny country. World journal of clinical pediatrics. 2017;6(1):1.
28. Moradzadeh K, Keshtkar A, HOSSEIN NA, Rajabian R, Nabipour I, OMRANI G, et al. Normal values of vitamin D and prevalence of vitamin D deficiency among Iranian population. 2006.

[DOI: 10.61186/iem.9.2.157]

29. Hennig BJ, Hall AJ. Host genetic factors in hepatitis B infection, liver cancer and vaccination response.
30. Kashi DS, Oliver SJ, Wentz LM, Roberts R, Carswell AT, Tang JC, et al. Vitamin D and the hepatitis B vaccine response: a prospective cohort study and a randomized, placebo-controlled oral vitamin D3 and simulated sunlight supplementation trial in healthy adults. European journal of nutrition. 2021;60(1):475-91.
31. Jafarzadeh A, Keshavarz J, Bagheri-Jamebozorgi M, Nemati M, Frootan R, Shokri F. The association of the vitamin D status with the persistence of anti-HBs antibody at 20 years after primary vaccination with recombinant hepatitis B vaccine in infancy. Clinics and Research in Hepatology and Gastroenterology. 2017;41(1):66-74.
32. Viard J-P, Assuied A, Lévy Y, Souberbielle J-C, Thiébaut R, Carrat F, et al. No positive association between vitamin D level and immune responses to hepatitis B and Streptococcus pneumoniae vaccination in HIV-infected adults. PLoS One. 2016;11(12):e0168640.
33. Gomes LC, Sanson MCG, Brainin P, de Melo MdCV, de Souza RM, Mazaro J, et al. Levels of hepatitis B antibody titers are affected by age and doses gap time in children from a high endemic area of the western Amazon. Plos one. 2021;16(7):e0253752.
34. Ruggieri A, Gagliardi MC, Anticoli S. Sex-dependent outcome of hepatitis B and C viruses infections: synergy of sex hormones and immune responses? Frontiers in Immunology. 2018;9:2302.

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