Jordan Medical Journal, Volume 56, No.3 2022
DOI: https://doi.org/10.35516/jmj.v56i3.358

Evaluation of Serum Vitamin D_3 Levels and Factors Associated with Chronic Hepatitis B: A Case-Control Study

***Masood Ziaee*[1], *Freshteh Osmani*[2, 3]✉**

Abstract

Introduction: Chronic hepatitis B (CHB) is caused by hepatitis B virus infection. It has been shown that vitamin D_3 has very important biological effects in the immune system and host response to viral infection. This study aimed to identify potential risk factors of vitamin D deficiency among CHB patients in Birjand, Iran.

Materials and Methods: In this case-control study, there were 292 patients with CHB and 304 healthy subjects as a control group in the outpatient clinic of the Infectious Diseases Department from January, 2017 to December, 2018. Of all the subjects, 62.7% of the control and 48.1% of the case groups were male. We quantified the levels of total vitamin D_3 in their serum samples. We employed *t*- and chi-square tests along with logistic regression to determine the association between the variables of interest and vitamin D3 deficiency related to CHB ($p<0.05$).

Results: The mean age and vitamin D_3 levels of the case group were 39.9 ±13.3 years and 20.76 ±15.53ng/ml, and for the control group 43 ±13.3 years and 19.07 ±12.41 ng/ml. No significant association between vitamin D_3 deficiency and CHB risk factors was found ($p>0.05$). The prevalence of vitamin D_3 inadequacy was high in both HBV patients (63%) and in the healthy group (52.9%). The frequency distribution for the vitamin D3 serum level was significantly different in the two groups ($p=0.001$).

Conclusions: According to the results, a high risk of vitamin D_3 deficiency related to CHB was not found among CHB patients in Birjand, in contrast to previous literature. Possible reasons for these findings were discussed, although future comprehensive studies are needed to clarify underlying mechanisms and real association.

Keywords: Vitamin D_3 level, liver disease, chronic HBV infection.

(J Med J 2022; Vol. 56 (3):226-234)

Received *September, 8, 2020* *Accepted* *June, 7, 2021*

Introduction

The liver is a major site for vitamin D_3 synthesis, where 25-hydroxylation occurs and a large portion of vitamin D_3 binding protein is manufactured [1]. Vitamin D_3 is an important immune modulator that plays an emerging role in inflammatory and metabolic liver diseases. Due to its immunomodulatory role, there is growing evidence on the interrelationship between vitamin D_3 and different chronic liver diseases in various stages [2–3]. Worldwide, almost 240 million individuals are chronically infected with HBV [4]. It has been shown that vitamin D_3 has very important biologic effects

[1] Infectious Disease Research Center, Birjand University of Medical Sciences, Birjand, Iran
[2] Assistant Professor of Biostatistics, Faculty of Health, Birjand University of Medical Sciences, Iran
[3] Dentistry Clinical Research Development Center, Birjand University of Medical Sciences, Birjand, Iran
✉Corresponding author: Dentistry Clinical Research Development Center, Birjand University of Medical Sciences, Birjand, Iran. dr.osmani68@gmail.com

[5–6].Vitamin D_3 levels may affect the immune system and host response to viral infections, such as HBV infection. In contrast, the relationship between vitamin D_3 metabolism and CHB is less well characterized [7].

Recent evidence from various studies has suggested that low levels of vitamin D_3 are associated with high levels of HBV replication in CHB infection. However, a study on spontaneous seroclearance of HBs-Ag found a positive correlation between HBs-Ag seroclearance and vitamin D_3 levels [8]. Another study demonstrated a significant association between higher levels of HBV replication and low levels of serum vitamin D_3 in CHB patients [9]. The relationship between liver diseases such as hepatitis B may be of great interest to clinicians. In addition, the role of vitamin D_3 may also affect disease progression in patients with HBV infection. Given this information, we hypothesized that vitamin D_3 level could be a factor in the very low serum levels of CHB patients, and so we aimed to determine the pattern, associated factors, and symptoms related to vitamin D_3 deficiency in CHB patients in Birjand city compared to healthy individuals.

Materials and Methods

Study Design

This case-control study was carried out in Khorasan Jonoobi province of Iran in 2018 in the outpatient clinic of the Infectious Diseases Department.

Sampling

We randomly selected 292 patients with CHB (Hbs-Ag positive, anti-HBs negative) from clinic according to a calculated sample size with a power of 90% by the following formula:

$$n = \left(\frac{z_{1-\frac{\alpha}{2}} + z_{1-\beta}}{0.5 \ln(\frac{1+r}{1-r})} \right)^2 + 3$$

In addition, 304 naturally immunized persons (HBsAg negative, anti-HBs with normal liver enzymes) who had not had antiviral treatment were included. These subjects had been referred to clinic for other reasons.

Vitamin D_3 Level Classification

Total vitamin D_3 levels were measured in serum samples. The WHO considers that a level above 30 ng/mlor is sufficient [10)]. Thus, vitamin D_3 status was classified as normal (≥30 ng/ml), insufficient (20–29.9 ng/ml), or deficient (<20 ng/ml).

Laboratory Tests

For laboratory tests, 10 cc of venous blood was taken from patients and healthy controls. The serum levels of vitamin D_3 were measured using a COBAS e411 analyzer, manufactured by Mannheim Roch Diagnostic Gmbh in Germany, with the Elecsys kit (REF 0589413). Other tests were performed on patients and healthy controls according to laboratory routines. Levels of alanine transaminase (ALT), aspartate transaminase (AST), HbA_1C (glycated hemoglobin) and the bilirubin levels of liver enzymes were measured by the ARCHITECT i System biochemical auto-analyzer. Levels of total cholesterol, LDL, HDL, FBS and BMI were also measured.

The inclusion criteria for CHB patients were: patients admitted to the Infectious Diseases Outpatient Clinic with a diagnosis of CHB with the approval of the infectious diseases specialist, according to clinical and

serological signs; willingness to participate in the study; not in receipt of supplementation or injection of calcium and vitamin D_3 in the last six months; and, aged ≥18 years. In addition, 304 healthy subjects without a history of hepatitis B who had come to the clinic for common infectious diseases such as the common cold served as the healthy control group. The exclusion criteria for both the control and healthy groups were: severe renal disease, history of cardiovascular disease, co-infection with cancer, pregnancy, diabetic disease, thyroid disorders, other viral hepatitis (HCV, HDV, HIV), and other causes of liver disease such as alcohol consumption. The variables of interest were age, sex, body mass index (BMI) and clinical symptoms such as fasting blood sugar (FBS), HbA_1C, blood pressure (BP) and HBs-Ag.

Statistical Analysis

Statistical analysis was performed using SPSS version 21.0 and descriptive statistics were used to describe the data. A chi-square test was performed to determine the significant differences of the presence of symptoms related to vitamin D_3 deficiency between the study groups. Logistic regression was performed to determine the significant association between the variables of interest and the presence of vitamin D_3 deficiency related to CHB. The significance in all these tests was two-tailed with a 5% significant level.

Ethics Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Birjand University of Medical Sciences' ethics board, reference number: IR.BUMS.REC.1398.324.

Results

Of all subjects in the study, 48.6% were male in the case group, with a mean age of 29 ±5.3; and 52.2% female, with a mean age of 31.5 ±7.8; among the 304 healthy subjects, 22.1% were male with a mean age of 23.1 ±7.7, and 77.8% were female, with a mean age of 29.1 ±11.7. Gender distribution was thus similar in the patient groups (Table1). Healthy controls were younger than patients but no significant difference between them was observed (p> 0.05). The mean ages of the CHB and control groups were: 32.9 ±12.3 years and 28.18 ±11.21 years, respectively. There was no significant difference in HbA_1C level between genders at 5.71% vs. 5.43% (p=0.343) (Table 1).

Mean vitamin D_3 serum levels in the CHB and control groups were 20.76 ±15.53ng/ml and 19.07 ±12.41ng/ml, respectively, with no significant differences (p=0.31) (Table 2), when categorized as deficient, insufficient, or sufficient. Among the healthy subjects, vitamin D_3 levels were classified as 52.8%, 25.96%, and 21.15%, respectively. Of 292 patients, 184 (63.1%), 56 (19.2%), and 52 (17.8%) had vitamin D_3 deficiency, insufficiency, or sufficient vitamin D_3 serum levels, respectively. The prevalence of vitamin D_3 insufficiency was high among healthy individuals (26%) as well as in CHB patients (19.2%). Vitamin D_3 levels frequency distribution showed a significant difference in two groups (p=0.001). Vitamin D_3 serum levels in the CHB patients were significantly lower in the sufficient category (p<0.001), but the healthy group had a higher prevalence of patients falling into the insufficient category. In contrast, deficient vitamin D_3 levels were significantly higher in the CHB group (Table 3).

A negative correlation was found between

vitamin D_3 levels and BMI and HbA_1C in patients. Also, a positive correlation was seen between age and vitamin D_3 levels, but in the control group none of these had significance values (p>0.05) (Table 4).

The results of the comparison of the laboratory characteristics of the CHB patients and healthy controls showed that the mean ALT in patients was 32.82 IU/ML (8–117) and AST was 32.21 IU/ML (10–167). Of all patients, 21.04% had ALT and 17.54% had AST higher than 40 IU/ML, while in the healthy group, only 15% had ALT and 5% had AST higher than 40 IU/ML. Also, there was a significant difference in ALT and AST between the two groups (p=0.001) (Table 5).

Based on the results, between other variables such as LDL, HDL, BMI, age, sex and vitamin D_3 levels, no significant difference was observed (Table 5].

According to the logistic regression results, the risk of vitamin D_3 deficiency in men is 45% higher than in women, which is not statistically significant (OR=1.54, p=0.114). Among these patients, 75.3% were urban and 24.7% were rural residents. The distribution of vitamin D3 deficiency in rural people is more than in the urban population, and was significant in both groups (OR=2.321, p=0.004). There was no significant difference in the BMI distribution between the two groups (p=0.1.3). In general, 47.3% of the patients had a desirable weight, 35.5% were overweight and 8.2% had a BMI of more than 30 (Table 6).

Discussion

Conducted for the first time in this province (in the east of Iran), this study of vitamin D_3 patterns in patients with CHB also investigated factors associated with vitamin D_3 deficiency in CHB. In a study covering the period 1990–2010, the prevalence of vitamin D_3 deficiency in Iranian society was, according to the results for both sexes, either moderate or significant [11]. Nghiem et al. showed that vitamin D_3 deficiency existed in many CHB patients, and this deficiency had a relationship with the complications and outcome of the disease. Decreased liver function due to HBV-induced injuries to liver cells can be one of the causes of vitamin D_3 deficiency in CHB [12–13]. In our study, the results showed that different degrees of prevalence of vitamin D_3 deficiency existed in the patients and healthy controls. The duration of exposure to sunlight is an important factor in the changes in vitamin D_3 levels [14]. In some studies, vitamin D_3 levels were found to be inversely proportional to HBVDNA viral load and sufficient levels of vitamin D_3 [15].

Previous studies have shown an association between D_3 level and CHB [16–18]; however, this study showed that, in healthy subjects, the vitamin D_3 level was lower than CHB patients, but this difference was not significant. The low serum vitamin D_3 level may be of great advantage to persistent infections of HBV [19]. In our study, vitamin D_3 insufficiency/deficiency accounted for 82.1% of patients, which was lower than the incidence reported from Japan and Germany [20–21]. The key difference between this study and those from these nations is that a significant percentage of the control group in our study had severe vitamin D_3 deficiency. However, our results indicate that the relatively high prevalence of vitamin D_3 deficiency is similar to those in [22], possibly because sunshine hours differ with latitude, and sunlight-related ultraviolet rays are a substantial factor in vitamin D_3 synthesis [22]. However, our study showed no association between serum

vitamin D_3 levels and biological factors in both study groups, and this failure could be due to variations in age, HBV genotype, and racial background. Moreover, a higher than normal BMI is considered an effective factor in the level of vitamin D_3 stores [23], but in our study no significant relationship was found between the serum levels of vitamin D_3 and BMI. This result is not in line with other studies [21–22].

In the current study, no association was found between the biochemical parameters and the serum vitamin D_3 level by sex. It is unclear whether vitamin D_3 deficiency is effective in CHB [16]. We assume that sunlight exposure time was shorter in the healthy subjects since they may go outside less frequently than patients with CHB.

A high prevalence of vitamin D_3 insufficiency (26%) in healthy individuals compared with CHB patients (19.2%) could be associated with the consumption of foods with low levels of vitamin D_3 and lower sun exposure than in the other group. Also, the results of Tabrizi et al., in a systematic review, showed a high prevalence of vitamin D_3 deficiency in the Iranian population [26], possibly due to spending more time at home, and/or because of the type of clothing worn, which may result in reduced vitamin D3 synthesis. Also, the vitamin D_3 content is low in the Iranian diet [24].

In the published records on the prevalence of vitamin D_3 deficiency showed that vitamin D_3 deficiency prevalence was significantly different based on geographical regions in Iranian population [10]. In this study, however, vitamin D_3 deficiency was not significantly correlated with liver function parameters, probably due to the fact that vitamin D_3 serum levels are affected by multiple factors. Roughly, one billion people worldwide are apparently vitamin D_3 deficient [11, 25]. In line with a previous study [3], our results confirm an inverse correlation between BMI and HbA_1C loads and vitamin D_3 levels in the patient groups.

Limitations

The limitations of this study were the influence of several factors on serum vitamin D_3 levels, such as seasonal variation, diet, and geographical habitation. However, any information about these affecting factors for study subjects was unavailable.

Conclusion

We found in our study that it is likely that the Birjand population suffers from an insufficiency of vitamin D_3. This was confirmed by the finding that only 21% of all the subjects (n=304) had sufficient levels of vitamin D_3, indicating the need to consume foods rich in vitamin D_3, be exposed to the sun for longer durations, and/or supplement vitamin D_3 in healthy people and patients with CHB. It is noteworthy that although vitamin D_3 deficiency was apparent in these patients, this deficiency was also noticeable in the healthy group. Therefore, a comprehensive study is recommended to identify the causes.

Acknowledgements

We would like to thank all the study subjects for their participation.

Competing interests

All authors declare that they have no competing interests.

Table 1. The baseline characteristics of CHB group by sex

	Male	Female	*p* value
HbA_1C (mmol/mol**)**	5.71 ±1.24	5.43 ±1.11	0.343
BMI (kg/m^2)	25.6 ± 3.1	24.7 ±2.9	0.595
Age (year)	23.33 ±7.76	29.79 ±11.77	0.033
BP1[1] (mmHg)	12.38 ±1.75	11.96 ±2.0	0.243
BP2[2] (mmHg)	7.9919 ±1.0	7.68 ±.861	0.084
FBS[3] (mg/dL)	97.08 ±24.5	121.44 ±123.82	0.131

[1]-blood pressure (systolic)
[2]- blood pressure (diastolic)
[3]-Fasting blood sugar

Table 2. Comparison between serum vitamin D levels in the study groups (patients vs. control)

Group	N	Serum vitamin D (ng/mL)	*p* value
		Mean ± SD	
Patients	292	20.74 ±15.19	*p*=0.312
Control	304	19.06 ±12.41	

Table 3. Distribution frequency of serum vitamin D level in the study groups

Group	n	Serum vitamin D level			*p* value
		Deficiency n (%)	Insufficiency n (%)	Sufficient n (%)	0.001
Patients	292	184 (63)	56 (19.2)	52 (17.8)	
Control	304	157 (52.9)	79 (26.0)	68 (21.2)	

Table 4. Correlation between serum vitamin D levels and assessed variables in the study groups

Variables	serum vitamin D			
	Case		Control	
	r	*p*	*r*	*p*
Age (year)	0.371	0.07	0.159	0.189
BMI (kg/m^2)	-0.039	0.69	0.106	0.382
HbA_1C (mmol/mol**)**	-0.04	0.78	-	-

Table 5. Comparison of laboratory properties and biochemical variables analysis of HBV patients and healthy controls

Variables		CHB	Healthy control	*p* value
ALT (IU/L)	Mean ±SD	34.12 ±7.03	26.12	0.001
	Range	(11–117)	(9–135)	
AST (IU/L)	Mean ±SD	29.98 ±12.19	23.54	0.001
	Range	(14–176)	(9–135)	
HB (g/dl)	Mean ±SD	13.31 ±9.13	12.93	0.931
	Range	(8.60–17.30)	(9.10–16.70)	
HDL (mg/dl)	Mean ±SD	162.72 ±31.2	182.27	<0.003
	Range	(94–284)	(98–352)	
LDL (mg/dl)	Mean ±SD	95.39 ±18.43	113.04	0.052
	Range	(23–239)	(18-345)	

Table 6. Presence of vitamin D deficiency symptoms against the variables of interest in the study groups

Item		CHB	Healthy control	Odds ratio (95% CI)	*p* value
Gender	**Male**	182 (62.7%)	150 (48.1%)	OR = 1.540	0.114
	Female	110 (37.3%)	154 (51.9%)	*	
Residence	**Urban**	197 (70.9%)	175 (62.1%)	*	
	Rural	95 (29.1%)	129 (37.9%)	2.321 (1.31–4.11)	0.004
BMI	**Mean ± SD**	24.39 ±4.60	25.26 ± 3.79		
(kg/m2)	**Normal (18.5–24.9)**	47.3%	44.4%	*	
	Overweight (25–29.9)	35.5%	40.04%	0.72 (0.568–1.07)	0.135
	Obese (>30)	8.2%	11.1%	0.86 (0.38–1.93)	0.081
BP1		12.28 ±1.29	11.42 ±1.3	0.39(0.16–1.78)	0.363
BP2		7.49 ±.971	7.23 ±.368	0.09(0.031–0.41)	0.671
Age group	**18–24**	23 (8.6)	22 (9.3)	0.12 (0.58, 1.07)	0.133
n (%)	**25–34**	76 (25.9)	64 (18.3)	0.997 (0.565–1.759)	0.091
	35–44	84 (29.3)	91 (28.1)	1.21(0.84, 1.98)	0.073
	45–54	44 (15.5)	72 (21.7)	1.079 (0.851–1.367)	0.341
	55–64	35 (12.1)	39 (11.6)	1.015 (0.691–1.492)	0.282
	>65	21 (7.1)	18 (3.9)	*	

* Reference group

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التقييم بين مستويات فيتامين(د3) في الدم والعوامل المرتبطة بالتهاب الكبد (ب) المزمن (دراسة الحالات والشواهد)

مسعود ضياء [1]، فرشتة عثماني [2، 3]

[1] مركز أبحاث الأمراض المعدية، جامعة بيرجند للعلوم الطبية، بيرجند، إيران.

[2] أستاذ مساعد في الإحصاء الحيوي، كلية الصحة، جامعة بيرجند للعلوم الطبية، إيران

[3] مركز تطوير البحوث السريرية لطب الأسنان، جامعة بيرجند للعلوم الطبية، بيرجند، إيران

الملخص

مقدمة: التهاب الكبد المزمن (CHB) (B) ناتج عن عدوى فيروس التهاب الكبد (B) وقد ثبت أن لفيتامين (D3) تأثيرات بيولوجية مهمة جدًا في جهاز المناعة، واستجابة المضيف للعدوى الفيروسية، لذا تهدف هذه الدراسة إلى تحديد عوامل الخطر المحتملة لنقص فيتامين(د) بين مرضى (CHB) في بيرجند، إيران.

المواد والطرق: في دراسة الحالات والشواهد هذه تم اختيار (292) مريضًا مع (CHB) و(304) من الأشخاص الأصحاء بوصفهم مجموعة ضابطة في العيادة الخارجية لقسم أمراض العدوى من يناير (2017) إلى ديسمبر (2018)، وقام الباحثان بتحديد مستويات إجمالي عينات مصل فيتامين (D3) في كلٍّ منهم، وتم إجراء اختبار (t-test) و(chi-square) جنبًا إلى جنب مع الانحدار اللوجستي لتحديد الارتباط المعنوي بين المتغيرات ذات الأهمية، ونقص فيتامين (D3) المرتبط بـ

النتائج: من بين جميع الأشخاص الذين شملتهم الدراسة، كان(62.7٪) من المجموعة الضابطة و(48.1٪) من مجموعات الحالة من الذكور، وكان متوسط العمر ومستويات فيتامين **(د3)** لحالة ومجموعات المراقبة: (39.9 ± 13.3) سنة، و(43.0 ± 13.3) سنة، (20.76 ± 15.53) نانوغرام / مل، (19.07 ± 12.41) نانوغرام / مل، على التوالي، ولا يوجد ارتباط كبير بين نقص فيتامين (D3) وعوامل الخطر (CHB) (P <0.05)

وكان انتشار عدم كفاية فيتامين **(د3)** مرتفعًا بين مرضى التهاب الكبد الوبائي (63.0٪)، وكذلك بين المرضى الأصحاء (52.9٪)، و أظهر توزيع تواتر مستويات فيتامين**(د3)** في الدم فرقًا معنويًا في مجموعتين.(P = 0.001)

الاستنتاجات: حسب النتائج لم يتم العثور على مخاطر عالية لنقص فيتامين (D3) المرتبط بـ (CHB) مقارنة بالأدبيات السابقة بين مرضى (CHB) في هذه المدينة ونوقشت الأسباب المحتملة لهذه النتائج على الرغم من أن هناك حاجة لدراسات شاملة في المستقبل لتوضيح الآليات الكامنة والارتباط الحقيقي.

الكلمات الدالة: مستوى فيتامين **(د3)** أمراض الكبد، العدوى المزمنة بفيروس التهاب الكبد بي.