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Hepatitis B Serological Tests and Vitamin D Values: A Seasonal Assessment

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ABSTRACT

Objective: Hepatitis B virus (HBV), a significant global health concern, remains a source of infection despite the availability of effective vaccines. The progression of the disease is influenced by HBV antigens and autoimmune reactions. A deficiency in the immunomodulatory vitamin D is associated with the severity of various illnesses. The purpose of this research was to explore the relationship between HBV serological test results and 25-hydroxyvitamin D levels.

Materials and Methods: The study included 120,004 HBV serological tests (Hepatitis B surface antigen (HBsAg), Hepatitis B e-antigen (HBeAg), anti-HBsAg, anti-HBeAg, antibodies to Hepatitis B core immunoglobulin G (anti-HBc IgG), and anti-HBc IgM) and 62.835 25-hydroxyvitamin D tests.

Results: In spring, summer, and fall, 25-hydroxyvitamin D levels in HBsAg-positive individuals were lower compared to HBsAg-negative individuals. Conversely, 25-hydroxyvitamin D levels in individuals positive for anti-HBsAg were higher than those in anti-HBsAg-negative individuals across all seasons. Furthermore, in both cases of 25-hydroxyvitamin D deficiency and optimal 25-hydroxyvitamin D levels, individuals positive for anti-HBsAg showed higher 25-hydroxyvitamin D values than those negative for anti-HBsAg. Additionally, individuals positive for anti-HBc IgG demonstrated higher 25-hydroxyvitamin D levels compared to anti-HBc IgG-negative individuals during winter and fall. Moreover, 25-hydroxyvitamin D levels in individuals negative for anti-HbeAg were found to be below the optimal range.

Conclusion: In conclusion, there may be a relationship between 25-hydroxyvitamin D levels and hepatitis B serological test positivity. Therefore, vitamin D levels should be monitored in populations affected by HBV.

Keywords: Anti-hepatitis B surface antigen, hepatitis B virus, hepatitis B surface antigen, serological test, vitamin D deficiency.

INTRODUCTION

A partially double-stranded DNA virus known as the hepatitis B virus (HBV) can cause both chronic and acute liver diseases. Acute HBV infection is characterized by the presence of HBV core antigen and HBV surface antigen (HBsAg), whereas the persistence of HBsAg for more than six months indicates chronic HBV infection.[1] Each year, approximately 800,000 people worldwide die from liver disorders caused by HBV-induced inflammation. Despite the availability of a highly effective childhood vaccine against HBV for over three decades, these mortality statistics are alarming. This highlights that the HBV problem is still unresolved.[2]

HBV-specific CD8+ T cells eliminate infected hepatocytes, while HBV-specific B cells generate antibodies to inhibit HBV inflammation. Exhaustion of virus-specific immune cell function and the inability to effectively activate adaptive immunity causes HBV infection to become chronic. The function of immune cells can be inhibited by viral antigens such as HBV surface antigen, core antigen, and e antigen.[3] The immune response to HBV requires the removal of HBV and HBsAg from the blood. This removal process depends on the ability of B cells, supported by helper T cells, to produce antibodies.[4] HBsAg is a valuable viral biomarker for clinical evaluation and detection of HBV. However, the accumulation and expression of HBsAg are observed in chronic HBV infection. These HBsAg molecules have been shown to play a significant role in the severity of HBV-related liver illnesses.[5]

Low levels of vitamin D, a hormone that helps regulate the body's immune system, have been linked to an increased risk of infection and the onset of autoimmune disorders.[6,7] This vitamin is a key regulator of innate immune responses and plays an important role in antiviral defenses. Deficiency in 25-hydroxyvitamin D is associated with an increased risk of viral diseases.[8] In addition to its effects on the immune system, vitamin D also exhibits antioxidant, apoptotic, and antiproliferative properties.[9,10]

Vitamin D levels are influenced by seasonal changes in both women and men. These levels are lower in winter than in other seasons and higher in summer.[11] Deficiency, which is widespread globally, is more prevalent in spring and winter. Therefore, seasonal changes are considered one of the key factors affecting vitamin D status.[12]

The severity of infection is related to HBV antigens and the immune response. Immunodeficiency and the accumulation of HBV surface antigens contribute to a poor prognosis. Vitamin D levels, which are immunomodulators, are affected by seasonal changes. Conversely, deficiency in these levels influences the prognosis of many diseases. Serological tests are used in the diagnosis and follow-up of HBV infection and in evaluating antigen presence and immune status. This research aimed to examine the relationship between HBV serological tests and vitamin D levels. For this purpose, HBV serological test positivity and vitamin D levels were analyzed across four different seasons over the course of a year. Additionally, vitamin D concentration status and HBV serological test positivity were evaluated.

KEY MESSAGES

- In spring, summer, and fall, 25-hydroxyvitamin D values of HBsAg-positive individuals were lower than those of HBsAg-negative individuals.
- In all seasons, 25-hydroxyvitamin D values of anti-HBsAg-positive individuals were higher than those of anti-HBsAg-negative individuals.
- In cases of vitamin D deficiency and optimal 25-hydroxyvitamin D concentration, the vitamin D values of anti-HbsAg-positive individuals were higher than those of anti-HbsAg-negative individuals.

MATERIALS AND METHODS

Ethics

This study was approved by the Ethics Committee of Samsun University Faculty of Medicine (Approval Number: GOKA/2020/7/13).

Study Design

This research was a retrospective, single-center study. HBV serological tests and 25-hydroxyvitamin D levels analyzed at Samsun University Faculty of Medicine Microbiology and Biochemistry Laboratory in 2022 were retrospectively reviewed and included in the study. Vitamin D values of 100 ng/mL and above were excluded from the study. Samples that were rejected due to preanalytical errors were also excluded. Hepatitis B e-antigen (HBeAg), HBsAg, anti-HBeAg, anti-HBsAg, anti-Hepatitis B core immunoglobulin M (anti-HBc IgM), and anti-HBc IgG serological tests were included as HBV serological tests.

Laboratory findings included in the research were classified according to seasons. Findings analyzed in December, January, and February were categorized under the winter season; those analyzed in March, April, and May under the spring season; findings from June, July, and August under the summer season; and findings from September, October, and November under the fall season.

Serum 25-hydroxyvitamin D values below 20 ng/mL are considered deficient; values between 20–30 ng/mL are considered suboptimal; and values between 30–50 ng/mL are considered within the optimal concentration range.[13] Based on these classifications, the laboratory findings in the research were grouped according to vitamin D concentration status. Using these groupings, the 25-hydroxyvitamin D values of individuals with HBV serological test-positive results were compared to those with HBV serological test-negative results.

Laboratory Analysis

HBV serological tests were conducted using an Abbott Architect i2000 analyzer (Chicago, Illinois, USA). HBsAg, HBeAg, and anti-HBsAg serological tests were performed using electro-chemiluminescence immunoassay method. Anti-HBc IgG, anti-HBc IgM, and anti-HBeAg serological tests were determined using the microparticle immunoassay method.

An HBsAg serological test result of 0–1 IU/L was considered negative, while a result greater than or equal to 1.0 IU/L was considered positive for HBsAg. An anti-HBsAg serological test result between 0–10 IU/L was considered negative, and a result equal to or greater than 10 IU/L was considered positive for the anti-HBsAg test.

An HBeAg serological test result of 0–1 IU/L was considered negative, while a result greater than or equal to 1.0 IU/L was considered positive for HBeAg. Conversely, an anti-HBeAg serological test result between 0–1 IU/L was considered positive, whereas a result equal to or greater than 1 IU/L was considered negative for the anti-HBeAg test.

An anti-HBc IgG serological test result of 0–1 IU/L was considered negative, while a result greater than or equal to 1.0 IU/L was considered positive for anti-HBc IgG. Similarly, an anti-HBc IgM serological test result of 0–1 IU/L was considered negative, while a result greater than or equal to 1.0 IU/L was considered positive for anti-HBc IgM.

Serum 25-hydroxyvitamin D values were tested using the chemiluminescence immunoassay method with a Roche Cobas 8000 instrument.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 22.0 (IBM SPSS Statistics for Windows, Version 22.0, IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation, while categorical variables were presented as percentages and frequencies. The Chi-square test was used to evaluate categorical variables, and the independent samples t-test was applied to compare continuous variables. P values less than 0.05 were considered statistically significant. Effect sizes for statistically significant results were calculated and expressed as "h" for categorical variables and "d" for continuous variables.

RESULTS

Descriptive Information

A total of 63.365 HBsAg serological test results, 36.144 anti-HBsAg serological test results, 3.583 HBeAg serological test results, 3.286 anti-HBeAg serological test results, 10.258 anti-HBc IgG serological test results, 3.368 anti-HBc IgM serological test results, and 62.835 25-hydroxyvitamin D test results analyzed at Samsun Training and Research Hospital between January 1, 2022 and December 31, 2022, were included in the research. Of these 25-hydroxyvitamin D tests, 43.954 (69.95%) were from female individuals. The mean age of individuals whose 25-hydroxyvitamin D test results were analyzed was 43.71±22.93 years. The mean 25-hydroxyvitamin D value of these individuals was 19.65±11.12 ng/mL. Descriptive information for individuals with these serological test results is presented in Table 1.

HBV Serological Test Positivity and 25-Hydroxyvitamin D Values According to Gender

The HBsAg positivity rate in male individuals was higher than the HBsAg positivity rate in female individuals ($p<0.001$, $h=0.079$) (Table 1). There was no significant difference in the rate of anti-HBsAg positivity between female and male individuals ($p=0.939$) (Table 1). Similarly, there was no significant difference in the rate of HBeAg positivity between male and female individuals ($p=0.551$) (Table 1). The anti-HBeAg positivity rate in male individuals was higher than the anti-HBeAg positivity rate in female individuals ($p<0.001$, $h=0.196$) (Table 1). Additionally, the anti-HBc IgG positivity rate in male individuals was higher than that in female individuals ($p=0.002$, $h=0.061$) (Table 1). The anti-HBc IgM positivity rate in male individuals was also higher than that in female individuals ($p=0.017$, $h=0.084$) (Table 1). The mean 25-hydroxyvitamin D value in male individuals was 21.16±10.60 ng/mL, compared to 19.00±11.28 ng/mL in female individuals. Male individuals had higher 25-hydroxyvitamin D values than female individuals ($p<0.001$, $d=0.197$).

HBV Serological Test Positivity and 25-Hydroxyvitamin D Values According to Seasons

Groups were created to evaluate these results based on seasonal changes. The data for these groups are presented in Table 2.

It was observed that all serological tests were most frequently performed in the fall and least frequently in the summer. The HBsAg serological test positivity rate was lowest in the summer

Table 1. Descriptive information about patients undergoing hepatitis B virus serological tests

	HbsAg (n=63.365)	Anti-HBsAg (n=36.144)	HbeAg (n=3.583)	Anti-HBeAg (n=3.286)	Anti-HBc IgG (n=10.258)	Anti-HBc IgM (n=3.368)
Age, Mean±SD	48.64±19.35	49.18±19.29	53.66±17.19	52.86±16.19	49.23±17.26	52.21±17.38
Gender, n (%)						
Male	28.334 (44.72)	17.786 (49.21)	1.782 (49.73)	1.606 (48.87)	4.728 (46.09)	1.525 (45.28)
Female	35.031 (55.28)	18.358 (50.79)	1.801 (50.27)	1.680 (51.13)	5.530 (53.91)	1.843 (54.72)
Result, n (%)						
Positive	2.178 (3.44)	16.299 (45.09)	89 (2.48)	1.292 (39.32)	2.730 (26.61)	16 (0.48)
Negative	61.187 (96.56)	19.845 (54.91)	3.494 (97.52)	1.994 (60.68)	7.528 (73.39)	3.352 (99.52)
Positive results by gender, n (%)						
Male	1.199 (4.23)	8.017 (45.07)	47 (02.64)	710 (44.21)	1.326 (28.04)	12 (0.79)
Female	979 (2.79)	8.282 (45.11)	42 (2.33)	582 (34.64)	1.404 (25.36)	4 (0.22)
p	<0.001	0.939	0.551	<0.001	0.002	0.017
h	0.079	–	–	0.196	0.061	0.084

SD: Standard deviation; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e-antigen; HBc IgG: Hepatitis B core immunoglobulin G; HBc IgM: Hepatitis B core immunoglobulin M; n: Sample size; h: Effect size.

and highest in the spring. The positivity rate in the summer was statistically lower than in the spring (p=0.028, h=0.027) (Table 2). The anti-HBsAg serological test positivity rate was lowest in the fall and highest in the summer. The positivity rate in the summer was statistically higher than in the fall (p=0.045, h=0.030) (Table 2). The HBeAg serological test positivity rate was lowest in the fall and highest in the winter. The positivity rate in the fall was statistically lower than in the winter (p=0.009, h=0.117) (Table 2). The anti-HBeAg serological test positivity rate was lowest in the summer and highest in the spring. The positivity rate in the spring was statistically higher than in other seasons (p<0.001, h=0.165 [winter], h=0.181 [summer], h=0.171 [fall]) (Table 2). The anti-HBc IgG serological test positivity rate was lowest in the fall and highest in the spring. The positivity rate in the spring was statistically higher than in the fall (p=0.015, h=0.066) and winter (p=0.023, h=0.065) (Table 2). The anti-HBc IgM serological test positivity rate was lowest in the winter and highest in the summer. However, no statistical difference was detected between seasons regarding anti-HBc IgM serological test positivity (p=0.546) (Table 2).

It was found that 25-hydroxyvitamin D analysis was performed most frequently in the fall and least frequently in the summer. The mean 25-hydroxyvitamin D value was highest in the summer and lowest in the spring. The mean 25-hydroxyvitamin D value detected in the summer and fall was statistically higher than the mean value detected in the spring and winter (p<0.001, d=0.415 [summer-spring], d=0.357 [summer-winter], d=0.404 [fall-spring], d=0.345 [fall-winter]) (Table 2).

25-Hydroxyvitamin D Values of Individuals Who Underwent HBV Serological Testing

It was found that 11.76% (n=7,453) of the HBsAg serological tests, 22.50% (n=8,133) of the anti-HBsAg serological tests, 18.14% (n=596) of the HBeAg serological tests, 18.47% (n=607) of the anti-HBeAg serological tests, 25.12% (n=2,577) of the anti-HBc IgG serological tests, and 32.87% (n=1,107) of the anti-HBc IgM serological tests included in the study were analyzed together with 25-hydroxyvitamin D values.

Of the 7.453 HBsAg serological tests, 248 (3.33%) were considered positive, while 3.736 (45.94%) of the 8,133 anti-HBsAg serological tests were positive. The mean 25-hydroxyvitamin D value in individuals with a positive HBsAg serological test was 18.27±9.68 ng/mL, compared to 18.86±10.71 ng/mL in individuals with a negative HBsAg serological test. No significant difference in 25-hydroxyvitamin D values was observed between these two groups (p=0.230). The mean 25-hydroxyvitamin D value of individuals with a positive anti-HBsAg serological test was 20.33±11.08 ng/mL, while the mean value for those with a negative anti-HBsAg serological test was 18.91±10.59 ng/mL. In addition, the mean 25-hydroxyvitamin D value in individuals with a positive anti-HBsAg serological test was higher than that in individuals with a negative anti-HBsAg serological test (p<0.001, d=0.131).

Among the 596 HBeAg serological tests, 3 (0.50%) were considered positive, while 174 (28.67%) of the 607 anti-HBeAg serological tests were positive. The mean 25-hydroxyvitamin D value in individuals with a positive HBeAg serological test was 22.06±12.77

Table 2. Hepatitis B virus serological test positivity and 25-hydroxyvitamin D levels by season

	Winter n (%)	Spring n (%)	Summer n (%)	Fall n (%)	p	Effect size
HBsAg						
Positive	544 (3.54)	529 (3.63)	405 (3.15)	700 (3.40)	0.028*	0.027
Negative	14.802 (96.46)	14.038 (96.37)	12.456 (96.85)	19.891 (96.60)		
Total	15.346	14.567	12.861	20.591		
Anti-HBsAg						
Positive	3.931 (45.54)	3.751 (45.19)	3.252 (45.79)	5.365 (44.30)	0.045†	0.030
Negative	4.701 (54.46)	4.549 (54.81)	3.850 (54.21)	6.745 (55.70)		
Total	8.632	8.300	7.102	12.110		
HBeAg						
Positive	32 (3.62)	22 (2.51)	15 (2.18)	20 (1.76)	0.009#	0.117
Negative	853 (96.38)	854 (97.49)	673 (97.82)	1.114 (98.24)		
Total	885	876	688	1.134		
Anti-HBeAg						
Positive	319 (37.75)	338 (45.86)	215 (36.94)	420 (37.43)	<0.001‡	0.165 (Winter),
Negative	526 (62.25)	399 (54.14)	367 (63.06)	702 (62.57)		0.181 (summer),
Total	845	737	582	1.122		0.171 (fall)
Anti-HBc IgG						
Positive	680 (25.71)	668 (28.58)	551 (27.06)	831 (25.65)	0.015§,	0.066§,
Negative	1.965 (74.29)	1.669 (71.42)	1.485 (72.94)	2.409 (74.35)	0.023§§	0.065§§
Total	2.645	2.337	2.036	3.240		
Anti-HBc IgM						
Positive	3 (0.34)	4 (0.48)	5 (0.83)	4 (0.38)	0.546	–
Negative	881 (99.66)	835 (99.52)	598 (99.17)	1.038 (99.62)		
Total	884	839	603	1.042		
25-Hydroxyvitamin D (ng/mL)						
Mean±SD	17.75±10.97	17.03±11.34	21.66±10.95	21.47±10.60	<0.001	0.415**, 0.357††,
Total	15.046	13.724	13.700	20.365		0.404‡‡, 0.345##

SD: Standard deviation; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e-antigen; HBc IgG: Hepatitis B core immunoglobulin G; HBc IgM: Hepatitis B core immunoglobulin M; n: Sample size; *: Spring-summer; †: Summer-fall; #: Fall-winter; ‡: Spring-other seasons; §: Fall-spring; §§: Spring-winter; **: Summer-spring; ††: Summer-winter; ‡‡: Fall-spring; ##: Fall-winter.

ng/mL, compared to 18.70±10.82 ng/mL in individuals with a negative HBeAg serological test. The mean 25-hydroxyvitamin D value in individuals with a positive HBeAg serological test was higher than that in individuals with a negative HBeAg serological test (p<0.001, d=0.290). The mean 25-hydroxyvitamin D value of individuals with a positive anti-HBeAg serological test was 20.87±11.00 ng/mL, while that of individuals with a negative anti-HBeAg serological test was 20.37±11.17 ng/mL. No significant difference in 25-hydroxyvitamin D values was observed between these two groups (p=0.379).

Of the 2,577 anti-HBc IgG serological tests, 644 (24.99%) were considered positive. The mean 25-hydroxyvitamin D value of individuals with a positive anti-HBc IgG serological test was 20.36±11.40 ng/mL, compared to 18.83±10.57 ng/mL in individuals with a negative anti-HBc IgG serological test. In addition, the mean 25-hydroxyvitamin D value in individuals with a positive anti-HBc IgG serological test was higher than in individuals with a negative anti-HBc IgG serological test (p=0.002, d=0.140). The mean 25-hydroxyvitamin D value in individuals with a negative anti-HBc IgM serological test was

Table 3. 25-hydroxyvitamin D levels of individuals undergoing hepatitis B virus serological testing across seasons

	25-Hydroxyvitamin D (ng/mL) Mean±SD			
	Winter (n=15.046)	**Spring (n=13.724)**	**Summer (n=13.700)**	**Fall (n=20.365)**
HBsAg				
Positive	17.88±10.61	14.33±5.51	19.36±7.49	20.67±8.96
Negative	18.56±10.98	16.15±10.81	22.69±9.19	21.66±9.54
p	0.808	<0.001	<0.001	0.007
d	–	0.212	0.364	0.104
Anti-HBsAg				
Positive	20.13±11.16	18.82±12.93	23.79±9.58	22.77±9.96
Negative	18.61±10.99	15.90±10.23	22.55±9.47	21.42±9.03
p	<0.001	0.004	<0.001	<0.001
d	0.137	0.252	0.130	0.143
HBeAg*				
Positive	36.67	–	16.51	13.00
Negative	17.78±10.52	16.99±11.54	19.03±10.65	20.24±10.52
Anti-HBeAg				
Positive	20.74±11.99	18.09±12.32	22.03±9.96	21.56±10.37
Negative	18.21±10.99	19.05±11.48	22.43±10.84	22.02±11.01
p	0.002	0.274	0.658	0.489
d	0.222	–	–	–
Anti-HBc IgG				
Positive	18.88±10.97	17.72±11.37	21.46±9.90	22.43±12.21
Negative	17.79±11.16	17.12±10.93	20.53±9.76	20.00±9.98
p	0.028	0.236	0.057	0.004
d	0.098	–	–	0.219
Anti-HBc IgM*				
Positive	–	–	–	–
Negative	17.83±11.15	17.09±10.34	21.37±10.57	20.27±10.48

SD: Standard deviation; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e-antigen; HBc IgG: Hepatitis B core immunoglobulin G; HBc IgM: Hepatitis B core immunoglobulin M; n: Sample size; d: Effect size; *: Since the number of HbeAg-positive or anti-HBc IgM-positive individuals with measured 25-hydroxyvitamin D values was insufficient, statistical analysis could not be performed for these groups.

18.86±10.75 ng/mL. There were no individuals with a positive anti-HBc IgM serological test who were analyzed together with 25-hydroxyvitamin D values.

25-Hydroxyvitamin D Values of Individuals Who Underwent HBV Serological Testing According to Seasonal Changes

The 25-hydroxyvitamin D values of individuals who underwent HBV serological testing according to seasonal changes are presented in Table 3.

In spring, summer, and fall, the 25-hydroxyvitamin D values of HbsAg-positive individuals were found to be lower than those of HbsAg-negative individuals (Table 3). In all seasons, the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBsAg serological test were higher than those of individuals with a negative anti-HBsAg serological test (Table 3).

Since the number of HbeAg-positive or anti-HBc IgM-positive individuals whose 25-hydroxyvitamin D

Table 4. 25-hydroxyvitamin D levels according to vitamin D status in individuals undergoing hepatitis B virus serological testing

	25-Hydroxyvitamin D (ng/mL) Mean±SD			
	Deficiency (<20 ng/mL) (n=36.187)	**Suboptimal (20–30 ng/mL) (n=17.417)**	**Optimal (30–50 ng/mL) (n=8.104)**	**Supra-optimal (>50 ng/mL) (n=1.127)**
HBsAg				
Positive	12.54±4.69	23.88±2.61	35.83±4.61	60.62±4.56
Negative	12.19±4.52	24.42±2.81	36.05±4.96	62.72±12.61
p	<0.001	<0.001	0.462	0.300
d	0.077	0.194	–	–
Anti-HBsAg				
Positive	12.73±4.48	24.48±2.76	36.12±4.89	60.88±9.80
Negative	12.08±4.54	24.42±2.83	35.88±4.86	61.38±11.01
p	<0.001	0.159	0.028	0.453
d	0.144	–	0.049	–
HBeAg*				
Positive	14.76±2.48	–	36.67	–
Negative	12.23±4.51	24.48±2.88	36.25±5.02	57.82±6.46
Anti-HBeAg				
Positive	9.46±7.89	27.53±1.60	36.35±4.98	60.59±7.09
Negative	12.70±4.55	22.56±1.52	–	–
p	<0.001	<0.001	–	–
d	0.530	3.190	–	–
Anti-HBc IgG				
Positive	12.49±4.52	24.72±2.60	36.16±4.81	62.67±11.20
Negative	12.25±4.36	24.52±2.84	36.06±4.66	61.90±10.16
p	0.017	0.0257	0.641	0.609
d	0.055	0.072	–	–
Anti-HBc IgM*				
Positive	–	–	–	–
Negative	12.05±4.47	24.31±2.72	35.66±4.73	57.92±7.49

SD: Standard deviation; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e-antigen; HBc IgG: Hepatitis B core immunoglobulin G; HBc IgM: Hepatitis B core immunoglobulin M; n: Sample size; d: Effect size; *: Due to the insufficient number of HbeAg-positive or anti-HBc IgM-positive individuals with measured 25-hydroxyvitamin D values, statistical analysis could not be conducted for these groups.

values were measured was insufficient for statistical analysis, statistical analysis could not be performed for these groups (Table 3). No significant difference was observed in spring, summer, and fall between the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBeAg serological test and those with a negative anti-HBeAg serological test (Table 3).

No significant difference was found between the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBc IgG serological test and those with a negative anti-HBc IgG serological test in spring and summer (Table 3). However, the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBc IgG serological test in winter and fall were higher than those of individuals with a negative anti-HBc IgG serological test (Table 3).

25-Hydroxyvitamin D Values According to Vitamin D Concentration Status of Individuals Who Underwent HBV Serological Testing

Individuals whose 25-hydroxyvitamin D values were examined along with serological tests were divided into groups based on their 25-hydroxyvitamin D concentration status. The 25-hydroxyvitamin D values according to vitamin D concentration status of individuals who underwent HBV serological testing are presented in Table 4.

In cases of vitamin D deficiency and suboptimal vitamin D concentration, a significant difference was found between the mean 25-hydroxyvitamin D values of individuals with a positive HBsAg serological test and those with a negative HBsAg serological test (Table 4). In cases of vitamin D deficiency and optimal vitamin D concentration, the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBsAg serological test were higher than those of individuals with a negative anti-HBsAg serological test (Table 4). In other cases of vitamin D concentration, no significant difference was observed between the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBsAg serological test and those with a negative anti-HBsAg serological test (Table 4).

Since the number of HbeAg-positive or anti-HBc IgM-positive individuals whose 25-hydroxyvitamin D values were measured was insufficient for statistical analysis, statistical analysis could not be performed for these groups (Table 4). In cases of vitamin D deficiency and suboptimal 25-hydroxyvitamin D concentration, there was a significant difference between the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBeAg serological test and those with a negative anti-HBeAg serological test (Table 4). In cases of optimal and supra-optimal vitamin D concentrations, there were no individuals with a negative anti-HBeAg serological test (Table 4).

In cases of vitamin D deficiency and suboptimal 25-hydroxyvitamin D concentration, there was a significant difference between the mean 25-hydroxyvitamin D values of individuals with a positive anti-HBc IgG serological test and those with a negative anti-HBc IgG serological test (Table 4). There were no individuals with a positive anti-HBc IgM serological test in any case of vitamin D concentration (Table 4).

DISCUSSION

Hepatitis B, a significant global public health issue affecting approximately 290 million individuals, is more prevalent in countries with low and middle incomes.[14] A study modeling data from 170 countries estimated that the global prevalence of HBV infection in 2022 was 3.2%, with up to 257.5 million (216.6–316.4 million) HBsAg-positive individuals.[15] Similarly, 25-hydroxyvitamin D deficiency is another global public health concern, particularly affecting the elderly, pregnant women, and non-Western immigrants. Vitamin D insufficiency is observed in nearly 50% of the world's population, especially during winter months.[16]

In this study, the HBsAg positivity rate was found to be 3.44%, while the anti-HBsAg positivity rate was 45.09%. Additionally, the mean 25-hydroxyvitamin D value was below the 20 ng/mL, the threshold for vitamin D deficiency.[13]

The incidence of HBV infection is higher in males than in females, a trend that mirrors the incidence of hepatitis C virus (HCV) infection and hepatocellular carcinoma. Moreover, HBV infection progresses more rapidly in males, and their overall survival rates are lower.[17] Males tend to exhibit less robust innate, cellular, and humoral immune responses to hepatitis B and C viral infections and vaccines compared to females. Sex hormones play a significant role, as they selectively bind to hormone receptors on immune cells, modulating immunological responses to hepatitis B and C viruses in distinct ways. Estrogens generally have an immune-stimulating effect, while androgens exert an immunosuppressive effect.[18] Additionally, estrogens appear to act synergically with vitamin D, providing beneficial effects against autoimmune processes. Rather than focusing solely on gender-related differences in vitamin D levels, it is crucial to consider the role of gender-related factors influencing vitamin D action.[19]

In this study, the HBsAg positivity rate detected in male individuals was higher than that in female individuals. However, no significant difference was found in terms of HBeAg and anti-HBsAg positivity rates. Additionally, 25-hydroxyvitamin D values in male individuals were higher than those in female individuals. Furthermore, anti-HBc IgG, anti-HBc IgM, and anti-HBeAg positivity rates detected in male individuals were higher than those in female individuals.

A study examining seven years of data from China reported that hepatitis B cases tend to follow a uniform growth trend with seasonal and periodic fluctuations. It is also noted that these fluctuations peak in March each year.[20] Conversely, a study analyzing five years of data in Qatar found no specific trend or seasonality in hepatitis B cases.[21] The lack of seasonality reported in the Qatar study regarding hepatitis B cases may be attributed to the composition of the study population. It has been noted that the population in Qatar predominantly consists of seasonal immigrants from Africa and Asia rather than local residents.

In this research, it was found that 25-hydroxyvitamin D values detected in winter and spring were below 20 ng/mL, the upper limit defined for vitamin D deficiency. HBsAg serological test positivity was highest in the spring months, when vitamin D values were at their lowest. Additionally, HBeAg serological test positivity was highest in the winter months, when vitamin D values were the second lowest. Conversely, anti-HBsAg serological test positivity was highest in the summer months, when vitamin D values were at their highest.

Several studies have found that 25-hydroxyvitamin D can reduce HBV replication through various mechanisms, including interfering with HBV viral protein synthesis and reducing viral transcription. However, some researchers have reported that vitamin D supplementation after vaccination does not affect vaccine response.[22] In an animal study, three groups were created, and it was found that vitamin D concentration showed a positive correlation with anti-HBsAg IgG levels in two of the three groups (the control group and the vitamin D-supplemented group). This correlation was not observed in the third group (the ultraviolet B (UVB)-exposed group). The lack of correlation in the UVB group was attributed to the dual effects of UVB radiation, which can both increase vitamin D levels and exert immunosuppressive effects. Additionally, it was noted that the vitamin D levels in groups showing a positive correlation between 25-hydroxyvitamin D concentration and anti-HbsAg IgG levels were below 20 ng/mL, the upper limit defined for vitamin D deficiency.[23] Furthermore, Ahluwalia et al.[24] identified vitamin D receptors as a novel regulator of HBV core promoter activity.

In this study, it was found that 25-hydroxyvitamin D levels detected in winter and spring were below 20 ng/mL, the threshold for vitamin D deficiency, whereas levels in summer and fall were above this threshold. Additionally, the 25-hydroxyvitamin D levels of individuals with positive anti-HBsAg serological tests were higher than the average 25-hydroxyvitamin D levels of individuals with negative anti-HBsAg serological tests in all seasons. In addition, the 25-hydroxyvitamin D values of individuals with a positive anti-HBc IgG serological test were higher than those of individuals with a negative anti-HBc IgG serological test in winter and fall. Furthermore, the 25-hydroxyvitamin D values of HBsAg-positive individuals were lower than those of HBsAg-negative individuals in spring, summer, and fall.

A multicenter study noted that 25-hydroxyvitamin D deficiency is associated with poor clinical outcomes in individuals with chronic HBV inflammation.[25] Another study conducted with children reported a positive correlation between 25-hydroxyvitamin D concentration and anti-HBsAg titer, with only 14.5% of the children having optimal 25-hydroxyvitamin D concentrations.[26] Additionally, a study of 737 individuals with untreated active chronic hepatitis B found that abnormally low vitamin D values were quite common.[27]

In this study, in cases of 25-hydroxyvitamin D deficiency and suboptimal vitamin D concentrations, the 25-hydroxyvitamin D values of individuals with a positive anti-HBsAg serological test were higher than those of individuals with a negative anti-HBsAg serological test. However, no significant differences were observed in other cases. Additionally, 25-hydroxyvitamin D values were either deficient or suboptimal in 433 individuals with negative anti-HBeAg serological tests.

CONCLUSION

In conclusion, 25-hydroxyvitamin D values may be associated with hepatitis B serological test positivity, particularly in cases of 25-hydroxyvitamin D deficiency. Monitoring 25-hydroxyvitamin D levels may enhance the likelihood of antibody formation against hepatitis B infection, and vitamin D supplementation should be recommended in cases of deficiency.

Ethics Committee Approval: The Samsun University Clinical Research Ethics Committee granted approval for this study (date: 27.05.2020, number: GOKA/2020/7/13).

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