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Relation of Vitamin D Status to Persistence of anti-HBs Antibody 20 Years after Compulsory Mass Vaccination and the Response to Booster Vaccination

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ABSTRACT

Background: Anti-HBs antibody levels can wane over years after hepatitis B vaccination. Vitamin D may influence the immune response to booster vaccination in previously seronegative individuals. *Objective:* to evaluate the association between vitamin D status and persistence of anti-HBs antibodies 20 years after primary infant HBV vaccination, and the response to a booster dose in seronegative individuals, with exploratory assessment of vitamin D supplementation.

Patients and Methods: A prospective cohort study was conducted on 300 healthy adults who had completed primary HBV vaccination in infancy. Anti-HBs titers were measured. Seronegative subjects (anti-HBs <10 IU/L, n=114) had 25(OH)D testing. Sixty deficient subjects (≤20 ng/mL) were non-randomly allocated to oral vitamin D supplementation (n=30) or no supplementation (n=30). A single 20 μg HBV booster was given to 112 seronegative subjects. Anti-HBs was re-measured at 4 weeks.

Results: The mean age was 20.5 ± 0.96 years; 60% were males. 53% of the cohort had anti-HBs <10 IU/L. Among those tested, 52.6% were vitamin D deficient. Post-booster, 99% of participants achieved protective anti-HBs titers (>10 IU/L). Vitamin D supplementation was associated with significantly higher post-booster antibody titers (mean 107.19 ± 43.84 IU/mL vs. 90.84 ± 134.47 IU/mL, $p<0.001$). A moderate positive correlation was observed between serum vitamin D level and post-booster anti-HBs titers ($r=0.388$, $p<0.001$).

Conclusion: vitamin D status may influence the post-booster response in seronegative individuals.

Keywords: anti-HBs antibody, hepatitis B (HB) vaccine, vitamin D, booster dose

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major global public health problem despite widespread implementation of vaccination programs worldwide. Chronic HBV infection affects approximately 254 million individuals globally and remains a leading cause of liver cirrhosis and hepatocellular carcinoma (1). Universal infant vaccination has markedly reduced HBV prevalence, HBV-related morbidity, and mortality in endemic regions including Egypt (2).

The recombinant hepatitis B vaccine induces protective anti-HBs antibody

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levels in more than 90% of immunocompetent individuals following completion of the standard three-dose schedule (3). Although vaccine-induced immune protection persists for many years, circulating anti-HBs antibody titers gradually decline over time and may eventually become undetectable (4). Nevertheless, several studies demonstrated persistence of immunological memory despite declining antibody levels, as evidenced by robust anamnestic responses following booster vaccination (5).

Long-term persistence of HBV vaccine immunity has become an important issue, particularly among healthcare workers and young adults vaccinated during infancy who may become exposed to HBV infection later in life (6). Recent studies reported that 35–60% of adults vaccinated during infancy may have anti-HBs titers below the protective threshold two decades after primary immunization (7,8).

Vitamin D is increasingly recognized as an important immunomodulatory hormone affecting both innate and adaptive immune responses (9). Vitamin D receptors are expressed on dendritic cells, macrophages, T lymphocytes, and B lymphocytes, suggesting a significant role in regulation of immune activation and antibody production (10). Emerging evidence suggests that vitamin D deficiency may impair vaccine immunogenicity and reduce antibody responses to several vaccines including influenza, COVID-19, and hepatitis B vaccines (11,12).

The present study aimed to evaluate persistence of anti-HBs antibodies approximately 20 years after compulsory infant HBV vaccination and to investigate the possible association between vitamin D status and post-booster anti-HBs response among seronegative individuals.

PATIENTS AND METHODS

This prospective cohort study was done at Endemic medicine department, Kasr-Alainy hospital, Faculty of Medicine, Cairo University in a time frame from January 2022 till November 2024. The study was conducted on 300 healthy subjects (eighty-five participants were excluded). They were selected after fulfilling the following criteria & after obtaining an informed consent & ethics committee approval.

Inclusion Criteria

Adult healthy individuals of both genders participants above 18 years. According to registered documents of Egyptian Ministry of Health centers, the participants had received three doses of recombinant HB vaccine, ten microgram each, during the first year of life, administered into the quadriceps muscle during infancy (first dose within two days of birth and the subsequent doses at 1 and 6 months of life). 20-21 years after primary HBV vaccination.

Exclusion Criteria

Participants were excluded from statistical analysis if their medical records documented booster dose of hepatitis B vaccination. Anti HCV or anti HIV1,2 or anti HBc total or IgM antibodies positive participants. Pregnant females were excluded. Those with elevated ALT or AST or whose abdomianl ultrasound showed evidence of significant chronic parenchymal liver disease.

Ethical Consideration

An approval of the study was obtained from Cairo University Academic and Ethical Committee. Written informed consent of all the participants was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Sample Size

Using clinCalc sample size calculator for analytic study; with 0.05 alpha error and power of the 0.80, CI of 95%. According to literature, mean serum levels of anti-HBs antibody in sero-protective subjects with Vit. D concentrations of ≥ 10 ng/mL was significantly higher than in those with deficient concentrations of Vit. D (58.91 ± 79.43 ng/mL vs. 16.16 ± 3.10 ng/mL) Similarly, the mean serum levels of anti-HBs antibody in sero-protective subjects with normal Vit. D concentrations (≥ 30ng/mL) was significantly higher than in those with low concentrations of Vit. D (68.26±87.07ng/mL vs. 22.38±14.74ng/mL). Sample size calculated to relation of Vit. D status to persistence of anti-HBs antibody 20 years after compulsory mass vaccination and the response to booster vaccination is 114 persons.

Clinical Assessment

A total of 300 healthy subjects submitted to baseline assessment during the first visit, *fig. 1*. History taking, clinical examination including BMI Lifestyle factors previously shown to influence the vaccination response were assessed; including alcohol or drug intake, smoking and history of booster vaccination, of

which eight subjects were excluded because they reported receiving HBV vaccine booster dose recently.

Laboratory investigation

Blood samples were obtained from every individual at enrollment. All tests were conducted according to the manufacturers' instructions at the department of clinical pathology of Cairo University hospitals. Blood samples were collected for:

- Serum levels of ALT, AST which revealed elevated AST in thirteen subjects and ALT in fifteen participants and they were excluded for the following steps, yet they were furtherly investigated for the cause of elevation of liver enzymes and followed up.
- HBsAg, anti HBc total, anti HBc IgM, anti HCV and anti HIV1&2 antibodies, were tested by commercial ELISA kits (Monolisa HBs Ag ULTRA, catalog no. 72346, Monolisa Anti-HBc EIA ULTRA, catalog no.26186, Monolisa Anti-HBc IgM EIA ULTRA, catalog no.26174, Monolisa HCV AgAb Ultra V2, catalog no. 72562, and Genscreen Ultra HIV Ag-Ab kit, catalog no. 72386, respectively (BioRad Laboratories, France).
- One participant showed positive HBc total, he was excluded from proceeding to the further steps but he also was furtherly investigated and followed up.
- Anti-HBs antibodies were detected using commercial enzyme-linked immunosorbent assay (ELISA) kits, Monolisa Anti-HBs PLUS, catalog no.72566 (BioRad Laboratories, France). Anti-HBs anti-body was measured using standard samples with known concentrations of anti-HBs antibody expressed as mIU/mL, provided by the manufacturer.

After baseline assessment

Eighty-five participants were excluded from the study due to receiving HBV vaccine booster dose (8 subjects); elevated liver enzymes (ALT and AST) (twenty-eight subjects); positive anti HBc total antibody (one subject); and fatty liver by abdominal US (forty-eight subjects).

The remaining 215 subjects were enrolled according to their anti-HBs titer into two groups:

- 1-Seropositive (101 subjects): when the anti-HBs titer is ≥10 IU/L.
- 2- Seronegative (114 subjects): when the anti-HBs titer is <10 IU/L.

Measurement of serum vitamin D level

The seronegative group was tested for serum vitamin D level using ELISA Kit designed for detection of human Vit. D level, human 25-Dihydroxy Vit. D ELISA Kit catalog number E1981Hu (Bioassay technology laboratory, Zhejiang, China) and expressed as ng/ml. They were categorized according to Central and Eastern

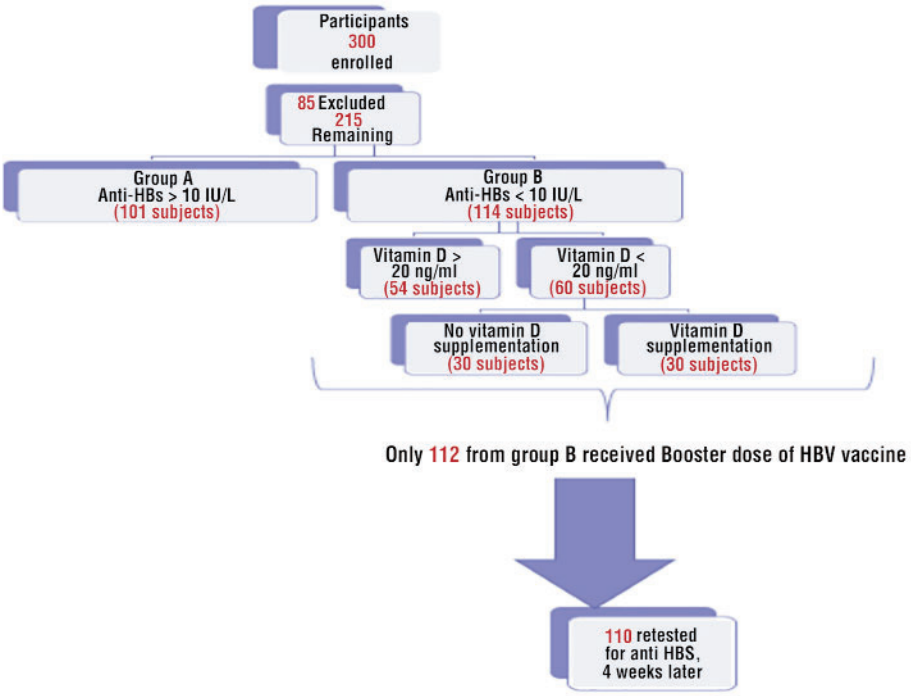

Figure 1 - Study participants' flow chart.

European Expert Consensus Statement into two groups:

1-Vitamin D level less than 20 ng/mL (50 nmol/L) (sixty subjects).

2- Vitamin D level more than 20 ng/mL (50 nmol/L) (fifty-four subjects).

Vitamin D supplementation

Sixty subjects, whom Vit. D level is less than 20 ng/mL (50 nmol/L), were divided into two equal groups:

Group A:

Thirty participants were offered Vit. D supplementation according to Central and Eastern European Expert Consensus Statement (Vidrop®), a 15 ml oral drops contain 2800 IU cholecalciferol/ml, for 12 consecutive weeks then monthly.

Treatment success may be evaluated after at least 6-12 weeks in certain risk groups, we selected all healthy participants, that is why retesting of serum Vit. D after Vit. D supplementation was not performed.

Group B:

The other thirty subjects did not receive Vit. D supplementation during the study but were instructed later on to receive supplementation.

Booster dose of HBV vaccine

All 114 subjects in the 2nd group (subjects with anti-HBs <10 IU/L) were offered counseling on vaccination and those who accepted booster vaccination (112 subjects) were given a single 20 microgram booster dose of recombinant HB vaccine (GeneVac-B®) administered intramuscularly in the deltoid region of the upper arm, except two cases (form Vit. D deficient & Vit. D sufficient groups that refused taking booster dose).

Follow up

The 112 participants who received booster dose of HBV vaccine were retested for anti-HBs antibody after four weeks except for two participants who lost follow up of anti-HBs titer testing after booster dose.

Statistical Analysis

Data were entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and relative frequency for categorical data. Comparisons between quantitative variables using Mann-Whitney test. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient. P-values less than 0.05 were considered as statistically significant.

RESULTS

Baseline Demographic and Clinical Characteristics

The mean age of the studied participants was 20.5 ± 0.96 years, with a median age of 20 years (IQR: 20–21 years). Males represented 60.0% of the study population, whereas females represented 40.0%. The mean BMI was 24.1 ± 3.2 kg/m². Additional demographic and clinical characteristics are summarized in *table 1*.

Pre- and Post-Booster anti-HBs Titers

Among seronegative participants, the mean baseline anti-HBs titer was 4.1 ± 2.3 mIU/mL, with a median value of 3.8 mIU/mL (IQR: 2.1–5.9 mIU/mL). Following booster vaccination, post-booster anti-HBs titers increased significantly, with a mean value of 97.6 ± 121.5 mIU/mL and a median value of 65.4 mIU/mL (IQR: 28.2–145.8 mIU/mL) ($p < 0.001$). Detailed pre- and post-booster anti-HBs titers are presented in *table 2*.

Association Between Vitamin D Status and Post-Booster Response

Vitamin D deficiency was identified in 60/114 participants (52.6%). Participants receiving vitamin D

Table 1 - Baseline demographic characteristics of the study population

Variable	Value
Age (years), mean ± SD	20.5 ± 0.96
Median age (IQR)	20 (20–21)
Minimum–maximum	18–23
Male sex, n (%)	129 (60%)
Female sex, n (%)	86 (40%)
Urban residence, n (%)	135 (62.8%)
Rural residence, n (%)	80 (37.2%)
Smokers, n (%)	17 (7.9%)
BMI (kg/m²), mean ± SD	24.1 ± 3.2
BMI median (IQR)	23.8 (21.7–26.2)

Table 2 - Pre-and post-booster anti-HBs titers among seronegative participants

Variable	Mean ± SD	Median (IQR)	Min–Max	p-value
Baseline anti-HBs (mIU/mL)	4.1 ± 2.3	3.8 (2.1–5.9)	0.1–9.8	
Post-booster anti-HBs (mIU/mL)	97.6 ± 121.5	65.4 (28.2–145.8)	1.4–742	<0.001

Table 3 - Vitamin D status and post-booster anti-HBs response

Variable	Supplemented group	Non-Supplemented Group	p-value
N	30	82	—
Vitamin D level (ng/mL), mean ± SD	14.2 ± 3.1	26.8 ± 5.7	< 0.001
Post-booster anti-HBs (mIU/mL), mean ± SD	107.19 ± 43.84	90.84 ± 134.47	0.001
Median post-booster anti-HBs	110.0	59.4	—
Minimum–maximum	18–224	2–742	—

supplementation demonstrated significantly higher post-booster anti-HBs titers compared with non-supplemented participants (107.19 ± 43.84 vs 90.84 ± 134.47 mIU/mL, respectively; p = 0.001).

Detailed comparison between supplemented and non-supplemented participants is presented in *table 3*.

Table 4 - Correlation analysis for post-booster anti-HBs titers

Variable	Correlation coefficient (r)	p –value
Vitamin D level	0.388	< 0.001
BMI	0.236	0.013
Age	0.140	0.145
ALT	0.184	0.054
AST	0.159	0.096

Correlation Analysis

Serum vitamin D levels demonstrated a moderate positive correlation with post-booster anti-HBs titers (r = 0.388, p < 0.001). BMI also demonstrated a weak positive correlation with post-booster anti-HBs titers (r = 0.236, p = 0.013). No statistically significant correlations were detected between post-booster anti-HBs titers and age, ALT, or AST levels.

Correlation analysis results are summarized in *table 4* and *fig. 2*.

DISCUSSION

The present study evaluated post-booster anti-HBs response among seronegative young adults approximately 20 years after compulsory infant hepatitis B vaccination and investigated the possible association between vitamin D status and booster response. More than half of the studied cohort demonstrated anti-HBs levels below the protective threshold, highlighting the progressive decline in detectable circulating antibodies over time despite completion of primary vaccination during infancy. Nevertheless, nearly all seronegative participants mounted a successful anamnestic response following administration of a single booster dose, supporting persistence of immune memory despite waning antibody titers.

The decline in anti-HBs levels observed in this cohort is consistent with recent reports evaluating long-term HBV vaccine immunity. Hanafi et al. demonstrated that a substantial proportion of young adults vaccinated during infancy lost measurable anti-HBs antibodies nearly two decades after vaccination;

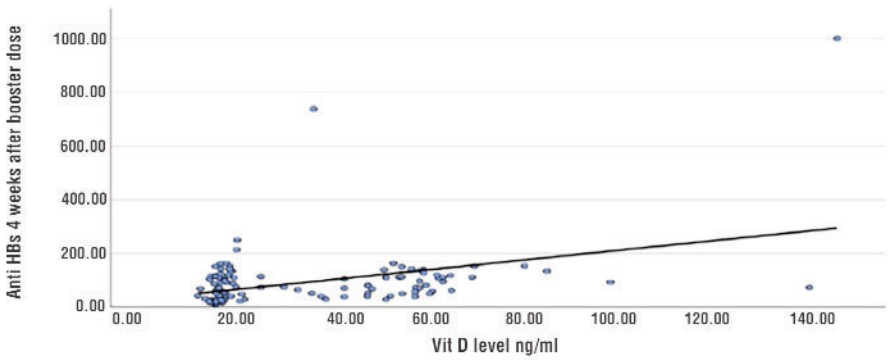

Figure 2 - Scatter plot demonstrating the correlation between serum vitamin D levels (ng/mL) and post-booster anti-HBs titers (mIU/mL)

however, most retained effective anamnestic responses following booster vaccination (7). Similarly, recent international studies reported declining seroprotection rates over time while preserving immunological memory among immunocompetent individuals (8,15).

In the present study, 53% of participants demonstrated anti-HBs titers below the protective threshold. Comparable findings were reported by previous studies evaluating long-term vaccine persistence among adults vaccinated during infancy (7,8). Variability among studies may be related to ethnicity, nutritional status, vaccine type, host genetic factors, and environmental influences.

A major finding of the current study was the observed association between vitamin D status and post-booster anti-HBs response. Participants with higher serum vitamin D levels demonstrated significantly higher post-booster antibody titers, and a moderate positive correlation was observed between serum vitamin D concentration and post-booster anti-HBs titers. Furthermore, vitamin D supplementation was associated with significantly higher post-booster immune responses compared with non-supplemented participants.

These findings are biologically plausible given the established immunomodulatory role of vitamin D. Vitamin D receptors are expressed on antigen-presenting cells and activated lymphocytes, where vitamin D influences cytokine production, antigen presentation, and B-cell differentiation (9,10). Several recent studies suggested that vitamin D deficiency may impair vaccine immunogenicity and antibody production (11,12).

Our findings are consistent with Kashi et al., who demonstrated that lower vitamin D levels were associated with weaker hepatitis B vaccine responses among healthy adults (13). Likewise, Youssry et al. reported enhanced HBV vaccine immunogenicity following vitamin D supplementation and ultraviolet B exposure in experimental models (14). In contrast, some studies failed to demonstrate a consistent association between vitamin D supplementation and vaccine responsiveness, suggesting that the effect may depend on baseline deficiency severity, timing of supplementation, and host immune characteristics (16).

The current study demonstrated that protective post-booster anti-HBs titers were achieved in 111/112 participants (99.1%), strongly supporting persistence of immunological memory despite declining circulating antibodies. Similar booster response rates exceeding 95% were reported in several recent studies evaluating long-term HBV vaccine efficacy (7,15). These findings support current recommendations indicating that routine booster doses are generally unnecessary in immunocompetent individuals who completed primary HBV vaccination during infancy.

Regarding demographic variables, BMI demonstrated a weak but statistically significant correlation with post-booster anti-HBs titers. Obesity has previously been associated with impaired vaccine responsiveness through chronic low-grade inflammation and immune dysregulation (17). However, the observed correlation in the present study remained modest.

The present study has several strengths, including evaluation of long-term HBV vaccine immunity approximately two decades after compulsory infant vaccination and assessment of vitamin D status as a potential modulator of vaccine response. Nevertheless, several limitations should be acknowledged.

Limitations

Several limitations should be acknowledged. First, vitamin D levels were measured only among seronegative participants and not in the entire cohort, limiting interpretation regarding the association between vitamin D status and long-term persistence of anti-HBs antibodies in all vaccinated individuals. Second, post-supplementation vitamin D levels were not reassessed. Third, vitamin D supplementation was not randomized, introducing potential selection bias. Fourth, the relatively small subgroup sample size and near-universal booster response limited multivariable statistical modeling. Finally, the observational design precludes causal inference.

CONCLUSION

Vitamin D status may be associated with post-booster anti-HBs response among previously seronegative young adults vaccinated during infancy. Protective post-booster anti-HBs titers were achieved in nearly all participants, supporting persistence of long-term immunological memory despite declining circulating antibody levels. Further prospective randomized studies are warranted to clarify the relationship between vitamin D status and HBV vaccine responsiveness.

Author's Contributions

Authors contributed equally in the study.

Conflict of Interest

The authors declare no conflict of interest.

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