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RESEARCH ARTICLE

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Efficacy of HBV booster dose administration in Italian medical students in relation to health determinants

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ABSTRACT

We evaluated the efficacy of a booster dose of HBV in Italian medical students. We conducted a prospective observational study in students who had received a full course of anti-HBV vaccination for at least 15 y. Those with an anti-HBs titer <10 mIU/mL were offered a booster dose of the HBV vaccine and the antibody titer was reevaluated after 1 month. The participants were classified into three categories: with anti-HBs titer >100 mIU/mL, between 10 and 100 mIU/mL and <10 mIU/mL. The study population was n. 625 medical student and 355 (56.8%) with anti HBs titer <10 mIU/mL were offered a booster dose. A total of 166 of them received the booster dose and 92.77% (38 + 116/166) achieved an anti-HBs titer ≥10 mIU/mL. The post-booster anti-HBs titer response was higher, i.e. >100 mIU/mL, in subjects who had a pre-booster anti-HBs titer between 1.00 and 9.99 mIU/mL (84.38%, 81/96), compared to those with titer <1 mIU/mL (50.00%, 35/70). Subjects with a titer <1.00 mIU/mL at enrollment showed no anamnestic response (post-booster anti-HBs <10 mIU/mL, RRR 0.23, 95% CI 0.06–0.84) and to a low anamnestic response (post-booster anti-HBs 10–100 mIU/mL, RRR 0.16, 95% CI 0.07–0.38). Physical activity was linked to a better antibody response to vaccination (post-booster anti-HBs 10–100 mIU/mL: RRR 2.39, 95% CI 1.05–5.59). Immune protection following primary vaccination against HBV tends to wane over time. Booster dose induces anamnestic responses, especially in individuals who maintain titer HBsAg >1 mIU/mL and do physical activity.

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Introduction

Hepatitis B virus (HBV) infection is a serious health problem worldwide. The hepatitis B virus (HBV) is a major cause of acute and chronic liver disease. Viral hepatitis B has a variable clinical course, including acute self-limiting hepatitis, fulminant liver failure, progression to cirrhosis and hepatocellular carcinoma.[1]

The WHO estimates that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year. In the same year, hepatitis B caused approximately 820,000 deaths, mainly from cirrhosis and hepatocellular carcinoma.[1]

According to data reported by the European Centre for Disease Prevention and Control (ECDC) during 2022 in 30 European Union (EU) and European Economic Area (EEA) countries 28,855 cases of hepatitis B were reported and excluding three countries that only reported acute cases, the number of cases (28 420) corresponds to a crude rate of 8.5 cases per 100,000 population. Of all cases, 7% were reported as acute, 40% as chronic, 47% as 'unknown' and 6% as 'could not be classified' due to lack of information. From 2013 to 2020, a decrease in acute hepatitis B cases has been observed, from 0.7 to 0.3 per 100,000 population, likely due to national vaccination programs. From 2020 to 2022 the acute notification rate increased to 0.5 per 100,000 population, although changes in health care-seeking behavior and testing during the COVID-19 pandemic could have affected recent trends.[2]

In Italy, the Integrated epidemiological System of Acute Viral Hepatitis (SEIEVA) surveillance, coordinated by the National Institute of Health (ISS), supports and integrates PREMAL, a disease notification computer system, providing annual data on the incidence of reported cases of acute viral hepatitis, characteristics of reported cases and the presence of main risk factors. From 1991 to 2021 the incidence of acute hepatitis B cases decreased from 5 cases per 100,000 population to 0.18 per 100,000. From 2022 to 2023 there is a slight increase in cases (up to 0.29 per 100,000 population). During 2023, as reported in the latest SEIEVA Bulletin No. 14, 153 cases of acute hepatitis B were reported in Italy mainly from central-northern regions such as Emilia-Romagna, Lombardy and Tuscany. The most affected individuals remain those aged between 35 and 54 y and are predominantly men (78.4%). The main risk factors included cosmetic treatments such as manicures, pedicures, piercings and tattoos (38% of cases), dental care (28.7%), risky sexual behaviors (25.2%) and nosocomial exposure (hospitalization, surgery, hemodialysis or blood

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transfusion) (19.9%). Eleven cases were observed in vaccinated or incompletely vaccinated subjects. Regarding the groups at increased risk, nine cases were observed in cohabitants of chronic carriers, seven in drug addicts and four in men who have sex with men (MSM).[3]

Safe and effective vaccines have been available since the early 1980s, enabling the prevention of HBV infection and its serious complications. Initially, vaccination strategies in industrialized countries were aimed at immunizing people at higher risk of exposure to HBV infection.[4] Vaccination of these had an individual benefit, but little or no impact in terms of control and prevention of hepatitis B in the general population, because hepatitis b virus infection in the occupational category was responsible for only a small part of the cases of infection found (about 5%).[5] For this reason, in 1991, the WHO Global Advisory Group recommended that all countries integrate the hepatitis B vaccine into their national immunization program by 1997.[4]

In Italy by Law No. 165 of 27 May 1991, due to the availability of the recombinant DNA vaccine, HBV vaccination became compulsory and was extended to the entire population through a universal 'two-cohort' vaccination strategy that included routine vaccination of all newborns and 12-y-olds. In addition, compulsory screening for HBsAg was introduced for pregnant women in the third trimester of pregnancy, in order to identify children who needed treatment with immunoglobulin and hepatitis B vaccine at birth, while the right to free vaccination of adults belonging to high-risk infection groups remained unchanged. Vaccination of 12-y-olds was discontinued in 2003, while compulsory vaccination of newborns continued.[6] With the introduction of compulsory vaccination, morbidity was drastically reduced, especially in the most vulnerable age groups, and Italy went from being a country of medium endemic rate to a country of low endemic rate with a prevalence of hepatitis B antigen (HBsAg) in the general population <2%.[7] The Italian HBV vaccination program has indeed achieved important results in terms of prevention and control of HBV infection.[7]

Immunization strategies against HBV are based on a three-dose primary vaccination cycle. Sero-protection rates are close to 100% in healthy children and adolescents and exceed 95% in healthy adults.[4,8,9]

Although the HBV vaccine has been shown to be safe and effective, between 5% and 10% of healthy individuals do not respond satisfactorily to vaccination. Several factors such as host genetics, age, body weight, smoking, alcoholism, concomitant diseases and the use of immunosuppressive drugs have been shown to influence the vaccine response rate.[10,11] Among unprotected individuals, one must distinguish 'non-responders,' i.e. those who, as indicated by the Centers for Disease Control and Prevention (CDC), do not develop protective surface antibodies after completing two rounds of HBV vaccine and are therefore susceptible to infection.[2]

As already observed in one of our previous studies and in agreement with data from the scientific literature, the anti-HBs titer decreases over time in vaccinated subjects 18–20 y after vaccination.[8,9] The persistence of anti-HBs correlates with the antibody peak reached after vaccination. It has been found, however, that a good percentage of subjects with a low anti-HBs titer express an anamnestic response to a booster dose, demonstrating that immune memory can persist even if the anti-HBs titer is below 10 mIU/mL.[6,8,9,12]

In Italy, according to Legislative Decree 81/2008, workers exposed to biological risk from HBV infection must have their anti-HBs titer checked during the preventive medical examination to assess that it is protective.[13] In fact, healthcare workers and students are exposed to a non-negligible occupational risk of infection and the biological risk of blood-borne infection transmission due to sharps injuries represents the main injury risk for healthcare workers, and in particular, the risk of infection with hepatitis B, C and HIV viruses.[14–16] For these professional categories, despite all the technical and organizational measures in place to date (work procedures, protective devices, needle and sharp devices) there are currently no adequate protection measures. The risk of cuts remains high, representing the main cause of accidents at work in hospital, and it is therefore essential to implement prevention measures by vaccinating against hepatitis B and measuring its effectiveness over time through serological tests aimed at analyzing the persistence of the antibody response over time.

Serological tests are not recommended to assess acquired immunity after primary vaccination against HBV in infants and adolescents, given the high protection achieved and the positive cost-effectiveness profile of vaccination. Conversely, in specific occupational groups at high risk of HBV infection, such as healthcare workers, anti-HBs testing is recommended to assess the acquisition of immunity to HBV. The Italian Ministry of Health, through the National Plan for Vaccine Prevention (PNPV), recommends, in addition to universal vaccination, the screening of healthcare workers by measuring anti-HBs in order to verify sero-protection. Furthermore, the PNPV recommends the administration of a booster dose of the vaccine to subjects with a non- protective antibody titer (<10 mIU/mL) followed, after 1 month, by a further blood test to see if immunological memory gives a secondary response. While subjects with anti-HBs levels ≥10 mIU/mL after the primary vaccination cycle are considered protected and do not require further action, those with anti-HBs <10 mIU/mL are considered unprotected and must undergo further investigation. In the latter cases, the administration of a booster dose of vaccine and serological testing at 1 month make it possible to discriminate those who present a physiological drop in antibody levels after successful immunization from those who do not respond to the initial vaccine course. If the anti-HBs titer remains below 10 mIU/mL, it is necessary to complete the second vaccine cycle with two further doses to try to obtain an effective response by stimulating immunological memory. Those who do not develop a protective anti-HBs titer after a second complete HBV vaccination course are considered 'non-responders.[17,18] The same recommendations for anti-HBV prophylaxis also apply to medical school students, who are on an equal footing with health care workers as they are exposed to a similar biohazard during their training activities.[19]

The main objective of the study was to evaluate the efficacy of a booster dose of HBV vaccine in a population of students enrolled in the Faculty of Medicine at the University of

L'Aquila who had not shown adequate antibody titer despite having received a primary vaccination in infancy or adolescence.

Furthermore, we analyzed the association between anti-HBs titer after booster dose administration and variables, such as gender, age at vaccination, smoking habits, alcohol consumption, physical activity, presence of concomitant diseases, and Body Mass Index (BMI).

Materials and methods

We conducted a prospective observational study in a population of students enrolled in the Faculty of Medicine at the University of L'Aquila and subjected to Health Surveillance, as required by Article 41 of Legislative Decree No. 81 of 9 April 2008, from January 2021 to March 2023. During this period, 750 Italian medical students of the University of L'Aquila, were examined for professional risk during Occupational Health Surveillance Program at occupational medicine clinic. All of which were invited to participate in the study, and there were 625 respondents. The 625 Italian medical students were born in Italy between February 1980 and March 2003 and all were fully vaccinated during infancy or adolescence. The vaccination status was verified through the immunization records provided by medical student.

In accordance with Law No. 165 of 27 May 1991, subjects who received the primary HBV vaccination in infancy will be defined as 'vaccinated during infancy, while subjects born before 1991 who received the vaccination during adolescence will be defined as 'vaccinated during adolescence.' Age, gender, province of residence, smoking habits, alcohol consumption, physical activity, chronic medical condition and BMI were also recorded through the review of medical records completed during the medical surveillance visit. In relation to physical activity, we only asked if they had sedentary life or they practiced physical actitity, in relation to smoking habits we only asked the actual state of smoking.

Italian medical students of University of L'Aquila who had received a full course of anti-HBV vaccination at least 15 y prior to enrollment were included in the study, those who had recently received an anti-HBV booster doseand those with personal or maternal HBsAg positivity were excluded.

Informed consent for participation and processing of personal data was obtained from the participants.

During the preventive medical examination, the anti-HBs titer was assessed, assayed by the chemiluminescence method (CLIA), and the antibody level was expressed in mIU/mL. Based on the antibody titer, the sample was divided into two groups: one with an anti-HBs titer ≥10 mIU/mL and the other with an anti-HBs titer <10 mIU/mL. The latter was offered a booster dose of HBV vaccine and had its antibody titer reevaluated after one month. The participants were then classified into three categories: those with anti-HBs >100 mIU/mL, those with anti-HBs between 10 and 100 mIU/mL and those with anti-HBs <10 mIU/mL. Subjects with anti-HBs titer >10 mIu/mL did not receive any other measure of prophylaxis.

Students with anti-HBs persistently <10mIU/mL were proposed to complete the second vaccination cycle with two further administrations (one immediately and one after four to 6 months) in order to induce immunological response.

The survey belongs to a research project relating to the risk factors and frequency of transmittable and non-transmissible diseases among workers at the University of L'Aquila, with analysis of health surveillance and preventive interventions pursuant to Legislative Decree 81/2008. The study protocol was approved by Internal Review Board (IRB 31/2020) (University of L'Aquila) and informed consent was obtained by subjects enrolled to the study.

Statistical analysis

A descriptive study of the quantitative and qualitative variables was performed and the normality of them was tested with the Shapiro–Wilk test. Nominal variables were reported as absolute frequencies and percentages, and continuous variables were expressed as mean values and standard deviations (SDs). The outcome of interest was the anti-HBs titer measured after a booster of the HBV vaccine in subjects with a non-protective anti-HBs titer at about 23 y after a full course of vaccination. The outcome variable was categorized into three levels: a <10 mIU/mL anti-HBs titer, 10–100 mIU/mL anti-HBs titer, and >100 mIU/mL anti-HBs titer after a booster dose of the HBV vaccine.

BMI was used to classify participants in the following weight categories, using the current WHO BMI cutoffs: Underweight (<18.50), Normal (18.50–24.99), and Overweight (≥25.00).[20]

The differences between categorical variables were assessed with the $\chi 2$ test or Fisher's exact test, as appropriate and a one-way ANOVA test for quantitative variables.

Independent variables with p-value <.25 in the univariate analysis were kept in a multinomial logistic regression model (Anti-HBs titer before booster dose, age at HBV vaccination, smoking status, chronic medical condition, geographical location, physical activity), according to the Zhongheng Zhang strategy to identify the factors independently associated with the anti-HBs titer measured after a booster of the HBV vaccine and presented relative risk ratios (RRRs) with 95% confidence intervals (95% CIs), corrected for the other factors present in the model.[21] We used a > 100 mIU/mL anti-HBs titer after a booster dose of the HBV vaccine, considered as an optimal anamnestic response, as the reference category, so in the study partecipants' values of <10 mIU/mL anti-HBs titer and between 10 and 100 mIU/mL anti-HBs titer after a booster dose of the HBV vaccine were compared to those who achieved >100 mIU/mL anti-HBs titer.

A p-value of <0.05 was the criterion for statistical significance. The data was processed using the STATA/BE software program, version 18.

Results

Characteristics of the study population

The study population was n. 625 and subjects with antiHBs titer <10 mIU/ml were at the beginning of our study n. 355 (56.80%). It was not possible to dose the antibody titer after the second dose of vaccine in subjects who had not responded adequately to the booster dose, as they were students and were

Table 1. Distribution of subjects based on demographic and HBV vaccination characteristics.

	N = (625)
Gender, n (%)	
Male	221 (35.36)
Female	404 (64.64)
Age at testing, years	25.28±3.53
Mean ± SD	
Age at HBV vaccination, n (%)	
In infancy (0–3 y)	596 (95.36)
During adolescence (11–14 y)	29 (4.64)
Anti-Hbs titre at enrolment	
In infancy (0–3 y), n (%)	
<10 mIU/mL	351 (58.90)
≥10 mIU/mL	245 (41.10)
During adolescence (11–14 y), n (%)	
<10 mIU/mL	4 (13.80)
≥10 mIU/mL	25 (86.20)
Time since primary vaccination, years	23.33 ± 2.41
Mean ± SD	
Geographical location, n (%)	
North	74 (11.84)
Center	201 (32.16)
South and Islands	350 (56.00)

SD: standard deviation.

lost from our monitoring. The mean age of the study population was 25.28 (SD ± 3.53); there were 404 (64.64%) females and 221 (35.36%) males. The majority of the students (596, 95.36%) had received a course of primary HBV vaccination during infancy and 29 (4.64%) during adolescence. The time since primary vaccination is not significantly different between the two groups (24.37 ± 3.54 vs 24.02 ± 2.58, respectively, for infancy and adolescence). An anti-HBs titer at enrollment <10mIU/mL was found in 351 (58.90%) students vaccinated in infancy and in 4 (13.80%) students vaccinated during adolescence. An anti-HB titer at enrollment ≥10mIU/mL was found in 245 (41.10%) students vaccinated in infancy and in 25 (86.20%) students vaccinated during adolescence. The mean time since administration of the last vaccine dose was 23.33 y (SD ± 2.41). The majority of study participants (56.00%) were from southern Italy and the islands based on the ISTAT Demo geographical breakdown[22] (Table 1).

With regard to lifestyle, 72.48% of the students at the time of the medical examination reported that they were nonsmokers, 24.8% did not consume alcohol and 61.76% practiced regular physical activity.

Of the subjects included in the study, 403 (64.48%) had no conditions interfering with the immune system; the remainer had one or more concomitant diseases involving the immune system, which in order of frequency turned out to be allergic diseases, autoimmune thyroiditis, celiac disease, psoriasis, diabetes mellitus, insulin resistance (Table 2). A 61.76% of them did regularly physical activity and only 14.56% of them were overweight.

Table 2. Habits and state of health among study population.

	N = (625)
Smoking status, n (%)	
Never smoker	453 (72.48)
Current smoker	172 (27.52)
Drinking habits, n (%)	
Do not drink alcohol	155 (24.80)
Rarely/occasionally/Often	470 (75.20)
Physical activity, n (%)	
No	239 (38.24)
Yes	386 (61.76)
Chronic medical condition, n (%)	
No	403 (64.48)
Yes	222 (35.52)
BMI, n (%)	
Underweight	42 (6.72)
Normal weight	492 (78.72)
Overweight	91 (14.56)

Medical students who received the HBV dose booster

Of the 355 participants, 189 withdrew from the study. The remaining 136 (38.31%) had a pre-booster anti-HBs titer <1.00 mIU/mL and 219 (61.69%) between 1.00 and 9.99 mIU/mL. A flow chart describing the study population is reported in Figure 1 and Table 3.

Of the remaining 166 who continued and are the subject of the present analysis, the 92.77% (38 + 116/166) of subjects that were boosted had a ≥ 10 mIU/mL post-booster anti-HBs titer, whereas 7.23% (12/166) did not mount an anamnestic response (Table 3).

Among the subjects with a non-protective anti-HBs titer at enrollment, 58.90% (351/596) had received the full course of vaccination during infancy, while only 13.80% (4/29) had received it during adolescence ($p < .001$). Of the 351 students vaccinated during infancy, 134 (38.18%) had anti- HBs <1.00 mIU/mL and 217 (61.82%) between 1.00 and 9.99 mIU/mL (Table 3).

The anti-HBs titer was higher, i.e. >100 mIU/mL, in subjects who had a pre-booster anti-HBs titer between 1.00 and 9.99 mIU/mL (81/96 = 84.38%), compared to those with a pre-booster anti-HBs titer <1 mIU/mL (35/70 = 50.00%). Furthermore, the percentage of students who maintained a non-protective anti-HBs titer 1 month after administration of the booster dose was higher among those with a pre-booster

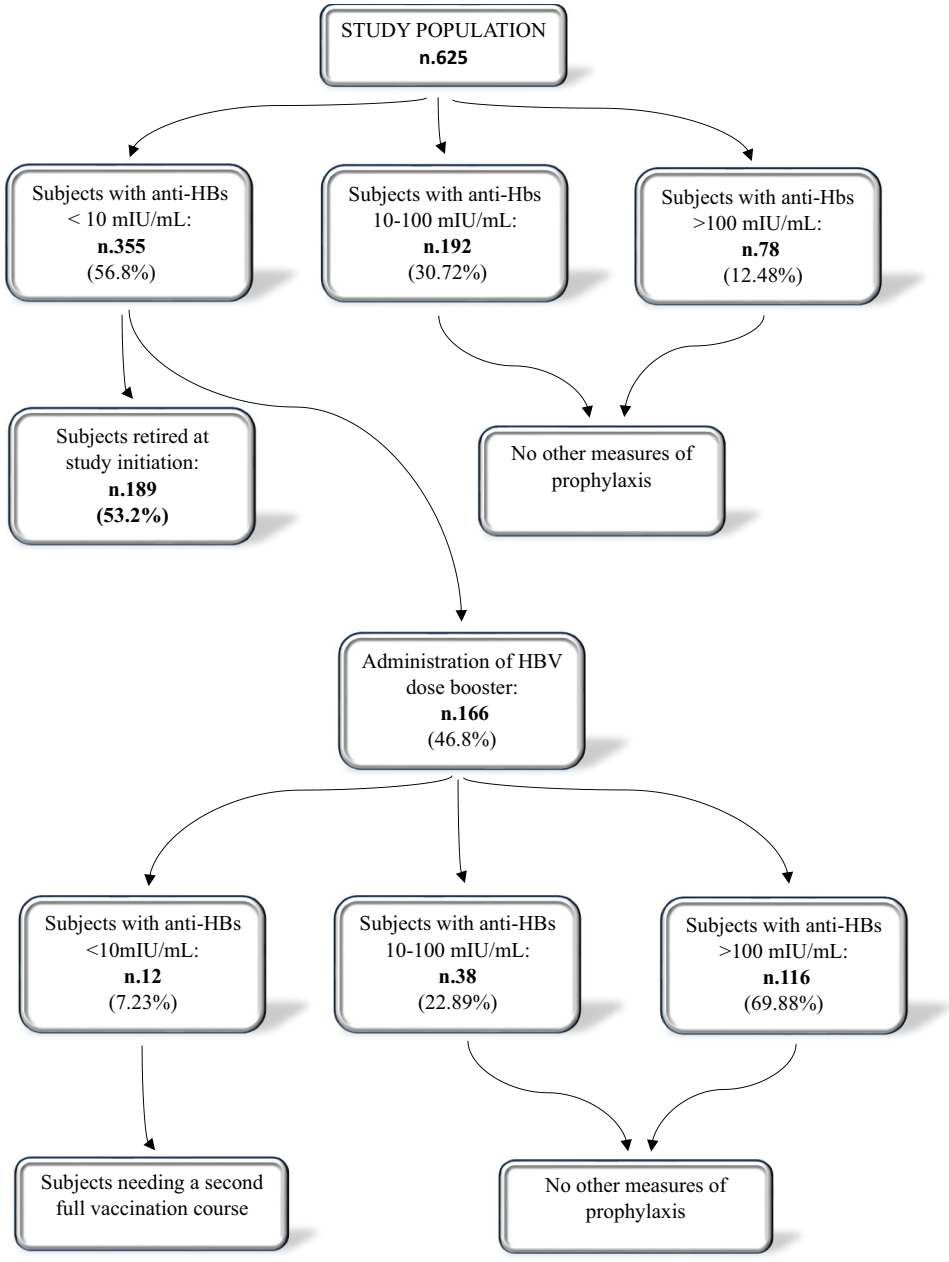

Figure 1. Flow-chart of the study.

Table 3. Booster dose response stratified by the pre-booster anti-Hbs titre.

Anti-HBs titre before booster dose (mIU/mL)	Subjects with <10 mIU/mL at Enrolment n. (%)	Lost to follow-up n. (%)	After Booster Anti-HBs Titre (mIU/mL)			
			<10 n. (%)	10–100 n. (%)	>100 n. (%)	Total n. (%)
Total subjects						
<1.00	136 (38.31)	66 (34.92)	8 (66.67)	27 (71.05)	35 (30.17)	70 (42.17)
1.00–9.99	219 (61.69)	123 (65.08)	4 (33.33)	11 (28.95)	81 (69.83)	96 (57.83)
Total	355 (100)	189 (100)	12 (100)	38 (100)	116 (100)	166 (100)
Vaccinated in infancy						
<1.00	134 (38.18)	65 (34.57)	7 (63.64)	27 (71.05)	35 (30.70)	69 (42.33)
1.00–9.99	217 (61.82)	123 (65.43)	4 (36.36)	11 (28.95)	79 (69.30)	94 (57.67)
Total	351 (100)	188 (100)	11 (100)	38 (100)	114 (100)	163 (100)
Vaccinated during adolescence						
<1.00	2 (50.00)	1 (100)	1 (100)	–	–	1 (33.33)
1.00–9.99	2 (50.00)	0 (0.00)	–	–	2 (100)	2 (66.67)
Total	4 (100)	1 (100)	1 (100)	–	2 (100)	3 (100)

Table 4. Subjects tested after the booster dose stratified by a post-booster anti-HBs titre.

	Total		Post-Booster Anti-HBs Titre					
			<10 mIU/mL		10–100 mIU/mL		>100 mIU/mL	
	N (166)	%	n. (12)	7.23%	n. (38)	22.89%	n. (116)	69.88%
Anti-HBs Titre before booster dose								
<1.00	70	42.17	8	11.43	27	38.57	35	50.00
1.00–9.99	96	57.83	4	4.17	11	11.46	81	84.38
	χ^2= 22.80, 2 df, p **<0.001**							
Gender, n (%)								
Male	56	33.73	5	8.93	9	16.07	42	75.00
Female	110	66.27	7	6.36	29	26.36	74	67.27
	χ2= 2.37, 2 df, *p*=.305							
BMI, n (%)								
	N (162)		n. (11)		n. (37)		n. (114)	
Underweight	13	8.02	0	0.00	2	15.38	11	84.62
Normal weight	125	77.16	9	7.20	29	23.20	87	69.60
Overweight	24	14.81	2	8.33	6	25.00	16	66.67
	Fisher's test; *p*=.864							
Age at testing, years Mean ± SD	25.28±3.53		25.83±3.41		25.13±2.36		25.14±2.50	
	F=0.42; *p*= .658							
Age at HBV vaccination, n (%)								
In infancy (0–3 y)	163	98.19	11	6.75	38	23.31	114	69.94
During adolescence (11–14 y)	3	1.81	1	33.33	0	0.00	2	67.67
	Fisher's test; *p*=.214							
Time since primary vaccination, years Mean ± SD	23.33±2.41		23.08±1.78		23.5±2.47		23.32±2.09	
	F=0.42 =, 2 df, *p*= .658							
Smoking status, n (%)								
Never smoker	117	70.48	6	5.13	25	21.37	86	73.50
Current smoker	49	29.52	6	12.24	13	26.53	30	61.22
	χ2= 3.56, 2 df, *p*=.168							
Chronic medical condition, n (%)								
No	106	63.86	5	4.72	29	27.36	72	67.92
Yes	60	36.14	7	11.67	9	15.00	44	73.33
	χ2= 5.27, 2 df, *p*=.071							
Geographical location, n (%)								
South and Islands	91	54.82	7	7.69	20	21.98	64	70.33
Center	57	34.34	3	5.26	12	21.05	42	73.68
North	18	10.84	2	11.11	6	33.33	10	55.56
	χ2= 2.33, 4 df, *p*=.071							
Drinking, n (%)								
Do not drink alcohol	36	21.69	1	2.78	9	25.00	26	72.22
Rarely/occasionally/Often	130	78.31	11	8.46	29	22.31	90	69.23
	Fisher's test; *p*= .633							
Physical activity, n (%)								
Do not physical activity	75	45.18	5	6.67	22	29.33	48	64.00
Yes	91	54.82	7	7.69	16	17.58	68	74.73

χ2= 3.21, 2 df, *p*=.200.
SD: Standard deviation; χ2 test; Fisher's test; F: one way ANOVA; df: Degree of freedom.

anti-HBs titer <1.00 mIU/mL (8/70 = 11.43%), compared to those with a pre-booster anti-HBs titer between 1.00 and 9.99 mIU/mL (4/96 = 4.17%) (χ^2 = 22.80, 2 df, p < .001). No other statistically significant associations were found between the post-booster anti-HBs titer and the other variables considered (Table 4). However, only 66.67% of the overweight subjects achieved a response >100 mIU/mL to the booster dose, whereas 84.62% of the underweight subjects achieved an antibody concentration >100 mIU/mL after the additional dose, although these differences are not statistically significant (p = .241).

The results of the multinomial logistic regression analysis used to determine the association between independent variables with p-value <.25 in the univariate analysis as anti-HBs titer before booster dose, Age at HBV vaccination, smoking status, chronic medical condition, geographical location, physical activity and the anti-HBs titer measured after a booster of the HBV vaccine showed that subjects with a pre-booster titer <1.00 mIU/mL at enrollment were significantly associated to no anamnestic response (Post-Booster anti-HBs <10 mIU/mL, RRR 0.23, 95% CI 0.06–0.84, p-value = .027) and to a suboptimal anamnestic response (Post-Booster Anti-HBs 10–100 mIU/mL, RRR 0.16, 95% CI 0.07–0.38, p-value <.001), compared to an optimal anamnestic response after the booster dose (>100 mIU/mL). Furthermore, the presence of physical activity increased the probability of having an anamnestic

Table 5. Results of the multinomial logistic regression analysis used to determine the association between the anti-HBs titre measured after a booster of the HBV vaccine.

Outcome: Anti-HBs Titre Measured after a Booster of HBV Vaccine	Post-Booster anti-HBs <10 mIU/mL		Post-Booster Anti-HBs 10–100 mIU/mL	
	RRR (95% CI)	p-Value	RRR (95% CI)	p-Value
Anti-HBs titre at enrolment with <1.00 mIU/mL (1.00–9.99 mIU/mL as reference)	.23 (0.06–.84)	**.027**	.16 (.07–.38)	**<.001**
Chronic medical condition (absence as reference category)	.45 (.12–1.62)	.226	1.87 (.75–4.66)	.174
Smoking status (do not smoke as reference category)	.38 (.11–1.38)	.142	.63 (.26–1.49)	.298
Physical activity (do not physical activity as reference category)	1.35 (.36–5.02)	.593	2.39 (1.05–5.59)	**.047**

RRR: Relative risk ratio.

Statistically significant differences in bold.

response Post-Booster Anti-HBs 10–100 mIU/mL: RRR 2.39, 95% CI 1.05–5.59, p-value = .047 (Table 5).

Discussion

Several studies have been performed over the years to assess the persistence of HBV seroprotection in risk groups adequately vaccinated in infancy or adolescence and the anamnestic response to the booster dose of the vaccine HBV. These studies showed an adequate response to the booster dose in at least 80% of subjects with pre-booster anti-HBs titer <10 mIU/mL.[8,18,23–26]

A recent systematic review and meta-analysis on the long-term efficacy of hepatitis B vaccination in healthcare workers in highly developed countries, which analyzed 46 articles, including our previous study, showed that the prevalence of seroprotection in healthcare students at the pre-exposure assessment was 73.8% and that the prevalence of anamnestic response after administration of a booster dose was 90.9%.[8,9,23,26] These results confirm that the majority of health students vaccinated with the full three-dose primary course maintain effective humoral immunity for more than two decades and that in most cases, there is a reactivation of the immune system against the virus after receiving a booster dose. The high response rate confirms the evidence of a previously established immunological memory. The decline in peripheral blood immunity, especially of the humoral branch, is not a completely reliable indicator of an actual reduction in immunity. In fact, only a small fraction of lymphocytes circulates constantly in the blood, while the rest are deposited in tissues and organs. Memory B-cells and plasma cells, in fact, are mostly housed in the bone marrow and lymphoid organs.[27] Our study also showed that the probability of achieving an adequate antibody titer was lower in subjects who had an undetectable anti-HBs titer at enrollment. In fact, only 50% of participants who had an undetectable antibody titer at enrollment (<1 mIU/mL) developed an optimal anamnestic response to booster dose, otherwise about 84% of students with antibody pre-booster titer between 1 and 9.99 mIU/mL showed a post-booster titer >100 mIU/mL.

In a study performed at the 'Magna Grecia' University of Catanzaro, involving over 1300 participants, it was emphasized that anamnestic response to the booster dose was significantly related to basal anti-HBs levels. Indeed, a low post-booster response (anti-HBs 10–100 mIU/mL) was significantly more likely in subjects with a pre-booster titer <2.00 mIU/mL, compared to those with a pre-booster titer between 2.00 and 9.99 mIU/mL. Specifically, the development of an inadequate anamnestic response after booster dose administration was found in 11% of those with an undetectable baseline antibody titer and only 2.3% of those with a detectable baseline antibody titer.[8]

Our study showed that approximately 57% of the students had non-protective anti-HBs levels 23 y after their last HBV vaccine dose. Among the subjects with a non-protective anti-HBs titer at enrollment, those who had received the full course of vaccination during infancy were less protected than those who had received it during adolescence (58.90% vs 13.80%). These data are consistent with the results of our previous study including 342 medical students and postgraduates Medical Doctors at the University of L'Aquila, which showed that among the subjects with suboptimal anti-HBs levels, those vaccinated at 1 y were less protected than those vaccinated at age 12 y (40.10% vs 16.50%), suggesting that vaccine administration in adolescence is the main predictor of a protective antibody titer.[9]

Another study comparable with our results performed on 717 students in northern Italy in 2017 showed that 70.5% of them maintained a good antibody response 20 y after the first administration and 95% of non-responders showed a good antibody response to the booster dose.[23] A further study performed in 2023 in South Africa on 180 students showed similar results to ours with 56% of the students having an insufficient antibody titer against hepatitis B and a 39% increase in seroprotection after the booster dose.[28]

Both the studies by Bini et al. and Stefanati et al. point out that the primary course of HBV vaccination during infancy is more frequently associated with the finding of non-protective anti-HBs titer after about 20 y than vaccination during adolescence. However, the results confirm that immunological memory appears to persist after the decline in anti-HBs titer, as demonstrated by the anamnestic response to the booster dose in infancy immunization cases.[18,24]

A study published in 2021, involving more than 11,000 undergraduate students of the Faculty of Medicine of the University of Padua, also showed that the year of birth, and consequently the age of administration of the first dose of anti-HBV vaccine, is the variable that most influences the antibody response. If the primary vaccination is administered before 1 y of age, there is about a 50% probability of having an antibody titer below 10 mIU/mL compared to subjects vaccinated after 1 y of age, where this probability is reduced to 13%. Vaccination

after 1 y of age induces a higher antibody titer probably related to the development of acquired immunity.[7] Indeed, the immune system in infancy is characterized by impaired *T*- cell function due to fewer B- and T-cell interactions, a reduced immunoglobulin assortment and a low-affinity antibody response.[9,24]

The persistence of long-term immunity was shown to be greater in young students who had received their first vaccination during adolescence vs infancy.[29,30]

In the scientific literature, many factors, such as host genetics, male gender, smoking, obesity, chronic diseases, immunosuppression, etc., have been found to be associated with a lower immunological response to HBV vaccine.[10,24] We evaluated the possible role of some of them and found that practicing regular physical activity increases the likelihood of developing an adequate post-booster response. On the basis of our knowledge, the possible role of the physical activity factor was found in only one previous study performed in 2008. In this case–control study, which included 21 elite football players and 30 control non-athlete males, 100% of football athletes developed good immunological response to HBV vaccination with a better response for younger athletes. None of the athletes was without response to the vaccine. One of the subjects from the control group did not develop it. The group of athletes was with better mean values of antibody titer and with a greater deal of subjects who developed a very good immune response (titer over 2000 mIU/ml). Younger football players had better immune reaction than older.[31]

Overweight and obesity, defined by a body mass index (BMI) $\geq 25\,kg/m^2$, are known to impair the strength of the immune response. This aspect became clear soon after the development and implementation of the HBV vaccine and has been largely validated by subsequent studies.[10] Adipose tissue plays a role in modulating the immune system through various pathways, inducing a chronic pro-inflammatory state, which is ultimately associated with immune system dysfunction. This includes chronic activation of innate immune system cells and subsequent local and systemic inflammation. Our data showed no differences between normal-weight and overweight subjects, which is probably related to the low percentage and young age of overweight subjects in our sample (approximately 14.8% of the entire sample).

The main limitation of this study is the lack of data on the antibody titer after the second complete vaccination cycle in subjects who did not respond adequately to the booster dose, due to the fact that being students and progressing in the course of studies, they graduated and left the study; this data is fundamental to identify the non-responders to HBV vaccination. Another potential limitation of the study is that no standardized measurement tool was used to estimate levels of physical activity. Furthermore, several students left the study without receiving booster vaccination, because the booster dose was recommended, not mandatory prior to entering the work environment.

Conclusions

Vaccination against the hepatitis B virus remains a cornerstone of preventive measures for the health of all citizens and workers at risk. Medical students represent a category at risk of exposure to biological agents hazardous to their health and safety. Health surveillance by the occupational physician offers the possibility to monitor the status of immunity against hepatitis B and to take control measures aimed at enhancing the antibody response, in individuals who, having received the vaccination decades earlier, are more likely to have a lower antibody titer.

In these cases, the administration of a booster dose acts as an effective stimulus in determining and reactivating the immune response. A more effective immune response is seen in individuals who showed a titer >1 mIU/mL at the last antibody measurement.

Certain factors such as activity and regular exercise, in addition to the already known facts described in the literature such as normal weight, a normal immunological state, and the absence of smoking, determine a better antibody response to vaccine administration. The importance of implementing health measures to increase people's physical well-being is therefore also confirmed in an area such as vaccination against hepatitis B. Our study showed the importance of monitoring against the hepatitis B virus for the medical students. It is necessary to promote in the future a higher awareness on the relevance of anamnestic response to HBV vaccination. Further studies will need to be carried out to confirm the role of physical activity in determining a better antibody response to vaccine administration.

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the formal analysis. S.B., L.F., R.F.Z., C.C., A.C., A.I.S. contributed to writing, reviewing, and editing manuscript. A.M. contributed to the statistical analysis and interpretation of results. All authors have read and accepted the published version of the manuscript.

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