

Predictors of Hepatitis B Surface Antigen Titers two decades after vaccination in a cohort of students and post-graduates of the Medical School at the University of Palermo, Italy

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Abstract

Introduction and objective. The introduction of a vaccine against hepatitis B virus (HBV) for newborn babies in Italy in 1991, extended to 12-year-old children for the first 12 years of application, has been a major achievement in terms of the prevention of HBV infection. The objective of this study was to analyse the long-term immunogenicity and effectiveness of HBV vaccination among healthcare students with different working seniorities.

Materials and method. A cross-sectional observational study of undergraduate and postgraduate students attending the Medical School of the University of Palermo was conducted from January 2014 – July 2016. HBV serum markers were performed with commercial chemiluminescence assays. Categorical variables were analyzed using the chi-square test (Mantel–Haenszel), whereas means were compared by using the Student's t test. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated by a multivariable logistic regression, using a model constructed to examine predictors of anti-HBs titer above 10 mIU/mL, assumed as protective.

Results. Of the 2,114 subjects evaluated – all vaccinated at infancy or at the age of 12 years and were HBsAg/anti-HBc negative – 806 (38.1%) had an anti-HBs titre <10 IU/L. The latter were younger, more likely to be attending a healthcare profession school (i.e., nursing and midwifery), than a medical postgraduate level school, and more likely to have been vaccinated in infancy ($p < 0.001$, 95% CI 2.63–5.26, adjusted OR 3.70).

Conclusion. The results of the study suggest that assessment of HBV serum markers in workers potentially exposed to hospital infections is useful for identifying small numbers of unvaccinated subjects, or vaccinated subjects with low antibody titre, all of whom should be referred to a booster series of vaccinations.

Key words

HBV infection, HBV vaccination, Anti-HBs titre, Healthcare students, postgraduate medical students

INTRODUCTION

Hepatitis B infection has a variety of clinical course, including self-limited acute hepatitis, fulminant hepatic failure, chronic hepatitis, and progression to cirrhosis and hepatocellular carcinoma. HBV is transmitted by percutaneous or mucosal exposure to infected blood or other body fluids. In July 2016, the World Health Organization (WHO) estimated that 240 million people are chronically infected with HBV, and more than 686,000 people die every year due to complications of hepatitis B, including cirrhosis and liver cancer [1]. The WHO's data reported that the hepatitis B prevalence is

highest in sub-Saharan Africa and East Asia, where between 5–10% of the adult population is chronically infected. High rates of chronic infections are also found in the Amazon and the southern parts of Eastern and Central Europe. In the Middle East and the Indian subcontinent, an estimated 2–5% of the general population are chronically infected. Less than 1% of the population of Western Europe and North America is chronically infected [1].

Worldwide, two billion people are infected with HBV (1/3 of the world's population), and there are four million new cases of acute hepatitis per year, with almost 400 million chronic carriers [2].

In countries where large-scale vaccination efforts were made, the epidemiology of hepatitis B has been transformed. In Italy, the epidemiology of this infection changed after the introduction in 1991 of the vaccination of newborn babies that was extended to 12-year-old children for the first 12 years of application.

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In Italy, from 1985–2014 in Italy, there has been a reduction in new notified cases: from 12 per 100,000 inhabitants to <1, as reported by the Integrated Epidemiological System for Acute Viral Hepatitis (SEIEVA) [2]. The Italian recommended adult/adolescent immunization schedule involves HBV vaccination doses at months 0, 1, and 6, whereas infant vaccination starts from the third month of life for infants, with 2nd and 3rd doses at 5 and 11 months. The vaccine is 95% effective in preventing infection and the development of chronic disease and liver cancer due to hepatitis B. In Italy, HBV vaccination is also recommended for people at risk of acquiring HBV infection, including those with important occupational risk as health workers [3–5]. In Italy, according to the national laws, students are also considered as workers, and therefore, if they are exposed to physical, chemical, biological or psychological risks, they are evaluated by an occupational health physician.

Of the population undergoing to HBV vaccination, 95% develop an effective immune response evaluated by the level of antibodies antiHBsAg > 10 mIU/mL. Several studies confirm that the acquired immunity persists for at least 10 years after vaccination with level of antibodies > 10 mIU/mL, but probably not longer, if vaccination had been performed at neonatal age [6–7]. In several countries in the world, students of faculties of medicine are examined to establish if the vaccination performed in infancy is still protective several decades after HBV vaccinations, because this is a population occupationally-exposed to a higher risk of acquiring HBV infection [8–20].

OBJECTIVE

The main aim of this study was to evaluate the persistence of long-term immunogenicity of HBV in students of the School of Medicine at the University of Palermo. A second aim was to identify possible predictive factors of long-term immunogenicity, such as age of individuals when they were vaccinated, gender and race.

MATERIALS AND METHOD

In this cross-sectional observational study, the levels of serum HBsAg, anti-HBs, and anti-HBc were evaluated of students attending schools of the health care professions, or postgraduate medical schools of the University of Palermo, Italy, who were examined for professional risks from January 2014 – July 2016. For each student, a standardized medical record was compiled, including socio-demographic (age, gender, country of origin) and clinical information (relatives' diseases and personal remote and proximate pathologies). A personal objective exam was additionally conducted for each subject before blood sampling.

Arbitrarily excluded from the study were subjects who met at least one of the following exclusion criteria: a) HBsAg personal or maternal positivity, chronic diseases or immunosuppression; b) absence of primary documentation of vaccination for HBV; c) recent booster dose of HBV vaccine.

According to Italian law, the subjects were requested to provide written informed consent to the processing of data. Moreover, although it is not required in Italy for observational studies, approval of the Local Ethics Committee was also obtained [21].

Serological tests. Serological analyses were performed with commercial chemiluminescence assays (VITROS anti-HBs assay on the Vitros ECI Immunodiagnostic system, Ortho-Clinical Diagnostics, UK). In particular, the antibody to the hepatitis B surface antigen (anti-HBs) levels were expressed as mIU/mL. Dynamic range of quantification is 10–1000 mIU/ml. The level of anti-HBs above 10 mIU/mL was considered as protective against HBV infection.

Statistical analysis. Statistical analysis was performed with R software version 3.3.2 (October 2016). The significance level chosen for all analyses was .05, 2-tailed. Absolute and relative frequencies were calculated for qualitative variables, whereas normally distributed quantitative variables were summarized as mean (standard deviation). Data normality was verified by the Shapiro–Wilk test for normality. Categorical variables were analyzed using the chi-square test (Mantel–Haenszel), means were compared by using the Student's t test. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated by a multivariable logistic regression model constructed to examine predictors of anti-HBs titer above 10 mIU/mL assumed as protective. All variables found to have a statistically significant association ($P < .05$) with anti-HBs titer > 10 mIU/mL were entered in multivariate logistic regression model in order to check for independence. In the multivariate analysis, age was included as a continuous variable.

RESULTS

The main characteristics of the 2,114 subjects included in the study are shown in Table 1.

Table 1. General characteristics of subjects (N=2,114) included in the study

	Total of students
Total, n (%)	2,114 (100)
Sex, n (%)	
– Males	1,275 (60.3)
– Females	839 (39.7)
Age in years, mean ± SD	26.6 ± 5.4
Country of birth, n (%)	
– Italy	2,112 (99.9)
– Poland	1 (0.05)
– Madagascar	1 (0.05)
University course, n (%)	
– Healthcare profession School	1,096 (51.8)
– Postgraduate medical School	1,018 (48.2)

All enrolled subjects were vaccinated for HBV and HBsAg/anti-HBc negative. 2, 870 (41.1%) students received a course of 3 paediatric doses (10 µg) of recombinant hepatitis B vaccine at their 3rd, 5th and 11th month of postnatal life, and 1,244 (58.9%) received a course of 3 adult doses (20 µg) of the same vaccine when they were 12 years old, as required by current law in Italy [22]. The majority (61.9 %) of the students had an anti-HBs titre >10 mIU/mL (Tab. 2).

Students with protective anti-HBs titre were statistically significantly older (27.9 vs. 24.4 years, $p < 0.001$), with fewer years after HBV vaccination (19.2 vs. 20.1, $p < 0.001$),

vaccinated at age 12 years (76.0% with \geq vs. 41.7% among vaccinee at infancy, $p < 0.001$) and more frequently attending postgraduate medical school (77.4% with \geq vs. 47.4% among healthcare profession school, $p < 0.001$). No statistically significant differences were observed in antibody titer between males and females.

Table 2. Variables associated with persistence of protective Hepatitis B surface antibody titers (≥ 10 mIU/mL)

	Hepatitis B surface antibody titers		p-value
	≥ 10 mIU/mL	< 10 mIU/mL	
Total, n (%)	1,308 (61.9)	806 (38.1)	-
Sex, n (%)			
- Males	513 (61.1)	326 (37.6)	0.58
- Females	795 (62.4)	480 (38.9)	
Age in years, mean \pm SD	27.9 \pm 5.01	24.4 \pm 5.47	<0.001
Years since HBV vaccination, mean \pm SD	19.2 \pm 3.57	20.1 \pm 3.24	<0.001
Vaccination period, n (%)			
- Vaccinated at infancy	363 (41.7)	507 (58.3)	<0.001
- Vaccinated at age 12 years	945 (76.0)	299 (24.0)	
University course, n (%)			
- Healthcare profession school	520 (47.4)	576 (52.6)	<0.001
- Postgraduate medical school	788 (77.4)	230 (22.6)	

The multivariable logistic regression model (Tab. 3) shows that after controlling for confounding, HBV vaccination at age of 12 years and attending postgraduate medical school, were significantly associated with increased odds of having protective Hepatitis B surface antibody titers (OR=3.70, 95% CI =2.63–5.26 and OR=1.40, 95% CI =1.02–1.94, respectively). In particular, a protective anti-HBV titer was about 4-fold more frequent among subjects vaccinated during adolescence than those vaccinated at infancy.

Table 3. Multivariable logistic regression model including variables statistically significantly associated with Hepatitis B surface antibody titers ≥ 10 mIU/mL

	Adjusted OR	(95% CI)
Age in years	0.81	(0.40 – 1.65)
Years since HBV vaccination	1.02	(0.99 – 1.05)
Vaccination period		
- Vaccinated at infancy	referent	
- Vaccinated at age 12 years	3.70	(2.63 – 5.26)
University course		
- Healthcare profession school	referent	
- Postgraduate medical school	1.40	(1.02 – 1.94)

DISCUSSION AND CONCLUSIONS

Health-care-related transmission has long been recognized as an important source of new HBV infections worldwide. It is estimated that in the United States, 12,000 health care workers were infected per year in the prevaccine era. A health care worker's risk of infection has been shown to correlate with level of blood and needle exposure [22].

It is internationally recognized that the healthcare profession and postgraduate medical students have a high occupational risk for HBV infection, also in countries with a low incidence of the disease [10–11,14]. In Italy, several studies have demonstrated a low endemic level (prevalence of HBsAg in the general population $< 2\%$) with an incidence of HBV infections of about 1 per 100,000 individuals, suggesting that healthcare workers and students could have a risk that is low but not negligible. Despite such evidence, for health professionals as well as for students and volunteers, to-date there is no obligation of vaccination, which is recommended only [23,24]. Fortunately a large majority of young Italian students have been vaccinated according to the national immunization programme that, since 1991, has included HBV vaccination as compulsory for infants and adolescents aged 12 years. Adolescent's vaccination was restricted to the first 12 years of the implementation of the vaccination law and, thus, in 2004, vaccination of 12-year-olds was stopped, but retained for infants.

As demonstrated by several studies, administration of HBV as part of a combination vaccine or as a monovalent vaccine induces long-lasting immune memory against HBV with long term antibody persistence. Several studies have reported that 85–90% of those vaccinated as adolescents have anti-HBs levels > 10 mIU/mL when measured 10 years after vaccination. This percentage was 40–60% for those vaccinated as infants, as measured 15–20 years after vaccination [25–26]. Both these data coincide with results obtained by the authors of the presented study, since more than 60% of their students had anti-HBV titers above 10 mIU/mL, also more than 20 years after vaccination. Moreover, it should be pointed out that none of them received a booster dose after the primary vaccination programme, and in the primary documentation of vaccination for HBV no date was indicated for its administration. In the experience of the authors, they observed that this habit is also common among the general population.

Despite declining serum levels of antibody, international evidence shows that vaccine-induced immunity continues to prevent clinical disease or detectable viremic HBV infection. The long-term efficacy of HBV vaccination is confirmed when one considers that none of vaccinated subjects in the current study was found to be HBsAg/anti-HBc positive.

As already reported by several authors, including those of the presented study, a relatively high percentage of students (about 40%) were revealed as having anti-HBV titers below 10 mIU/mL.

The CDC recommend pre-exposure assessment of current or past anti-HBs results upon matriculation, followed by one or more additional doses of HBV vaccine for subjects with anti-HBs < 10 mIU/mL, if necessary, helps to ensure HBV protection after contacts with blood or body fluids [27].

The administration of an HBV challenge dose after the primary schedule, induces strong anamnestic responses and is well tolerated [28]. In Italy, for Medical School students it is necessary to assess anti-HBs titre before making stages in hospital, in order to identify subjects with levels < 10 mIU/mL. In fact, although the current WHO view is that subjects with an anti-HBs titre < 10 mIU/mL still retain memory immunity, and no booster dose is necessary as part of a routine immunization programme, it could be beneficial to have a more protective approach for healthcare workers who are at significantly higher risk of exposure to HBV, administering a booster dose and rechecking titre after 1–2

months to verify whether or not they are responders [29]. The screening in the healthcare categories is also fundamental to identify students and workers older than 35 years old, that have not been vaccinated, neither at birth or at 12 years of age, and immigrants from countries without universal immunization. This approach would allow identification of non-responders to the primary vaccination cycle or subjects with incomplete vaccination cycle.

Moreover we have found that two variables could help in predicting subjects at higher risk of having anti-HBs titre <10 mIU/mL. These variables are HBV vaccination at infancy and attending healthcare professional courses.

The possible causes of the low anti-HBs titres in adult subjects vaccinated in infancy could be due to the immaturity of the immune system in infants, at an age of life when there could be a lower interaction between B and T lymphocytes. Otherwise, it is less clear the reason for which students attending healthcare professional courses could have a higher risk of non-protective HBV titers', also after adjustment for confounding due to age, years after vaccination and vaccination period. Further analyses could be needed for answering to this intriguing question and some variables, as socio-demographic characteristics could play a role in this association.

In this sense, the lack of more information about students (e.g. anti-HBV vaccine used for primary cycle, socio-demographic characteristics, immunological status, previous blood exposure etc) represents a major limit of the presented study. Despite these limitations, the presented study enriches the literature on HBV vaccination, highlighting that although anti-HBV vaccination is associated with long persistence of protective titers, several students and post-graduates of the Medical School could benefit from a booster dose.

Moreover, some factors were found that could contribute to identify susceptible subjects and that could be associated with immune regulation pattern. Long-term follow-up studies several decades after vaccination administration will be needed to confirm the duration and persistence of immune memory.

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