Hepatitis B Virus Protection 20 Years Post-Vaccination In Healthcare Students: An Observational Retrospective Study at the University of Rome Tor Vergata

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Abstract

Immunization of health care workers and students is still a major issue for infection control in healthcare facilities. Maintenance of immunity is therefore an essential part of prevention and infection control programs for Health care workers (HCWs).

The aim of this study was to assess the persistence of anti-HBV immunity in medical and nursing school students vaccinated in infancy (median time from vaccination to current testing = 20.4 years) and to update the Occupational Medicine protocol for HBV risk management at Tor Vergata University Hospital.

The total number of subjects was 806, of which 549 females (68%) and 257 males (32%). The time frame of serological testing (anti-HBs concentration) was from January 2010 to September 2015. The baseline anti-HBs values allowed the students to be grouped as follows: 478/806 (59.3%) group 1 students with antibody concentration > 10 mIU/mL and 328/806 (40.7%) group 2 students with antibody concentration < 10 mIU/mL. 147 of the group 2 students received a booster dose of recombinant anti-hepatitis B vaccine and had their anti-HBs measured 1 month after booster administration. Over 90% of students who received the booster dose had anti-HBs > 10 mIU/mL 1 month after administration. The results of this study stimulated a review of the current protocol for HBV management in HCWs and students at Tor Vergata University Hospital.

ABBREVIATIONS

HBV: Hepatitis B Virus; WHO: World Health Organization; HCW: Health Care Workers; CDC: Centers for Disease Control and Prevention; NHIS: National Health Interview Survey; SIMLII: Società Italiana di Medicina del Lavoro e Igiene Industriale; PNPV: Piano Nazionale di Prevenzione Vaccinale; PTU: Tor Vergata University Hospital UOSD Unità Operativa Semplice Dipartimentale; ECLIA: Electro Chemi Luminescence ImmunoAssay.

INTRODUCTION

Hepatitis B Virus (HBV) is recognized worldwide as an important issue for Public Health. Globally, around 2 billion subjects (1/3 of world population) exhibit serologic evidence of HBV infection [1,2]. Key epidemiological features of HBV infection are: about 4 million new annual cases, approximately 400 million chronic carriers, and 0.7-1.0 million deaths per year [3]. Vaccine administration has deeply modified the HBV epidemiology. After mass vaccination programs, World Health Organization (WHO)
has estimated a average reduction of chronically infected infants from 15% to 1% in countries where the disease is endemic. According to WHO estimates, anti-HBV vaccine coverage rate at 90% in healthy subjects, would prevent 537,000-660,000 deaths each year worldwide [4,5].

HBV is currently considered as an occupational risk for HCWs due to the high transmissibility of the virus. In the absence of vaccination, the risk of developing hepatitis B is around 18% -30% in HCWs [6,7]. Healthcare activities represent a working environment at high risk for occupational HBV infection, related to direct or indirect contact with blood or fluids from infected subjects; the risks are particularly high in surgical wards, hematology or hemodialysis centers. WHO estimates that every year more than 300,000 HCWs are exposed to accidental percutaneous contact with contaminated fomites, and that about 66,000 infections take place [6-8]. Thus, the Centers for Disease Control and Prevention (CDC) recommend anti-HBV vaccination for all HCWs, independently of job duty [9].

From 1983 to 2010, HBV infections in HCWs were reduced by about 98% [9,10]. In 2010, data from National Health Interview Survey (NHIS) demonstrated a different HBV vaccination coverage among HCWs depending on their specific duty. In particular, HBV vaccine coverage was 74% for HCWs with direct patient contact, and 46% for HCWs without direct patient contact [9,11]; both these values are substantially below the target of 90% anti-HBV vaccination coverage among HCWs established by “Healthy People 2020” (objective no. IID-15.3) [9,12]. In 2011, NHIS evidenced a ≥3-dose anti-HBV vaccination coverage of 63.8% among HCWs aged ≥19 years [9,13]. The decrease in acute HBV infection among HCWs probably resulted from both routine anti-HBV vaccination and reduced risk of exposure [9].

The vaccination for all health professionals has been recommended and offered free of charge in Italy since the 1980’s [14]. In Italy, a national vaccination program against HBV infection has been introduced and made compulsory since 1991 for two cohorts of children (3 doses in infants at 3, 5 and 11 months of age and 3 doses in 12 years old), which have merged since 2003, and strongly recommended for subjects at high risk of contagion, such as HCWs [15,16]. In 2005, the guidelines for HBV screening in HCWs were drafted by the Italian Society of Occupational Medicine and Industrial Hygiene (SIMLI); the need for a booster dose was considered useless in the presence of evidence of an effective seroprotection (anti-HBs concentration > 100 mIU/mL) following the fourth dose of anti-HBV vaccine administration. However, anti-HBs concentration is subject to decrease over time and can reach values < 10mIU/mL 5-20 years after vaccination. Nonetheless, these subjects seem to be protected against HBV infection due to the presence of anamnestic memory [17]. Due to the compulsory nature of HBV vaccination of infants since 1991, the coverage level for HBV vaccination in Italy is over 90%; this condition differs from other European countries, such as France and Greece, where HBV vaccination is only recommended in infants [18,19].

HBV exposure for HCWs remains an important subject of debate in Italy. The current National Vaccine Prevention Plan (PNPV 2012-2014), has established the immunization schedule for HCWs, both pre- and post-exposure [20]. In facilities where the risk of occupational infection is high, the anti-HBV vaccination is strongly recommended for all health care workers and medical students, before starting the activities at risk. For all subjects not previously vaccinated, three doses of anti-HBV vaccine are offered at 0, 1 and 6-12 months. A rapid schedule with 4 doses (0, 1, 2, 12 month) is administered in case of exposure to infectious material [21-23]. The seroconversion should be verified after the third or the fourth dose to confirm the protection (as mandated by the Ministerial Decree DM 20/11/2000, Article 4) [20-22]. For all subjects born from 1980 onwards that have presumably been vaccinated against HBV it is recommended to perform anti-HBs concentration measurement before starting the activities at risk. HCWs with anti-HBs < 10 mIU/mL are strongly recommended to have a booster dose and anti-HBs dosage one month after booster administration [20]. In Italy, healthcare students are considered as HCWs for the purposes of Occupational Health Law and controlled by the Occupational Medicine Unit of teaching hospitals [24]. In a previous study performed in Italy in a similar population (healthcare students at a major Italian university), protective antibody levels were found in 49.6% of students [25].

The aim of this study was to verify immunity against HBV in medical and nursing school students born after July 1991 in order to create a picture of the local epidemiological situation to provide basis for an update of the Occupational Medicine protocol for HBV risk management at Tor Vergata University Hospital.

MATERIALS AND METHODS

All healthcare students have to undergo medical examination before attending the internship at the Tor Vergata University Hospital (FTV); the Occupational Medicine Unit (UOSD – Unità Operativa Semplice Dipartimentale) is in charge of the evaluation of healthcare workers (including students) and of the final decision on the ability to attend clinical activities. In particular, one of the several tests that are performed is a serological screening for anti-HBs. Those who have anti-HBs concentrations less than 10 mIU/mL are offered a booster dose of commercially available recombinant anti-hepatitis B vaccine and anti-HBs is measured 1 month after booster administration (see Figure 1 for the routine individual HBV serology and vaccination form).

The baseline anti-HBs concentration measured, between January 2010 and September 2015 (t0), in students born after July 1991 was obtained from the Department of Laboratory Medicine. Furthermore, anti-HBs concentration measured 1 month after booster dose was evaluated for evidence of immunological memory.

Inclusion criteria were: being a university student (in any healthcare course) born after July 31st 1991. Exclusion criteria were: being HBsAg positive, or anti-HBe positive.

For each subject the following data were recorded: gender, date of birth, anti-HBs concentration at the first occupational medicine visit, anti-HBs concentration after booster dose.

The subjects were categorized according to their serological test results as follows:
- Anti HBs > 10 mIU/mL considered as having protective levels of anti-HBs;
• Anti HBs <10 mIU/mL considered as having a non protective concentration of anti-HBs.

All subjects with anti HBs < 10 mIU/mL were offered counselling on vaccination; the subjects who accepted to be vaccinated were given a booster dose of monovalent HBV vaccine.

The serological tests were performed by ElectroChemiLuminescence ImmunoAssay (ECLIA).

The data were entered into Microsoft Excel® spreadsheets and were accessible only to the medical staff of the Occupational Health Unit and selected people within the Department of Biomedicine and Prevention; furthermore all personal identifying information was edited out of the analytic database.

The study was not interventional and therefore required no Ethics Committee Approval; all subjects gave their written informed consent before receiving vaccination; furthermore no additional costs over the routine activity of the Unit were incurred in performing the study.

**RESULTS AND DISCUSSION**

The total number of subjects was 806, of which 549 females (68%) and 257 males (32%); the age of the students

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**Figure 1** First page of routine Anti-Hepatitis B Vaccination Form.

<table>
<thead>
<tr>
<th>Date of blood test</th>
<th>HBs Ag</th>
<th>Anti-HBs concentration</th>
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<tbody>
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<th>Date</th>
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was categorized according to year of birth (Table 1 for study demographics). The median time from primary series vaccination after birth to anti-HBs testing at the University Hospital was 20.4 years (range: 18.9-23.4 years). Students' baseline serological tests were divided according to an antibody concentration cutoff of 10 mIU/mL (Table 2 for data stratified by concentration):

- Group 1: students with antibody concentration > 10 mIU/mL which were considered to be protected from infection following occupational exposure; this group was made up of 478/806 (59.3%) students, of which 313/549 females (57%) and 165/257 males (64%);

- Group 2: students with antibody concentration < 10 mIU/mL, which were considered to be at risk of infection following occupational exposure; this group was made up of 328/806 (40.7%) students, of which 236 females (72%) and 92 males (28%).

Overall, among 328 Group 2 students, 147 (45%) accepted the offer of a booster dose, whereas 181 (55%) did not accept. All 147 students who accepted the booster dose were invited to return for anti-HBs testing 4 to 6 weeks later, according to the recommendations of the Ministry of Health (DM 20/11/2000) and of the Italian Occupational Medicine Act [24].

Of 147 students who had the booster dose, only 63 returned for anti-HBs testing (43.3%); 84 students did not undergo anti-HBs test.

Of 63 students who returned for anti-HBs testing, 58 (92%) had anti-HBs concentrations greater than the cut-off for protective immunity of 10 mIU/mL and among them 47 (81%) had anti-HBs concentrations greater than 100 mIU/mL.

The remaining 5 students had anti-HBs lower than 10 mIU/mL and were considered to be non protected (see Figure 2 for participants flowchart). In particular, 2 had a measurable anti-HBs concentration (Anti-HBs from 2 to less than 10 mIU/mL) and 3 had an undetectable concentration (< 2 mIU/mL).

Overall, 5 out of 63 students (8%) who had the booster dose and the following anti-HBs testing did not respond. Among them, 3 had baseline (t0) anti-HBs < 10 mIU/mL and 2 had baseline (t0) detectable anti-HBs < 10 mIU/mL.

Extrapolating to the entire population the 8% of subjects with an anti-HBs < 10 mIU/mL after booster dose, 26/806 students would not be protected (3.2%; range 2.9%-3.3%). This percentage of non protected students is low and in line with published information [26].

The reason we decided to enroll students born after 31st July 1991 is that in the early months of the introduction of compulsory infant anti-HBV vaccination (Law 165/91 of 27th May 1991) the coverage was very uneven in the different regions of Italy.

Globally, around 60% of students presented protective anti-HBs levels 20 years after vaccination.

As is evident from Table 2, the percentage of protected students shows a decreasing trend from 67% (born in 1991) to 51% (born 1994-96); this finding represents an unexplained paradox, as immunity would be expected to decrease over time. A possible hypothesis could be the use of different vaccines with different immunogenicity over time.

Immunization of HCWs is still a major issue for infection control in healthcare facilities. Maintenance of immunity is therefore an essential part of prevention and infection control programs for HCWs. Optimal use of immunizing agents safeguards the health of workers and protects patients from becoming infected through exposure to infected workers. Relevant EU legislation reads: “Effective vaccine must be made available free of charge for workers not already immune to the biological agents to which they are (or are likely to be) exposed. The training shall be: (a) given at the beginning of work involving contact with biological agents, (b) adapted to take account of new or changed risks, and (c) repeated periodically if necessary” [27].

Our study is retrospective and highlights a situation of low compliance with HBV prevention (see Figure 2 – Flowchart)

Table 1: Demographic characteristics of the students.

<table>
<thead>
<tr>
<th>YEAR OF BIRTH</th>
<th>Number of students</th>
<th>Males</th>
<th>Females</th>
<th>Percentage of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>148</td>
<td>56</td>
<td>92</td>
<td>18%</td>
</tr>
<tr>
<td>1992</td>
<td>287</td>
<td>102</td>
<td>185</td>
<td>36%</td>
</tr>
<tr>
<td>1993</td>
<td>215</td>
<td>58</td>
<td>157</td>
<td>27%</td>
</tr>
<tr>
<td>1994</td>
<td>125</td>
<td>30</td>
<td>95</td>
<td>15.8%</td>
</tr>
<tr>
<td>1995</td>
<td>29</td>
<td>9</td>
<td>20</td>
<td>3%</td>
</tr>
<tr>
<td>1996</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.2%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>806</td>
<td>256</td>
<td>550</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2: Anti-HBs concentration (at t0) stratified for years of birth.

<table>
<thead>
<tr>
<th>YEAR OF BIRTH</th>
<th>Anti HBs&lt;2 mIU/mL (%)</th>
<th>2 mIU&lt; Anti-HBs&lt;10 mIU/mL (%)</th>
<th>Anti HBs&gt;10 mIU/mL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>17</td>
<td>16.2</td>
<td>66.9</td>
</tr>
<tr>
<td>1992</td>
<td>26.8</td>
<td>12.2</td>
<td>61</td>
</tr>
<tr>
<td>1993</td>
<td>30.7</td>
<td>11.6</td>
<td>57.7</td>
</tr>
<tr>
<td>1994-1996</td>
<td>36</td>
<td>13</td>
<td>51</td>
</tr>
</tbody>
</table>
recorded in our population of students; therefore one of the objectives for the near future is to revise the Occupational Health Unit protocol for HBV vaccination. From now on, in agreement with the University, healthcare students will be required to have a visit with the Occupational Medicine Unit at the time of enrolment into healthcare courses and not, as is customary now, during the third year. Students without evidence of protective anti-HBs concentrations (>10 mIU/mL) will not be issued the ID badge necessary to attend clinical activities before receiving the booster dose.

One of the objectives for the near future is to reinforce and reach an agreement between the Occupational Health Unit and the Department of Biomedicine and Prevention to set up initiatives of health promotion on risk perception for 1st year healthcare students. It is foreseen that these initiatives will be as follows:

- Update the current protocol for working ability establishment, in particular, as far as serological testing is concerned, the anti-HBs screening will take place at the time of enrolment into healthcare courses and not, as is customary now, during the third year. Furthermore, the ID badge necessary for student clinical activities will not be issued without evidence of anti-HBs > 10 mIU/mL or a booster dose. In case of non-responding subjects, a 3-dose course will be administered and the possibility of using an adjuvanted vaccine (currently approved for subjects undergoing hemodialysis) will be taken into consideration;

- Provide more information and training to 1st year healthcare students on biological risk and on recommended vaccinations for HCWs, focusing specifically on the risk related to HBV infection in order to create a correct perception of risk. It is foreseen that an ad-hoc lesson will be set-up and educational material will be distributed to 1st year healthcare students. Information will focus on the different tests that the students will undergo at the Occupational Medicine visits in order to minimize as much as possible the non compliance rate and future costs related to possible litigation issues;

After several decades of hepatitis B vaccination, the duration of protection is still subject to debate, post-immunization values (1-month after primary series vaccination) are often not available and the question whether a booster dose is ever needed continues to be open. These questions have been taken
into consideration since the early days of universal infant HBV vaccination. Indeed, in 1996, a population survey was conducted to assess the duration of anti-HBs level > 10 mIU/mL in vaccinees living in the same region where this study was performed 5 years after the introduction of compulsory vaccination of newborns and 11-year-old children. In 92.9% of children and 94.1% of adolescents anti-HBs concentration were protective (>10 mIU/mL) and antibody titres were significantly higher in adolescents [28].

In another study, performed in children and Italian Air Force recruits that had been vaccinated more than 10 years before, protective anti-HBs concentrations were retained in 64% of children and 89% of recruits [29].

In a study on Palestinian healthcare students vaccinated in infancy, over 75% of students had protective anti-HBs 18-22 years post-vaccination [30]. At 20 years after primary vaccination, an Iranian study analyzed the anti-HBs of 300 adults: 37.0% of participants had protective levels of antibody (> 10 mIU/mL). After booster vaccination, 97.1% developed protective levels of antibody. These values are in line with the findings from our study, particularly as far as booster dose response is concerned (97.1% vs 92% in our study) and support the persistence of long-term immune memory even in subjects with non protective levels of anti-HBs [25].

This study has some limitations; the design was observational and retrospective and no data were available on primary vaccine formulation and dosage. No post-vaccination anti-HBs test is recommended following universal mass immunization. Furthermore, the high rate of students who did not return for booster administration and subsequent evaluation could have distorted the results even if the study population appears to be representative of the general population of the same age range.

**CONCLUSION**

This study provides additional knowledge on the duration of anti-HBV immunity 20 years after immunization in persons vaccinated in infancy. The results support the current idea that the vast majority of individuals without protective anti-HBs concentration present an immunological memory response to HBV antigens and should therefore be protected against infections. On the other hand, monitoring of healthcare workers/students is still important to identify the few non-responders who may need to have a full 3-dose course or an adjuvanted vaccine. In this way the best results in protection can be reached and possible litigation issues can be avoided.

**CONFLICT OF INTEREST**

EF received reimbursement for participation to meetings from GSK and Sanofi-Pasteur MSD without any personal fee. All the other authors report no conflict of interest.

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