THE LONG-TERM IMMUNOGENICITY OF RECOMBINANT HEPATITIS B VIRUS (HBV) VACCINE: CONTRIBUTION OF UNIVERSAL HBV VACCINATION IN ITALY.

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Running head: Long-term immunogenicity of HBV vaccination in healthcare students

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ABSTRACT

Aim: To identify factors associated with long-term low protection against hepatitis B virus (HBV) 17 years after primary vaccination in students attending medical schools in Naples, Italy.

Methods: 1,704 students attending the school of medicine, schools of the healthcare professions, or postgraduate medical schools of the Second University of Naples, Italy, from September 2012 to December 2013 were enrolled in this cross-sectional study. Of these, 588 had been vaccinated against HBV at birth and 1,116 when 12 years old. Students with an anti-HBs titer >10 UI/mL were considered long-term responders to HBV vaccination, and those with a titer from 1–9 UI/mL were considered long-term low responders. Multivariate logistic regression analysis was used to identify factors associated with the level of long-term immune response.

Results: All subjects were HBsAg/anti-HBc negative: 270 (15.8%) were long-term low responders, and the remaining 1,434 were long-term responders. When compared with responders, low responders were younger (24±5.2 years vs. 26±4.9 years, p<0.000), more frequently students attending a healthcare school (59% vs. 47%, p<0.001), and more frequently had been vaccinated at birth (50% vs. 31.5%, p<0.0001). Multivariate logistic regression identified age at vaccination as the only factor independently associated with long-term low response (OR: 2.43; CI 95%: 1.57–3.76, p=0.001).

Conclusions: Universal HBV vaccination in Italy has been more effective in generating a prolonged protective response in subjects vaccinated during adolescence than at birth. Long-term low response students should be considered for a booster dose because most will be exposed to the risk of acquiring HBV for decades.

Keywords: HBV infection, HBV vaccination, anti-HBs titer, healthcare students
INTRODUCTION

Infection with hepatitis B virus (HBV) is a leading cause of acute and chronic liver disease worldwide [1]. The World Health Organization (WHO) estimates that, globally, about 2 billion people have been infected with HBV, more than 350 million are chronically infected, and nearly one million per year die from its acute or chronic sequelae, such as fulminant hepatitis, liver cirrhosis, and hepatocellular carcinoma [2]. The prevalence of HBV-related hepatitis varies across countries: in industrialized Western European countries and North America, the prevalence of HBV surface antigen (HBsAg) positivity in the general population is less than 2% (low endemicity); in most countries of the Mediterranean, Eastern Europe, and Asia it ranges between 2–8% (intermediate endemicity); whereas it is over 8% in some developing countries in Far-East Asia and Sub-Saharan Africa (high endemicity) [3,4].

In Italy, the epidemiology of HBV infection has changed substantially over the last 50 years: there has been a remarkable, progressive reduction in the incidence of acute hepatitis B (from 10/100,000 inhabitants in 1984 to 0.85/100,000 in 2012) and in the percentage of HBsAg-positivity in patients with chronic hepatitis (from 60% in 1975 to nearly 10% in 2001) [5,6]; moreover, the prevalence of chronic carriers of HBsAg in the general population has decreased from nearly 3% in the 1980s to 1% or less in 2010 [5,7]. The reasons for this may be due to a number of relevant events occurring in Italy over the last three decades, including an improvement in socio-economic conditions, a reduction in the size of families, the national educational campaigns against HIV infection, mandatory screening for women during pregnancy and/or at the time of delivery, and a mass vaccination campaign against HBV [8,9].

Universal HBV vaccination of newborn babies was introduced in Italy in 1991 and was extended to 12-year-old children during the first 12 years of application, a strategy that allowed to cover in a dozen years the Italian population aged 0–24 years. In Italy, HBV vaccination is also recommended for people at risk of acquiring HBV infection [10-12].
A debated issue is how long a protective antibody response may persist after vaccination. A study performed in Italy in 2003 showed that after primary vaccination of infants and adolescents, the antibody response persisted at protective levels (>10 IU/mL) for at least 10 years in most subjects [13]. However, the data on the persistence of the efficacy of vaccination for longer periods are scant and fragmentary [14-18]. Thus, the present study was carried out to evaluate the long-term (median: 17 years) protective value of HBV vaccination and to identify independent predictive factors of long-term efficacy. To this end, students attending the medical and healthcare schools of the Second University of Naples, Italy, were enrolled in this cross-sectional study.

MATERIAL AND METHODS

From September 2012 to December 2013, we actively screened serum HBsAg, anti-HBs, and anti-HBc in all students attending the 6th year of the medical school, the first year of the health profession schools (nursing, pediatric nursing, radiology, and midwifery), and of the postgraduate medical schools of the Second University of Naples, Naples, Italy.

A pre-coded questionnaire on demographics, previous exposure to HBV, and HBV vaccination was filled in by each student. Out of the total of 1,727 students examined, 23 had escaped HBV vaccination and 1,704 had been vaccinated and were enrolled in this cross-sectional study. Of the latter, 588 had received a course of 3 pediatric doses (10 µg) of recombinant hepatitis B vaccine at their 3rd, 5th, and 11th month of postnatal life, and are classified as “vaccinated at birth” in this paper; 1,116 had received a course of 3 adult doses (20 µg) of the same vaccine when 12 years of age, administered at 0, 1, and 6 month intervals. Information on HBV vaccination was always confirmed by the students’ vaccination cards.

HBV serum markers (HBsAg, anti-HBs, total anti-HBc) were determined using commercial immunoenzymatic assays (Abbott Laboratories, North Chicago, IL, USA). Anti-HBs titers were measured on a calibration curve generated with the WHO reference standard, and are expressed in
IU/mL: subjects with an antihBs titer >10 UI/mL were considered “long-term responders” to HBV vaccination, and those with an antibody titer <10 UI/mL were termed “long-term low responders”.

All procedures were performed in compliance with the Declaration of Helsinki and with the current healthcare standards indicated by the Italian Ministry of Health. This investigation was not submitted for approval to an ethics committee since this is not required by Italian law for observational cross-sectional studies. In full agreement with the rationale and the aim of the study, all students signed a written informed consent form. Personal information regarding the subjects included in the study was protected according to Italian law.

Statistical analysis was performed with StatGraph, version 3.0. Continuous variables are given as mean±standard deviation, and categorical variables as the absolute value and relative frequency. Differences in means were evaluated with unpaired Student t-test, and the chi-squared test was applied to categorical variables. A p-value < 0.05 was considered to be statistically significant. Odds ratios, with 95% confidence intervals (CI), were estimated with a logistic regression model to evaluate the relationship between the long-term low response to HBV vaccination and sex, age at vaccination, years from vaccination, and the type of school attended. A p-value < 0.05 was considered to be statistically significant.

RESULTS

Of the 23 students who had escaped HBV vaccination, 22 were HBsAg/antihBs/antihBc negative, and one was HBsAg positive (Figure 1). The 588 students vaccinated at birth and the 1,116 vaccinated during adolescence were all HBsAg/antihBc negative and were investigated to assess the long-term efficacy of HBV vaccination (Figure 1). The demographic and epidemiologic characteristics of the 1,704 students are given in Table 1. Most were females of Caucasian Italian ethnic background. The ages of the 525 and 829 students attending the school of medicine and a healthcare profession school were 26.5±3.7 and 23.8±4.9 years old, respectively; the 350 postgraduate medical school
students were older (29.7±2.2 years). The majority (84.2%) of the enrolled students were classified as long-term responders (range of anti-HBs titers: 1-9) (Table 1).

Compared with students vaccinated at their 12th year of age, those vaccinated at birth were younger, more frequently female, and more frequently attending a healthcare profession school than either the school of medicine or a postgraduate school (Table 2); overall, a longer period of time had elapsed since their vaccination, with a significantly higher number of individuals classified as long-term low responders (23% vs. 12%, p<0.0001). The difference in the frequencies of low responders was even more marked considering in both groups only the students vaccinated between 1991 and 1995, and thus rendering the time from vaccination comparable in the two groups (23% of 135 students vaccinated at birth vs. 6.4% of the 202 vaccinated at their 12 year of age; p<0.0001) (Table 2).

The characteristics of the vaccinated students stratified according to HBsAb titer are given in Table 3. Long-term low responders were younger, more frequently attending a healthcare profession school, and more frequently vaccinated at birth. Females were prevalent to a similar degree in both groups.

To identify the factors independently associated with long-term low response, a logistic regression analysis was performed with sex, age at vaccination, type of school attended, and years elapsed from vaccination as the variables (Table 4). The analysis identified age at vaccination as the only independent predictor of long-term low response (Odds Ratio: 2.43; Confidence Interval 95%: 1.57-3.76, p=0.00).

DISCUSSION

Universal vaccination against HBV infection was introduced in Italy in 1991: all newborn babies were vaccinated to prevent the risk of acquiring HBV infection by vertical and familial transmission, while all children aged 12 years were vaccinated to prevent HBV transmission by unsafe sexual activity or intravenous drug addiction. This strategy, chosen to cover the Italian population aged 0-24 within the first 12 years of application, has allowed us to compare the efficacy of the HBV vaccine in 588 students vaccinated at birth, and mostly attending a healthcare profession school, with 1,116 students vaccinated during adolescence, mostly attending medical school (either at the graduate or postgraduate level).
HBV vaccination has produced an excellent protective effect in all students, since none have been infected with HBV, as reflected by the absence of positivity to serum HBsAg or anti-HBc after a mean period of nearly 17 years. Only 1 (4.3%) of the 23 students escaping the mandatory HBV vaccination had acquired an HBV infection and become a chronic HBsAg carrier. The efficacy of HBV vaccination is also demonstrated by the observation that 88% of students vaccinated at 12 years old and 77% of those vaccinated at birth had an anti-HBs titer ≥10 IU/mL, commonly accepted as protective, and that even the long-term low responders had detectable anti-HBs with titers ranging from 1–9 IU/mL.

Students vaccinated at birth were more frequently long-term low responders. The highly significant statistical difference between the two groups was not due to a longer time elapsing from vaccination to screening: in fact, the difference was even more marked when the time effect was removed by considering in both groups, only those that had been vaccinated between 1991 and 1995. Indeed, multivariate analysis identified vaccination at birth as the only factor associated with long-term low response to HBV vaccine. Thus, vaccination in adolescence results in a more effective HBV immunogenicity than in infancy, most probably reflecting the progressive improvement of the immune system during childhood. Indeed, the immune system in infancy is characterized by impaired T cell function, lower interaction between B and T cells, restriction of the immunoglobulin repertoire, and low affinity antibody response [19]. These factors, along with the presence of serum anti-HBs in some mothers, might affect the response to HBV vaccine in newborn babies [20], and thus it has been suggested to reconsider the vaccination of newborn babies in order to obtain a more effective response.

It is common opinion that even subjects with an anti-HBs titer below <10 UI/mL could be considered protected against HBV infection, since the immunological memory to HBsAg may persist even in these cases, thereby securing a rapid rise of protective antibodies in the case of an HBV assault [13,18,21]. Nonetheless, considering that medical and healthcare profession students will become active workers in a few years and will probably be exposed to HBV for decades, a booster dose should be considered for all long-term low responders [22-25]. In fact, it has been estimated that 14.4% of hospital
workers (HCPs) are chronic HBV carriers in countries where vaccination is not mandatory, and that the incidence rate of HBV transmission from patients to HCPs is 0.42 considering only infection acquired through injuries sustained from sharp objects [26,27]. Moreover, according to Italian law, students and assistants in training are considered potentially exposed to hospital infections to a similar degree as HCPs [28]. The argument for a booster dose was also underlined by a study on 94 non responders administered plasma-derived HBV vaccine at birth, in which a single booster dose given 15 years later resulted in an anti-HBs response in about 20% of cases [29]. Our data are in good agreement with those of a previous investigation on the long-term effect of plasma-derived vaccine, which reported that the anti-HBs titer observed after vaccination (822 mIU/mL) was markedly decreased (to 27 mIU/mL) 15 years later, and that an older age at vaccination was a factor associated with the persistence of high anti-HBs titer [30].

There few Italian investigations compared the long-term antibody response to HBV vaccine administered at birth and during adolescence, and these were all conducted on small or particular study populations [16-18]. To the best of our knowledge, the present study is the first to evaluate long-term antibody response to HCV vaccine in a large study sample, identifying a predictive factor for long-term protective response. A quarter of students vaccinated at birth and nearly one eighth of those vaccinated during adolescence were found to be long-term low responders; age at vaccination was found to be the only independent predictor of long-term response. Although an immunological memory may persist years after vaccination even in subjects with a low or undetectable antibody titer, the immune system of subjects exposed to a high professional risk of HBV infection for 3–4 decades may become overwhelmed by a highly infectious inoculum. Consequently, the administration of a booster dose of HBV vaccine should be considered for all long-term low responders attending a medical or healthcare profession school.
Competing interests: All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

Author Contributions

NC, ML were responsible for the conception and design of the study and wrote the manuscript; ES and AN participated in the conception of the study and interpreted the data; ARC and EDF enrolled and followed up the patients; SDP and GS interpreted and analyzed the data and performed the statistical analysis.
REFERENCES


Figure legends

Figure 1: Flow chart of the students studied at the Second University of Naples from September 2012 to December 2013.
Figure 1

- Students observed: 1,727
  - Vaccinated students: 1,704
    - Students with anti-HBs titers ≥10: 1,434
    - Students with anti-HBs titers <10: 270
  - Non-vaccinated students: 23
    - HBsAg/anti-HBc positive students: 0
    - HBsAg/anti-HBc negative students: 22
    - HBsAg positive students: 1
Additional files provided with this submission:

Additional file 1: Table 1.doc, 46K
http://www.biomedcentral.com/imedia/2832900991466189/supp1.doc

Additional file 2: Table 2.doc, 43K
http://www.biomedcentral.com/imedia/8846304801466189/supp2.doc

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Additional file 4: Table 4.doc, 36K
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