

Serological Status and Vaccination for Hepatitis B Virus in Nursing Students during 1990-2006

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A total of 1,270 nursing students of Nagasaki University School of Health Sciences were examined for serum HBsAg and anti-HBs at their first year between 1990 and 2006. The prevalence of HBsAg was 0.39% (5/1,270); 0.52% (4/762) in 1990-1999, 0.27% (1/368) in 2000-2004, and 0% (0/140) in 2005-2006. The prevalence of anti-HBs was 1.81% (23/1,270); 1.97% (15/762) in 1990-1999, 0.82% (3/368) in 2000-2004, and 3.57% (5/140) in 2005-2006. A total of 650 students who were negative for HBsAg and anti-HBs in 1998-2006 received a primary series of hepatitis B vaccinations. Subsequently, 98.2% (638/650) acquired anti-HBs. The median titer of anti-HBs concentrations at 4 weeks after primary vaccination was 2,145.0 mIU/mL. Eleven among 12 students who did not acquire anti-HBs after a primary vaccination received a second series of vaccinations, after which eight (72.7%) acquired anti-HBs. Overall 99.4% (646/650) of the students vaccinated acquired anti-HBs. Among 286 students who acquired anti-HBs after the primary series of vaccinations, 17 (5.9%) became negative for anti-HBs in their third year. Nursing students should be educated about the risks for and prevention of blood borne infections, including the need to be vaccinated against hepatitis B. Moreover, nursing students who do not acquire anti-HBs after primary vaccination should receive a 3-dose revaccination series.

ACTA MEDICA NAGASAKIENSIA 52: 83 - 86, 2007

Keywords: Hepatitis B virus; Hepatitis B surface antigen; Vaccine; Nursing student

Introduction

Hepatitis B virus (HBV) infection has worldwide distribution. It is estimated that, globally, about 2 billion people have been infected with HBV, and more than 350 million are chronically infected.¹ Chronic HBV infection occurs in 90% of infants infected at birth, 30% of children infected at age 1-5 years, and 6% of persons infected after age 5 years, with continuing viral replication in the liver and persistent viremia.² Because babies who acquire the persistent HBV carrier state can develop liver diseases, such as liver cirrhosis, liver cancer and liver failure, there is a pressing need to efficiently prevent perinatal HBV infection. In Japan, immunoprophylaxis for babies born to carrier mothers with hepatitis B e antigen (HBeAg), by means of hepatitis B immune globulin (HBIG) and vaccine, was started in 1981. Immunoprophylaxis became mandatory as part of a national program implemented in 1986, and was passed on to social health insurance policies in 1995. Babies born in 1981 or 1986

reached 19 years of age in 2000 or 2005, respectively. At Nagasaki University, we have been examining serum hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs) in nursing students since 1990. In addition, we administered hepatitis B vaccine to the students who were negative for HBsAg and anti-HBs. In the present study, we have analyzed serological states and immunization sequences for HBV.

Subjects and Methods

Subjects

A total of 1,270 nursing students of Nagasaki University School of Health Sciences were examined for serum HBsAg and anti-HBs at their first year between 1990 and 2006. Subjects included 1,208 women and 62 men aged 18-35 years with the mean (standard deviation) of 18.3 (1.4) years.

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Received April 18, 2007; Accepted August 27, 2007

Serological tests for HBV markers

HBsAg and anti-HBs were determined by passive hemagglutination (PHA), in the April of the students' first year. Four weeks after hepatitis B vaccination, anti-HBs were determined by PHA from 1998-2001, or by chemiluminescent immunoassay (CLIA) from 2002-2006. Students attending Nagasaki University from 2000-2003 were retested for anti-HBs by PHA in their third year, 1.5 years after the primary vaccination. In addition, Hepatitis B core antibody (anti-HBc) were determined by radioimmunoassay (RIA) in the students who were positive for anti-HBs from 2004-2006.

Hepatitis B vaccination

Students who were negative for HBsAg and anti-HBs received a primary series of hepatitis B vaccinations. Three adult doses (10 µg) of hepatitis B vaccine were injected subcutaneously into the upper arm at months 0, 1 and 6. Serum anti-HBs were then examined 4 weeks after the third vaccination. The students who showed anti-HBs concentrations of less than 10 mIU/mL were offered an additional 3-dose vaccine series the next year (their second year). The hepatitis B vaccine used was yeast-derived recombinant hepatitis B vaccine in 1998-1999, or absorbed hepatitis B vaccine (huGK-14 origin) in 2000-2006. Students who acquired anti-HBs after the primary vaccination in their first year, but thereafter became negative for anti-HBs in their third year, were given two booster doses of hepatitis B vaccine. The study design conformed to the 1975 Declaration of Helsinki. Students and their legal guardians gave written informed consent, and verbal assent was obtained from every participant.

Statistical analysis

The difference in the prevalence of HBsAg or anti-HBs by factor was tested using Fisher's exact test for factor with two categories, while we used the Wilcoxon rank-sum test for factor with three or more ordinal categories. We compared anti-HBs titers between women and men using Wilcoxon rank-sum test. FREQ, NPARIWAY and UNIVARIATE in the SAS® system were used for the calculation.

Results

Prevalence of HBsAg and anti-HBs

The prevalence of HBsAg and anti-HBs was 0.39% (5/1,270) and 1.81% (23/1,270), respectively, in nursing students of Nagasaki University during 1990-2006. Although the prevalence of HBsAg showed a tendency to decrease with the periods of 1990-1999, 2000-2004 and 2005-2006, the difference by period was not significant in the prevalence of either HBsAg ($p=0.311$) or anti-HBs ($p=0.698$) (Table 1). HBsAg and anti-HBs were detected in women in 0.41% (5/1,208) and 1.90% (23/1,208), respectively, but either was not detected in men. Seven students who were positive for

Table 1. Prevalence of HBsAg and anti-HBs in nursing students by period

Period	Number of students examined	HBsAg (+)	Anti-HBs (+)
1990-1999	762	4 (0.52) ^a	15 (1.97) ^a
2000-2004	368	1 (0.27)	3 (0.82)
2005-2006	140	0	5 (3.57)
Total	1,270	5 (0.39)	23 (1.81)

^aNumber of students with percentage in parentheses.

anti-HBs in 2004-2006 were tested for anti-HBc, and three of them (42.9%) were positive.

Hepatitis B vaccination

A total of 650 students (604 women and 46 men) who were negative for HBsAg and anti-HBs received a primary series of hepatitis B vaccinations from 1998-2006; 158 and 492 of them were vaccinated by yeast-derived recombinant hepatitis B vaccine and absorbed hepatitis B vaccine (huGK-14 origin), respectively. Subsequently, 98.2% (638/650) acquired anti-HBs, and the two types of vaccine did not significantly differ ($p=0.157$, Fisher's exact test) in their efficacy; 96.8% (153/158) by yeast-derived recombinant hepatitis B vaccine and 98.6% (485/492) by absorbed hepatitis B vaccine (huGK-14 origin), respectively. No significant difference was observed in the frequency of acquiring anti-HBs between women (98.3%) and men (95.7%) ($p=0.191$, Fisher's exact test).

The anti-HBs concentrations at 4 weeks after the primary series of vaccinations with absorbed hepatitis B vaccine (huGK-14 origin) ranged from <10 mIU/mL to 55,000 mIU/mL with the median titer of 2,145.0 mIU/mL; 2175.0 mIU/mL in women and 1610.0 mIU/mL in men (Figure 1).

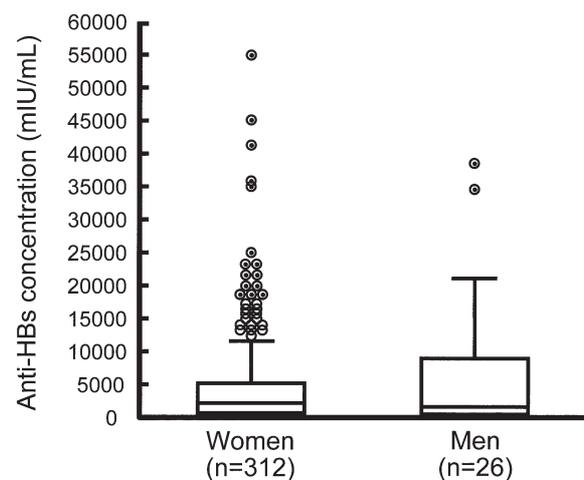


Figure 1. Box-and-whisker plots of anti-HBs concentration after primary series of hepatitis B vaccination by gender. The bottom and top ends of the box and the bar inside the box correspond to the 25th, 75th and 50th sample percentiles, respectively. The open circle and the double circle with black dot represent extreme values called "outside" and "far out," respectively.

Eleven of 12 students who did not acquire anti-HBs after the primary series of vaccinations received a second series of vaccinations, after which eight (72.7%) acquired anti-HBs, and the titers examined were from 273.0 mIU/mL to 788.8 mIU/mL. Overall 99.4% (646/650) of the students vaccinated acquired anti-HBs (Table 2). Among 286 students who acquired anti-HBs after the primary series of vaccinations, 17 (5.9%) became negative for anti-HBs in their third year, 1.5 years after the last vaccination in the primary series. The titer of anti-HBs at 4 weeks after the primary vaccination varied from 23.4 to 417.2 mIU/mL with the median of 175.4 mIU/mL. Three students were given two booster doses of vaccine, and anti-HBs concentrations increased to 320.0 mIU/mL, 56.5 mIU/mL and 236.0 mIU/mL, which were higher than those after the primary series of vaccinations (23.4 mIU/mL, 39.6 mIU/mL and 41.9 mIU/mL, respectively).

Table 2. Anti-HBs-positive rates after hepatitis B vaccinations

Series of vaccination	Number of vaccinated students	anti-HBs (+)
Primary	650	638 (98.2) ^a
Second	11	8 (72.8)
Total	650	646 (99.4)

^aNumber of students with percentage in parentheses.

Discussion

The frequency of HBV carriers recently estimated in Japanese was as follows: 0.23% in those aged 16-19 years, 0.52%—20-29 years, 0.84%—30-39 years, 1.19%—40-49 years, 1.50%—50-59 years, and 1.07%—60—69 years.³ Thus, about one million Japanese aged between 15 and 69 years are HBV carriers. Perinatal transmission from infected mothers is a significant route for establishing the persistent carrier state; therefore, prevention of perinatal HBV infection is very important. In Japan, immunoprophylaxis against perinatal transmission of HBV was started in 1981 and became mandatory as part of a national program in 1986. Koyama et al.,⁴ reported that the prevalence of HBsAg decreased from 0.75% (78/10,437) among children born in 1978-1980 to 0.23% (46/20,812) among those born in 1981-1985, and further to 0.04% (12/32,049) among those born in 1986-1990. The prevalence of anti-HBs was 1.52%, 0.79% and 0.85% in the above-mentioned three groups of children. In addition, they reported that the prevalence of anti-HBc among anti-HBs-positive children markedly decreased. Noto et al.⁵ also discussed the efficacy of immunoprophylaxis for preventing perinatal transmission of HBV. Although the frequency of HBsAg-positive students in Nagasaki University did not significantly differ among the periods of 1990-1999 (when most students were born before 1981), 2000-2004, and 2005-2006 (when all students were born in or after 1986), it showed a tendency to decrease. These data

suggest a decrease in naturally acquired HBV infection in people born after the start of immunoprophylaxis. However, the prevalence of anti-HBs in the students of Nagasaki University was higher in 2005-2006 than in 2000-2004, though the difference was not significant. The prevalence of anti-HBc among anti-HBs-positive students was 42.9% (3/7) in 2004-2006. These data suggest that there are two groups in anti-HBs-positive students, one is the students who acquired anti-HBs by hepatitis B vaccination and another is those who were infected to HBV. Of three anti-HBc-positive students, two had family members with liver disease, and one had a sex partner. Thus, the anti-HBc-positive students might be infected by a chronically infected person.

Horizontal transmission from infected household contacts or sexual contact is another source of HBV infection.^{6,7} In 1991, the WHO recommended that hepatitis B vaccine be introduced into the Expanded Programme of Immunization, and all countries were asked to introduce a program of universal immunization, in either infancy, adolescence or both.⁸ By 2004, 168 countries had implemented these immunization programs.⁹ In Japan, babies born to mothers with HBsAg are to be vaccinated; however, the other babies or children do not necessarily receive the hepatitis B vaccination.

HBV infection is a well-recognized occupational risk for healthcare workers.¹⁰ In studies of healthcare workers who sustained injuries from needles contaminated with blood containing HBV, the risk of developing clinical hepatitis was 22-31% if the blood was both HBsAg- and HBeAg-positive, and the risk of developing serologic evidence of HBV infection was 37-62%. By comparison, the risk of developing clinical hepatitis from a needle contaminated with HBsAg-positive and HBeAg-negative blood was 1-6%, and the risk of developing serologic evidence of HBV infection was 23-37%.¹¹ Percutaneous injuries are the most efficient modes of HBV transmission; however, most infected healthcare workers could not recall an overt percutaneous injury.¹² HBV is comparatively stable in the environment and remains viable for over 7 days on environmental surfaces at room temperature,¹³ and HBV at concentration of 10²-10³ virions/mL can be present on environmental surfaces in the absence of any visible blood and still causes transmission.¹⁴ The potential for HBV transmission through contact with environmental surfaces has been demonstrated in investigations of HBV outbreaks among patients and staff of hemodialysis units.¹⁵ Thus, HBV infections that occur in healthcare workers with no history of non-occupational exposure or occupational percutaneous injury might have resulted from direct or indirect blood or body fluid exposure that inoculated HBV into cutaneous scratches, abrasions, burns, other lesions, or onto mucosal surfaces.¹⁶

Since nursing students and nurses are at risk for HBV infection, individuals who do not have both HBsAg and anti-HBs should be vaccinated. The proportion of students who had a protective antibody response was 99.4%; 98.2% after a primary hepatitis B vaccination and 72.7% after a second series of vaccination. It has been reported that of individuals who did not respond to a primary 3-dose vaccine series, 25-50% responded to an additional vaccine dose, and

44-100% responded to a 3-dose revaccination series.¹⁷⁻¹⁹ Therefore, individuals who do not acquire anti-HBs after a primary vaccination should receive a 3-dose revaccination series.

Anti-HBs concentration acquired after vaccination declines rapidly within the first year and more slowly thereafter, and the persistence of detectable anti-HBs depends on the level of post-vaccination antibody.^{20,21} Among students who acquired anti-HBs after the primary series of vaccinations, 5.9% became negative for anti-HBs within 1.5 years after the last vaccination of the primary series. Three of them were given two booster doses of vaccine, and anti-HBs concentrations after the booster vaccination were higher than those after the primary series of vaccinations.

Zanetti et al.⁹ showed that more than 60% of children and nearly 90% of recruits maintained anti-HBs ≥ 10 IU/L at more than 10 years after vaccination. Because Italy is a country with lower endemicity, in which the presence of natural boosters is conceivably scarce, the rate of anti-HBs persistence suggests that the protective effects against hepatitis B infection could be due to the sole administration of vaccine. In addition, as HBV infections were rare over the 10-year study, it was concluded that the immune memory persists for longer than 10 years after primary immunization, and booster doses of hepatitis B vaccine may not be needed for at least 10 years.

The mechanism for continued vaccine-induced protection is thought to be the preservation of immune memory through selective expansion and differentiation of clones of antigen-specific B and T lymphocytes.²² Persistence of vaccine-induced immune memory among those who responded to the primary vaccine series, but then had anti-HBs concentrations of <10 mIU/mL, has been demonstrated by an anamnestic increase in anti-HBs concentrations in 74-100% of these individuals at 2-4 weeks after administration of an additional vaccine dose and by antigen-specific B and T cell proliferation.²³ Recent studies have indicated that periodic serologic testing to monitor antibody concentrations after completion of the vaccine series and booster doses of hepatitis B vaccine will not be necessary for individuals with normal immune status, because the 6-week to 6-month incubation period of HBV infection is much longer than the days required to mount the anamnestic anti-HBs response.^{9,24,25}

Exposure prevention is the primary strategy for reducing blood borne pathogen infections, however, occupational exposure will continue to occur. Healthcare workers and nursing students should be educated about the risks for and prevention of blood borne infections, including the need to be vaccinated against hepatitis B.

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