ORIGINAL ARTICLE

Stamford Journal of Microbiology, July 2011. Vol. 1, Issue 1
ISSN: 2074-5346

Persistence of Anti-HBs Antibody and Immunological Memory in Healthy Individuals Vaccinated with Hepatitis B Vaccine

Bidhan Chakraborty,[1] Tanvir Bashar,[1] Konok Roy,[2] Rashed Noor,[1‡] and M. Majibur Rahman[1]

[1]*Department of Microbiology, Stamford University Bangladesh,* [2]*LAB AID Hospital, Dhaka, Bangladesh*

Received 19 February 2011/Accepted 25 April 2011

Hepatitis B virus (HBV) infection is one of the major public health problems in the world. It is estimated that there are three hundred million HBV carriers and around one million deaths annually due to HBV infection worldwide. Vaccination is considered to be the best solution to this problem. The present study was conducted to investigate the efficacy of the vaccine administered against hepatitis B and to reveal the immunological memory against the vaccine. Samples were taken from both male (74 individuals) and female (37 individuals) from different age groups ranging from three to sixty three years. Among the population studied, both good (63.06%) and hypo (36.94%) responders were found. All the individuals (100%) showed a satisfactory result having an anti-HBs antibody titer above the protective level (≥ 10 IU/L).

Hepatitis B is an infectious illness caused by hepatitis B virus (HBV) which infects the liver of hominoidea, including humans, and causes an inflammation called hepatitis. Hepatitis B virus (HBV) infection is one of the major public health problems in the world (1). About 350 to 400 million people are chronically infected with hepatitis B worldwide. Originally known as 'serum hepatitis' (2), the disease has caused epidemics in parts of Asia and Africa, and it is endemic in China (3). Approximately 45% of the world population live in hyper-endemic areas where prevalence of hepatitis B surface antigen (HBsAg) is greater than 8%; 43% live in mid-endemic areas where HBsAg prevalence is 2% to 7%; and 12% live in hypo-endemic areas where HBsAg prevalence is less than 2% (1,4). The acute illness causes liver inflammation, vomiting, jaundice and rarely, death. Chronic hepatitis B may eventually cause liver cirrhosis and liver cancer- a fatal disease with very poor response to current chemotherapy (5). However, the infection is preventable by vaccination (6).

Vaccination against hepatitis B stimulates the body's immune defenses and protects most people. Effective control of HBV transmission in areas of high and intermediate endemicity would not be possible without vaccination of the vulnerable group of the population (7). The WHO strategy for effective control of HBV infection and its sequel is mass vaccination of neonates and children within the framework of Expanded Program on Immunization (EPI) (8). It has been recommended that all countries integrate hepatitis B vaccine into national immunization by the year 1997 (8).

‡Corresponding Author. Mailing address: Dr. Rashed Noor, Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka, Bangladesh. Phone: +88-02-8354577 (Ext-472), Fax: +88-02-9143531, Email: noor.rashed@yahoo.com .

Recent results reported in many countries clearly indicated that in areas of high endemicity such as some parts of Asia; highly effective vaccination program has shifted this pattern toward intermediate or low endemicity (9). However, people undergoing dialysis, people with cirrhosis, and people with an impaired immune system may result in less protection from vaccination. Vaccination is especially important for people at risk of contracting hepatitis B. Standard immune globulin provides immediate but weak protection for people exposed to hepatitis B. Hepatitis B immune globulin provides better protection. Infants born to mothers with hepatitis B are given hepatitis B immune globulin and are vaccinated; this combination prevents chronic hepatitis B in about 70%.

The immunity of HB vaccination is directly related to the development of anti-HBs antibodies with a minimum level of 10 IU/L and is considered as protective immunity (10). Most people develop antibody titer >100 IU/L within 6-8 weeks after completing vaccination (11). Some apparently healthy individual do not show an anti-HBs antibody response or respond poorly to the surface antigen component (HBsAg) and they are labeled as non-responders or hypo-responders with antibody titer <10 IU/L and 10-100 IU/L, respectively (12). It is estimated that about 5-15% of the vaccinees may be non-responders (13). However, dose, storage, site and route of administration, male sex, genetic factor, obesity, diabetes, and immunosuppression can all adversely affect the immune response. Post vaccination testing for antibody titer within 1 to 6 months after completion of vaccination series is recommended to detect non-responder or hypo-responder (14). Although antibody titer declines with time but it should be reasonably to the level of 10 IU/L at any time for ensuring immunoprotection among vaccinated individuals (10).

Although the practice of vaccination against HBV has been started in our country for several years but till date there is not much published data regarding the immunity level among the recipients of HB vaccines of different commercial brands available. Considering this, the present study undertook to evaluate the status of seroconversion by measuring anti-HBs antibody titer among the recipients of recombinant hepatitis B vaccine after completion of their vaccine schedule.

MATERIALS AND METHODS

Specimen Collection and Preparation. Human serum tubes were used in the anti-HBs assay. All blood samples were collected observing universal precautions for venipuncture using vacuum tube (Red top). The samples were then allowed to clot for 20 minutes before centrifugation. Following this, the samples were centrifuged at 4000 rpm for 10 minutes. Then the supernatant (400 µl) was transferred to sample cup. If the sample was needed to preserve, then it was capped prior to use and stored up to 72 hours at 2 ~ 8 ^{o}C.

Number of Samples. A total of 121 samples including 10 controls was considered in this present study irrespective of age and sex. Samples were collected from different persons after different duration of vaccination. The age range considered in this present study was between 3 to 63 years. Anti-HBs antibody was detected by Chemiluminescence Immunoassay using commercial kits (Roche Diagnostics Corporation USA). Anti-HBs antibody was quantified using appropriate dilution of a positive sample with a known concentration of anti-HBs expressed as IU/L, provided by the manufacturer. The assay determined IgG type of anti-HBs antibody and the protective level of antibody was considered >10 IU/L.

Measurement of Anti-HBs Antibody Titer. The measurement of the anti-HBs antibody titre was done with the Roche Elecsys 2010 immunoassay analyzer. To perform the test, chemicals provided by the supplier were added to the patients' blood sample. This produces a light reaction which was measured inside the analyzer. The amount of light produced shows the level of anti-HBs in the blood. A second device, the Elecsys PreciControl anti-HBs, was also used to ensure that the test was performed accurately.

RESULT

In this study, a total of 121 individuals including 10 controls (non-vaccinated individuals) from different age range and sex was studied. Based on the measurement of their anti-HBs titer, the population was categorized in three groups: non-responders (<10 IU/L), hypo-responders (10-100 IU/L) and good-responders (>100 IU/L). The results are summarized in the following table 1 and 2.

TABLE 1. Anti-HBs titer among vaccinated and non-vaccinated individuals

Study population	Anti-HBs antibody titer (IU/L)		Totals
	Protective (≥ 10 IU/L)	Non-protective (<10 IU/L)	
Vaccinated (111)	111 (100%)	0 (0%)	111
Control (10)	0 (100%)	10 (100%)	10
Total	111	10	121

TABLE 2. Distribution of good, hypo and non-responders among individuals

Population	Anti-HBs titer (IU/L)	Status
Group A (41 individuals)	10-100 IU/L	Hypo-responder
Group B (70 individuals)	>100 IU/L	Good-responder
Group C (Control)	<10 IU/L	Non-responder

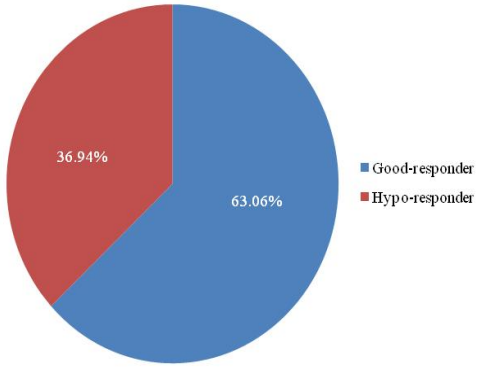

FIG. 1. Distribution of good and hypo-responders among the studied individuals. A total of 111 samples was studied excluding 10 controls. Among them, 63.06% samples were found to be good-responders (having an antibody titer of >100 IU/L) and 36.94% samples were found to be hypo-responders (having an antibody titer of 10-100 IU/L).

All the samples considered in this study were categorized both in the two sex groups- male and female; the percentage of which is shown graphically in Fig 2. The number of individuals of different age ranges is also given below.

TABLE 3. Number of samples according to sex considered in this study

Sex	Number of individuals
Male	74
Female	37
Total	111

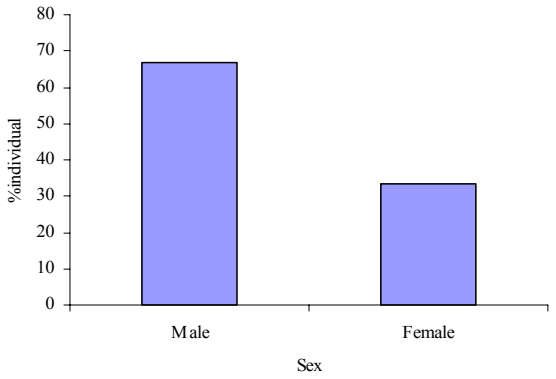

FIG. 2. Percentage of individuals according to sex. Among the 111 individuals excluding 10 control, 66.67% (a total number of 74) and 33.33% (a total number of 37) were male and female respectively.

TABLE 4. Number of individuals according to age considered in this study

Age range	Number of individuals
0-10	19
11-20	10
21-30	36
31-40	31
41-50	10
51-60	4
61-70	1
Total	111

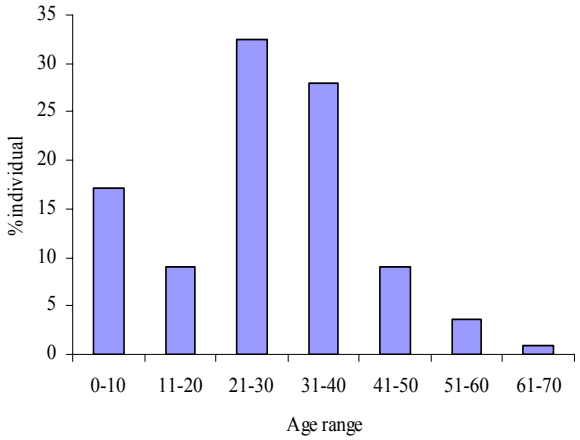

FIG. 3. Percentage of individuals according to age. The age of the individuals was categorized in five groups having an age range difference of 10 years. The maximum sample was collected from the age range 21-30 years (32.43%) and the minimum sample was collected from the age range 61-70 years (0.9%).

The anti-HBs antibody titer of the studied individuals was measured by chemiluminescence immunoassay technique through an automated analyzer. The antibody titer is presented below according to sex and age ranges. A total of 10 more individuals were also studied in this present study as control (non-vaccinated individuals) irrespective of age and sex. All non-vaccinated controls had anti-HBs titers below the protective level.

TABLE 5. Anti-HBs antibody titer of the individuals according to sex

Sex	Mean anti-HBs antibody titer (IU/L)
Male	529.35
Female	596.65

TABLE 6. Anti-HBs antibody titer of the individuals according to age

Age range	Mean anti-HBs antibody titer (IU/L)
0-10	618.05
11-20	378.86
21-30	676.78
31-40	409.29
41-50	623.03
51-60	467.01
61-70	565

DISCUSSION

Persistent hepatitis B virus (HBV) infection may lead to the development of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. It is estimated that there are 300 million HBV carriers and as many as 1 million deaths annually due to HBV infection worldwide (15). Various therapies for persistent HBV infection, such as interferon-α or antiviral agents, have been developed but the goal of eradicating HBV globally depends largely on efforts to prevent new infection. In an attempt to reduce the global impact of HBV infection, in 1991 the WHO recommended that HB vaccine should be integrated into national immunization programs by the year 1997 (16).

HBV infection is a significant health problem around the world but it is one of the oncoviruses that is vaccine preventable. HB vaccine induces anti-HBs response which can prevent HBV infection. Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and its subsequent complications. Studies have shown that childhood vaccination significantly reduced the rate of chronic HBV infection (17).

Since the introduction of hepatitis B vaccination in the early 1980s, many epidemiological studies have been done to determine the efficacy of the vaccine in eliciting protective immunity against HBV infection. The protective efficacy of a primary course comprised of 3 doses of vaccine is well established. However, the duration of protection is still unknown, especially when anti-HBs titers decline to low or undetectable levels. After booster vaccination, anti-HBs titers rise significantly within 3-5 days (18). The antibody response to HB vaccine has been found occurring in more than 90% of the healthy vaccines (19-23). Kinetic studies showed that the serum anti-HBs levels decreased in course of time following vaccination (24-27). Several demographic and behavioral factors have been found to be associated with a lower rate of antibody response to hepatitis B vaccine (28, 29).

The present study was conducted to investigate the efficacy of the vaccine administered against hepatitis B and to reveal the immunological memory against the vaccine. Demographic analysis of the samples was also done to find out any coorelation between the different demographic variable with the antibody titre. Blood samples were collected from the individuals at variable duration after booster dose ranging from 6 to 12 months. Samples were collected from both male and female candidates of different ages ranging from 3 to 63 years.

Among the studied 111 individuals, 66.67% (74 individuals) and 33.33% (37 individuals) samples were collected from male and female, respectively (Table 3). On the other hand, the maximim individuals were in the age range of 21 to 30 years (36 samples, 32.43%) followed by 31 to 40 years (31 samples, 27.93%) (Table 4). The least number of samples was collected from those individuals having the age range of 51 to 60 years (4 samples, 3.60%) other than the age range of 61 to 70 years; only 1 sample (90%) was considered from this age range.

Among all the studied individuals who received 3 doses or booster doses of Hepatitis B vaccine, all developed seroconversion with protective level of immunity in the current study while all 10 non-vaccinated individuals who served as controls in the present study had anti-HBs titer below the protective level (<10 IU/L) (Table 1). Regarding immune response developed among vaccinated individuals, 63.06% and 36.94% were good-responders and hypo-responders respectively (Table 2).

The avarage anti-HBs titer found among the male (529.35 IU/L) and female (596.65 IU/L) individuals revealed no co-relation between the immunological memory and sex. At the same time, the immonological memory against different ages varies randomly indicating no relationship with this demographic variable.

However, in a previous study, some associations between the immunological memory and socioeconomic status was found (30). Further study will be conducted to observe the effect of this variable.

REFERENCES

1. **World Health Organization**. 2001. Hepatitis B immunization.
2. **Barker, L. F., et al.** 1996. Transmission of serum hepatitis. Journal of the American Medical Association **276(10)**:841–844.
3. **Williams, R**. 2006. Global challenges in liver disease. Hepatology **44(3)**:521–526.
4. **Mast, E. E., et al.** 2005. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States; recommendations of ACIP part 1: immunization of infants, children, and adolescents. MMWR **54(16)**:1-23.
5. **Chang, M**. 2007. Hepatitis B virus infection. Seminars in fetal & neonatal medicine **12(3)**:160–167.
6. **Pungpapong, S., W. Kim, and J**. **Poterucha.** 2007. Natural History of Hepatitis B Virus Infection: an Update for Clinicians. Mayo Clinic Proceedings **82(8)**:967.
7. **Mass, R., and G**. **Delage.** 1998. Universal hepatitis B immunization: infants and plus adolescent immunization. Vaccine **16**:17-22.
8. **Kao, J. H., and D. S**. **Chen.** 2002. Global control of hepatitis B virus infection. Lancet Infect Dis**. 2(7)**:395-403.
9. **Andre, F**. 2000. Hepatitis B epidemiology in Asia, the Middle East and Africa. Vaccine **18:**20-22.
10. **Mahoney, F. J**. 1999. Update on diagnosis, management, and prevention of hepatitis B virus infection. Clin Microbiol Rev **12**:351-366.
11. **Siebert, D. and S**. **Locarnini.** 1998. Hepatitis B: issues in laboratory diagnosis and vaccination. Aust Prescr **21**:72-5.
12. **Zuckerman, J. N., et al.** 1997. Immune response to a new hepatitis B vaccine in healthcare workers who had not responded to standard vaccine: randomised blind dose- response study. Br Med J **314**:329-33.
13. **Zuckerman, A. J**. 1996. Hepatitis Viruses. *In* Baron S et al. (ed.), Baron's Medical Microbiology, 4th ed. Univ of Texas Medical Branch.
14. **Ronald, W. E**. 1993. Hepatitis B vaccine in clinical practice. Taylor & Francis, New York.
15. **Treadwell, T. L., et al.** 1993. Immunogenicity of two recombinant hepatitis B vaccines in older individuals. Am J Med **95**:584-588.
16. **World Health Organization**. 1991. WHO expanded programme on immunization. Report of 14th Global Advisory Group (Antalya, Turkey, Oct 14-18, 1991). Geneva: WHO.
17. **Harpaz, R., B. J. MacMohan, and H. S**. **Margolis.** 2000. Elimination of new chronic hepatitis B virus infection: results of the Alaska immunization program. J Infect Dis **181**:413-418.
18. **Jilg, W., M. Schmidt, and F**. **Deinhardt.** 1989. Four-year experience with a recombinant hepatitis B vaccine. Infection **17**:70-76.
19. **Liao, S. S., et al.** 1999. Long-term efficacy of plasma-derived hepatitis B vaccine among Chinese children: a 12-year follow-up study. World J Gastroenterol **5**:165-166.
20. **Kojouharova, M., et al.** 2001. Safety and immunogenicity of a yeast-derived recombinant hepatitis B vaccine in Bulgarian newborns. Infection **29**:342-344.
21. **Rendi-Wagner, P., et al.** 2001. Antibodyresponse to three recombinant hepatitis B vaccines: comparative evaluation of multicenter travel-clinic based experience. Vaccine **19**:2055-2060.
22. **Jain, A., et al.** 2000. A multicentric evaluation of recombinant DNA hepatitis B vaccine of Cuban origin. Trop. Gastroentero. **21:**14-17.
23. **Zeng, X. J., et al.** 1999. Survey of coverage,strategy and cost of hepatitis B vaccination in rural and urban areas of China. World J. Gastroenterol. **5**:320-323.
24. **Ozaki, T., et al.** 2000. Persistence of hepatitis B surface antibody levels after vaccination with a recombinant hepatitis B vaccine: a 3-year follow-up study. J. Oral Sci. **42**:147-150.

25. **Liu, H. B., et al.** 2000. A 12-year cohort study on the efficacy of plasma-derived hepatitis B vaccine in rural newborns. World J. Gastroenterol. **6**:381-383.
26. **Whittle, H. C., et al.** 1995. Long-term efficacy of continuing hepatitis B vaccination in infancy in two Gambian villages. Lancet **345**:1089-1092.
27. **Wismans, P. J., et al.** 1988. Is booster injection with hepatitis B vaccination necessary in healthy responders? A study of the immune response. J. Hepatol. **1**:1-5.
28. **Wood, R. C., et al.** 1993. Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. JAMA **270**:2935-2939.
29. **Roome, A. J., S. J. Walsh, M. L. Cartter, and J. L. Hadler.** 1993. Hepatitis B vaccine responsiveness in the Connecticut public safety personnel. JAMA **270**:2931-2934.
30. **Abhijit, G., et al.** 2010. Seroconversion after Recombinant Hepatitis B Vaccination. J. Medicine **11**:143-150.