PERSISTENCE OF ANTI-HBs ANTIBODY AND IMMUNOLOGICAL MEMORY IN CHILDREN VACCINATED WITH HEPATITIS B VACCINE AT BIRTH.

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Aims: Vaccination with the hepatitis B surface antigen (HBsAg) induces protective levels of antibody (anti-HBs = 10 IU/L) in majority of vaccinees. It has been shown that the levels of anti-HBs antibody do wane after vaccination. The aim of this study was to evaluate the persistence of anti-HBs antibodies in healthy Iranian children at 10 years after primary vaccination and the response to a booster dose using recombinant hepatitis B vaccine. **Materials and Methods:** Blood samples were collected from 146 healthy 10-11 years old children who received primary course of Hepatitis B vaccine at 0, 1.5 and 9 months of age. The sera were tested for anti-HBs, antibody to Hepatitis B core antigen (anti-HBc) and HBsAg by ELISA technique. A single booster dose of recombinant hepatitis B vaccine was administered intramuscularly to a total of 94 children, whose anti-HBs antibody was less than 50 IU/L (70 children with anti-HBs <10 IU/L and 24 subjects with anti-HBs 10-50 IU/L). The sera of children were re-tested for anti-HBs antibody levels at 4 weeks after booster vaccination. **Results:** At 10 years after primary vaccination 70/146 (47.9%) of children had protective levels of antibody with geometric mean titer (GMT) of 68.12 IU/ml. All children were negative for HBsAg, although anti-HBc antibody was positive in 11 (7.5%) of children. In the 94 subjects who received the booster dose the seroprotection and the GMT of anti-HBs antibody were 25.5% and 9.58 IU/L at pre-booster time and rose to 95.75% and 575.6 IU/L after the booster vaccination, respectively. Seroprotection rates and mean titer of antibody similarly expressed in males and females. **Conclusion:** The results of present study showed that at 10 years after primary vaccination with recombinant HB vaccine, 47.9% of the children had protective levels of anti-HBs antibody. Moreover we have demonstrated an anamnestic response to booster vaccination that confirms the persistence of an effective immunological memory in vaccinees.

Key words: Children, Hepatitis B vaccine, Anti-HBs antibody, Immunologic memory, Booster vaccination.

INTRODUCTION

Hepatitis B virus (HBV) is a serious public health problem and major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. It was estimated that approximately 2 billion people have serological evidence of past or present HBV infection and there are 350 million carriers of virus, worldwide [1]. The World Health Organization (WHO) strategy for effective control of HBV infection and its sequelae is mass vaccination of neonates and children within the framework of Expanded Program on Immunization (EPI) and recommended that hepatitis B vaccination should be included in national immunization system in all countries by 1997 [2]. This program has been incorporated in the national vaccination scheme in Iran since 1991 [3].

Vaccination with the HBsAg induces protection in the majority of vaccinees [8]. In a series of studies it has been demonstrated that 90-99% of healthy neonates, children, adolescents and adults developed protective levels of anti-HBs antibody following a standard vaccination course with hepatitis B vaccine [4-7]. The effectiveness of routine infant hepatitis B immunization in significantly reducing the prevalence of chronic HBV infection has been demonstrated in a variety of countries [1]. However, it has been shown that the levels of anti-HBs antibody do wane after vaccination. Accordingly, some investigators have suggested the need for a booster dose after 5-15 years [8,9]. However, the duration of protection has yet remained to be determined and it is important to evaluate whether booster vaccination might eventually be necessary to extend protection through adulthood.

This study was conducted for the first time in Iranian children to evaluate the persistence of anti-HBs antibody at 10 years after primary vaccination course with recombinant hepatitis B vaccine and the response to a booster dose.

MATERIALS AND METHODS

Study Design

This study was performed in two parts. The first part of investigation (as a historical cohort study) conducted to determination of the persistence of anti-HBs antibody ten years after primary vaccination

course. In this stage children received three doses of HB vaccine during first year of life, from February to October 1994. Serum specimens were collected 10 years after primary vaccination, in November 2004, and HBV markers such as anti-HBs antibody were determined. In the second part of study (booster vaccination stage and as a clinical trial study) the response to a booster dose of hepatitis B vaccine was assessed. In this stage 94 children with anti-HBs < 50 IU/L received a single booster dose of HB vaccine and the sera were re-tested for anti-HBs antibody levels at 4 weeks after booster vaccination.

This study was evaluated and approved by the Ethical Committee of Rafsanjan University of Medical Sciences. Moreover, parents were informed about the study by a letter and informed consents were obtained from parents of all children before enrollment.

Subjects

A total of 146 healthy children (58 males and 88 females) attending the health centers of Rafsanjan, (a city in Kerman province, located south-east of Iran) were included in this study, retrospectively. Subjects were born during February 1994 and had received the first dose of hepatitis B vaccine within 2 days of birth. Medical history of children and their family, especially HBsAg carriage in their mothers if mentioned in the health record was noted. All participants were basically healthy, with no acute or chronic illnesses. Indeed any individual with history of chronic or acute disease and use of any drug were excluded from the study.

Primary vaccination schedule

Data about primary anti-hepatitis B vaccination (doses of vaccine and date of administration) were obtained from public health centers. Originally, from February to October 1994, primary vaccination course of hepatitis B vaccine including triple 10 microgram doses of a recombinant HB vaccine (Engerix-B, SmithKline Beecham, Rixensart Belgium) was administered into the quadriceps muscle during infancy (first dose within 2 days of birth and at 1.5 and 9 months). Blood samples were collected in November 2004, at 10 years after completion of primary vaccination course. The sera of children were tested for anti-HBs and anti-HBc antibodies and HBsAg.

Booster vaccination

Totally, 94 children with anti-HBs < 50 IU/L (70 children with anti-HBs < 10 IU/L and 24 subjects with anti-HBs 10-50 IU/L) received booster vaccination. A single booster dose of the same concentration was administered to all children at 10 years after primary vaccination. Because previously used vaccine was no longer available, we therefore used another recombinant yeast derived HB vaccine (Heberbiovac, S.A. Havana, Cuba) for booster vaccination. Peripheral blood (2-3ml) was taken from all vaccinees at 4 weeks after booster vaccination and the sera were collected and stored at -20 °C. Sera of children were re-tested for anti-HBs antibodies.

Detection of HBV markers

HBs antigen, anti-HBs and anti-HBc antibodies were detected by enzyme-linked immunosorbent assay (ELISA) Using commercial kits (Radim, Italy). Anti-HBs antibody was measured by using standard samples with known concentrations of anti-HBs antibody expressed as IU/L, provided by the manufacturer.

Statistical Analysis

Differences in variables were analyzed using Mann-Whitney U-test, Chi-square and Fisher exact tests as appropriate and p values of less than 0.05 were considered significant.

RESULTS

At 10 years after completion of primary vaccination course 70/146 (47.9%) of children had protective concentrations of anti-HBs antibody (anti-HBs = 10 IU/L) with GMT of 68.12 IU/L. Both seroprotection rate and GMT were similarly expressed in male and female children (*Table 1*). The children could be arbitrary classified into different groups according to their serum titer of anti-HBs antibody. Accordingly, the proportions of subjects with antibody titer of <10 IU/L, 10-99 IU/L, 100-499 IU/L and 500-999 IU/L were 52.05%, 24.65%, 20.54 and 2.73%, respectively. Collectively, at 10 years after primary vaccination, the titer of anti-HBs antibody for 76.7% of children was <100 IU/L and none of them had a titer =1000 IU/L (*Table 2*).

At 10 years after completion of primary vaccination, all subjects were found to be HbsAg negative, although 11/146 (7.5%) of children were positive for anti-HBc antibody.

Seventy of the children, in whom the anti-HBs antibodies had fallen below 10 IU/L and 24 children with anti-HBs antibodies between 10 to 50 IU/L received a booster dose. The results of booster vaccination summarized in *Table 3*. After booster vaccination, 90/94 (95.75%) of vaccines developed protective levels of anti-HBs antibody. The seroprotection rate and the GMT of anti-HBs antibody in the 94 subjects given the booster dose were 25.5% and 9.58 IU/L at pre-booster time and rose to 95.75% and 575.6 IU/L after the booster vaccination, respectively. At post-booster vaccination, both seroprotection and the GMT of

anti-HBs antibody were found to be significantly higher in comparison with those at pre-booster vaccination time ($p<0.00001$). The gender of vaccinees did not significantly influence the post-booster seroprotection rate and mean concentration of anti-HBs antibody (*Table 3*). After booster vaccination, 4.25%, 17.02%, 19.14%, 46.8% and 12.76% of vaccinees had a titer of <10 IU/L, 10-99 IU/L, 100-499 IU/L, 500-999 IU/L and =1000 IU/L, respectively. Moreover, 78.7% vaccinees developed a titer above 100 IU/L after the booster vaccination (*Table 2*).

Table 1: Seroprotection rates and GMT of anti-HBs antibody in male and female vaccinees at 10 years after primary vaccination

Time	Sex	No. of vaccinees	Seroprotection rate (anti-HBs = 10 IU/L)	GMT ± SD (IU/L)
10 years after primary vaccination	Male	58	27 (46.6%)	86.2± 177.95
	Female	88	43 (48.9%)	56.23±123.47
	Total	146	70 (47.9%)	68.12±147.68

Anti-HBs: Antibody against hepatitis B surface antigen; GMT: Geometric mean titer; SD: Standard deviation; IU/L: International unit per liter

Table 2: Classification of vaccinees based on serum concentration of anti-HBs antibody.

Anti-HBs (IU/L)	Pre-booster			Post-booster		
	No. of vaccinees	%	GMT ± SD (IU/L)	No. of vaccinees	%	GMT ± SD (IU/L)
< 10	76	52.05	1.46 ± 2.35	4	4.25	5 ± 1.4
10-99	36	24.65	43.9 ± 23.87	16	17.02	48.93 ± 24
100-499	30	20.54	163.56 ± 63.73	18	19.14	288.8 ± 171.76
500-999	4	2.73	836.75 ± 98.87	44	46.8	797.5 ± 129.78
=1000	0	0	0	12	12.76	1096 ± 66.44
Total	146	100	68.12 ± 147.68	94	100	575.64 ± 386.13

Anti-HBs: Antibody against hepatitis B surface antigen; GMT: Geometric mean titer; SD: Standard deviation; IU/L: International unit per liter

Table 3: Comparison of seroprotection rates and GMT of anti-HBs antibody in vaccinees at pre- and post-booster vaccination.

Time	Sex	No. of vaccinees	Seroprotection rate (anti-HBs = 10 IU/L)	GMT ± SD(IU/L)
Pre-booster	Male	34	6 (18.2%)	10.15 ± 26.1
	Female	60	18 (29.5%)	9.27 ± 14.48
	Total	94	24 (25.5%)	9.58 ± 19.23
Post-booster	Male	34	32 (94.1%)	581 ± 401.6
	Female	60	58 (96.7%)	572.6 ± 380.5
	Total	94	90 (95.75%)	575.64 ± 386.13

Anti-HBs: Antibody against hepatitis B surface antigen; GMT: Geometric mean titer; SD: Standard deviation; IU/L: International unit per liter

Table 4: Outcome of booster vaccination with HB vaccine in healthy Children at 5-12 years after primary immunization

Population at primary vaccination	No. of Vaccinees	Primary vaccine and schedule	Time after primary vaccination	Booster vaccine	Seroprotection (GMT) Pre-booster	Seroprotection (GMT) Post-booster	Fold rise in titer	Ref.
Chines neonates	144	P-0,1,6 months	7 years	P	54.9 (14.7)	89.6% (190.6) at 1 month	13 x	14
US neonates	63	R-0,1,6 months	5 years	R	41%(35)	100% (1180) at 2 weeks	33 x	15
US neonates	14	P-0,1 months	12 years	R	100%(130)	100% (1050) at 1 week	8 x	16
Italian neonates	11	P-20 days, 2,12months	5 years	P	0% (N.S)	100% (400) at 1 month	N.S	17
Spanish neonates	34	P-0,1,6 months	7 years	R	85% (34)	100% (2985) at 1 month	88 x	18
Taiwanese neonates	35	P-2,6,10,50 weeks	7 years	R	86%(103)	100% (4566) at 1 month	44 x	9
Taiwanese neonates	40	P-2,6,10,50 weeks	7 years	P	92%(137)	100% (3579) at 1 months	26 x	9
Iranian neonates	81	R-0,1.5,9 months	5 years	R	81.5%(206)	100% (1278)at 1 month	6 x	10
Iranian neonates	*94	R-0,1.5,9 months	10 years	R	25.5% (9.6)	95.7% (575.6) at 1 month	30 x	P.S

*: represents 94 subjects out of 146 tested at 10 years who had anti-HBs < 50 IU/L. P: plasma derived vaccine, R: recombinant vaccine, N.S: not specified, P.S: present study. Anti-HBs: Antibody against hepatitis B surface antigen; GMT: Geometric mean titer; SD: Standard deviation; IU/L: International unit per liter

DISCUSSION

The results of present study show that at 10 years after completion of primary vaccination course 70/146 (47.9%) of children had protective levels of anti-HBs antibody with GMT of 68.12 IU/L. In previous study we have demonstrated that at five years after primary hepatitis B immunization 81.5% of children had protective levels of antibody with mean titer of 206 IU/L [10]. The persistence of protective levels of anti-HBs has been attributed to the peak of antibody at 1 month after completion of primary vaccination course [11]. We did not measure the peak of anti-HBs antibody level after primary vaccination. We expect that the peak of anti-HBs antibody concentration in this group was similar to others that we have measured [4-6].

In a series of studies among healthy children who received a complete hepatitis B immunization program, 50-100% of vaccinees had protective titer of anti- HBs antibody > 5 years after the last dose [8,12,13]. In the studies summarized in *Table 4* healthy children were given a booster dose of vaccine (usually of the recombinant type) at 5-12 years after the primary vaccination course. This *Table* represents the proportion of vaccinees with anti-HBs antibody level =10 IU/L and the associated GMT, just prior and after booster dose. Different results have been reported in these studies. This discrepancy may be attributed largely to differences in the age and race of vaccinees, age of initial vaccination, the primary vaccination schedule, the dosage, route and nature of vaccine (i.e., plasma derived or recombinant), time intervals between primary and booster vaccination and time intervals between vaccine administration and collection of blood samples. Accordingly, the

majority of vaccinees developed protective titer of anti-HBs antibody and the GMT measured at 1-4 weeks after booster vaccination ranged 6-88 fold above pre-booster levels.

It has been shown that vaccine induces active synthesis of anti-HBs antibody accompanied by immunological memory for HBsAg that afford ongoing protection in the absence of antibody [8,9]. The persistence of immunological memory over periods of 5 years or more is evident from rapid increases in antibody titer following booster vaccination, even in subjects who have lost antibody [14-18]. This phenomenon clearly reflects immunological memory residing in memory B lymphocytes that sensitized through an initial exposure to antigen, and upon a subsequent encounter with the same antigen, induces rapid proliferation, differentiation and production of specific antibody. Moreover, complementary studies, using an in vitro enzyme linked immunosorbent assay (spot- ELISA) show that the number of memory B lymphocytes able to produce anti-HBs antibody dose not diminish as the level of antibody decline [9,19]. In the present study 95.75% of children responded to a hepatitis B booster dose with sharp increase in anti-HBs antibody titer. This response was seen even in those subjects who had undetectable anti-HBs antibody titers before receiving the booster dose. In other word, loss of antibody does not necessarily means loss of immunity to HBV antigens, through the presence of immunological memory. Accordingly immunologic memory at 10 years after primary vaccination appeared to be good and it is possible that 4.25% may have been primary nonresponders.

Our results show that 52.05% of children lost protective antibody by early adolescence but in the majority of them the immunological memory remains intact. Breakthrough infection which may identified by detection of HBsAg and resulting clinical disease, has not been observed in present study. However, sub clinical infection that results in seroconversion for anti-HBc has occurred in 7.5% of vaccinees. It has been reported that those individuals who were vaccinated in the past and whose level of anti-HBs decline to low or undetectable levels over time, can mount an anamnestic response within a period as short as 4 days of viral exposure [20]. While HBV infection may be limited to a small number of hepatocytes, rapid antibody production by memory B cells can prevent spread of the virus to large areas of the liver, hence terminating infection before the person becomes at risk of development chronic HBV infection. In other word, since HBV infection has an incubation period of several weeks to months, exposure to natural infection and stimulation of memory cell by virus should rapidly trigger the production of antibody to prevent or markedly attenuate the infection. In fact serological studies over periods of 5 years or more in vaccinees who were frequently exposed to HBV, demonstrated that there have been very few clinically significant breakthrough infections. [9,20]

Optimally, booster vaccination should be recommended at a point in time when majority of vaccinees actually begin to lose protection. For children who received a primary course of hepatitis B vaccine in infancy, booster dose might be considered in conjugation with other preschool booster vaccination or at 11-12 years of age, if additional studies verify that immunologic memory in children vaccinated as infants, persist into adolescence. In that context the development of combination vaccine combining HBsAg with booster doses of tetanus and diphtheria toxoids is an attractive prospect.

In conclusion the results of present study shows that at 10 years after primary vaccination with recombinant HB vaccine, 47.9% of the children had protective levels of anti-HBs antibody. Moreover we have demonstrated an anamnestic response to booster vaccination, even in children who had lost anti-HBs antibody that confirms the persistence of an effective immunological memory in vaccines. Only additional follow-up studies in high and low risk groups determining the duration of immunological memory after primary hepatitis B vaccination course and the time that booster dose should be injected.

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