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The persistence of anti-HBs antibody and anamnestic response 20 years after primary vaccination with recombinant hepatitis B vaccine at infancy

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Abbreviations: Anti-HBs antibody, antibody to HBsAg; Anti-HBc antibody, antibody to HBcAg; HB, Hepatitis B; HBsAg, Hepatitis B surface antigen; HBcAg, Hepatitis B core antigen; HBV, Hepatitis B virus; ELISA, Enzyme-linked immunosorbent assay; EPI, Expanded Program on Immunization; GMT, Geometric mean titer; mIU/mL, milli-international units per milliliter; WHO, World Health Organization

Hepatitis B (HB) vaccine induces protective levels of antibody response (anti-HBs $\geq$ 10 mIU/mL) in 90–99% of vaccinees. The levels of anti-HBs antibody decline after vaccination. The aim of this study was to evaluate the persistence of anti-HBs antibodies and immunologic memory in healthy adults at 20 years after primary vaccination with recombinant HB vaccine. Blood samples were collected from 300 adults at 20 years after primary HB vaccination and their sera were tested for anti-HBs antibody by ELISA technique. A single booster dose of HB vaccine was administered to a total of 138 subjects, whose anti-HBs antibody titer was <10 mIU/mL. The sera of subjects were re-tested for the anti-HBs antibody levels at 4 weeks after booster vaccination. At 20 years after primary vaccination 37.0% of participants had protective levels of antibody with geometric mean titer (GMT) of 55.44 $\pm$ 77.01 mIU/mL. After booster vaccination, 97.1% of vaccinees developed protective levels of antibody and the GMT rose from 2.35 $\pm$ 6.49 mIU/mL to 176.28 $\pm$ 161.78 mIU/mL. 125/138 (90.6%) of re-vaccinated subjects also showed an anamnestic response to booster vaccination. At 20 years after primary vaccination with HB vaccine, low proportion of the subjects had protective levels of antibody. However, the majority of the re-vaccinated subjects developed protective levels of anti-HBs and showed an anamnestic response after booster vaccination. Additional follow-up studies are necessary to determine the duration of immunological memory.

Introduction

Hepatitis B virus (HBV) infection and its complications such as cirrhosis and hepatocellular carcinoma has remained a major public health problem throughout the world. Approximately, one third of the world population shows a previous history of infection and more than 350 million individuals have been estimated to be chronically infected.[1] In areas with high endemicity, especially in some parts of Africa and south-east Asia, over 8% of individuals are chronically infected and the infection is predominantly transmitted vertically during prenatal period from carrier mothers to their neonates. In regions of intermediate endemicity, the patterns of the disease transmission is mixed and disease occurs at all ages, but again the predominant period of transmission seems to be at younger ages.[2]

Effective control of HBV transmission in regions with high and intermediate endemicity, therefore, would not be possible without vaccination of the vulnerable groups of the population.[3] The WHO (World Health Organization) strategy for effective control of HBV infection and its complications is the mass vaccination of neonates and children within the framework of Expanded Program on Immunization (EPI). In 1991, the Global Advisory Group to the WHO recommended that all countries integrate hepatitis B vaccine into national immunization by 1997.[4,5] This program has been incorporated in the national immunization scheme in Iran since 1993.[6] As of 2008, 177

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Table 1. Seroprotection rates and GMT of anti-HBs antibody in vaccinees 20 years after primary vaccination

Time	Sex	No. of vaccinees	Seroprotection rate (anti-HBs $\geq$ 10 mIU/mL)	GMT $\pm$ SD (mIU/mL)	P value
20 years after primary vaccination	Men	142	57 (40.1%)	65.03 $\pm$ 85.03	* = 0.14 ** = 0.17
	Women	158	54 (34.2%)	45.33 $\pm$ 66.83	
	Total	300	111(37%)	55.44 $\pm$ 77.01	

* and ** represent the differences between men and women regarding the seroprotection rate and geometric mean titer (GMT) of antibody, respectively.

countries worldwide have implemented HB immunization into their national immunization program as a routine vaccine given to all infants that lead to substantial reduction in the global burden and transmission of HBV.[7]

HBV expresses 3 forms of overlapping envelope proteins including the small (S antigen), middle (pre-S2 antigen) and large (pre-S1 antigen) proteins. The 'S' antigen (HBsAg) is the predominant form of the surface antigens and constitutes the immunodominant 'a' determinant required for induction of protective antibody response in human.[8,9] The antibody response to HBsAg (anti-HBs) provides the immunity against HBV infection that appears after clearance of HBsAg or after immunization.[8]

Despite some differences in national vaccination programs between different countries, a 3 dose vaccination schedule (of 10 μg or 20 μg doses) of recombinant HBsAg are administered in most countries for vaccination of neonates and adults, respectively.[6,8,10] Vaccination with HBsAg induces protective antibody response (anti-HBs $\geq$ 10 mIU/mL) in the majority of vaccinees. The results obtained from several studies have indicated that vaccination of healthy neonates and adults with recombinant HBsAg induces a protective antibody response in 90-99% of vaccines.[6,8,10] We have previously reported a strong protective antibody response in the majority of healthy vaccinated neonates from Kerman and Urmia cities located in southeast and northwest of Iran, respectively.[11] However, a small proportion of vaccinees fail to respond, accounting for 1.7% and 3.9% of Urmian and Kermanian neonates, respectively.[11] We have also demonstrated that intramuscularly administration of a single supplementary low dose of HB vaccine induced high seroprotection rate in the non-responder neonates to primary course of vaccination.[12]

Although, vaccination with the HBsAg induces protection in the majority of vaccines, however, it has been shown that the anti-HBs level diminishes after vaccination. The results of the studies reported from different regions with varied hepatitis B endemicity have demonstrated the persistence of protection for at least 10–15 years after primary vaccination despite diminishing antibody levels.[13-16] Some investigators have also suggested the need for a booster vaccination at 15 years after the completion of primary vaccination.[17,18] However, the determination of the protection duration after primary vaccination against HBV is important to evaluate whether booster vaccination may be necessary to extend protection through adulthood.

This study was conducted for the first time in an Iranian population to evaluate the persistence of anti-HBs antibody 20 years after primary vaccination with recombinant hepatitis B vaccine and also the anamnestic response to a booster dose.

Results

The persistence of anti-HBs antibody 20 years after primary vaccination

At 20 years after completion of the primary vaccination course 111/300 (37%) of individuals had protective concentrations of anti-HBs antibody (anti-HBs $\geq$ 10 mIU/mL) with GMT of 55.44$\pm$ 77.01 mIU/mL. Both seroprotection rate and GMT were higher in men as compared to women but the differences were not statistically significant (**Table 1**).

The individuals have been arbitrary classified into different groups according to their serum titer of anti-HBs antibody. Accordingly, the proportions of subjects with antibody titer of $<$10 mIU/mL, 10-99 mIU/mL, 100-499 mIU/mL and $\geq$ 500 mIU/mL were 63%, 30.7%, 6.3% and 0.0% respectively. Collectively, 20 years after primary vaccination, the titer of anti-HBs antibody for 93.7% of subjects was $<$100 mIU/mL and none of participants had a titer $\geq$500 mIU/mL (**Table 2**).

None of the participants reported clinical symptoms of HBV infection during the 20 years after primary vaccination. Moreover, 20 years after the completion of primary HB vaccination course, all subjects were found to be negative for HBsAg and anti-HBc antibody. Accordingly, no breakthrough infection was observed in this investigation.

Table 2. Classification of vaccinees based on their serum concentration of anti-HBs antibody at 20 years after primary vaccination and post booster vaccination

Grouping of vaccinees	Pre-booster			Post-booster		
[based on anti-HBs levels (mIU/mL)]	No. of vaccinees	%	GMT $\pm$ SD (mIU/mL)	No. of vaccinees	%	GMT $\pm$ SD (mIU/mL)
$<$ 10	189	63%	2.46 $\pm$ 6.44	4	2.9%	8.47 $\pm$ 1.01
10-99	92	30.7%	18.98 $\pm$ 7.60	63	45.7%	43.15 $\pm$ 25.45
100-499	19	6.3%	206.97 $\pm$ 76.20	68	49.3%	294.46 $\pm$ 123.26
$\geq$500	0	0.0	0	3	2.2%	517.00 $\pm$ 12.28

Table 3. Seroprotection rates and GMT of anti-HBs antibody at pre- and post booster vaccination in vaccinees who received a booster dose

Sex	No. of vaccinees	Pre-booster		Post-booster		
		Seroprotection rate (anti-HBs ≥ 10 mIU/mL)	GMT ± SD (IU/L)	Seroprotection rate (anti-HBs ≥ 10 mIU/mL)	GMT ± SD (mIU/mL)	Anamnestic Response
Men	62	0 (0.0%)	0.70 ± 7.75	60 (96.8%)	150.78 ± 146.75	55 (88.7%)
Women	76	0 (0.0%)	3.70 ± 3.61	74 (97.4%)	197.08 ± 171.21	70 (92.1%)
Total	138	0 (0.0%)	2.35 ± 5.49	134 (97.1%)	176.28 ± 161.78	125 (90.6%)

The antibody response to a booster vaccination 20 years after primary vaccination

20 years after completion of the primary vaccination course 189/300 (63%) of individuals had no protective concentrations of anti-HBs antibody. Of these 51 subjects were not available for booster vaccination. Totally, 138 subjects received a booster dose. The results of the booster vaccination are summarized in **Table 3**. After booster vaccination, 134/138 (97.1%) of vaccinees developed protective levels of anti-HBs antibody with GMT 176.28 ± 161.78 mIU/mL. 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.

The seroprotection rate was similar in men and women after the booster vaccination (96.8% and 97.1%, respectively). After booster vaccination, however, the GMT of anti-HBs antibody was higher in women as compared to men but the difference was not statistically significant (197.08 ± 171.21 mIU/mL vs 150.78 ± 146.75 mIU/mL; P=0.09) (**Table 3**).

Following the booster vaccination, 2.9%, 45.7%, 49.3% and 2.2% of vaccinees had a titer of <10 mIU/mL, 10-99 mIU/mL, 100-499 mIU/mL and ≥500 mIU/mL, respectively. Moreover, 51.5% of subjects developed a titer above 100 mIU/mL after the booster vaccination (**Table 2**).

The anamnestic response to a booster vaccination 20 years after primary vaccination

Totally, 138 subjects received a booster dose. The results of the anti-HBs antibody concentrations at pre- and post- booster vaccination and also the anamnestic response in vaccinees who received a booster dose have demonstrated in **Table 3**. Collectively, 125/138 (90.6%) of revaccinated subjects showed an anamnestic response to booster vaccination. No significant differences was observed between men and women regarding the anamnestic response to booster vaccination [70/76 (92.1%) and 55/62 (88.7%), respectively, P = 0.49)]. Overall, the GMT of anti-HBs antibody increased by 75-fold, from 2.35 ± 6.49 mIU/mL at pre-booster time to 176.28± 161.78 mIU/mL after the booster vaccination.

Of the 138 subjects included for booster vaccination, 35 subjects were seronegative and 103 subjects were seropositive for anti-HBs antibody. All seronegative subjects showed an anamnestic response after booster vaccination. An anamnestic response was also observed in 90/103 (88.4%) of seropositive participants.

Discussion

The results of present study showed that at 20 years after completion of primary HB vaccination course 111/300 (37%) of individuals had protective levels of anti-HBs antibody with GMT of 55.44± 77.01 mIU/mL. In our previous studies in the same population we have demonstrated that 5 and 10 years after primary HB immunization 81.5% and 47.9% of individuals had protective levels of antibody with mean titer of 206 ± 354 mIU/mL and 68.12 ± 147.68 mIU/mL, respectively.[20,21] 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.[22] We expect that the peak of anti-HBs antibody levels in investigated group was similar to others that we have measured, previously.[6,11,23]

In a series of studies among healthy children and neonates who received a complete HB immunization program, 40%-95% of vaccinees had protective titer of anti- HBs antibody >5 years after the completion of the primary vaccination (**Table 4**). Different results have been reported in these studies. However, the results of the present study showed lower seroprotection rates year 20 after primary vaccination as compared with other studies (**Table 4**). It has been reported that the persistence of anti-HBs and response to booster vaccination may be related to the age of initial vaccination, the primary vaccination schedule, the vaccine dosage, route and nature of vaccine (i.e., plasma derived or recombinant), time intervals between primary and booster vaccination, time intervals between vaccine administration and collection of blood samples vaccine dosage and timing of the last vaccination of the primary series (ie, the interval time between last and preceding dose). The persistence of anti-HBs and response to booster vaccination may also be influenced by the prevalence of hepatitis B infection in the population and race of vaccinees.[24]

The results of the present study also showed that the booster vaccination induced protective levels of anti-HBs antibody in the 97.1% of vaccinees and the GMT of anti-HBs antibody increased by 75-fold, from 2.35± 6.49 mIU/mL at pre-booster time to 176.28± 161.78 mIU/mL after the booster vaccination. These results clearly represent that the immunological remains intact 20 years after the completion of primary vaccination with HB vaccine. The seroprotection rate after booster vaccination is similar to the results reported in studies from Malaysia (94.0%), Thailand (98.6%) and Germany (97.2%-99.6%).[14,16,25]

Moreover, 90.6% of the re-vaccinated subjects showed an anamnestic response to booster vaccination. Similar anamnestic

Table 4. Persistence of the anti-HBs antibodies after primary vaccination in different studies

Country	Subjects	NO.	Primary vaccination program	Primary vaccination doses (μg)	Time after primary vaccination (year)	Seroprotection (%)	GMT of antibody (mIU/mL)	Ref.
Germany	Neonates	300	0-1-6	10	5	83.3	55.7	14
		306	0-1-6	10	10	78.3	34.5	
Canada	Neonates	326	0-1-6	10	5	88.2	269	15
		326	0-1-6	10	10	86.4	169	
		326	0-1-6	10	15	76.7	51	
USA	Neonates	105	0-2-6	5, 2.5, 2.5	15	40%	NS*	35
Thailand	Neonates	25	0-1-2-12	10	20	64%	20.4	25
China	Children	35	0-1-6	10	23	60	39.1	19
		46	0-1-6	20	23	58.7	19.9	
Australia	Neonates	234	0-1-6	10	5	91/4%	100	36
Canada	Children	1129	0-1-6	10	5	94.7%	252	37
		1126	0-1-6	2.5	5	95.2%	66	
Malaysia	Neonates	402	NS*	NS*	24	67.9%	NS*	16
I.R. Iran	Neonates	81	0-1.5-9	10	5	81.5%	206	20
		146	0-1.5-9	10	10	47.9%	68.12	21
		729	0-1.5-9	10	5-6	84%	230.5	38
		300	0-1.5-9	10	20	37%	55.44	PS**

*NS: Not specified, **PS: present study.

response has been reported at 23 years after primary vaccination in a study from China.[19] These observations indicate that the primary vaccination has induced strong immune memory as observed by the anamnestic response to the challenge dose in 90.6% subjects. These findings are in accordance with results reported in other studies.[25]

It has been shown that vaccine induces active production of anti-HBs antibody accompanied by HBsAg-specific immunological memory that provide continuous protection in the absence of antibody.[17,18] The persistence of immunological memory over 5 years after primary vaccination is apparent from rapid increases in antibody level following booster vaccination, even in subjects who have lost antibodies.[26-29] This phenomenon clearly represents the presence of the immunological memory exist within the memory B lymphocytes that are induced during the first exposure to antigen, and upon a subsequent exposure to the same antigen, induces rapid production of specific antibody. Moreover, complementary studies using an in vitro cell enzyme linked immunosorbent assay (spot- ELISA) have demonstrated that the number of memory B lymphocytes able to produce anti-HBs antibody is not reduced as the level of antibody declines.[18,30] In other words, loss of antibody does not necessarily means loss of immunity to HBV antigens, due to the presence of immunological memory. Accordingly, the individuals whose anti-HBs levels decline to the <10 mIU/mL may not be at risk of hepatic disease since they have HBsAg-specific immunological memory. Following the exposure to HBV, the presence of the immunological memory rapidly leads to a robust anamnestic response, which prevents acute disease and chronic infection.

The prevalence of HBV infection in Iran was about 3.5% in 1990s, however, after implementation of the HB vaccination into the national immunization program, the prevalence of HBV infection has reduced to 2.14%.[31,32] Accordingly, the participants are living in a region with intermediate endemicity for HBV infection. However, none of the subjects of the present study were positive for HBsAg, anti-HBc antibodies or reported to have clinical symptoms of HBV infection during the 20 years after primary HB vaccination. These observations indicate that the participants were protected against HBV infection for 20 years post primary vaccination. Furthermore, these findings confirm the effectiveness of HB vaccination in preventing chronic infection after primary vaccination as also demonstrated in other studies.[25] A meta-analysis study also indicated that long-term protection provided by HB vaccine is sufficient to prevent HBV infection in healthy subjects for at least 20 years.[33]

Although, 63% of individuals in this study had lost protective levels of antibody but in the majority of them the immunological memory remains intact. Breakthrough infection which may be identified by detection of HBsAg and resulting clinical disease, has not been observed in present study. 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.[34] 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 words, 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 are frequently exposed to HBV demonstrated that there have been very few clinically significant breakthrough infection.[18,34]

In conclusion, the results of present study showed that at 20 years after primary vaccination 37.0% of participants had protective levels of antibody. After booster vaccination, 97.1% of vaccinees developed protective levels of anti-HBs antibody and

the GMT rose by 75-fold after the booster vaccination as compared to pre-booster time. 90.6% of the re-vaccinated subjects also showed an anamnestic response to booster vaccination. The participants were also protected against HBV infection for 20 years post primary vaccination. Additional follow-up studies are essential in high and low risk groups to determining the duration of immunological memory after primary hepatitis B vaccination course and the time that booster dose should be injected.

Methods

Study design and subjects: This retrospective cohort study was conducted to determine the persistence of anti-HBs antibody and also to evaluate the anamnestic response to a booster HB vaccine at 20 years after primary vaccination course. A total of 300 healthy subjects (142 men and 158 women) attending the health centers of Rafsanjan (a city in Kerman province, located southeast of Iran) were included in this study.

The participants had been vaccinated with 3 doses of HB vaccine during first year of life. Originally, from March to December 1993, primary vaccination course of hepatitis B vaccine including a 3 dose vaccination schedule (of 10 microgram doses) of a recombinant HB vaccine (Engerix-B, Smithkline Beechman, Rixensart Belgium) were administered into the quadriceps muscle during infancy (first dose within 2 days of birth and the subsequent doses at 1.5 and 9 months of life). Blood samples were collected in January 2014, 20 years after completion of primary vaccination course. The sera of individuals were tested for anti-HBs and anti-HBc antibodies and HBsAg. Moreover, a single 20 microgram booster dose of recombinant HB vaccine (Heberbiovac, Heberbiotec Co, Cuba) was administered intramuscularly (i.m) to subjects with anti-HBs <10 mIU/mL and the sera were retested for anti-HBs antibody levels at 4 weeks after booster vaccination.

The anamnestic response to booster vaccination was defined as anti-HBs antibody concentration ≥ 10 mIU/mL one month post-challenge dose as compared with pre-challenge anti-HBs concentration for seronegative subjects or at least a 4-fold increase in anti-HBs level at post-booster vaccination as compared with pre-challenge anti-HBs level for seropositive subjects.[14,19]

All participants were basically healthy, with no acute or chronic illnesses. Indeed each individual with history of chronic or acute disease and use of any drug were excluded from the study. This study was approved by the Ethical Committee of Rafsanjan University of Medical Sciences. Moreover, informed written consent was obtained from the participants before enrollment into the study.

Detection of HBV markers

HBs antigen, anti-HBs and anti-HBc antibodies were detected by enzyme-linked immunosorbent assay (ELISA) Using commercial kits (Behring, Germany). Anti-HBs antibody was measured by using standard samples with known concentrations of anti-HBs antibody expressed as mIU/mL, provided by the manufacturer. Seropositivity and seroprotection were defined as anti-HBs antibody concentrations ≥ 1.0 mIU/mL and ≥ 10 mIU/mL, respectively. Subjects with non-measurable levels of antibody or anti-HBs antibody concentrations <1.0 mIU/mL were considered as seronegative.

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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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