PERSISTENCE OF ANTI-HBs ANTIBODIES IN HEALTHY IRANIAN CHILDREN VACCINATED WITH RECOMBINANT HEPATITIS B VACCINE AND RESPONSE TO A BOOSTER DOSE

A. Jafarzadeh[*] and S. M. A. Sajjadi

Department of Microbiology and Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Abstract- Long-term protection against hepatitis B virus (HBV) is dependent on persistence of anti-HBs antibodies and/or strong immunological memory. In this study we evaluated the persistence of anti-HBs antibodies in healthy Iranian children 5 years after primary vaccination and the response to a booster dose using recombinant hepatitis B vaccine. Totally, 81 children who had received primary course of hepatitis B vaccine at 0, 1.5 and 9 months of age were included in this study. A booster dose of hepatitis B vaccine was administered at 5 years after completion of primary vaccination program. Children were tested for anti-HBs antibody just before administration of booster dose and at 4 weeks after booster vaccination. An 81.5% seroprotection rate (anti-HBs > 10 IU/L) was observed 5 years after primary vaccination. After administration of booster dose, 100% of the children developed protective level of anti-HBs antibody and geometric mean titer rose from 206 IU/L to 1278 IU/L. These results indicate the existence of an effective immunological memory over a period of 5 years after primary vaccination with recombinant hepatitis B vaccine in healthy Iranian children.

Acta Medica Iranica, 43(2): 79-84; 2005

Key words: Children, hepatitis B vaccine, anti-HBs antibodies, booster vaccination

INTRODUCTION

It is estimated that there are 350 million carriers of hepatitis B virus (HBV) worldwide (1). In areas of high endemicity, especially in some parts of Africa and South East Asia, 7-20% of individuals are chronically infected and more than 70% of the adults show evidence of prior infection (2). In these populations the vast majority of infections are transmitted vertically during perinatal period from carrier mothers to their neonates (1). In areas of intermediate endemicity, such as Iran (3), however, disease transmission is mixed and occurs at all ages, but again the predominant period of transmission seems to be at younger ages (4).

Received: 26 Feb. 2003, Revised: 21 June 2004 Accepted: 21 Aug. 2004

*** Corresponding Author:**
A. Jafarzadeh, Department of Microbiology and Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Tel: +98 391 8220086, Fax: +98 391 5225209
E-mail: gafarzadeh2@yahoo.com

Effective control of HBV transmission in areas of high and intermediate endemicity, therefore, would not be possible without vaccination of this vulnerable group of the population (5). The 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 it has been recommended that all countries integrate hepatitis B vaccine into national immunization by 1997 (6). This program has been incorporated in the national vaccination scheme in Iran since 1991 (7). 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 (2).

Vaccination with the major surface antigen of virus (HBsAg) induces protection in the majority of vaccinees (8). However, the emerging problem is to evaluate the duration of the immune protection and

whether booster dose is required. Antibody kinetic studies have shown a decrease in anti-HBs level after vaccination and some authors have suggested the need for a booster dose after 5-15 years (8-10).

This study was conducted for the first time in Iranian children to evaluate the persistence of anti-HBs antibody at 5 years after primary vaccination course with recombinant hepatitis B vaccine and the response to a booster dose.

MATERIALS AND METHODS

A total of 81 healthy children (47 males and 34 females) attending the health centers of Rafsanjan, (a city in Kerman province, located south-east of Iran) for receiving booster dose of hepatitis B vaccine were included in this study. We obtained informed consent from all parents.

All participants were basically healthy with no acute or chronic illnesses. Any individual with history of chronic or acute disease and use of any drug was excluded from the study. Primary vaccination course of hepatitis B vaccine had been administered to children at birth, 1.5 and 9 months of age. Originally, during the neonatal period, from February to October 1996, triple 10 microgram doses of a recombinant HB vaccine (Engerix-B, Smith Kline Beechman, Rixensart Belgium) had been administered into the quadriceps muscle at 0, 1.5 and 9 months of age. Booster vaccination was given 5 years after completion of primary vaccination program. Children were tested for anti-HBs antibody just before administration of booster dose and at 4 weeks after booster vaccination. Blood samples were collected in November 2001, 5 years after completion of primary vaccination course. Immediately, a single booster dose of the same concentration was administered to all children.

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 (2-3 ml) was taken from all vaccinees 4 weeks after booster vaccination and the sera were collected and stored at -20°C.

Anti-HBs and anti-HBc antibodies were detected by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Behring, Germany). Anti-HBs antibody was quantitated 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.

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 5 years after completion of primary vaccination course 66/81 (81.5%) of children had protective concentrations of anti-HBs antibody (anti-HBs >10 IU/L) with geometric mean titer (GMT) of 206 IU/L (Table 1). Both seroprotection rate and GMT were higher in females compared to males, but the differences did not reach statistical significance. After receiving the booster dose, 100% of vaccinated children developed protective levels of anti-HBs antibody and GMT increased from 206 IU/L to 1278 IU/L. The postbooster GMT of anti-HBs was found to be significantly higher in comparison with prebooster mean concentration ($P < 0.00001$). The sex of vaccinees did not significantly influence the postbooster mean concentration of anti-HBs, although this parameter was found to be higher in females compared to males (1360 IU/L vs 1219 IU/L). The children could be arbitrarily classified into different groups according to their serum titer of anti-HBs antibody (Table 2). After booster dose administration, 26% and 74% of 81 children had a titer of 100-1000 IU/L and > 1000 IU/L, respectively.

Moreover, 100% vaccinees developed titer of > 100 IU/L after the booster vaccination. Before booster dose administration the proportion of subjects with antibody titer of 0-10 IU/L, 10-100 IU/L, 100-1000 IU/L and > 1000 IU/L was 18.5%, 46.9%, 21% and 13.6%, respectively.

All subjects were still found to be anti-HBc antibody negative.

Table 1. Comparison of seroprotection rates and GMT of anti-HBs antibody in male and female vaccines

Time	Sex	No	Seroprotection rate	GMT±SD (IU/L)
Pre-booster	Male	47	36 (76.6%)	196±344
	Female	34	30 (88.2%)	214±365
	Total	81	66 (81.5%)	206±354
Post-booster	Male	47	47 (100%)	1219±628
	Female	34	34 (100%)	1360±513
	Total	81	81 (100%)	1278±383

Abbreviation: GMT, geometric mean titer.

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

Anti-HBs	Pre-booster		Post-booster	
(IU/L)	No (%)	GMT±SD	No (%)	GMT±SD
0-10	15(18.5)	3.5±3	0(0)	0
10-100	38(46.9)	30±22	0(0)	0
100-1000	17(21)	267±113	21(26)	404±195
> 1000	11(13.6)	1020±51	60(74)	1584±272
Total	81(100)	206±354	81(10)	1278±383

Abbreviation: GMT, geometric mean titer.

DISCUSSION

In this study we have demonstrated that 5 years after primary immunizations, 81.5% of children had protective levels of anti-HBs antibody with GMT of 206 IU/L. It has been shown that the level of anti-HBs antibody does wane rapidly within the first year after vaccination and more slowly thereafter (8). 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, since we had this parameter in other studies (12, 13). However, since it is clear that anti-HBs antibody concentrations fall about 90% in the first year, we expected that the peak of anti- HBs antibody concentration in this group to be similar to others that we have measured (13).

In a series of studies it has been demonstrated that 90-99% of healthy neonates, children, adolescents and adults develop protective levels of anti-HBs antibody following a standard vaccination course with hepatitis B vaccine (8, 9, 12-15). However, the duration of protection has yet remained to be determined. 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 (16-20).

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 (19, 20).

Furthermore, healthy vaccinees typically respond to the last dose of a hepatitis B vaccine series with a large secondary (anamnestic) rise in antibody. 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 (9). With specific regard to hepatitis B vaccine, 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 (19-21). 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 declines (22). In the present study, one month after the booster dose, 100% of the subjects had an anamnestic type of response with anti-HBs antibody titer > 100 IU/L. This response was seen even in those subjects who had undetectable anti-HBs antibody titers before receiving the booster dose. Our results indicate that, despite the decline of anti-HBs antibody titer and negativization in some cases, an immunological memory exists in these subjects. In the studies summarized in table 3 healthy subjects were given a booster dose of vaccine (usually of the recombinant type) at 4-12 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.

Table 3. Outcome of booster vaccination with HB vaccine in healthy subjects at 4-12 years after primary immunization

Population at primary vaccination	Primary vaccine and schedule	Time after primary vaccination	Booster vaccine	Seroprotection (GMT)		Fold-rise in titer	Ref
				Prebooster	Post booster		
Italian adults (n=302)	P-0,1,6 m	6 years	R	67%(142)	97% (9891) at 1 month	70×	23
Italian adults (n=653)	P-0,1,2,14 m	6 years	R	94%(1022)	99.2% (149961) at 1 month	147×	23
Spanish adults (n=462)	P-0,1,6	6.5 years	R (n=125)	85%(13)	98% (609) at 1 month	46×	24
Swiss adults (n=55)*	P-0,1,2,12 m	5.5 years	R (n=33), P (n=22)	0%(NS)	73% (NS) at 1 month	NS	25
Chines neonates (n=144)	P-0,1,6 m	7 years	R	54.9%(147)	89.6% (1906) at 1 month	13×	26
US neonates (n=63)	R-0,1,6 m	5 years	R	41%(35)	100% (1180) at 1 month	33×	27
Sweden adults (n=41)	R-0,1,6 m	4 years	R	59%(NS)	95% (NS) at 1 month	NS	28
New Zealand adolescents (n=71)	P-0,1,6 m	5.5 years	R	90%(123)	100% (8826) at 1 month	72×	29
New Zealand children (n=308)	P-0,1,2 m	5 years	R	85%(89)	99% (4777) at 1 month	54×	30
New Zealand children (n=17)	P-0,1,2 m	9 years	R	59%(2)	94% (158) at 1 month	79×	31
US neonates (n=14)	P-0,1 m	12 years	R	100%(130)	100% (1050) at 1 month	8×	20
Italian neonates (n=11)	P-20 d, 2,12 m	5 years	R	0%(NS)	100% (400) at 1 month	N.S	32
Spanish neonates (n=34)	P-0,1,6 m	7 years	R	85%(34)	100% (2985) at 1 month	88×	33
Taiwanese neonates (n=35)	P-2,6,10,50 w	7 years	R	86%(103)	100% (4566) at 1 month	44×	9
Taiwanese neonates (n=40)	P-2,6,10,50 w	7 years	R	92%(137)	100% (3579) at 1 month	26×	9
Iranian neonates (n=81)	R-0,1.5,9 m	5 years	R	81.5%(206)	100% (1278) at 1 month	6×	PS

Abbreviations: HB, hepatitis B, GMT, geometric mean titer; P, plasma derived vaccine; R, recombinant vaccine; NS, not specified; PS, present study; Ref, reference; m, months; w, week.
* represents 55 subjects out of 655 tested at 5.5 years who lacked any detectable anti-HBs before the booster.

Different results have been reported in these studies. This discrepancy may be attributed largely to differences in the age and race of vaccinees, 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- to 88-fold above pre-booster levels. These results show that immunological memory remains intact over periods of 5 or more years, even in subjects who retain little or no antibody. In other word, loss of antibody does not necessarily mean loss of immunity to HBV antigens, through the presence of immunological memory. Based on these observations, 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 (8, 9).

Optimally, booster vaccination should be recommended at a point in time when majority of vaccinees actually begin to lose protection. For children who have received a primary course of hepatitis B vaccine in infancy, booster dose might be considered at 4-6 years of age 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 persisted 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 show that at 5 years after primary vaccination with recombinant HB vaccine, 81.5% 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 vaccinees. 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 booster dose should be injected are recommended.

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