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Long-term Antibody Response and Immunologic Memory in Children Immunized with Hepatitis B Vaccine at Birth

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Four hundred and fifty three healthy children immunized with a course of hepatitis B vaccine beginning at birth were tested at 10-11 years of age for persistence of anti-hepatitis B-S antigen antibody (anti-HBs); and responses of children without protective antibody to different doses of hepatitis B vaccine booster were evaluated. Although nearly 42% of them were not seroprotected, but most of boosted subjects (87.3%) retained robust immunologic memory and rapidly retained a protective anti-HBs antibody titer of at least 10 IU/L after booster vaccination.

Key words: *Hepatitis B vaccine Booster, Immunologic memory, Long-term immunity.*

Hepatitis B virus (HBV) infection remains a worldwide health problem. There are an estimated number of 350 million hepatitis B carriers globally, who are faced with significant morbidity and mortality(1). Hepatitis B vaccine has been shown to be immunogenic and effective in preventing HBV infection(2). A potential problem of HBV immunization is that vaccine-induced anti-HBs antibody titers decline with time(3,4). Instances of late infection, occasionally resulting in HBV carriage, were documented in some long-term follow-up studies(4-6). The rapid decline of anti-HBs levels in children immunized at < 1 year of age poses some serious concern regarding the duration of immune response in this age group(3,5,7).

There is an increased risk for HBV infection during the sexually active years. Therefore, the duration of protection must last into these years. If the protection induced by HB vaccination at infancy dose not last until the adulthood, booster doses should be administered at the preschool entry ages, or during adolescence(3,4,8-11).

Our study monitored the persistence of anti-HBs antibody in a group of healthy 10-to-11 years old children who had been initially immunized with HB vaccine starting at birth, and the status of immunologic memory was assessed after administering different doses of booster HB vaccine in those children, who lost their protective levels of anti-HBs .

Subjects and Methods

In a community-based study between January to April 2003, sera were collected from 453 apparently healthy 10-11 years (mean = 10.7 years) old children [without histories of prematurity, birth weight of <2500 g or chronic diseases], who had earlier received the routine universal HB vaccine three doses of HB vaccine (recombinant Engerix-B Biotec-Havana, Cuba, 10 µg) during infancy (first dose; within 3 days of birth and 1.5, 9 months schedules) in Sari-Iran. Parents of all children were requested to sign an informed consent form and to provide vaccination histories based on the immunization records of their children.

Serum anti-HBs levels were measured by using an ELISA quantitative method

$$\left\{\frac{\text{HBs - Ab EIA - 96}}{\text{Paramex Labo Diagnostic Inc: CA-USA}}\right\}$$

and their concentrations were expressed as IU/L. All children with anti- HBs concentration >10 IU/L were considered seroprotected and excluded from further analysis. Children with anti-HBs levels of 2- 10 IU/L were considered seropositive but not seroprotected and children with anti-HBS levels of <2 IU/L were considered nonprotected (susceptible). Subjects with anti-HBs titer <10 IU/L were enrolled for the booster study. These children were divided into 3 groups according to 3 different booster of the same HB vaccine, and given three different doses. Group 1: 10µg intramusculary (IM), group II: 5µg IM, and group III: 2.5µg intradermally (ID). Detailed quantitative antibody responses were studied 10-14 days after administration of the booster dose. An anamnestic antibody response is defined as increase in anti-HBs concentrations to more than10 IU/L, 10-14 days after boosting. Children with no response to booster dose were tested for HBV infection markers (HBs-Ag with HBsAg kit EIA-95 paramex - CA USA , and Anti-HBc with anti-HBc kit , Capita TM, Biotech Ireland). The calculation of geometric mean concentrations (GMCs) of anti-HBs was based on the WHO standard. Differences in frequency between groups were examined by Chi square test. P <0.05 was considered statistically significant.

Results

Initial serologic status at 10 years

Four hundred fifty three children (242 boys, 211 girls) were enrolled in the study. The overall seropositivity rate (>2 IU/L) for anti- HBs was 81.5% (369 out of 453 with geometric concentration of GMCs: 39.45 (69.93). 42.2% (191 of 453) of children had anti-HBs concentrations <10 IU/L (GMCs: 3.84 (3.34). These results are presented in *Table I*.

Booster effects on children without protective anti-HBs

Of 191 children without seroprotective concentrations of antibody (<10 IU/L), 165 children (91 boys, 74 girls) were enrolled for the booster study. As shown in *Table II*. 144 out of 165 (87.3%) subjects responded to the booster doses of HB vaccine.

Group I

Fifty seven children (35 with anti-HBs levels 2-10, 22 with < 2 IU/L) were allocated to this group. All boosted children except

TABLE I–*Anti HBs Titers in Sari- Children 10 Years After HB Vaccination.*

	Pre booster	Post boost
No. of studied children	453	165
Gender M(%)	242 (53.4%)	91 (55.2%)
F(%)	211(46.6%)	74 (44.9%)
No. of children with anti HBs titer:		
: > 10 IU/L	262 (57.8%)	--
: 2-10 IU/L	107 (23.6%)	99 (60%)
: < 2 IU/L	84 (18.6%)	66 (40%)
GMCs of studied children	39.45 ± 69.93	--
GMCs of children with titers < 10 IU/L	3.84 ± 3.39	153 ± 163.84

3 (94.7%) had anamnestic responses with GMCs: 166.76 ± 105.61 2 weeks after boosting. Although statistically nonsignificant, children with anti- HBs concentrations of 2-10 IU/L before the booster acquired higher anti-HBs titers when measured 2 weeks after the booster dose compared to that in children with anti- HBs concentration <2 IU/L (GMCs: 175.50 ± 107.75 *vs* 158 ± 102.8, P >0.2).

Group II

After administration of the 5µg IM of the HB vaccine booster dose to 52 children (31 with anti HBs 2-10 and 21 with <2 IU/L), 89.3% (50 of 56) of them showed anamnestic antibody response (GMCs: 130.91 ± 107.18). Differences between these 2 subgroups were statistically significant (GMCs: 149.26 ± 111.19 *vs* 86.84 ± 94.78; P <0.02).

Group III

Fifty six children (33 with anti-HBs 2-10 and 23 with <2 IU/L). After administration of 2.5 µg ID of the booster, 78.6% (44 of 56) of the children had anamnestic response (GMCs: 103.2 ± 89.3). The post-booster anti- HBs GMCs differences between two subgroups were statistically nonsignificant (GMCs 124.87 ± 89.42 *vs* 82.79 ± 85.4, P >0.05).

It was noticed that by decreasing the concentration of the booster, the numbers of the children who did not respond to the booster increased significantly (3 of 56 in Group I *vs* 6 of 52 Group II *vs* 12 of 56 Group III, with P value: groups I to II: 0.616; I to III 0.011; and II to III 0.017; respectively).

Twenty one (12.7%) children, did not show any immunologic response. These children were investigated for HBV infection markers (HBs-Ag, anti-HBc) all of them were negative. They are going to complete 1-3 doses of HB vaccination.

Discussion

The results of this study showed that seropositivity rate in children vaccinated in the first year of implementation of the HB vaccine in Iran vaccination programs,"some 10 years ago" is 81.5%. However 42% of all these children at this age has lost their protective levels of anti-HBs (>10 IU/L). Literature is lacking to understand the trend of decline in the anti-HBs levels in children, as the original antibody levels immediately after the completion of the primary series of HB vaccination is unknown. Nevertheless,

TABLE II–*Response to Booster doses of Hepatitis B Vaccine.*

Total No. of boosted children	No. in each group / GMCs	No. of 2-10 IU/L / No of <2 IU/L	GMCs Pre boost	GMCs Post boost	Subgroup p value	No. of responded (%)*
165	G_1 = n = 57 / 166.76 ± 105.61	G_1a(n = 35)	7.2 ± 2.5	175.50 ± 107.75	>0.2	54/57
		G_1b(n = 22)	0.63 ± 0.53	158 ± 102.80		(94.7%)
	G_2 = n=52 / 130.9 ± 107.18	G_2a(n = 31)	6 ± 2.48	149.26 ± 111.19	>0.02	46/52
		G_2b(n = 21)	0.93 ± 0.73	86.84 ± 94.78		(89.3%)
	G_3 = n=56 / 103.2 ± 89.3	G_3a(n = 33)	6.5 ± 2.48	124.87 ± 89.4	>0.05	44/56
		G_3b(n = 23)	0.93 ± 0.73	82.79 ± 85.4		(78.6%)

* Totally 21 boosted children did not respond to the booster dose,of them 12 out of 99(12.1%) children were in subgroup with anti-HBs titer 2-10 IU/L and 9 out of 66(13.6%) children in subgroup<2 IU/L.

comparison can be made between the results of this study and finding of other studies carried out on the efficacy and immunogenicity of HB vaccine(with the same vaccine and schedules) on Sari {Saffar, *et al.*(12) seroprotective anti-HBs >10 IU/L and GMCs: 658 ± 378 in 94.4%] and Iranian children [Zamani, *et al.*(13) Pashapour, *et al.*(14) 94.8% and 100% respectively].

Two different studies by Xu, *et al.*(15) and Delage, *et al*.(3) showed that 20% and 16% of children (born from HBs-Ag positive mothers) lost their protective antibody levels after 5 years. In other studies (16,17), after 7 and 15 years follow-up, the seroprotective rate dropped to 57.2% and 50% respectively. In a recent study by Williams, *et al.*(18) after 9 years follow-up, only 37% of children lost their protective levels of anti-HBs antibody and the anti-HBs antibody levels were higher among those children who had received booster doses at the 5 years of follow-up.

Long- term immunity and memory of HB vaccination are evident from anamnestic rises of anti-HBs to HBs-Ag exposure despite the decline of anti- HBs with time. Two weeks after the booster dose, 88% of the subjects with antibody titers of 2-10 IU/L and 86.4% those with titers of <2 IU/L showed an anamnestic responses with anti- HBs titer beyond protective levels. Previous studies on the efficacy of HB vaccine in children, shows that protection against HBV infection lasts for at least 10-15 years despite a decline in anti-HBs concentration to less than protective levels (<10IU/L)(16,18). Immunologic memory networks of sensitized T and B cells to HBs-Ag during the primary vaccination series are thought to provide the capacity to mount a rapid antibody response to a HBV expose(8-11,19). The rapid anti-HBs responses observed in boosted children provided indirect evidence that immunologic memory was present.

On the basis of this study result, it can also be concluded that immunologic memory may decline in some children overtime, and that higher dosage of HBs-Ag may be needed in

Key Messages

- Immunological memory to hepatitis B may decline in some children over time and these children may require restimulation with higher doses of HBsAg.

order to re-stimulate immunologic memory cells. These findings are supported by various earlier studies(7-9,11,18).

According to the absence of HBs-Ag and anti-HBc in 21 nonresponders children to the booster, anamnestic responses to different dose of vaccine in 87.3% of children (144 of 165) without seroprotection, persistence of anti- HBs levels >10 IU/L in 57.8% of vaccines 10 year after routine infant HB vaccination and review of the other studies, we conclude that routine adminstration of booster doses, serologic testing for HBV infection, and serologic testing for anti- HBs status are not necessary in healthy immuno-compotent vaccinees at least within 10 years after primary HB vaccination at infancy. Further follow-up studies until adolescence and adulthood are needed to verify whether this immunologic memory will persist and protect against significant breakthrough infections when these children begin sexual activities and other high- risk behaviors that increase the risk of HBV infection.

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