Original article

Long-term protection of hepatitis B vaccination among Egyptian children

Background: *Hepatitis B Vaccination is the most effective way to prevent transmission of hepatitis B virus (HBV).*
Objective: *to detect the long-term immunogenicity of the vaccine in Egyptian children after five and ten years of vaccination.*
Methods: *Two hundreds healthy children were recruited. They were divided into two groups according to their age. Group A included 100 child, around 6 years old, vaccinated 5 years ago. Group `B` included 100 child, around 11 years old, vaccinated 10 years ago. Hepatitis B surface antibody (HBsAb) titre was tested, booster dose of the vaccine was given to children whose HBsAb was < 10 mIU/ml, then one and half month later, they were retested for HBsAb to evaluate the response.*
Results: *Both groups had a wide range of HBsAb (2-1000 mlU/ml), and there was a significant difference in the level of the two groups. Our data proves the decline of antibody titre with time. In group A, 19 children needed a booster dose, 14 of them were vaccinated, and 10 were retested after one and half month. The results showed that 9 (90%) responded by increased level of HBsAb, with six (66.6%) showing an adequate response. In group B, 52 children had antibody titre < 10, 48 of them were vaccinated and 34 were retested one and half months later. Two out of the 34 did not respond and 32 (94.2%) responded by an increase in the antibody titre. Of those who responded, 19 had adequate response (HBsAb ≥ 100) and 13 had hypo-response (HBsAb = 10 -100). Eighty percent (80%) of boys versus 51.7% of girls responded adequately.*
Conclusion: *Hepatitis B vaccine is an effective and successful way for preventing HBV infection. No need for booster dose at least for 5 years after vaccination .*

Keywords: HBV- HB vaccine- long term immunity

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INTRODUCTION

Immunization is the most effective way to prevent transmission of hepatitis B virus (HBV) and, hence, the development of acute and chronic hepatitis B. Sero-protection after vaccination, defined as HBsAb ≥ 10 mIU/mL, is achieved in over 95% of all vaccinees[1]. Ideally, the antibody response is determined within one to three months after the last dose of the vaccine in persons with risk factors for lack of response or those at high risk for exposure to blood or bodily fluids. Unfortunately, the antibody response is frequently tested years after completion of vaccination series, in which case a true non-response (an antibody level of <10 mIU/mL after appropriate vaccine series) must be distinguished from ″ waning″ antibody levels (those levels of antibody that are initially protective but that become undetectable over time)[2]. Few data are available concerning the long term immunogenicity of the pediatric doses of hepatitis B vaccines but the immunity persists for at least 5 years after the primary vaccination[3]. The aim of the work is to determine the level of HBsAb in children who were primarily vaccinated by the three doses of HBV vaccine after five and ten years of vaccination, also to test for anamnestic reaction, in those with declining level of antibody[4], to determine whether or not a booster dose is needed.

METHODS

Two hundreds normal children were recruited and their data were included. They were divided into two groups according to age (each group contains 100 children). Group A was around 6 years old, and included 53 males and 47 females. Group B was around 11 years old, and included 27 males and 73 females.

Selection criteria of cases: - Normal children - No hepatic or any other chronic illness - Normal growth and nutritional status - Received their three

doses of HBV vaccine at 2, 4 and 6 months according to the EPI in Egypt.

Hepatitis-B Vaccine, B.P (r-DNA), genetically engineered recombinant vaccine, VACSERA, manufactured by VACSERA under license of SHANTHA BIOTECHNIC. Pediatric dose = 0.5 ml IM, one ml contains 20µg HBsAg (purified). This dose is given to test for anamnestic reaction, in those with declining level of antibodies[4], and the level of antibodies was measured again after one and half month.

All children were subjected to:

1. History taking:
- Detailed history was taken from the parents of each child.
- Immunization history was included to be sure that every child had received the three doses of HBV vaccine.

2. Clinical examination:
- Anthropometric measures were taken for each child: weight, height and mid-arm circumference (MAC).

  Anthropometric measures of growth are among the most sensitive and commonly used indicators of child health[5]. Body weight is an indicator of general nutritional status[6]. Measurement of mid-arm circumference is a practical screening tool for assessment of malnutrition[7].

3. Laboratory Investigations :

  A blood sample (4ml) was collected from each child, centrifuged and serum was stored at -4°C until testing for liver function and viral markers were performed. Another blood sample (2ml) was also collected in special tubes for CBC.

  The following tests were performed for each child:

  - CBC: analyzed via Sysmex (KX-21)(Automated hematology analyzer) ─ Roche Diagnostics
  - ALT, AST and albumin were analyzed via COBAS INTEGRA 400 ─Roche Diagnostics.
  - Viral markers including: HBsAg and HBcAb (IgG and IgM) were tested by ELISA (enzyme-linked immuno-sorbent assay) via COBAS INTEGRA 400 ─ Roche- Diagnostics, to confirm that the child is free from viral hepatitis.
  - HBsAb titre was determined using chemi-luminence immunoassay intended for use on the ELECSYS 2010, immunoassay analyzer —Roche Diagnostics.

Statistical analysis:

The results were analyzed using the Statistical Package of Social Science (SPSS) computer software program, version 10.0 (Chicago, IL, USA). To study the relationship between two variables Pearson's and/or Spearman's correlation coefficients were calculated. All tests were two tailed and considered statistically significant at $p<0.05$.

RESULTS

No significant difference in weight, height, MAC or serum albumin were observed between males and females of group A. Females in group B were significantly taller than males, otherwise both groups were comparable (Table 1). When testing the serum of the children in both groups for HBsAb titre, there was significant difference between them ($p = 0.000$). While the median level of HBsAb in group A was 36.22, it was 9.8 in group B. Both groups had a wide range of HBsAb level (2 - 1000) mlU/ml). In group A: the median values of HBsAb for males was 37.43, and for females was 35.02 ($p= 0.462$), while in group B: the median values were 14.88 and 6.63 respectively ($p= 0.000$) (Table 2,3).

Children of group A having HBsAb titre ≥ 10 were 81 child (44 males i.e. 83% of males and 37 females i.e. 78% of females) and those having titre <10 were 19 child (9 males i.e. 17% of males and 10 females i.e. 22% of females) with no significant difference between boys and girls (Table 4,5).

In group B: 52 children had titre < 10 (6 males i.e. 22.2% of males and 46 females i.e. 63% of females). Forty eight children had titre of ≥ 10 (21males i.e. 77.8% of males and 27 females i.e. 37% of females). There is significant difference between boys and girls in group B ($p= 0.000$) (Table 4,5).

On comparing children of each sex in both groups who had HBsAb titre < 10 to those with titre ≥10 as regards their nutritional status and growth, the weight, height, MAC and albumin were not significantly different.

In group A, of the 19 children who needed an additional vaccination dose, 14 were vaccinated (5 children were lost to follow up). One and half month after vaccination another sample was taken from 10 children (4 children were lost to follow up). Out of these ten children, 9 (90%) responded by increased level of HBsAb and only one child did not respond. Six children (66.6%) of the nine showed an adequate response (i.e. HBsAb ≥ 100), 3 were hyporesponders (i.e. HBsAb ranges from 10-100). There was no significant difference in the response of group B ($p=0.814$). Fifty two children in this group had antibody titre < 10, forty eight only were vaccinated. After one and half month, 34 children were tested again for HBsAb (14 children were lost to follow up). Two out of thirty four children did not respond (5.8%) and 32 (94.2%)

responded by increase in the titre. Of those who responded, 19 had adequate response (HBsAb ≥ 100) and 13 had hypo-response. Therefore, 80% of boys and 51.7% of girls who were retested for HBsAb after vaccination, responded adequately. (Table 4,5).

Table 1. Description of groups A and B regarding growth and nutritional status.

Parameter	Group A			Group B		
	Males	Females	P	Males	Females	P
Weight (kg) Range Mean	(21─30) 27.24±3.31	(22–35) 27.76±3.12	0.423	(27–71) 36.44±8.59	(25–66) 39.78±10.27	0.136
Height (cm) Range Mean	(121–139) 129±4.44	(122–139) 128.59±4.48	0.362	(126–149) 139.4±5.08	(129–160) 143.16±7.92	0.007*
MAC (cm) Range Mean	(14–23) 17.45±1.8	(15–22) 17.95±1.62	0.147	(17–32) 21 ± 3.02	(14–32) 21.31±3.05	0.647
Albumin (g/dl) Range Mean	(3.8–5.6) 4.49±0.32	(3.8–5.9) 4.51±0.41	0.767	(3.9–5.5) 5.00 ±0.39	(4.1–6.0) 5.10±0.38	0.268

MAC = mid-arm circumference. *= significant

Table 2. Distribution of children in group A and B according to HBsAb titre (mIU/ mL).

HBsAb titre	Group A	Group B	P
< 10	N=19 (19%)	N=52 (52%)	0.000*
≥ 10	N=81 (81%)	N=48 (48%)	0.000*
Range Median	2-1000 36.22	2-1000 9.8	0.000*

N= number of children, *= significant value

Table 3. Comparison between males and females in each group regarding level of HBsAb in mIU/ mL.

Age group		Male	Female	P
Group A	Range Median	(4.2- 1000) 37.43	(2.2- 1000) 35.02	0.462
Group B	Range Median	(3.3- 1000) 14.88	(2.0- 99) 6.63	0.000*

*= significant value

Table 4. Level of HBsAb after a booster dose of vaccination in the study groups.

	GroupA (n=10)	Group B (n=34)	P
HBsAb after vaccination (mIU/ mL) Range Median	(8.9-1000) 579.65	(3.23-1000) 230.95	0.887

Table 5. Level of HBsAb after a booster dose of vaccination in males versus females.

Group	Sex (number)	HBsAb (mIU/ mL) after vaccination Mean (range)	p
Group A	M= 3	588.6 (65.42-1000)	0.648
	F =7	570.70 (8.9-1000)	
Group B	M =5	1000 (18-1000)	0.311
	F =29	144.6 (3.23-1000)	

M= males, F= females

DISCUSSION

HBV infection is preventable with safe and effective vaccine that has been available since 1982[8] . In 1992, Egypt started a program of universal immunization in infancy. The schedule which adopted by Egyptian Ministry of Health, was three doses of yeast-recombinant hepatitis B vaccine and administered to all infants at 2, 4 and 6 months of age to coincide with other compulsory vaccines[9] .

Two hundreds healthy children were recruited in the study. Before enrollment screening was done to ensure that they are free from HBV infection, liver enzymes, HBsAg, HBcIgM and HBcIgG were tested. The results of our study showed statistically significant difference in the HBsAb titre between children tested at 5 and at 10 years (p= 0.000) indicating a decline of the titre with time. This is in concordance with other investigators. Dentinger et al[10] conducted a study to evaluate the long-term protection of hepatitis B vaccination among children and found that HBsAb concentration dropped rapidly among all participants. They reported that five years after vaccination, 6% only of all children had HBsAb ≥10mIU/ml and 3% of them at 10 years retained the protective titre. In our study 81% and 48% retained immune protective levels of ≥ 10mIU/ ml at 5 and 10 years of age respectively. These differences may be attributed to the less endemic community of their study .

On comparing growth and nutritional status of children with HBsAb <10mIU/ ml, with those having titre ≥ 10 and also by assessing growth of children who didn't respond to the additional dose of vaccine, the results showed no statistically significant difference. On the contrary, Keating et al[11] reported that the greater the body mass index, the less the immune response, while Ingardia et al[12] showed that: advancing age, obesity and smoking in adults have negative influence on the efficacy of hepatitis B vaccination and explained that effect by the deposition of the vaccine in fat rather than in muscle resulting in higher failure rates. It was reported that many host and immunization factors affect the immune response, and duration of immunity. Host factors include age, weight, immunocompetence of the host, smoking habits, body mass index, genetics and socioeconomic state[13]. Wang et al[14] reported that universal hepatitis B vaccination program (UHBVP) was less effective in socio-economically disadvantaged area and the long-term efficacy and immunogenicity of vaccination were modified by host factors and factors associated with urbanization, but none of these factors show significance if correlated with levels of HBsAb .

When testing the presence of immunologic memory by an additional dose of the vaccine, no statistically significant difference between both groups: group A and B (p= 0.887). Children who didn't respond to testing dose of vaccination are either primary non-responders or may have lost their immunologic memory, this was in agreement with some investigators[14].

Puvacic et al[4] reported that after five years of vaccination, long term immunogenicity remained at 88.89% (81% in our study) and that vaccination against viral hepatitis B results in immunologic memory response among the vaccinated. Even after a decrease of HBsAb level following the third vaccine dose inoculation, a booster dose is not needed, nor recommended. Sjogren[15] stated that the distinction between true non-response (after adequate immunization) and waning HBsAb level is important. The latter is not uncommon in populations in areas of the world with low endemicity of HBV infection. Data from subjects with waning anti-HBs levels show that immunologic memory may still protect these individuals against acute HBV infection or may prevent chronic infection with HBV for < or =10 years after immunization. In a long study for 18 years, Yuen et al[16] stated that the long-term immunogenicity and efficacy of HBV vaccination remain to be defined. They concluded that a booster dose was not necessary at least up to 18 years after the primary vaccination. Dentinger et al[10] reported that up to age 16 years, booster doses of HB vaccine are not required. The results of a small study revealed that approximately one-fourth of successfully vaccinated infants with anti-HBs<10mIU/ml by early adolescence, fail to mount an anamnestic response to a booster dose of HB vaccine. Whether booster doses of the vaccine are needed for long term protection into late adolescence and adulthood need to be clarified. Wang et al[17] stated that routine booster vaccination may not be necessary before age 15 years, as the maintenance of HBsAg-specific memory confers protection against a clinical breakthrough infection, even in the absence of detectable antibodies. However, the possibility of a need for a booster dose exists, particularly when the child becomes adolescent. El-Sawy and Mohamed[18] evaluated the long-term immunogenicity and efficacy of HBV vaccination in children whose time lapse since last vaccination varied between 1 month and 5 years. They found that there were low initial HBsAb concentrations and it declined rapidly by time. They

recommended booster inoculations for all previously vaccinated children and a new vaccination schedule at 1, 2 and 9 months .

Lastly this study detected that males may retain HBsAb titres of higher values than females. There was statistically significant difference in levels of HBsAb between boys and girls and there was less number of boys with HBsAb < 10 mIU/ ml (22.2% of males versus 63% of females). Also boys who received the additional vaccine dose responded more adequately than girls (HBsAb ≥ 100mIU/mL). This agrees in part with some investigators. McMahon et al[19] stated that male sex were associated with persistence of higher anti-HBs levels at 15 years.

It is concluded that hepatitis B vaccine is an effective and successful way for preventing HBV infection. No need for booster dose at least for 5 years after vaccination .

RECOMMENDATION

Where no need for booster dose at least for 5 years after vaccination, persistence of immunogenicity of HBV vaccine is recommended to be studied for longer periods of time to detect the need of a booster dose in adolescence or early adulthood.

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