Immunogenicity of compulsory and booster doses of hepatitis B vaccine among children in Cairo, Egypt

Original Article

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ABSTRACT

Background: Although Egypt had adopted implementation of routine infant hepatitis B virus (HBV) vaccination in 1992, its effectiveness is not evaluated on a national scale. Assessment of early and long-term seroprotection after compulsory vaccination is an important measure for monitoring the success of the vaccination program.

Aim: The aim of this study was to assess HBV seroprotection and immune memory in children and adolescents who were vaccinated during infancy in Cairo Governorate.

Materials and Methods: The study was carried out in two phases. The first phase was a cross-sectional study carried out in five districts in Cairo Governorate, recruiting 819 children in the age range of 9 months to 16 years. All children had received full doses of the compulsory HBV vaccination. Serum samples were taken from each child and assessed for antibody against hepatitis B virus surface antigen (anti-HBs) titer; total antibodies against HBV core antigen, and HBV surface antigen. HBV DNA was investigated by real-time PCR for those who were HBV core antigen or HBV surface antigen positive. In the second phase, nonseroprotected children (anti-HBs <10 IU/l) received HBV booster dose. Anti-HBs titer was reassessed after 4 weeks to identify anamnestic response. Individuals showing antibody concentrations of less than 10 IU/l were then given an additional complete course of vaccination.

Results: Four out of 819 children had HBV breakthrough infection. The seroprotection rate was 60.7%, and was significantly higher among children aged less than 5 years compared to the older age groups and among boys compared to girls. Multivariate logistic analysis showed age as the only independent predictor of low anti-HBs titer. About 95% of nonseroprotected children developed anamnestic response postbooster. Anti-HBs geometric mean titer (GMT) increased significantly from pre-booster (13.8±16.9IU/L) compared to post-booster (307±6.0IU/L, P<0.001). Anti-HBs GMT was significantly higher among children with prebooster anti-HBs level ≥1 IU/l (424.9±4.4 IU/l) compared to children with undetectable level (178.3±8.3).

Conclusion: Despite waning of anti-HBs over time, long-term protection still exists. The high anamnestic response rate signifies the existence of immune memory and giving a booster dose is not recommended. However, we suggest that prolonged follow up and surveillance of vaccinees immunized at an early age should be continued.

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INTRODUCTION

Hepatitis B virus (HBV) infection is considered a global health problem. Worldwide, it is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC)[1]. The percent of patients with acute HBV Infection who progress to chronic infection varies according to age and immune status. About 90% of HBV infected infants at birth or in infancy become chronically infected and 30% to 50% of children are infected between 1 year and 5 years of age. By adulthood, the risk of acquiring chronic HBV infection is ~5%[2].

The available r-DNA HBsAg vaccine produced a robust immunity against HBV infectivity and will consequently prevent all the post HBV infection complications, including HCC[3]. Implementation of a universal HBV vaccination program has proven successful in eliminating infection and related complications[4]. Although long-term reduction of chronic HBV infection after HBV vaccination has been documented, the decreasing levels of HBV surface antigen (anti-HBs) over time can be alarming. Many controversies over the long-term persistence of post vaccination immunity to HBV and the need for booster doses of the vaccine were present[5]. HBV prevalence has decreased

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dramatically in Egyptian population since 1992 with the start of the mass vaccination program. The schedule adopted by the MOHP was a series of three doses of yeast-recombinant HBV vaccine administrated at 2, 4, and 6 months of age[6]. According to the National Demographic and Health Survey, 2014, the coverage of dose three of HBV vaccine was 94.9%[7].

The present study was carried out to assess the HBV seroprotection after HBV primary compulsory vaccination among children and adolescents in Cairo Governorate, Egypt, and to evaluate immune memory through early anamnestic response following receiving a booster dose of HBV vaccine.

MATERIALS AND METHODS

The current study is considered part of a national community-based multistage cluster sampling design conducted from July 2010 to June 2014 in six governorates representing all geographic areas of Egypt. It was carried out in two phases. The first phase was a cross-sectional study to assess the seroprotection rate of HBV vaccine. The second phase was one-group pretest post-test intervention design to assess the persistence of immunological memory toward HBV of nonseroprotected studied children by assessing their anamnestic response to booster dose.

Sample size

For the sampling process and selecting the clusters, probability proportional to size sampling was used. The sample frame for the present work was based on the 2006 population census. According to the population size in each governorate, the number of participating clusters was identified. First, implicit stratification by geographic location in each governorate, lists of shiakhas, medinas, cities, and villages were arranged geographically in a serpentine order. This stratification was carried out for urban and rural areas independently. A sampling interval was calculated and accordingly a random number was selected, using a table of random numbers. From these lists, areas such as villages or city blocks were selected. In each selected area, lists of maternal and child health center, kindergarten, and school facilities were identified and five facility clusters were randomly selected.

The current work presented the results of the project concerning Cairo governorate where 819 children were recruited from five cluster areas that were randomly chosen from urban areas (El-Mataria, El-Nozha, El-Sayeda Zeinab, El-Sahel, and El-Basatien). In each area, the targeted children were randomly selected from five facilities according to the age. There were 23 children from maternal and child health center, 32 children from kindergarten, 41 children from primary school, 36 children from preparatory school, and 32 children from secondary schools.

Data collection

A closed-ended questionnaire was designed and tested. For quality assurance, training sessions for supervisors, interviewers, and MOHP staff in each governorate were carried out. A barcode was used in order to prepare peel-off barcode sheets. Inclusion criteria included children aged from 9 months to 16 years who had received the full three compulsory doses of HBV vaccine during infancy. A face to face interview was carried out with the parents or caretakers of the children. Adolescents above 10 years of age were also interviewed after their verbal consent. The questionnaire covered data about the child's age, sex, date of birth, and other demographic and socioeconomic status (SES) variables. Data regarding child HBV vaccination were also collected and the available vaccination card was reviewed for the date and dose intervals of HBV vaccine. Child HBV vaccination for all studied children (819) was confirmed by certification for 420 children (51.3%) and by mother's recall for 399 (48.7%) children. The SES was determined according to Fahmy and El-Sherbiny[8]. It depended on the parents' educational profile, maternal working status, source of water, sewage disposal, electricity, and family income (some modification was done).

Children proved to have nonseroprotective levels of hepatitis B virus surface antigen (anti-HBs) titer (<10 IU/l) were given a booster dose of 10 μg of monovalant Euvax (Euvax-B®) (LG chemical Ltd., Seoul, Korea) HB vaccine intramuscularly in the deltoid muscle. A blood sample was withdrawn 4 weeks later from children for postbooster anti-HBs quantitative evaluation to assess their early anamnestic response. A nonresponder for the booster dose was further given two additional doses of HBV vaccine 1 and 6 months following the first dose and assessed for their HBV antibody level.

Blood sampling and laboratory analysis

A blood sample of about 3–5 ml was collected under aseptic conditions from each participant. Serum samples were aliquoted into two labeled sterile cryo tubes and stored at -20°C until being used. Quantitative detection of serum anti-HBs and qualitative determination of serum total hepatitis B virus core antibody (anti-HBc) and hepatitis B surface antigen (HBsAg) were assessed at the Microbiology Department, Faculty of Medicine (for Girls), Al Azhar University, Cairo, Egypt. This was conducted using commercially available enzyme-linked immunoassays (ELISA; DiaSorin, Italy Qiagen, Germany) and followed the manufacturer's guidelines. According to the international standards, anti-HBs of at least 10 IU/l was considered to be protective against HBV infection[9,10]. Vaccinated children who developed anti-HBs level of between 10 and 100 IU/l after the full vaccination dose were recognized as low responders and for those more than 100 IU/l as good responders[11]. Breakthrough infection was

defined as anti-HBc seropositivity in vaccinated participants who were not chronically infected[12]. Postbooster anti-HBs level was quantitatively assessed to evaluate early anamnestic response. An anamnestic response was defined by Zanetti *et al*.[10] as rise in anti-HBs to at least 10 U/ml. Individuals showing antibody concentrations of less than 10 IU/l were then offered an additional complete course of vaccination.

Samples that were repeatedly positive for either anti-HBc or HBsAg were subjected to quantification of HBV genome by real-time PCR of HBV genome using an automated system. Viral DNA was extracted from serum samples using QIAxtractor and VX kit as recommended by the manufacturer. PCR setup was automated via QIAgility (Qiagen, Germany). HBV real-time assays were performed using Artus HBV RG PCR Kit (Artus; GmbH, Hamburg, Germany) and the real-time PCR instrument, Rotor-Gene Q (Qiagen). The thermal profile was set based on the manufacturer's guideline. Detection limit of HBV DNA in the current study assay is 3.8 IU/l assessed by the WHO international standard (97/750)[13]. At least two negative controls, one nontemplate control and four standards (provided by the manufacturer) were added per run. Strict precautions were considered to avoid possible contamination. Only data that showed no false positive results in the negative controls and that were reproducible were used.

Data management

Data entry was carried out on Excel sheet and statistical analysis was conducted using the statistical package for the social sciences (SPSS) software program version 18.0 (SPSS Inc., Chicago, Illinois, USA). The geometric mean titer (GMT) was calculated to specify the central tendency of anti-HBs titers taking into consideration the skewed distribution of anti-HBs level. For calculating the GMT, children who had an undetectable anti-HBs titer were allocated a nominal serum anti-HBs titer value of 0.05 IU/l[14]. χ^2 was implemented for qualitative data which was presented by numbers and percentages. *t*-Test was used to compare between two means and one-way analysis of variance for comparing more than two means. Multivariate logistic analysis was done to predict the risk factors significantly associated with nonseroprotection and postbooster nonresponders. A *P* value of less than 0.05 was considered statistically significant.

Ethical considerations

The study protocol was approved by the ethics committee of Ministry of Health and Population, Medical Research Ethics Committee of National Research Centre and Ministry of Education. Signed written consents were also obtained from each guardian.

RESULTS

A total of 819 participants were enrolled in the current study. Four children (one boy and three girls) had breakthrough infection showed by positive total anti-HBc and HBV DNA (4/819, 0.49%). All of them developed anti-HBs (439, 210, 24, and 15 IU/l) and only one of them was HBsAg positive (Table 1).

Table 1: Demographic characteristics and laboratory findings among hepatitis B virus infected children, Cairo, Egypt, 2014

			Baseline HBV				Follow-up HBV markers (6 months)			
Serial number	Age (years)	Sex	Anti-HBs (IU/L)	HBsAg	Anti-HBc	HBV DNA level (IU/L)	HBsAg	Anti-HBc	Anti-HBs	HBV markers among family
11	9.3	Girl	439	-	+	48	-	-	994	All family negative
12	9	Girl	210	-	+	3920	-	-	306	All family negative
13	9.8	Girl	24	+	-	2440	Lost to follow-up			NA
14	3.3	Boy	15	-	+	209	Lost to follow-up			NA

Anti-HBc, hepatitis B virus core antigen; Anti-HBs, hepatitis B virus surface antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; NA, not available.
+ = Positive
- = Negative

Among the noninfected children, the percent of nonseroprotection was significantly higher with increasing age of children (14.5% among children <5 years ≤72% among children ≥15 years) (*P<0.01*). In the postbooster dose, the total GMT of anti-HBs was 307±6 IU/l. Out of the 150 children who had received the booster dose, nine (5%) failed to develop response and 82% showed good anamnestic response. Those who failed to generate anamnestic response (5%) were mostly in the age group 10–15 years, but the difference between age groups was statistically insignificant (*P>0.05*) (Table 2).

Table 2: Distribution of prebooster and postbooster hepatitis B virus surface antigen titers by age of the studied children, Cairo, Egypt, 2014

Age (years)	Total	Level of Anti HBs IU/L			
		<10 n (%)	10-99 n (%)	≥100 n (%)	Geometric mean titer ±SD
<5	283	41 (14.5)	95 (33.6)	147 (51.9)	77.6±7.0
5–<10	148	42 (28.4)	62 (41.9)	44 (29.7)	24.1±11.2**
10–<15	201	105 (52.3)	73 (36.3)	23 (11.4)	5.3±13.6**
≥15	183	132 (72.1)	41 (22.4)	10 (5.5)	1.8±15.6**
P value			<0.0005		
Total	815	320 (39.2)	271 (33.3)	224 (27.5)	13.8±16.9
Postbooster					
<5	22	0 (0)	2 (9.1)	20 (90.9)	512.6±2.7
5–<10	27	1 (3.7)	1 (3.7)	25 (92.6)	390.3±6.8
10–<15	56	3 (5.3)	10 (17.9)	43 (76.8)	262.0±7.5
≥15	45	3 (6.6)	7 (15.6)	35 (77.8)	255.6±5.5
P value			0.471		
Total	150	7 (4.7)	20 (13.3)	123 (82)	307.9±6.0

**P<0.01.

At the baseline, the risk of non sero-protection was significantly higher among girls compared to boys ($P < 0.05$, odds ratio 1.3). No significant difference in seroprotective rate as regards the SES was detected (*P>0.05*) (Table 3).

Table 3: Hepatitis B virus immunity at baseline in relation to sex and socioeconomic status of studied children, Cairo, Egypt, 2014

	Total (N=815)	Level of anti-HBs IU/L		Odds ratio (95% confidence interval)
		<10	≥10	
Sex				
Boys	431	155 (36.0)	276 (64.0)	Reference group
Girls	384	165 (43.0)	219 (57.0)	1.3 (1.1–1.7)*
P value		0.041		
Socioeconomic status[a]				
Very low	160	57 (35.6)	103 (64.4)	Reference group
Low	134	52 (38.8)	82 (61.2)	1.1 (0.7–1.8)
Middle	180	72 (40.0)	108 (60.0)	1.2 (0.8–1.9)
High	331	132 (39.9)	199 (60.1)	1.2 (0.8–1.9)
P value		0.814		

[a]Data available for socioeconomic status was among 805.
*P<0.05.

Following receiving the booster dose, boys showed higher risk of no response compared with girls, but this relation was statistically insignificant [*P*>0.05, relative risk (RR): 1.6]. The rate of anamnestic response was higher among children with middle and high SES, *P*>0.05. Children with undetectable level of prebooster anti-HBs were four times at risk of having nonseroprotective levels of anti-HBs after the booster dose than those with anti-HBs level more than or equal to 1 in the prebooster dose, but this relation was statistically insignificant (*P*=0.05, RR: 4.2). The GMT of anti-HBs postbooster dose was significantly higher among children with prebooster anti-HBs level more than or equal to 1 IU/l than those with undetectable levels (*P*=0.009) (Table 4).

Table 4: Early anamnestic response in relation to sex, socioeconomic status, and baseline level of hepatitis B virus surface antigen, Cairo, Egypt, 2014

		Level of post booster Anti HBs IU/L			
	Total	<10 n (%)	≥10 n (%)	Relative risk	Geometric mean titer (mean±SD)
Sex					
Boys	69	4 (5.8)	65 (94.2)	1.6 (0.3–7.4)	299.1±6.5
Girls	81	3 (3.7)	78 (96.3)	Reference group	312.0±5.7
P value		0.704			0.887
Ssocioeconomic status[a]					
Very low	26	2 (7.7)	24 (92.3)	3.0 (0.4–22.8)	308.2±7.7
Low	14	1 (7.1)	13 (92.9)	2.8 (0.2–33.3)	366.0±5.0
Middle	32	2 (6.3)	30 (93.8)	2.4 (0.2–24.0)	258.3±8.4
High	75	2 (2.7)	73 (97.3)	Reference group	322.3±5.0
P value		0.647			0.924
Level of pre booster Anti HBs IU/L					
<1	56	5 (8.9)	51 (91.1)	4.2 (0.84–20.9)	178.3±8.3
≥1	94	2 (2.1)	92 (97.9)	Reference group	424.9±4.4
P value		0.056			0.009**

[a]Data available for socioeconomic status was among 147.
**P<0.01.

Variables entered in the logistic analysis included the studied districts, sex, age groups, and SES. The nonsignificant variables were included in the logistic regression to confirm absence of any role of these variables even after adjustment. At baseline, age was the only significant predictor variable for having nonseroprotective level; the risk of nonseroprotective level was significantly higher among children aged 5–10 years and older compared with younger children with a P value of less than 0.01 (Table 5).

Table 5: Logistic regression to determine the predictors for risk of nonseroprotection in Cairo, Egypt, 2014

Variable	Nonseroprotection rate	Univariate odds ratio	Adjusted odds ratio (95% confidence interval)
Age (years)			
<5	41 (14.5)	Reference group	Reference group
5–	42 (28.4)	2.3 (1.4–3.8)**	2.4 (1.4–4)**
10–	105 (52.2)	6.5 (4.2–9.9)**	6.4 (4.0–10.1)**
≥15	132 (72.1)	15.3 (9.6–24.3)**	13.9 (8.5–22.6)**

**P<0.01.
The upper limit for age: 16 years old

On the other hand, after receiving the booster dose, none of the studied variables were found to be predictors for the development of anamnestic response.

Six children were nonresponders for the booster dose and were further given two additional doses of HBV vaccine 1 and 6 months following the first dose. The results showed that one child remained nonseroprotected (anti-HBs response <10 IU/l); three children developed poor anti-HBs response (10–99 IU/l), and two had good response (>100 IU/l) (data not tabulated).

DISCUSSION

HBV vaccines are considered one of the most effective ways to produce protective immunity against HBV infections. Nowadays, the widely used r-HBsAg vaccines are a viral subunit produced by yeast after being transfected with a plasmid that contains the S gene (codes for HBsAg). It is either present as a single preparation or in a combined form[3]. Global neonatal vaccination is considered the most effective strategy in the prevention of HBV infection[15].

The protection rate after a full course of primary vaccination series is more than 95% in children; however, the anti-HBs antibody titer shows rapid decline in the first year and then gradually after 1 year[16]. It is estimated that 13–60% of initial responders to HBV vaccine may lose detectable anti-HBs in subsequent years[17].

The current study revealed that 60.7 % of the fully vaccinated children had antibodies levels ≥ 10 IU/mL. Protective antibody level was detected in 85.5% of the children aged up to 5 year, 71.6% of children aged between 5 and 10 years, and 47.7% among older children. A previous Egyptian study reported that 81 and 48% of children aged up to 5 years and up to 10 years, respectively, had protective antibody level[18].

Similar results were reported in two studies in Italy among children aged less than 5 years (83.2%)[19,20]. Moreover, Jafarzadeh and Montazerifar (2006) found that 47.9% of children who received primary vaccination had protective antibody level after 10 years of vaccination[21].

A higher prevalence of seroprotection rate was reported by Gilca *et al*.[22], they found that 88.2, 86.4, and 76.7% of cases had a titer more than or equal to 10 IU/l at 5, 10 and 15 years postvaccination, respectively.

A lower sero protection rate was detected in 65% of children in a study in Iran one year after vaccination that declined significantly over time to 24% after 15 years of vaccination[23]. In Saudi Arabia, Al Faleh *et al*.[24] found that only 38% of the studied cohort at 16–18 years postvaccination retained protective anti-HBs. This wide variation among studies may be related to the difference in HBV endemicity, occurrence of natural infection, type of HBV vaccine used, dose, and vaccination.

As regards gender, most studies reported no gender differences among the sero-protective rate to HBV vaccine[25-27]. On the contrary, the present study found that the risk of non sero-protection was significantly higher in girls compared to boys ($p < 0.05$, OR= 1.3) at baseline, while there was no significant difference following the booster dose.

Some studies associate the SES with vaccine response[27]. However, in the present study it did not significantly influence the seroprotection rate. Similar results were reported by Zanetti *et al*.[20] and Yazdanpanah *et al*.[26].

To evaluate the long-term protection of HBV vaccine, four methods were usually used: the anamnestic response following administration of a booster dose, in-vitro B cell and T-cell activity evaluation, infection rate in vaccinated populations, and seroepidemiological studies[28]. In the present study, 4/819 children (0.49%) developed HBV breakthrough infection, and one of them was positive for HBsAg. Similarly, Hashemi *et al*.[15] and Salama *et al*.[25] showed that the prevalence of breakthrough HBV infection was about 0.5% among HBV-vaccinated children. While higher percentage (1%) was reported in Taiwan among children less than 18 years[29], in Italy, none of the HBV-vaccinated subjects have been infected with HBV[30].

Following HBV vaccination, persistent memory lasting for 5 years or more is recognized from the large and fast increases in anti-HBs level after giving a booster vaccination, even in those who have shown undetectable anti-HBs as measured by the available commercial kits. In vitro studies showed that the number of memory B lymphocyte cells which is able to induce anti-HBs does not decrease with the decline of anti-HBs level[31]. In the present study, while the total anti-HBs GMT was 13.8±16.9 IU/l at baseline, it increased to 307.9±6.0 IU/l postbooster. This vigorous response of the immune system to the booster dose suggested that the immunologic memory was active. These results were comparable with the findings of Yazdanpanah *et al*.[26], who found that the mean postbooster anti-HBs increased significantly to about 67 times and Teoharov *et al*.[32], who showed that the mean anti-HBs titer increased significantly from 63.57 to 337.38 IU/l after the booster dose. Several studies conducted up to 20 years following the original vaccination showed that long-term protection against HBV infection depends on the persistence of a strong immunological memory. No booster dose is needed in immune competent individuals who have completed their full vaccination[28].

The present study suggested that baseline undetectable level of antibodies was a risk factor of developing poor postbooster response (RR: 4.2, P=0.05). Children who did not respond to HBV booster vaccination might be either true nonresponders or initial responders, who had lost the immunological memory to HBV vaccine[33].

In the current study, multivariate logistic regression showed that age was the only significant predictor variable

for nonseroprotective anti-HBs level. The risk increased significantly with the increase in age as it was higher among children 10–15 and more than 15 years (Adjusted Odds Ratio (AOR) =6.4 and 13.9, respectively). This is in accordance with a previous Egyptian study, where they found that in children less than 4 years, 65% had protective anti-HBs level that decreased to 45% at age more than 20 years[6].

LIMITATIONS

There were some concerns related to the current study that need to be mentioned. First, the vaccination history of the included children was obtained from their parents especially among adolescents. However, it is reasonable to assume that there was minimal influence of parents' recall bias as there was no significant difference in the distributions of pre- and post-booster anti-HBs titers between participants with and without an official vaccination record. Consequently, our findings are internally valid to demonstrate the response to a booster dose of HB vaccine in anti-HBs seronegative children and adolescents who were vaccinated 1–16 years before.

Secondly, due to the lack of the initial response to primary HB vaccination during infancy, participants who did not respond to booster vaccination might be true non-responders or initial responders but had lost their immunological memory to HB vaccine.

CONCLUSION AND RECOMMENDATIONS

The current study provided additional evidence that HBV vaccine can offer long-term immunity supported by the findings of our study in which vaccinated individuals who lost protective level of antibody showed rapid anamnestic response when vaccinated, suggesting the presence of fully effective immunologic memory. Therefore, although HBV breakthrough infections might occur in vaccinated individuals, yet those infections do not seem to be a substantial threat to the whole population. However, we suggest that prolonged follow-up and surveillance of vaccinees immunized at an early age should be continued.

CONFLICT OF INTEREST

There are no conflicts of interest.

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