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ORIGINAL ARTICLE

Observational Study

Effectiveness of hepatitis B virus vaccination program in Egypt: Multicenter national project

Iman I Salama, Samia M Sami, Zeinab Nabil Ahmed Said, Manal H El-Sayed, Lobna A El Etreby, Thanaa M Rabah, Dalia M Elmosalami, Amany T Abdel Hamid, Somaia I Salama, Aida M Abdel Mohsen, Hanaa M Emam, Safaa M Elserougy, Amal I Hassanain, Naglaa F Abd Alhalim, Fatma A Shaaban, Samia A Hemeda, Nihad A Ibrahim, Ammal M Metwally

Iman I Salama, Lobna A El Etreby, Thanaa M Rabah, Dalia M Elmosalami, Amany T Abdel Hamid, Somaia I Salama, Aida M Abdel Mohsen, Samia A Hemeda, Nihad A Ibrahim, Ammal M Metwally, Community Medicine Research Department, National Research Center, Cairo 12311, Egypt

Samia M Sami, Amal I Hassanain, Fatma A Shaaban, Child Health Department, National Research Center, Cairo 12311, Egypt

Zeinab Nabil Ahmed Said, Naglaa F Abd Alhalim, Microbiology and Immunology Department, Faculty of Medicine (for girls), Al-Azhar University, Cairo 11754, Egypt

Manal H El-Sayed, Pediatrics Department, Faculty of Medicine, Ain-Shams University, Cairo 11566, Egypt

Hanaa M Emam, Dermatology and Venereology Department, National Research Center, Cairo 12311, Egypt

Safaa M Elserougy, Environmental and Occupational Medicine Department, National Research Center, Cairo 12311, Egypt

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Correspondence to: Zeinab Nabil Ahmed Said, Professor in Microbiology and Immunology Department, Faculty of Medicine (for girls), Al-Azhar University, Yousef Abbas Str, Nasr City, Cairo 11754, Egypt. znabil58@yahoo.com
Telephone: +2-01-6602418

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Abstract

AIM: To assess the effectiveness of hepatitis B virus (HBV) vaccination program among fully vaccinated children.

METHODS: A national community based cross-sectional study was carried out in 6 governorates representing Egypt. A total of 3600 children aged from 9 mo to 16 years who were fully vaccinated with HBV vaccine during infancy were recruited. Face to face interviews were carried out and sera were evaluated for hepatitis B surface antigen (HBsAg), anti-HBV core antibodies (total) and quantitative detection of hepatitis B surface antibody using enzyme linked immunoassays techniques. Samples positive to HBsAg/anti-HBV core antibodies were subjected to quantitative HBV-DNA detection by real time polymerase chain reaction with 3.8 IU/L detection limit.

RESULTS: Sero-protection was detected among 2059 children (57.2%) with geometric mean titers 75.4 ± 3.6 IU/L compared to 3.1 ± 2.1 IU/L among non-seroprotected children. Multivariate logistic analysis revealed that older age and female gender were the significant predicting variables for having non sero-protective level, with adjusted odds ratio 3.3, 9.1 and 14.2 among children aged 5 to < 10, 10 to < 15 and ⩾ 15 years respectively compared to those < 5 years and 1.1 among girls compared to boys with P < 0.01. HBsAg was positive in 0.11% and breakthrough infection was 0.36% and 0.39% depending on positivity of anti-HBc and DNA detection respectively. The prevalence of HBV infection was significantly higher among children aged ⩾ 7 years (0.59%) compared to 0.07% among younger children with odds ratio equal to 8.4 (95%CI: 1.1-64.2) and P < 0.01.The prevalence was higher among girls (0.48%) than boys (0.29%) with P > 0.05.

CONCLUSION: The Egyptian compulsory HBV vaccination program provides adequate protection. Occult HBV infection exists among apparently healthy vaccinated children. Adherence to infection control measures is mandatory.

Key words: Hepatitis B virus; Immunization; Sero-protection; Breakthrough infection; Children

Core tip: To assess the effectiveness of hepatitis B virus (HBV) vaccination program, a national community based survey was carried out in six governorates representing Egypt on 3600 children aged 9 mo to 16 years (received 3 doses HBV vaccine during infancy). Anti-hepatitis B surface (anti-HBs) titer, anti-HBc and HBs antigen were assessed. HBV DNA detection was done for suspected cases. Prevalence of HBV sero-protection, breakthrough HBV infection, and chronic carrier were 57.2% 0.39% and 0.11% respectively. Multivariate analysis revealed that older age and girls were the significant predictor variables for non sero-protection. Despite waning of anti-HBs over time, HBV vaccination program is effective in Egypt.

Salama II, Sami SM, Said ZNA, El-Sayed MH, El Etreby LA, Rabah TM, Elmosalami DM, Abdel Hamid AT, Salama SI, Abdel Mohsen AM, Emam HM, Elserougy SM, Hassanain AI, Abd Alhalim NF, Shaaban FA, Hemeda SA, Ibrahim NA, Metwally AM. Effectiveness of hepatitis B virus vaccination program in Egypt: Multicenter national project. *World J Hepatol* 2015; 7(22): 2418-2426 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i22/2418.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i22.2418

INTRODUCTION

The hepatitis B vaccine is the mainstay of hepatitis B prevention. In 1992, the World Health Organization (WHO) recommended the implementation of universal childhood vaccination worldwide and by the end of 2012, 181 countries had adopted this measure[1]. The complete vaccination series induces protective antibody levels in more than 95% of infants, children and young adults[2]. Persistence of hepatitis B surface antibody (anti-HBs) and thus the protection against infection and carrier state depends on the peak anti-HBs concentration achieved after primary vaccination. However, anti-HBs decay exponentially with length of time since vaccination[3].

Hepatitis B virus (HBV) is considered moderately endemic in Egypt with 4% of the population having evidence of chronic HBV infection[4]. A key goal of HBV immunization program is to reduce the prevalence of hepatitis B surface antigen (HBsAg) among cohorts born since the program implementation. A practical means to determine the long term protection provided by HB vaccine is to estimate the incidence of break-through infection (positive anti-HBc) as well as chronic carrier state (positive HBsAg) among previously vaccinated individuals[5]. In Egypt, the HBV vaccination program was applied in 1992 with a schedule of 2, 4 and 6 mo of age, while routine screening of pregnant women for HBsAg was not applied[6]. There have been no sero-surveys among children born since the introduction of the vaccine in Egypt; however, the finding of acute disease transmission in these cohorts indicates there is ongoing HBV transmission and more in-depth evaluation of the immunization program is needed[7]. Although several studies have been done in Egypt to measure the effectiveness of HBV vaccination, yet these studies were done on a relatively small scale and in certain areas of Egypt, from which arose the need for a large national study including numerous areas to be representative of all Egypt to give a clear picture of the situation. The present study aimed to assess the effectiveness of compulsory HBV vaccination on national basis and to determine health disparities and risk factors associated with non-seroprotective levels among Egyptian children aged from 9 mo to 16 years. It also aimed to assess the prevalence of breakthrough HBV infection and carrier state among the studied children.

MATERIALS AND METHODS

This is a community based national project using a multi-stage cluster sampling technique. It was carried out in 6 representative governorates in Egypt from July 2010 to June 2013. These governorates included the Capital (Cairo), two in Lower Egypt (Gharbeya and Dakahleya), two in Upper Egypt (Assiut and Beni-Suef), and one Frontier (Red Sea). The age of the participating children ranged from 9 mo to 16 years. They were fully vaccinated by the 3 compulsory HBV vaccine doses during infancy. Probability proportional to size sampling was used for the sampling process and selection of clusters. The design effect used was considered equal to 2. From previous Egyptian studies carried out on small scales, the prevalence of sero-protection level among fully vaccinated children ranged from 95% among infants aged 9 mo to 35% among children aged 11 years[8,9]. We assumed that it might be about 25% among older children. The sample size allowed an estimated precision (margin of error) of 5% and 95% confidence level of being within 5% of the true value, with response rate estimated to be 90%. The sample frame for the survey was based on the most recent population census of 2006. List of cities and villages were arranged in serpentine order after the implicit stratification by geographic location independently for urban and rural areas of each governorate. After calculating a sampling interval, a random number was selected from the table of random numbers. Out of this list, the number of participating areas in each governorate was identified according to its population size. So, we identified 5 urban areas from Cairo governorate, 4 areas from Gharbeya governorate (1 city and 3 villages), 5 areas from Dakahleya governorate (2 cities and 3 villages), 3 areas from Assuit governorate (1 city and 2 villages), 2 areas from Beni-Suef governorate (1 city and 1 village), and one city area from Red Sea governorate. In each selected area, one maternal and child health center in urban areas or health unit (in rural areas) was randomly selected. Then according to the age of the targeted children within the catchment areas, one kindergarten and 3 schools (primary, preparatory and secondary) were randomly selected.

The study was reviewed and approved by the ethical committees of Ministry of Health, National Research Center and Ministry of Education. All the legal guardians of the study participants were provided informed written consent prior to study enrollment. In addition, children aged above 10 years were enrolled after getting their verbal assent. Through face to face interview, children's personal data, demographic and socioeconomic variables and current and past medical history were collected through a designed pre-tested close-ended questionnaire. Children's HBV vaccination was confirmed by taking a full detailed vaccination history from parents as well as revising the vaccination cards available with their parents or in the child's school file. For quality assurance, Ministry of Health staff, supervisors and interviewers attended several training sessions before the study implementation in each governorate. To ensure tracing blood samples and linking laboratory results with other survey, data peel-off barcode sheets were used. To assess the nutritional status, anthropometric measurements including height and weight were also taken.

Laboratory analysis

A blood sample (3-5 mL) was withdrawn from each child aseptically and serum was aliquoted into two labeled sterile cryotubes and stored at -20 ℃. Detection of HBV markers was performed in the Virology lab - Microbiology and Immunology Department-Faculty of Medicine (for girls), Al-Azhar University, Cairo. Serum total anti-HBc, HBsAg and anti-HBs were assessed using commercially available enzyme linked immunoassays (Dia Sorin-Italy) according to the manufacturer instructions. Anti-HBs $\geqslant$ 10 IU/L, was considered to be protective against HBV infection[10].

Repeatedly positive samples for either anti-HBc or HBsAg were subjected to quantification of HBV DNA by Real-time PCR using automated system. Viral DNA was extracted from serum samples using QIAxtractor®, and VX kit as recommended by the manufacturer (QIAGEN, Germany). PCR setup was automated *via* QIAgility (QIAGEN, Germany). HBV real-time assays were performed in combination of Artus HBV RG PCR Kit (Artus™ GmbH, Hamburg, Germany) and the Real time PCR instrument, Rotor-Gene Q (QIAGEN, Germany). Thermal profile was set according to manufacturer's guideline. Detection limit of HBV DNA in the current study assay was 3.8 IU/L assessed by the WHO international standard (97/750)[11]. At least two negative controls, one non template control and four standards (provided by the manufacturer) were added per run. Strict precautions were taken to avoid possible contamination. Only reproducible data that revealed no false positive results in the negative controls was used.

Statistical analysis

Data entry and statistical analysis were done using SPSS software program version 18.0. Anti-HBs geometric mean titer (GMT) was calculated to estimate the central tendency of anti-HBs level in consideration to its skewed distribution. Children who had an undetectable anti-HBs titer were assigned a titer value of 0.05 IU/L[12]. For qualitative data that presented by numbers and percentages, χ^2 was done. For comparison between two means, *t*-test was done while one way ANOVA was used for more than two means. Multivariate logistic analysis was carried out to predict risk factors significantly associated with non-serprotection. $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered statistically highly significant.

The statistical methods of this study were performed by first author Iman I Salama, professor of public Health and Preventive Medicine at National Research Center and she is a Bio-statistical reviewer in Medical

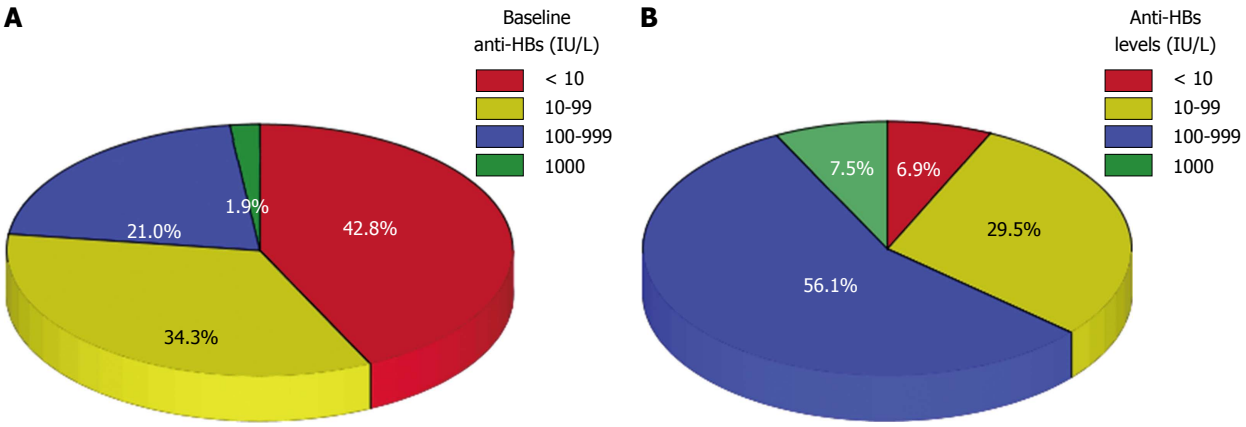

Figure 1 Prevalence of hepatitis B surface antibody levels among all the studied children (A) and among children aged ⩽ 1 year (B). Anti-HBs: Hepatitis B surface antibody.

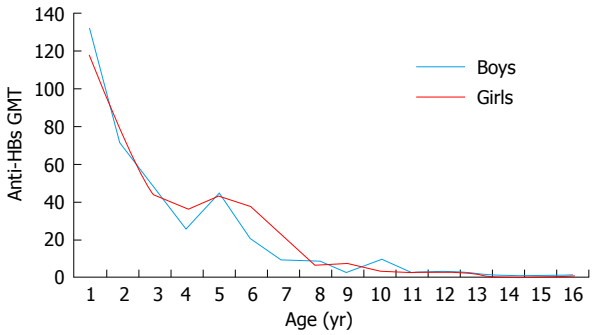

Figure 2 Hepatitis B surface antibody geometric mean titer among the studied children according to age and gender. Anti-HBs: Hepatitis B surface antibody; GMT: Geometric mean titer.

Research J.

RESULTS

Short and long term HBV sero-protection among the fully vaccinated children

The present study was carried out on 3600 children and adolescents from six Egyptian governorates, 1909 (53%) from urban and 1691 (47%) from rural areas. There were 1743 (48.4%) boys and 1857 (51.6%) girls with a mean age of 9.1 ± 5.5 years. Overall sero-protection rate among the studied children was 57.2% (95%CI: 55.6% to 58.8%). Figure 1A shows anti-HBs level among all the studied children, while Figure 1B shows anti-HBs level among children ⩽ 1 year representing the primary response to HBV vaccine 3-6 mo after receiving the 3 compulsory doses. The GMT of the anti-HBs was significantly lower among children ⩾ 5 years compared to younger, among girls than boys, in lower socioeconomic status, in Assuit and Red Sea compared to other governorates and in rural compared to urban areas ($P < 0.05$), Table 1. The table also showed that the distribution of anti-HBs levels was significantly different as regards all studied socio-demographic characteristics, $P < 0.05$. Antibody concentrations decline more quickly during the first 4 years after vaccination than they do later on (Figure 2). There was no significant difference between boys and girls in the different governorates except in Gharbeya and Assuit, where males had significantly higher sero-protection rate (66.4% and 59.3%) compared to females (49.9% and 48.4%) respectively, $P < 0.01$.

Children with history of hospital admission, abscess incision, surgical operation, regular medical injection, blood transfusion and rheumatic fever had significantly higher non sero-protective rates compared to children with no such history ($P < 0.001$). Children with Height for age percentile (HAP) and Weight for age percentile (WAP) < 5th percentiles had significantly lower non sero-protection rate compared to normal children with odds ratio 1.3 for both, $P < 0.05$ (Table 2). Multivariate logistic analysis revealed that older age and female gender were the significant predicting variables for having non sero-protective level, with adjusted odds ratio 3.3, 9.1 and 14.2 among children aged 5 to < 10, 10 to < 15 and ⩾ 15 years respectively compared to those < 5 years and 1.1 among girls compared to boys, $P < 0.01$ (Table 3).

HBV breakthrough infection among the fully vaccinated children

Fourteen children (9 females and 5 males) showed HBV breakthrough infection; 14/3600 (0.39%) were positive for HBV-DNA, 13/3600 (0.36%) had positive anti-HBc and 4/3600 (0.11%) had positive HBsAg. HBV infection was not found among children aged < 3 years (Table 4). The prevalence of HBV infection was significantly higher among children aged ⩾ 7 years (0.59%) compared to 0.07% among younger children, odds ratio 8.4 (95%CI: 1.1-64.2), $P < 0.01$. The prevalence was higher among girls (9/1857; 0.48%) than boys (5/1743; 0.29%), $P > 0.05$. Table 5 shows the demographic characteristics and HBV markers of breakthrough infection among the studied children. Five out of thirteen children with positive anti-HBc had anit-HBs above 100 IU/L and 11 out of 14 children presented HBV DNA ⩾ 200 IU/L. Three children, positive for HBsAg, had a family history

Table 1 Prevalence of hepatitis B virus anti-hepatitis B surface in relation to some socio-demographic characteristics among 3586[1] studied children *n* (%)

	Total	Anti-HBs IU/L			
	n = 3586	Mean GMT	< 10	10-99	⩾ 100
			n = 1535	*n* = 1229	*n* = 822
Gender					
Boys	1738	220.4 ± 13.4	681 (39.2)	643 (37.0)	414 (23.8)
Girls	1848	161.7 ± 11.2	854 (46.2)	586 (31.7)	408 (22.1)
P value		< 0.05	< 0.001		
Age in years					
< 5 yr	1114	64.0 ± 8.0	152 (13.6)	435 (39.0)	527 (47.3)
5 to < 10 yr	625	18.7 ± 13.8	206 (33.0)	240 (38.4)	179 (28.6)
10 to < 15 yr	1026	3.0 ± 15.0	606 (59.1)	346 (33.7)	74 (7.2)
⩾ 15 yr	821	1.5 ± 17.0	571 (69.5)	208 (25.3)	42 (5.1)
P value		< 0.001	< 0.001		
Socio-economic status[2]					
Very low	992	22.3 ± 6.3	465 (46.9)	345 (34.8)	182 (18.3)
Low	650	24.4 ± 6.2	301 (46.3)	220 (33.8)	129 (19.8)
Middle	934	37.2 ± 6.4	349 (37.4)	324 (34.7)	261 (27.9)
High	916	33.3 ± 6.5	376 (41.0)	308 (33.6)	232 (25.3)
P value		< 0.001	< 0.001		
Governorate					
Cairo	815	32.3 ± 6.8	320 (39.3)	271 (33.3)	224 (27.5)
Dakahleya	898	29.6 ± 5.8	380 (42.3)	328(36.5)	190 (21.2)
Gharbeya	762	29.8 ± 6.5	328 (43.0)	256 (33.6)	178 (23.4)
Beni-Suef	358	35.3 ± 6.2	155 (43.3)	126 (35.2)	77 (21.5)
Assuit	564	22.3 ± 6.7	264 (46.8)	191 (33.9)	109 (19.3)
Red Sea	189	21.2 ± 7.0	88 (46.6)	57 (30.2)	44 (23.3)
P value		< 0.01	< 0.05		
Residence					
Urban	1902	11.0 ± 18.4	784 (41.2)	649 (34.1)	469 (24.7)
Rural	1684	7.3 ± 20.8	751 (44.6)	580 (34.4)	353 (21.0)
P value		< 0.001	0.021		

[1]Breakthrough infected children were excluded; [2]Data for Socio-economic status was fulfilled for only 3492 children. Anti-HBs: Hepatitis B surface antibodies; GMT: Geometric mean titer.

Table 2 Hepatitis B virus immunity in relation to the child's medical history *n* (%)

Risk factors	Total	Level of anti-HBs		Odds ratio (95%CI)
		< 10 IU/L	⩾ 10 IU/L	
		n = 1535	*n* = 2051	
Hospital admission				
Yes	1196 (33.4)	558 (46.4)	638 (53.6)	1.3 (1.0-1.5)[b]
No	2390 (66.6)	977 (40.9)	1413 (59.1)	®
Open abscess				
Yes	280 (7.8)	150 (53.5)	130 (46.5)	1.6 (1.2-2.0)[b]
No	3306 (92.2)	1385 (41.6)	1921 (58.4)	®
Surgical operation				
Yes	683 (19.0)	339 (49.6)	344 (50.4)	1.4 (1.2-1.7)[b]
No	2903 (81.0)	1196 (41.2)	1707 (58.8)	®
Regular medical injection				
Yes	112 (3.1)	59 (52.7)	53 (47.3)	1.5 (1.0-2.2)[a]
No	3474 (96.9)	1476 (42.5)	1998 (57.5)	®
Blood transfusion				
Yes	90 (2.5)	48 (53.3)	42 (46.7)	1.5 (1.0-2.4)[a]
No	3496 (97.5)	1487 (42.5)	2009 (57.5)	®
Rheumatic fever				
Yes	140 (3.9)	74 (52.9)	66 (47.1)	1.5 (1.1-2.1)[a]
No	3446 (96.1)	1461 (42.3)	1985 (57.6)	®
HAP (total = 3256)				
< 5th percentile	569 (17.5)	218 (38.3)	351 (61.7)	1.3 (1.1-1.6)[b]
⩾ 5th percentile	2687 (82.5)	1208 (45.0)	1479 (55.0)	®
WAP (total = 3317)				
< 5th percentile	240 (7.2)	89 (37.1)	151 (62.9)	1.3 (1.0-1.7)[a]
⩾ 5th percentile	3077 (92.8)	1341 (43.6)	1736 (56.4)	®

[a]$P < 0.05$, [b]$P < 0.01$. ®: Reference group; Anti-HBs: Hepatitis B surface antibodies.

Table 3 Univariate and multivariate logistic analysis to determine predictors for risk of non sero-protection *n* (%)

Variable	Non-seroprotection rate	Crude odds ratio (95%CI)	AOR (95%CI)
Age in years			
< 5	152 (13.6)	®	®
5 to < 10	256 (34.7)	3.4 (2.7-4.2)[b]	3.3 (2.5-4.2)[b]
10 to < 15	606 (59.1)	9.1 (7.4-11.3)[b]	9.1 (7.3-11.2)[b]
⩾ 15	571 (69.5)	14.5 (11.5-18.1)[b]	14.2 (11.3-17.9)[b]
Gender			
Boys	681 (39.2)	®	®
Girls	854 (46.2)	1.3 (1.2-1.5)[b]	1.1 (1.0-1.4)[b]

Variables entered in model: Age group, gender, socio-economic levels and history of rheumatic fever, diabetes mellitus, surgical operation, regular medical injection, blood transfusion, hospital admission, open abscess. [b]$P < 0.01$. AOR: Adjusted odds ratio; ®: Reference group.

Table 4 Hepatitis B virus breakthrough infection among the studied children in different age groups

Age group (yr)	Total	Sero-protection rate Anti-HBs ⩾ 10 IU/L		HBV infection markers		
		n (%)	95%CI	Anti-HBc %	HBsAg %	HBV-DNA %
< 3	702	633 (90.2)	88.0-92.4	0 (0.0)	0 (0.0)	0 (0.0)
3-	705	557 (79.0)	76.0-82.0	1 (0.14)	0 (0.0)	1 (0.14)
7-	493	282 (57.2)	52.9-61.6	4 (0.81)	2 (0.41)	5 (1.00)
11-	875	335 (38.3)	35.1-41.5	4 (0.46)	1 (0.11)	4 (0.46)
⩾ 15	825	252 (30.5)	27.4-33.6	4 (0.48)	1 (0.12)	4 (0.48)
Total	3600	2059 (57.2)	55.6-58.8	13 (0.36)	4 (0.11)	14 (0.39)

HBV: Hepatitis B virus; Anti-HBs: Hepatitis B surface antibodies; Anti-HBc: Hepatitis B core antibody; HBsAg: Hepatitis B surface antigen.

Table 5 Demographic characteristics and hepatitis B virus markers of breakthrough infection among the studied children

N	Age (yr)	Gender	Residence	Governorate	Base line HBV markers			
					Anti-HBs (IU/mL)	HBsAg	Anti-HBc	HBV DNA
1	10	Boy	Urban	Beni-Suef	0	+	+	10000
2	15.8	Girl	Urban		37	-	+	1280
3	16.8	Girl	Urban		3	+	+	866
4	11	Girl	Rural		0	-	+	24100
5	11	Girl	Urban	Assuit	992	-	+	953
6	10	Boy	Urban		404	-	+	2850
7	11.8	Girl	Rural	Dakahleya	4	-	+	4170
8	15.8	Girl	Rural		3	-	+	455
9	16	Boy	Rural		559	-	+	781
10	12	Boy	Rural		0	+	+	26
11	9.3	Girl	Urban	Cairo	439	-	+	48
12	9	Girl	Urban		210	-	+	3920
13	9.8	Girl	Urban		24	+	-	2440
14	3.3	Boy	Urban		15	-	+	209

HBV: Hepatitis B virus; Anti-HBs: Hepatitis B surface antibodies; Anti-HBc: Hepatitis B core antibody; HBsAg: Hepatitis B surface antigen.

of positive HBV infection where two mothers and one father were positive. None of the studied children had elevated liver enzymes or was hepatitis B e antigen (HBeAg) positive. Follow up after one year showed that six children only retained anti-HBc positivity, three of them were also positive for HBsAg while the other three children had isolated anti-HBc indicating occult HBV infection.

DISCUSSION

HBV vaccine is the first vaccine against a major human cancer[13]. The present study has the greatest sample size to study the effectiveness of HBV vaccine among Egyptian children (n = 3600). Subjects were relatively homogenous: All had received the same recombinant HBV vaccine with the same schedule during infancy. The

overall sero-protection rate among the studied children was 57.2%, which decreased significantly from 90.2% among children < 3 years to 30.5% among children ⩾ 15 years. Similarly, other Egyptian studies carried out on smaller sample sizes[8,14], reported 54% and 39.7% sero-protection rate among vaccinated children aged 6-12 years respectively. In Slovakia, 10-11 years after primary vaccination, 48.4% children had persisting sero-protection anti-HBs[15]. Similar to the present study, Afifi *et al*[9], found that the mean anti-HBs level decreased significantly with increasing age, being 426.8, 79.2 and 32.1 IU/L at 9 mo, 6 years and 11 years post vaccination respectively. On the other hand, in Italy higher sero-protection rate (64% of children aged over 10 years) was reported by Zanetti *et al*[16]. Similar to our results, anti-HBs concentrations decline more quickly during the first few years after vaccination than they do later on which was mentioned in a Turkish study on children aged 2 to 12-years[17]. Using multivariate logistic analysis, the current study showed that age and gender were the only two risk factors for non-seroprotection among the studied children. The risk of non-sero-protection was significantly slightly higher among girls than boys with odds ratio of 1.1. However, other studies in the United States and Iran found no gender difference[18,19].

Some investigators correlate the socioeconomic status (SES) with vaccine response[20]. In this study, the percentage of non sero-protection was significantly high among very low and low SES when compared to non sero-protection among middle SES, with odds ratio 1.5 and 1.4 respectively. Wang *et al*[21] reported that HBV vaccination program was less effective in socio-economically disadvantaged area and was affected by factors associated with urbanization[22]. However, Zanetti *et al*[16] showed that socioeconomic factors such as residential location, family size, fathers' level of education did not affect the level of protective antibody concentrations.

In the current study, it was found that children with HAP and WAP < 5th percentiles had significantly lower non sero-protection rate compared to normal children with odds ratio 1.3 for both. These results are in accordance to Karimi *et al*[23]. However, another Egyptian study done on 200 children showed no difference in sero-protection rates as regards children's growth and nutritional status[22].

The fall in the antibody titer does not necessary indicate loss of immunity. Protection against clinically important disease outlasts the presence of detectable antibodies[24]. No HBV infection was detected among children aged < 3 years, indicating absence of perinatal infection. However, three older children found positive for HBsAg had a family history of positive HBV infection (2 mothers and 1 father were positive). Transmission from chronically infected women to their infants during delivery is one of the most common routes of HBV infection worldwide. The risk of perinatal infection is 5%-20% in infants born to HBsAg-positive mothers and 70%-90% if the mother is HBeAg positive[25,26]. However, it was previously shown that combined active and passive immunization of newborns of HBsAg$^+$ mothers against HBV demonstrates persistent protection up to adolescence despite a frequent waning of anti-HBs antibodies[27]. A recent meta analysis showed that HBV vaccine alone seems to be equally effective to a combination of HBIG and hepatitis B vaccine for neonates of HBsAg$^+$/HBeAg$^-$ mothers in preventing infection[28]. Currently, WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 h. The birth dose should be followed by 2 or 3 doses to complete the primary series. The complete vaccination series induces protective antibody levels in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is possibly lifelong[29].

None of the studied children aged < 7 years was a carrier for HBsAg. This was in accordance with a recent Egyptian study which did not find HBsAg positive sera among 180 children < 5 years[30]. Whereas another Egyptian study in 2003 reported higher prevalence (0.8%) of 6-year-old children having positive HBsAg[31]. In Taiwan, anti-HBc was detectable among 33% of vaccinated children aged 15 years *vs* 0.48% of children of the same age in the present study, and only one child had detectable HBsAg in both the Taiwanese and the present study[32]. In Italy, 3 out of 1543 vaccinated children aged 5 years were found to be anti-HBc positive[10].

However in long-term follow-up studies, breakthrough infections do occur, illustrated by the sero-conversion of anti-HBc, but few clinically significant infections are diagnosed and few new carriers are reported[33,34].

A meta-analysis revealed that the overall cumulative incidence of HBV breakthrough infection 5-20 years post-primary vaccination was 0.007 with a variation among studies ranging from 0 to 0.094[5]. In the present study, the highest prevalence of HBV infection detected by DNA (0.59%) was found in the age group ⩾ 7 years compared to 0.07% in the age group < 7 years and it was higher among girls (9/1857; 0.48%) than boys (5/1743; 0.29%). Results also suggested that monitoring the presence of HBV DNA (by using qPCR) is a better diagnostic parameter than anti-HBc for detecting viral infection. The effect of increasing age on the prevalence of breakthrough infection was also detected by other studies[35-37]. On the contrary as regards to gender, infection was higher among males than females in both Gambia[37] and in Iran[38].

In the current study, five out of thirteen children with positive anti-HBc had anti-HBs above 100 IU/L. It was reported that immunological responses to exposure to HBV, so-called breakthrough infections, have been observed in successfully vaccinated individuals who were later exposed to HBV. Such an exposure may simply boost the titer of anti-HBs[39]. The results also showed that one year later, six children retained anti-HBc positivity, three of them were also positive for

HBsAg while the other three children had isolated anti-HBc indicating occult HBV infection. Similar results were obtained by Su *et al*[40], who recommended a single HBV booster dose of vaccine for those with isolated anti-HBc who were fully vaccinated with HBV vaccine as infants.

From this study it can be concluded that the Egyptian national HBV vaccination in infancy produces adequate protection 1 to 16 years post vaccination. Successful implementation of universal vaccination policies in Egypt with a good coverage rate, together with the general improvement in infection control measures and safety blood donation can minimize the hepatitis B disease burden. Strict adherence to infection control measures and safe blood transfusion are needed especially for high-risk infants to augment the effectiveness of the vaccine.

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COMMENTS

Background

Hepatitis B virus (HBV) is considered moderately endemic in Egypt with 4% of the population having evidence of chronic HBV infection. World Health Organization recommended in 1992 to implement universal childhood vaccination worldwide. The complete vaccination series induces protective antibody levels in more than 95% of infants, children and young adults. A key goal of HBV immunization program is to reduce the prevalence of hepatitis B surface antigen (HBsAg) among cohorts born since program implementation.

Research frontiers

Although several studies have been done in Egypt to measure the effectiveness of HBV vaccination, yet these studies were done on a relatively small scale and in certain areas of Egypt from which arose the need for a large national study including numerous areas to be representative and give a clear picture of the situation in Egypt.

Innovations and breakthroughs

There have been no sero-surveys among children born since the introduction of the vaccine in Egypt. However, the finding of acute disease transmission in these cohorts indicates ongoing HBV transmission, thus the need for more in-depth evaluation of the immunization program. The present study had the greatest sample size (n = 3600) and aimed to assess the prevalence of breakthrough HBV infection (positive anti-HBc) as well as chronic carrier state (positive HBsAg) among the previously vaccinated studied children as a practical means to determine the long term protection provided by hepatitis B vaccine. It also aimed to determine health disparities and risk factors associated with non-seroprotective levels among Egyptian children aged from 9 mo to 16 years.

Applications

Successful implementation of universal vaccination policies in Egypt with a good coverage rate, together with the general improvement in infection control measures especially for high risk infants and safe blood donation can minimize the hepatitis B disease burden.

Terminology

Hepatitis B is a serious disease caused by a virus that attacks the liver. The virus, which is called HBV, can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death; Sero-protection: Following a standard hepatitis B vaccination course, antibody to HBsAg is established in the bloodstream, the antibody is known as antibodies to hepatitis B surface (anti-HBs). About 90%-99% of healthy neonates, children, adolescents and adults develop protective levels of anti-HBs; Breakthrough infection: (Positive anti-HBc): HBV infection in previously vaccinated subjects. Vaccinated subjects with anti-HBs antibody titers below the protective level are still susceptible to HBV infection, especially if they are exposed to a high viral load; HBV infections positive HBsAg: Chronic carrier state among previously vaccinated individual.

Peer-review

The manuscript is well written and is based in a large and well selected cohort that represents the Egypt young population. The conclusions and statements are well made in face of the obtained results. HBV vaccine is worldwide used and other studies have demonstrated its effectiveness in other populations. This study was focused in Egypt population and evaluated the behavior of anti-HBV response build after HBV vaccination. The expected result "The Egyptian compulsory HBV vaccination program has produced adequate protection" was correctly placed and support by the data collect.

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