Follow-up of hepatitis b virus vaccine response in healthy individuals

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Background The long-term protective effect of hepatitis B virus (HBV) vaccine and the need for booster dose vaccination remain unclear. Detection of nonresponders to HBV vaccine and search for factors that lead to nonresponse will help in prevention of HBV infection, hepatocellular carcinoma related to hepatitis B infection and hepatitis D virus infection.

Aim The aim of this study was to assess the benefit of follow-up of hepatitis B vaccine response and evaluate the persistence of seroprotection after HBV vaccination to determine the necessity of a booster dose in healthy individuals after 5 and 10 years of vaccination.

Patients and methods Serum samples were tested for quantitative detection of hepatitis B surface antibodies (HBsAb) using ELISA for 30 individuals who received HBV vaccine of less than or equal to 5 years (group I) and 30 individuals who received HBV vaccine for more than or equal to 10 years (group II), and if the results were negative or less than 10 IU/ml, evaluations of hepatitis B surface antigen and hepatitis B core antibodies (total) were done.

Results HBsAb was positive among 66.7% of each group, and the median HBsAb level was 59.73 and 51.21 in groups I and II, respectively.

Conclusion Approximately 33% of the studied groups were nonresponders of HBV vaccine regardless of postvaccination years (5–10).
Hepatitis B infection and occult hepatitis B infection results were negative in all of nonresponders.

Recommendations Follow-up of HBsAb levels in vaccinated individuals after having completed three doses of hepatitis B vaccination on a large scale is important to detect nonresponses and revaccinate them.

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Introduction

Hepatitis B virus (HBV) is considered moderately endemic in Egypt, with 4% of the population having evidence of chronic HBV infection [1].

In 1992, the WHO recommended the implementation of universal childhood vaccination worldwide, and by the end of 2012, 181 countries had adopted this measure [2]. In Egypt, the HBV vaccination program was applied in 1992 with a schedule at 2, 4 and 6 months of age, whereas routine screening of pregnant women for hepatitis B surface antigen (HBsAg) was not applied [3].

The complete vaccination series induces protective antibody levels in more than 95% of infants, children and young adults [4].

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 [5]. Seroprotection is ensured when HBsAb levels are at least 10 mIU/ml [6].

Decreased serum level of HBsAb with age has been reported in some studies [7]. If the immunity induced by hepatitis B vaccine decreases in older adolescents, young adults and high-risk groups, then HBV infection could occur in adolescence and adulthood [8].

Routine assessment of HBsAb levels after vaccination to detect immunity response was not applied.

Aim

The aim of this study was to assess the benefit of follow-up of hepatitis B vaccine response and evaluate the persistence of seroprotection after HBV vaccination to determine the necessity of a booster dose in healthy individuals after 5 and 10 years of vaccination.

Patients and methods

Study setting

The study was conducted at the outpatient clinic of Tropical Medicine Department, Al-Zahraa University Hospital, Cairo, from March 2016 to July 2016.

This was a cross-sectional study conducted on 60 individuals who received hepatitis B vaccine as a part of Expanded Program of Immunization during infancy in our country (three doses of recombinant hepatitis B vaccine were given during infancy according to Ministry of Health recommendations in Egypt) (Euvax B; LG Life Science, Seoul, Korea) (administered to all infants at 2, 4 and 6 months of age). The study also included medical persons who received hepatitis B vaccine (three successive doses) as a part of vaccination program inside Al-Zahraa University Hospital.

Inclusion criteria

Healthy individuals who received three doses of hepatitis B vaccine and who accepted to participate were included in the study.

Exclusion criteria

Immune-compromised individuals, healthy individuals but having any infection during the period of the study (respiratory tract, urinary tract, or gastrointestinal tract infections) and individuals who refused to participate in the study were excluded.

The participants were divided into two groups according to duration (in years) after hepatitis B vaccine completion.

(1) Group I included 30 individuals who received HBV vaccine of less than or equal to 5 years (11 males and 19 females).
(2) Group II included 30 individuals who received HBV vaccine of more than or equal to 10 years (17 males and 13 females).

All studied groups were subjected to the following:

History taking, clinical examination and data collection (including name, age, sex, residence, date of receiving HBV vaccine in their vaccination cards, past history of blood transfusion or surgical operations, visiting dentist, and history of HBV infection in their parents or their brothers and sisters) were done.

Blood samples (5 ml) were withdrawn under strict sterile condition and then centrifuged for quantitative detection of anti-HBs using ELISA. When the results were negative or less than 10 IU/ml, assessments of HBsAg and hepatitis B core antibodies (total) (HBcAb) were done.

Hepatitis B surface antibody

HBsAb was detected by enzyme immunoassay for quantitative determination of antibodies to the surface antigen of HB virus in human plasma and sera. For in-vitro diagnostic use, only after washing, captured antibodies are detected by HBs antigen labelled with peroxidase that specially binds the second available binding site of this antibody. The enzymes specifically bind to wells, by acting on the substrate/chromogen mixture, and generates an optical signal that is proportional to the amount of (HBs) antibody in the samples, which is detected by ELISA reader. The amount of antibodies may be quantitated by means of standard curve against WHO reference preparation.

Hepatitis B surface antigen

HBsAg was detected by a solid-phase enzyme immunoassay based on the sandwich principle. The solid phase of microtiter plate is made of polystyrene wells coated with mouse monoclonal antibodies specific for HBsAg. Then, a serum or plasma specimen containing HBsAg is added to the anti-HBs antibody-coated wells, which is followed by addition of guinea pig polyclonal antibodies purified by affinity chromatography together with peroxidase conjugated anti-HBs antibody, and then the antibody-HBsAg-antibody-peroxidase complex that forms on the wells is incubated.

After washing the microtiter plate to remove the unbound materials, a solution of tetramethylbenzidine (TMB) substrate was added to the wells and incubated. The color develops in proportion to the amount of HBsAg bound to anti-HBs antibody. The peroxidase reaction is stopped by addition of sulfuric acid, and then read by ELISA reader.

Hepatitis B core antibody

ELISA is a solid-phase enzyme immunoassay based on the principle of anti-HBcAb. The solid phase of microtiter plate is made of polystyrene wells coated with anti-human Ab. When a serum or plasma specimen containing anti-HBcAb is added to the anti-human Ab-coated wells and incubated, antibodies present in the specimen bind to anti-human Ab on the wells. After addition of HBcAg-containing reagent and solution containing peroxidase conjugated anti-HBc, incubation is done, during which anti-human-Ab, anti-HBcAb) (HBcAg) (anti-HBc peroxidase) complex is formed in

the wells. After washing, the microtitre plate to remove unbound material. A solution of substrate is added to the wells and incubated. If anti-HBcAb is present in the specimen after washing, the activity of peroxidase on the wells reflects the content of HBcAb in a specimen.

Another direct interview was done by one of the authors with all participants to inform them about their immune response. HBV vaccine booster dose is suggested for individuals with absence of anti-HBs antibody. However, none of the nonresponders acknowledged regarding repeated HBV vaccination.

Statistical analysis

Data collected were reviewed and coded, and statistical analysis of the collected data was done by using statistical package of social science program (SPSS; SPSS Inc., Chicago, Illinois, USA) version 16 for Microsoft Windows. Frequency of occurrence was calculated to describe qualitative data, and median percentiles for quantitative nonparametric ones. For comparison of qualitative data, χ^2-test was used, and Mann–Whitney *U*-test was used to determine the significance in the difference between two quantatative nonparametric variables. The level of significance was taken at *P* value of less than 0.05 with confidence level 95%, and the results were represented in tables.

Results

The results of the current study revealed that no statistical significant difference was seen regarding age of both groups, with mean age of noncompulsory individuals in group I and group II being 38.85±8.67 and 39.33±7.57 years, respectively.

Males accounted for 36.7% of group I and 56.7% of group II. Large percentages of both groups were residing in suburban areas (70.0 and 86.6%, in group I and II, respectively); these differences were statistically insignificant ($P>0.05$) (Table 1).

Regarding risk factors for hepatitis B infection, in group I, there were five male and two female participants who had a history of repeated dentist visits, whereas there were four male and five female participants who had a history of repeated dentist visits in group II.

There was a history of surgical operations in one male and two female participants in group I and in two male and four female participants in group II.

None of the participants in both groups had a history of blood transfusion.

Regarding HBV serology, no statistically significant differences were recorded between the two groups, as HBsAb was positive among 66.7% of each group, and the median of HBsAb was 59.73 and 51.21 IU/ml in groups I and II, respectively (Table 2).

Among group I, female participants accounted for 65.0% of those with positive HBsAb compared with 60.0% of those with HBsAb negative, and 70.0% of each groups were residing in suburban areas (Table 3). However, among group II, females accounted for 60.0% of those with positive results of HBsAb compared with 10.0% of those with negative results, with statistically significant difference, and 70.0% each with either results were residing in suburban areas, with no statistical significant difference observed (Table 4).

Multivariate regression analysis revealed that age, sex, duration of vaccination and residence had insignificant association with HBsAb (Table 5).

Regarding the results of HBsAb and hepatitis B core antibody for nonresponders, all of them were negative.

The results of the current study revealed that among group I (duration of vaccination ≤5 years), 17 were having the vaccine as a part of compulsory schedule of

Table 1 Demographic data of the studied groups

	Groups [*n* (%)]		Significance test	*P* value
	Group I (duration of vaccination ≤5 years)	Group II (duration of vaccination ≥10 years)		
Sex				
Male	11 (36.7)	17 (56.7)	2.41 (χ^2)	0.121
Female	19 (63.3)	13 (43.3)		
Residence				
Suburban	21 (70.0)	26 (86.6)	3.07 (χ^2)	0.215
Urban	9 (30.0)	4 (13.4)		
Age of noncompulsory individuals (mean±SD)	38.85±8.67	39.33±7.57	0.09 (Student's *t*-test)	0.930

Table 2 Hepatitis B virus serology among the studied groups

Hepatitis B virus serology	Groups		Significance test	P value
	Group I (duration of vaccination ≤5 years)	Group II (duration of vaccination ≥10 years)		
Hepatitis B surface antibodies [*n* (%)]				
Positive	20 (66.7)	20 (66.7)	0.00 (χ^2)	1.000
Negative	10 (33.3)	10 (33.3)		
Hepatitis B surface antibodies level (IU/ml)				
Median	59.73	51.21	*Z*=0.44 (Mann–Whitney *U*-test)	0.657
Interquartile range	6.49–92.49	3.96–143.50		

Table 3 Hepatitis B surface antibodies results among group I regarding sex and residence

	Hepatitis B surface antibodies results [*n* (%)]		Significance test (χ^2)	*P* value
	Positive (20)	Negative (10)		
Sex				
Male	7 (35.0)	4 (40.0)	0.072	0.789
Female	13 (65.0)	6 (60.0)		
Residence				
Suburban	14 (70.0)	7 (70.0)	00.00	1.000
Urban	6 (30.0)	3 (30.0)		

Table 4 Hepatitis B surface antibodies results among group II regarding sex and residence

	Hepatitis B surface antibodies results [*n* (%)]		Significance test (χ^2)	*P* value
	Positive (20)	Negative (10)		
Sex				
Male	8 (40.0)	9 (90.0)	6.78	0.009
Female	12 (60.0)	1 (10.0)		
Residence				
Suburban	16 (80.0)	10 (100.0)	3.00	0.223
Urban	4 (20.0)	0 (0.0)		

Table 5 Logistic regression analysis of factors affecting hepatitis B surface antibodies level

	B	Standard error	Wald	Significant	Exp(*B*)
Age	0.058	0.040	2.134	0.144	1.060
Sex	–1.291	0.738	3.062	0.080	0.275
Duration of vaccination	–0.063	0.121	0.274	0.601	0.939
Residence	1.767	1.206	2.147	0.143	5.851
Constant	–4.062	3.984	1.039	0.308	0.017

infant vaccination whereas 13 were having the vaccine outside of such schedule (median level of HBsAb 20.00 and 72.45 IU/ml, respectively). Meanwhile, among group II (duration of vaccination ≥10 years), 27 were having the vaccine as part of compulsory schedule of infant vaccination whereas three were having the vaccine outside of such schedule (median level of HBsAb was 30.84 and 240.90 in compulsory and noncompulsory groups, respectively).

Group I (duration ≤5 years)

	Schedule of vaccination ≤5 years [*n* (%)]		Significance test	*P* value
	Compulsory (17)	Noncompulsory (13)		
HBsAb				
Positive	11 (64.7)	9 (69.2)	χ^2=0.07	Corrected
Negative	6 (35.3)	4 (30.8)		*P*=1.00
HBsAb level			Mann–Whitney *U*	
Median	20.00	72.45	*Z*=–1.82	0.069
Interquartile range	2.60–67.87	7.16–283.00		

Group II (duration ≥10 years)

	Schedule of vaccination ≤10 years [*n* (%)]		Significance test	*P* value
	Compulsory (27)	Noncompulsory (3)		
HBsAb				
Positive	17 (63.0)	3 (100.0)	χ^2=1.67	Corrected
Negative	10 (37.0)	0 (0.0)		*P*=0.532
HB s Ab level			Mann–Whitney *U*	
Median	30.84	240.90	*Z*=–2.42	0.016*
Interquartile range	3.90–117.50	173.50–1000.0		

*$P<0.05$, significant difference.

Discussion

A key goal of HBV immunization program is to reduce the prevalence of 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 [9].

The complete vaccination series induces protective antibody levels in more than 95% of infants, children and young adults [4].

The current study aimed to assess the benefit of follow-up of hepatitis B vaccine response and evaluate the persistence of seroprotection after HBV vaccination to determine the necessity of a booster dose in healthy individuals after 5 and 10 years of vaccination. The current study revealed that protective level of HBsAb was found (>10 IU/ml) among 66.7% of each group and the median of HBsAb level was 59.73 and 51.21 IU/ml of both groups, respectively.

These results coincides with several Egyptian studies that discussed the long-term efficacy of hepatitis B vaccine in children. The overall seroprotection rate among the studied children was 57.2% [10]; other Egyptian studies carried out on smaller sample sizes Shaaban *et al.* [11] and El Sherbini *et al.* [12] reported 54 and 39.7% seroprotection rates, respectively, among vaccinated children aged 6–12 years.

Persistence of anti-HBs and thus the protection against infection and carrier state depends on the peak anti-HBs concentration achieved after primary vaccination [5]. However, anti-HBs decay exponentially with length of time since vaccination [5].

These results are against the current study as the median level of HBsAb in group I (schedule of vaccination ≤5 years) was 20 in compulsory vaccinated individuals whereas it was 30.84 in group II (schedule of vaccination ≤10 years) in compulsory vaccinated individuals, which might be owing to small sample size.

Regarding the current study, none of the nonresponders have evidence of active infection or carrier state (HBsAg and or HBcAb) which raises the importance of follow-up of seroprotection after hepatitis B vaccine and booster dose administration for protection against hepatitis B infection.

Conclusion

Approximately 33% of the studied groups were nonresponders of hepatitis B vaccine regardless of the postvaccination years (5–10).

Hepatitis B and occult hepatitis B infection were negative in all of nonresponders.

Recommendations

Follow-up of anti-HBs levels in vaccinated individuals after complete three doses of hepatitis B vaccination in large scale is important to detect nonresponders and revaccinate them, and it is essential to search for factors that lead to nonresponse.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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