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Need and Response to Hepatitis B Virus Booster Immunization among Egyptian Type 1 Diabetic Students 10-17 Years after Initial Immunization: A Quasi-Experimental Comparative Study

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Abstract

Background: Insufficient immune response to hepatitis B virus (HBV) vaccine in diabetics is frequently encountered and anti-HBs levels may persist for a shorter time than among immune competent persons. The present study was conducted to determine long–term persistence of anti-HBs among Egyptian diabetic school students and to assess the need and response to a subsequent challenge with vaccine booster doses.

Methods: The study included two phases, a comparative (screening) phase followed by a quasi-experimental (boosting) phase. A baseline serologic screening for anti-HBs titre was carried out among 260 school students aged 10-17 years (130 diabetics and 130 healthy non-diabetics, matched for age and sex) who received the full three-dose regimen of HBV vaccine under the Expanded Program of Immunization (EPI) in Egypt. Ninety participants (45 diabetic and 45 healthy ones) with anti-HBs<10 m IU/ml consented to be enrolled in the second phase to receive additional booster doses of the vaccine.

Results: The median value of anti-HBs titre was significantly lower among diabetics (3.0 m IU/ml) as compared to non-diabetics (6.8 m IU/ml). Age was the only significant risk factor associated with poor response. Adequate protection (anti-HBs>100 mIU/ml) was achieved after receiving two doses of the vaccine for healthy students and three doses for diabetics. BMI and history of hospitalization due to diabetes were the only significant factors affecting the response to boosting among diabetic students. In conclusion, type 1 DM adolescents express hyporesponsivness to HBV vaccination and more rapid decline of protective anti-HBs compared to healthy ones. A booster dose of HBV vaccine would be recommended at age 12 years for diabetic students.

Keywords: HBV; Vaccine response; Children; Booster; Diabetes mellitus

Introduction

Hepatitis B virus (HBV) is a serious public health problem worldwide and a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). HBV vaccination is the most effective measure for prevention of HBV infection. Published data concerning the long-term immunogenicity of HB vaccine in Egyptian children indicated that the vaccine was effective in preventing HBV infection with an efficacy of more than 90% and that booster doses are unnecessary possibly due to protective anamnestic response to antigenic challenge [1]. However, other authors recommended that further follow-up studies for longer duration than 10 years are still needed especially during adolescence with the onset of sexual activity and among certain groups such as immune-compromised patients among whom lower efficacy of HB vaccine was found [2].

Type 1 Diabetes Mellitus (DM) is a clinical condition that influences the cellular and humoral immune system, as that the immunological response to HB vaccine could be less optimal and lasts for a shorter duration than in non-diabetic individuals [3]. Considering both the low seroconversion rate [4] and the several risk factors for enhancing HBV exposure among type 1 DM patients, these diabetic non responders could constitute an important reservoir that is potentially capable of modifying the epidemiology of HBV disease. The main priority should be directed to continue protecting vaccinated children. It was suggested that immune-suppressed patients may be the only subgroup in which it is necessary to maintain protective antibody level (anti-HBs titer >10 mIU/ml) [5].

Maintaining of such protection depends on immunological memory which can be demonstrated by administering an additional booster dose of the vaccine. A rapid increase in anti-HBs level represents an anamnestic response and indicates the presence of HBV-specific immune memory [6]. Anamnestic antibody response to booster doses in vaccinees has been demonstrated up to 15 years post neonatal vaccination [7]. Being one of immune-compromising diseases the response among diabetics was the concern of a quite many studies [5,8]. There are insufficient data on long-term protection durability provided by HB vaccine especially among this vulnerable group and guidelines for booster administration for immune-compromised patients when antibody to HBsAg titer falls, than 10 mIU/ml has not been elucidated. Therefore, the present study aims to determine how long immunity persists in Egyptian diabetic school students after 10-17 years of vaccination compared to non-diabetic peers, whether boosters are needed, and, if so, when and in whom they should be administered.

Subjects and Methods

The study started in October 2010 till May 2013 and included two phases, a comparative-case-control (screening) phase followed by a quasi-experimental (boosting) phase. The sample size of the screening phase was estimated based on a previous study on protective immunity after HB vaccination, [9] and assuming the percent of anti-HBs seropositivity among diabetic students to be 10% and of healthy students equal to 59.5% using an alpha error of 0.01 and a power of 95%. A minimum sample size required was calculated to be 80 for each group [10].

A baseline serologic screening for anti-HBs titre was carried out among 260 school students aged 10-17 years (130 diabetics and 130 healthy non-diabetics, matched for age, sex and socio-economic condition and had normal fasting serum glucose (FSG) who had received the full three-dose regimen of HBV vaccine (at 2-4-6 months of age) under the EPI in Egypt confirmed by vaccination card. Children suffering from other chronic diseases or reported a history of corticosteroid or immunosuppressive drugs intake were excluded. Diabetic children were diagnosed by specialists and under insulin therapy.

The subjects were chosen by a simple random sampling technique from those attending the outpatient clinics of EL-Shatby Pediatrics University hospital and Insurance Sporting students' hospital in Alexandria after taking the required approvals.

Ninety participants (45 diabetic and 45 healthy ones) with anti-HBs <10 mIU/ml were included in the quasi-experimental phase after getting informed consent. These numbers are sufficient to detect a difference between vaccinated diabetics and controls in the percentage of participants with anti-HBs <10 mIU/ml of 25% [5] with 90% power and alpha level of 0.05. Sample size calculation was performed using MedCalc software version 11.3.1.0.

All participants were subjected to an interview using a pre-designed questionnaire including inquiries about socio-demographic data, risk factors for virus hepatitis and diabetes mellitus status. Calculation of boby mass index (BMI) for age [11] was done and blood samples were drown from participants to be tested for fasting and post-prandial blood sugar and glycosylated hemoglobin for diabetics [12].

All sera were tested for anti-HCV using rapid test (J. Mitra and Co. Pvt. Ltd. INDIA, New Delhi) and anti-HBs levels (mIU/ml) using quantitative enzyme linked immunosorbent assay (ELISA). (Dialab, Austria, Wiener Neudorf). Sera with anti-HBs titre <10 mIU/ml were further tested for HBsAg and total anti-HBc using the same latter ELISA kit.

In the boosting phase, a 1st booster dose of 0.5 ml containing 10 µg of HBsAg (pediatric dose) of recombinant HBV vaccine [Euvax B, Sanofi Pasteur LTd.] was given for students aged up to 15 years, while a dose of 1.0 ml containing 20 µg of HBsAg (adult dose) was given to those above 15 years. The vaccine was given intramuscularly in the deltoid muscle. Four weeks after vaccine administration, anti-HBs was quantitatively measured.

Participants who lacked an anamnestic reaction (anti-HBs concentrations ≥ 10 mIU/ml in subjects who were sero-negative before the challenge or an increase of at least 4-fold in anti-HBs concentrations in subjects who were sero-positive before the challenge) [13] received a 2nd booster dose using the same vaccine doses and technique. One month after the second booster dose, participants who still failed to express an anamnestic reaction or showed inadequate response (anti-HBs<100 mIU/ml) were given a third booster dose 6 months after the 1st boosting and anti-HBs level was assessed one month later.

Ethical consideration: The study followed strictly the Declaration of Helsinki and was approved by the High Institute of Public Health (HIPH) Ethics Committee. Children assents and their parents' written informed consent were mandatory before enrollment in the study.

Data were analyzed using SPSS version 16. Two tailed tests and alpha error of 0.05 p value were used. The mean and standard deviation were used for normally distributed numeric data, median for skewed numeric data and percent to describe the scale and categorical data.

Independent sample t-test was used to compare means of two independent samples which follow a normal distribution. Man Whitney test was used to compare ranks (medians) for two independent groups of cases. Wilcoxon test was used to compare the mean ranks of two related groups. For categorical data, Pearson's chi square test was used to test for the association of two independent samples. Mont Carlo exact test and Fishers exact test were alternatives for the Pearson's chi square test if there were many small expected values. Friedman test was used to compare the responses for the rate for the categories occurrence at the different stages for the same individuals. Multiple associations were evaluated in a multiple regression models based on stepwise methods. An adjusted odd (OR) with a 95% confidence interval (CI) that did not include 1.0 was considered significant.

Results

Screening phase

After 10-17 years of vaccination, anti-HBs levels decreased dramatically among healthy and diabetic children (Table 1). The median value of anti-HBs titre was significantly lower among diabetics and the frequency of poor response (anti-HBs<10 mIU/ml) was higher among diabetics as compared to non-diabetics.

Anti-HBs titre (mIU/ml)	Diabetics (n=130)		Non diabetics (n=130)		TOS	p
	No.	%	No.	%	X2=3.331	0.068
<10	92	70.8	78	60.0		
≥ 10	38	29.2	52	40.0		
Median	3.0		6.8		Z=-3.026	0 .002*

Table 1: HBV vaccine long-term immune response in the studied groups measured by anti-HBs titre. TOS: test of significance, *P<0.05.

Table 2 summarizes the stepwise logistic regression analysis which included all risk factors significantly affecting the immune response among participants when keeping all other factors constant.

It showed that those who were diabetics have 60% more risk for expressing poor response (anti-HBs <10 mIU/ml) than non-diabetics. The only significant risk factor associated with poor response was the age. One year increase in age was associated with about 30% higher risk for being poor responder.

Variables	B	S.E.	p	OR	95.0% C.I. for OR	
					Lower	Upper
age	0.265	0.095	0.005	1.303	1.083	1.569
DM	0.479	0.263	0.069	1.614	0.964	2.703
p value for model=0.001						
Classification accuracy 83.4%						

Table 2: Stepwise logistic regression analysis of factors affecting immune status among studied children. B: regression co-efficient, SE: standard error, OR: odds ratio, CI: confidence interval, DM: Diabetes Mellitus.

Boosting phase

The immune response among the studied students after vaccine boosting is illustrated in Table 3. After the first and the second booster doses of HB vaccine, the diabetic students had a much lower minimum level of anti-HBs compared to non-diabetics. Moreover, the diabetic students showed a lower mean titre of anti-HBs than that of the non-diabetic students. This difference was statistically significant after the first booster dose only. Comparing the mean titre of anti-HBs among diabetics after first, second and third booster doses revealed a statistically significant difference. In contrast to non-diabetics whose difference in anti-HBs means did not reach a statistically significant level after the first and second booster doses.

When comparing anti-HBs titre categories after HBV vaccine boosting as shown in Table 4, we found a significantly lower immune response among diabetic group. After the 1st booster dose, 7(15.6%) of the diabetics still remained non-protected (anti-HBs<10 mIU/ml) versus none of the control. A significantly lower percentage of diabetics showed protective levels (anti-HBs=10-100 and >100 mIU/ml) as compared to non-diabetics (4.4, 80% versus 8.9, 91.1% respectively).

Anti-HBs titre (mIU/ml)	Diabetics	Non-diabetics	Z	P
After first booster	n= 45	n= 45	-2.406	0.016*
Minimum	0.0	21.0		
Maximum	160.0	160.0		
Mean	124.3	149.3		
SD	60.4	32.0		
Median	160.0	160.0		
After second booster	n= 9	n= 4	-1.542	0.123
Minimum	11.0	146.0		
Maximum	160.0	160.0		
Mean	104.2	156.5		
SD	63.0	7.0		
Median	140.0	160.0		
After third booster	n= 4			
Minimum	132.0			
Maximum	160.0			
Mean	153.0			
SD	14.0			
Median	160.0			
TOS P	X2= 8 P= 0.018*	Z=1.8 P= 0.068		

Table 3: Immune response among the studied students after vaccine boosting. Z: Wilcoxon test of two related groups, X2: Friedman test for related samples, * P<0.05 (significant).

Anti-HBs titre categories (mIU/ml)	Diabetics		Non-diabetics		MCP
	No.	%	No.	%	
After first booster	n=45		n=45		0.028*
<10	7	15.6	0	0.0	
10-100	2	4.4	4	8.9	
>100	36	80.0	41	91.1	
After second booster	n= 9		n= 4		0.109!
10-100	4°	44.4	0	0.0	
>100	5˜	55.6	4	100.0	
After third booster	n= 4				NA
>100	4	100.0	0	0.0	
Z(P)	-2.428 (0.015*) £		-2.00(0.046*)		

Table 4: Anti-HBs titre categories of HBV immune response among the studied students after booster doses measured in mIU/ml. Z: Wilcoxon test of two related groups, MCP: P value based on Mont Carlo exact probability, ! P value based on Fisher exact probability P£: P value based on Friedman test of several related samples, 4° : Three from the 7 unprotected after the 1st boosting and one from the 2 having anti-HBs level of 10-100 mIU/ml after the 1st boosting, 5˜ : Four from the 7 unprotected after the 1st boosting and one from the 2 having anti-HBs level of 10-100 mIU/ml after the 1st boosting.

Diabetic cases with anti-HBs titre <10 mIU/ml and participants with titre=10-100 mIU /ml were offered a second booster dose. All the 4 non-diabetics had reached a high protective level of anti-HBs (>100 mIU/ml) in contrast to only 55.6% of diabetics.

Comparing the anti-HBs level among the diabetics after the 1st, 2nd and 3rd booster doses and among non-diabetics after the 1st and 2nd booster doses revealed statistically significant difference (Table 4).

We examined the factors that affected the immune response to boosting in diabetics by comparing the poor and good responders after administration of the 1st booster. The mean BMI and the history of hospitalization due to DM were the only significant factors affecting the response to boosting among diabetic students. Non-protected diabetics had a significantly higher mean of BMI than that of the

protected diabetics. Although the gender was not significantly associated with the level of anti- HBs ,yet six of the seven non-responders were males. The percentage of non-protected diabetics increased in the presence of DM complications and increasing insulin dose. The evidence of metabolic control was detected among 5 of the seven non-responders diabetics. All four diabetics who were exposed to HBV infection gained a protective level of anti-HBs. Having only one diabetic student seropositive for antibody to HCV did not allow us to judge its effect.

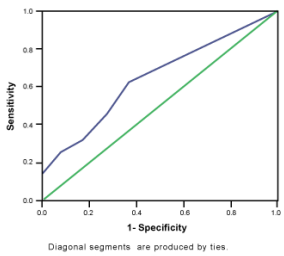
Factors	**Anti-HBs after 1st booster dose**					**X2**	**P**
	No. examined	**< 10 mIU/ml**		**> 10 mIU/ml**			
		No.	%	No.	%		
Age (years)						-	0.303Δ
10-	9	1	11.1	8	88.9		
12-	17	1	5.9	16	94.1		
14-	6	1	16.7	5	83.3		
16-17	13	4	30.8	9	69.2		
Mean ± SD		14.1 ± 2.6		13.5 ± 2.2		T=1.1	P=0.280
Gender						-	0.103Δ
Male	26	6	23.1	20	76.9		
Female	19	1	5.3	18	94.7		
BMI						1.147	0.766
Underweight	2	0	0.0	2	100.0		
Normal R	32	5	15.6	27	84.4		
Overweight	8	1	12.5	7	87.5		
Obese	3	1	33.3	2	66.7		
Mean ± SD		24.4 ± 5.9		19.8 ± 3.3		t=3.283	P=0.001*
Duration of DM						0.400	0.940
<2	10	2	20.0	8	80.0		
2-	11	2	18.2	9	81.8		
4-	15	2	13.3	13	86.7		
7-12	9	1	11.1	8	88.9		
Mean ± SD		3.1 ± 4.5		4.5 ± 3.3		t=-1.015	P=0.316
Insulin dose						-	0.936Δ
< 30	8	1	12.5	7	87.5		
30-	14	2	14.3	12	85.7		
40+	28	4	17.4	19	82.6		
Mean ± SD		50.7 ± 23.5		40.9 ± 15.9		t=1.4	P=0.173
HBA1c						-	0.118Δ
Less than 7	20	5	25.0	15	75.0		
More than 7	25	2	8.0	23	92.0		
Complications of DM						0.773	0.379

No	42	6	14.3	36	85.7		
yes	3	1	33.3	2	66.7		
Hospitalization						8.198	0.017*
No	18	1	5.6	17	94.4		
Once	14	1	7.1	13	92.9		
Frequent	12	5	41.7	7	58.3		
Anti-HBc						0.809	0.368
Negative	41	7	17.1	34	82.9		
Positive	4	0	0.0	4	100.0		
Anti-HCV						0.188	0.664
Negative	44	7	15.9	37	84.1		
Positive	1	0	0.0	1	100.0		

Table 5: Factors affecting the immune response to 1st boosting among diabetics. *P<0.05 (significant), Δ: P value based on Mont Carlo exact probability, DM: Diabetes Mellitus.

Number of vaccine booster doses	Diabetics (n=45)		Non-diabetics (n=45)		X2	P
	No.	%	No.	%		
One	36	80.0	41	91.1	4.436	0.109
Two	5	11.1	4	8.9		
Three	4	8.9	0	0.0		

Table 6: Number of HBV vaccine booster doses needed for protection of diabetic and non-diabetic students.



Age of boosting	Area under the curve	Sensitivity	Specificity
Diabetic	0.645	65.1	62.6
Healthy	0.649	75.0	43.6

Figure 1: ROC curve of the appropriate time of boosting

To reach full protection, 80.0% of diabetics needed only one booster dose compared to 91.1% of non-diabetics while two doses were needed by 11.1% of diabetics compared to 8.9% of non-diabetic students. Four (8.9%) diabetic students needed a third booster dose versus none of the control. ROC curve analysis was applied to forecast the age at which a booster dose may be applied in healthy and diabetic children. With considerable sensitivities and areas under the curves, 13.5 years for healthy students and 12 years for diabetics appeared appropriate. But this forecast for healthy individuals had a low specificity.

Discussion

DM is a major global emerging public health threat and is a marker for increased risk of HBV transmission [14]. HB vaccine, one of the safest vaccines available in the world [15,16], despite being highly efficacious, long term maintenance of its immune-protection evidenced by adequate immunological memory is one of the recent healthcare challenges among this immune compromised vulnerable group [5].

Vaccination of diabetic adults [17] and children [5] was repeatedly reported to result in low anti-HBs titres compared to normal subjects. A clear association between DM and impaired response was proved among hemodialysis (HD) patients [18] and non-renal failure DM subjects [19]. Similarly, in the present study, diabetic students revealed hypo-responsiveness to HB vaccine 10-17 years after initial vaccination during infancy as compared to non-diabetics, a finding that confirms the decline of anti-HBs titre overtime and matches well the data of most published follow-up studies regarding HB vaccine long-term immune response [5]. Studying children from HBV high endemic countries revealed that from four to ten years after vaccination, about 50% of participants had anti-HBs levels less than 10 mIU/ml. Studies conducted in Taiwan showed that the post-vaccination seropositivity rate dropped from 99% at 1 year to 83% at 5 years [20] and from 71.1% at 7 years to 37.4% at 12 years [21]. In Alaskan study, 88% of children who were vaccinated at birth lost their anti-HBs at the age of 5 years [22]. Likewise, 80-95% lost their protective anti-HBs when tested 13-15 years after vaccination [23]. The stepwise logistic regression analysis results in the present study also indicated that the age was the only significant factor associated with poor response, one year increase

in age was associated with about 30% higher risk of being non-responder (Table 2).

Nevertheless, persistence of anti-HBs at a concentration of ≥ 10 m IU /ml is not necessary for protection, because it is the immune memory that matters as it can outlast the presence of vaccine-induced antibodies, conferring effective protection against acute disease and the carrier state. The presence of HBV- specific immune memory can be demonstrated by administering an additional (booster) dose of the vaccine and measuring anti-HBs responses [24]. Dentinger et al. [24] approved that 13-22 years after primary vaccination, one booster was sufficient to elevate anti-HBs titre to the protective level in 93-100% of the vaccinees. In addition, 89-100% of booster recipients 4-13 years old children responded to a booster dose of HB vaccine in another study [25].

The boosting phase in the present quasi-experimental comparative study further confirms hyporesponsiveness of diabetic students to booster vaccination as compared to non-diabetic healthy controls. Although one booster dose resulted in seroconversion in 100% of our studied seronegative healthy adolescents, yet it was not sufficient to protect all the diabetics with 15.6% (7/45) of them left non-protected with significant lower mean of anti-HBs titre (124.3 ± 60.4 mIU/ml) than that of the non-diabetic students (149.3 ± 32.0 mIU/ml) (P=0.016). A finding that is comparable to another study conducted by El desoky et al. [9] who revealed that after one booster dose, 94.4% of diabetic vs. 100% of non-diabetic children turned seroprotected. Another study revealed that 78.9% of diabetic and 96.6% of non-diabetic hemodialysis patients produced protective anti-HBs levels after the administration of booster doses. The rate of the protective anti-HBs levels in non-diabetics was 1.2 times higher than that in diabetics [26].

Moreover, our data indicated that to reach an adequate protection (anti-HBs>100 mIU/ml) against HBV infection among all diabetic and healthy unprotected students, two booster doses were needed by 11.1% of diabetics in comparison to 8.9% of non-diabetic students. Four (8.9%) diabetic students needed a third booster dose to reach full protection in comparison to none of the normal students. Likewise, Ocak and Eskiocak [26] gave evidence that more than one booster dose was needed to reach protection in diabetic hemodialysis adult patients. Although up to three booster doses led to a significant improvement in protective response rates, it left 21.1% of diabetic vs. 3.4% of non-diabetics unprotected.

In addition to age, male gender is one of host factors that contributed to decreased immunogenicity [27]. In the present study, it is worth to mention that six of the seven non-responders were males. Abdolsamadi et al. [28] also observed a better vaccine response in women.

The exact mechanism of lower immunity rates after vaccinating obese subjects is not clear. Arslanoğlu et al. [17] and Ocak and Eskiocak [26] failed to link obesity with non-response to HBV vaccine among diabetic children and diabetic hemodialysis patients respectively. In contrast, in the present study non-protected diabetics had significantly higher mean BMI as compared to the protected ones. Therefore, studying immune response of obese children to booster doses of vaccine is an urgent issue of public health importance in an HBV endemic area.

In accordance with published data, [29,30] frequent hospitalization among diabetics was the only revealed significant factor associated with decreased immune response to vaccine boosting in the present study. The authors attributed this to a worse diabetic status with subsequent lower immunity and the fact that frequent hospitalization exposes diabetic patients to sharing insulin vials and spring triggered devices as well as frequent injections and needle pricks made them more prone to nosocomial infections with subsequent impairment of the immunity.

Published results indicated that a high proportion of vaccinated infants and children retain protective concentrations of anti-HBs in adolescence and exhibit a booster response if revaccinated which might be because of natural boosting from exposure to HBV-infected persons in the household and community [23]. Marseglia et al. [29] mentioned that the fact that three diabetics and five controls with anti-HBs median value of 150 mIU/ml reached a level of >1000 mIU/ml 4 years later is consistent with the hypothesis of anamnestic response after a natural exposure. The above justification matched well our results as it was found that all diabetics (n=4) who were exposed to HBV infection (anti-HBc positive) gained a protective level of anti-HBs after a single booster. Moreover, all the low responders (4 healthy and 4 diabetics) after either 1st or 2nd booster doses were anti- HBc negative.

The question that remains to be answered is how long immune memory will last and hence when to provide a booster dose. Bialek et al. [23] recommended the application of the extra booster dose at age of 4-5 years, while others considered it is unnecessary until 10 years of age [21]. Moreover, John and Cooksley [31] did not recommend booster vaccination before 15 years of age. Optimally, booster vaccination should be recommended at a point in time when majority of vaccinees actually begin to lose protection [32]. The results of present study verified that incorporating a booster dose of HBV vaccine into the routine childhood vaccination schedule would be best timed at 12 years old for diabetic students while it remains a debatable issue at 13.5 years old for healthy students.

In conclusion, type 1 diabetic adolescents are hyporesponsive to HBV vaccination and show more rapid decline of protective anti-HBs compared to healthy ones. Adequate protection against HBV infection among students with unprotective anti-HBs level could be achieved after two booster doses for healthy students and three booster doses for diabetic ones. Therefore, we recommend a booster dose for diabetic children 12 years after initial vaccination in Egypt.

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