ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Protective immunity after hepatitis B vaccination: Cross-sectional study among school children attending Benha university hospital.

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Manuscript Info

Manuscript History:

Received: 14 December 2015
Final Accepted: 25 January 2016
Published Online: February 2016

Key words:
protective immunity - hepatitis B vaccine – postvaccination HBs Abtitre (seroprotected ,seronegative)

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Abstract

Background: Few data are available concerning the long term immunogenicity of the pediatric doses of hepatitis B vaccines.

The aim: was to evaluate the immune response among school children after hepatitis B vaccination according to Expanded Program on Immunization (EPI) in Egypt, and to determine factors influencing the immune response to hepatitis B vaccine.

Subjects and Methods: This cross-sectional study included 300 healthy school children who had been given three doses of hepatitis B vaccine during obligatory immunization schedule in Egypt. HBs antibody concentration was quantitated using Enzyme Linked ImmunoSorbent Assay (ELISA) and for seronegative cases (Anti-HBs Ab<10 mIU/ml), hepatitis B surface Antigen (HBs Ag)was done to exclude hepatitis B virus (HBV) infection.

Results: Among 300 school children, 43.7% of them were seroprotective. HBs Ab titre decrease significantly with increasing age and with increase duration post vaccination as seroprotection rates ($\geq$10 mIU/ml)in studied subjects from 6-10 y, 10-13 y and 13-17 years old were 69.5%, 25.2% and 5.3% respectively, also significant decrease was found in males than females. There was no significant association between HBs Ab titre and other factors that affect the immune response to HB vaccine. HBs Ag was negative in 100% of studied seronegative children.

Conclusion: Our results revealed that 43.7%of the studied children were HBV seroprotective. HBs Ab titre decrease significantly with increasing age, duration after vaccination and with male gender. Booster dose of hepatitis B vaccine should be considered to enhance the immune protection of the vaccine especially in our endemic area.

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Introduction:-

An estimated 2 billion persons worldwide have been infected with Hepatitis B Virus (HBV), and more than 350 million persons have chronic, lifelong infections [1]. There are approximately 620,000 HBV related deaths each year. In addition, approximately 4.5 million new HBV infections occur worldwide each year [2].

Worldwide, chronic hepatitis B causes 80% of all liver cancer, which is the 9th leading cause of death. Therefore, a vaccine that protects against hepatitis B infection can also help prevent liver cancer [3].

In 1992, Egypt started a program of universal immunization in infancy. The schedule adapted by the Egyptian Ministry of Health was three doses of a yeast- recombinant hepatitis B vaccine administered to all infants at 2, 4, 6 to coincide with other compulsory vaccines (Diphteria , Tetanus, Pertussis and oral polio(DPT-OPV).

Seroprotection against HBV infection was defined as having an anti- HBs level of ≥ 10 mIU/ml after having received a complete immunization schedule [4].

Anti-HBs antibody is the only easily measurable correlate of vaccine induced protection. Immunocompetent persons who achieve anti-HBs concentrations of >10 mIU/ml after preexposure vaccination have nearly complete protection against both acute disease and chronic infection, even if anti-HBs concentrations decline subsequently to <10 mIU/ml. After primary immunization with hepatitis B vaccine, anti-HBs levels decline rapidly within the first year and more slowly thereafter [5].

The aim:-

It was to evaluate the immune response among school aged children after hepatitis B vaccination according to EPI in Egypt, and to determine factors influencing the immune response to hepatitis B vaccine.

Subjects and methods:-

This cross-sectional study included 300 healthy school aged children attending Benha university hospital during the period from July 2014 to October 2015 after obtaining informed consent from parents of those children before the study. Selection of the sample was of systematic random sampling including every third student attended the clinic. Sample size was calculated using the formula $\{N = 4\sigma^2 (Zcrit + Zpowr)^2/D^2\}$(N= Total sample size which is the sum of the sizes of both comparison groups, σ = The standard deviation of each groups, assumed to be equal in both groups, Zcrit= The desired significance criterion, Zpowr = Desired statistical power and D = The minimum expected difference)[6].

All subjects were school age children (6-17 years old) who previously given three doses of recombinant hepatitis B vaccine at 2, 4 and 6 months during obligatory immunization schedule in Egypt, healthy with no history or clinical evidence of hepatitis B infection with exclusion of children who didn't complete the three doses of Hepatitis B vaccine and children with history or clinical evidence of symptomatic hepatitis B infection.

They were divided according to their HBs Ab level into group I [positive HBs Ab (seropositive=seroprotective) whose Anti-HBs Ab≥10 mIU/ml] and group II [Negative HBs Ab (seronegative) whose Anti-HBs Ab<10 mIU/ml].

All subjects included in this study were subjected to full history taking (age ,sex, residence, socioeconomic status according to Fahmy and El-Sherbiny scale[7],history of completion the 3doses of hepatitis B vaccine with emphasis on factors influencing the immune response to vaccine as smoking, obesity and any medical condition that compromise the immune system such as diseases or drugs).

Full clinical examination (weight and height to calculate Body Mass Index (BMI)[body weight(kg) \ height (m^2)] and a cutoff point of 30 is recognized internationally as a definition of obesity [8]) and laboratory investigations *(* Five ml blood was collected by venipuncture and allowed to clot, serum was separated by centrifugation then was kept frozen (-20˚C<onemonth) until used. HBs Ab was detected by ELISA using ETI-AB-AUK-3,Diasorin Kit for quantitative determination of HBs Ab, and for seronegative cases (Anti-HBs Ab<10 mIU/ml) HBs Ag was detected by ELISA using SURASE B-96 (TMB), Medical Technology Promedt Consulting Gmbh[9] to exclude hepatitis B virus infection) were performed to all subjects.

Statistical Analysis:-

Results were organized, tabulated and statistically analyzed using statistical package for social sciences (SPSS) software statistically computer package version 16. For quantitative data, the mean and standard deviation were calculated; the difference between two means was statistically analyzed using the student (t) test.

For qualitative data the number and percent distribution were calculated. Chi square was used as a test of significance and when found inappropriate fisher exact test was used. Interpretation of results was significant when p value<0.05.

Results:-

Three hundred healthy children were recruited in the study to evaluate the response to hepatitis B vaccine by measuring HBsAb titre. As regard demographic data(age, sex, residence and social class), there was significant

inverse association between age and HBsAb titre as HBsAb titre decreases with increasing age(P<0.001), significant decrease in HBsAb titre among males than females(P <0.05), no significant difference between positive and negative HBsAb titre groups as regard residence but we observed increase percentage of negative HBsAb subjects among rural (75.1%) than urban areas(24.9%) and no significant difference between the two groups as regard social class but we observed increase percentage of negative HBsAb subjects among low class(49.7%) followed by middle class (41.4%) then high class (8.9%)***(Table 1)***.

In our study negative HBsAb titre group (HBs Ab<10 mIU/ml) represented 56.3 % and positive HBsAb titre group (HBs Ab ≥ 10 mIU/ml) represented 43.7% ***(Table 2 &Fig 1).***

There was significant association between the duration from last dose of HB vaccine and HBs Abtitre as HBs Abtitre decrease with increasing duration from last dose of HB vaccine(P <0.001) ***(Table 3)***.

No significant difference between the two groups as regard obesity, smoking and medical conditions compromise immune system was detected (P >0.05) ***(Table 4).*** HBs Ag was negative in all children with negative HBs Ab titre.

In our study HBs antigen using ELISA was done for seronegative cases (Anti-HBs Ab<10 mIU/ml) to exclude hepatitis B virus infection and HBs Ag was –ve in 100% of studied seronegative children.

Discussion:-

Hepatitis B vaccine (both plasma derived and recombinant) has been shown to be highly efficacious in preventing infection with hepatitis B virus. Immunization with this vaccine starting at birth has dramatically reduced the subsequent development of chronic hepatitis B in young children from perinatal or early childhood exposure to HBV [10].

The study results revealed that 43.7% of the studied children were seroprotective (HBs Ab ≥ 10 mIU/ml) while 56.3% were seronegative (HBs Ab<10 mIU/ml). It was found that 47.9% of Iranian children had protective level of HBs Ab ≥ 10 mIU/ml at ten years after primary vaccination [11]. Other study reported that 53.8% were seroprotective and 46.2% were seronegative among 91 screened preschool children [12].

This study revealed that HBsAb titre decrease significantly with increasing age and with increase duration after vaccination as seroprotection rates (≥10 mIU/ml) in studied subjects from 6-10 years old, from 10-13 years old and from 13-17 years old were 69.5%, 25.2% and 5.3% respectively. This finding was in accordance with a study who showed that the seroprotection rates in those less than 5 years old, from 5 to 10 years old and more than 10 years old were 85%, 53.3% and 33.3% respectively [13] also in accordance with another study who showed statistically significant difference in the HBsAb titre between children tested at 5 and at 10 years indicating a decline of the titre with time [14].

As regard the relation between the gender and the antibody responses to HB vaccination, statistically significant decrease was found in males than females. These findings were in accordance with studies observed that males achieved lower geometric mean titers of antibodies and lower seroprotective rates compared to females[15] [16] [17]. In contrary other studies detected that males may retain HBsAb titres of higher values than females [14] and women were significantly more likely than men to experience a poor response to hepatitis B vaccine [18]. "Gender has no effect on the rate of positivity of HBsAb titer" was the result of a study showed that there was no statistical difference between boys and girls [4].

Our study showed that there was no statistically significant association between residence or social class and HBs Abtitre but we observed increase percentage of negative HBs Ab subjects among rural than urban areas and among low class followed by middle class then high class. Universal hepatitis B vaccination program(UHBVP) was less effective in socio-economically disadvantaged area and the long-term efficacy and immunogenicity of vaccination were modified by host factors and factors associated with urbanization, but none of these factors show significance if correlated with levels of HBs Ab [19].

Regarding other factors that affect the immune response to HB vaccine, our results revealed insignificant association between HBs Ab titre and obesity, smoking and medical conditions compromise the immune system, as few subjects

in our study had a BMI greater than 30, previous history of smoking or immunocompromised and this low number could explain why we were unable to demonstrate any effect of these factors on HBsAb titre.

A study compared growth and nutritional status of children with HBsAb<10mIU/ ml, with those having titre ≥ 10 and also by assessing growth of children who didn't respond to the additional dose of vaccine showed no statistically significant difference [13]. In contrary to that BMI were associated with reduced immune response [19].
Smoking is strongly associated with a poor response rate to hepatitis B vaccine [20].

Diseases associated with a reduced immune response are known to impair the vaccine response. Patients with end-stage kidney disease, irrespective of whether they are on dialysis or not, have a poorer immune response to hepatitis B vaccine [21].

Table (1):Comparison between our two groups positive HBs Abtitre mIU/ml and negative HBs Abaccording to some demographic data.

Parameter	Negative HBs Abtitre(<10mIU/ml) (169)	Positive HBs Abtitre(≥10mIU/ml) (131)	Test	p-value
Age mean± S.D (in years)	10.50±3.18	9.09±2.59	^4.2	<0.001*
Sex n(%) Male Female	98(57.9%) 71(42.1%)	61(46.6%) 70(53.4%)	$^{\epsilon}$3.9	<0.05*
Residence n(%) Rural Urban	127(75.1%) 42(24.9%)	103(78.6%) 28(21.4%)	$^{\epsilon}$0.5	>0.05
Social class n(%) High Middle Low	15(8.9%) 70(41.4%) 84(49.7%)	20(15.3%) 48(36.6%) 63(48.1%)	$^{\epsilon}$3.1	>0.05

^=Student t test ϵ= Chi square test *=significant n(%)= number(percentage)

Table (2): Distribution of subjects according to response to vaccine.

HBs Ab level	No.	%
Inadequate responders = Negative HBs Abtitre (below 10 mIU/ml)	169	56.3
Weak responders (10-100 mIU/ml)	101	33.7
High responders (100-1000 mIU/ml)	30	10
1000-10000 mIU/ml	0	0
above 10,000 mIU/ml	0	0

Table (3): Effect of duration from last dose of HB vaccine on HBs Abtitre

Age of studied subjects	Negative HBs Abtitre (<10mIU/ml)	Positive HBs Abtitre (≥10mIU/ml)	X^2	p-value
6-10 years old	90(53.3%)	91(69.5%)	15.7	<0.001*
10-13 years old	44(26.0%)	33(25.2%)		
13-17 years old	35(20.7%)	7(5.3%)		

*=significant

Table (4): Factors affecting immune response to HB vaccine

Parameter	Negative HBs Abtitre(<10mIU/ml)	Positive HBs Abtitre(≥10mIU/ml)	X^2	p-value
Obesity No Yes	167(98.8%) 2(1.2%)	129(98.5%) 2(1.5%)	0.1	>0.05
Exposure to smoking No Yes	167(98.8%) 2(1.2%)	129(98.5%) 2(1.5%)	0.1	>0.05
Medical conditions compromise immune system No Yes	159(94.1%) 10(5.9%)	124(94.7%) 7(5.3%)	0.1	>0.05

Conclusion:-

Our results revealed that 43.7%of the studied children were HBV seroprotective (HBs Ab≥10 mIU/ml). HBs Ab titre decrease significantly with increasing age, duration after vaccination and with male gender. Booster dose of hepatitis B vaccine should be considered to enhance the immune protection of the vaccine especially in our endemic area.

Recommendations:-

Strict emphasis on EPI including HB vaccine to avoid side effects and complications of hepatitis B virus infection. Booster dose of HB vaccine should be considered to enhance immune protection of the vaccine especially in our endemic area and to immunocompromised patients if their HBs Ab titre decreases below 10 mIU/ml. Further studies should be recommended to determine the recommended time for giving booster dose and to evaluate the response to booster dose in subjects with negative HBs Abtitre and the duration of protection after booster dose.

Acknowledgment:-

The authors thank parents of children for sharing in this scientific medical research.

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