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EVALUATION OF THE EFFICACY OF HEPATITIS B VACCINE IN DIFFERENT AGE GROUPS OF IMMUNIZED CHILDREN IN GAZA STRIP

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ABSTRACT: The objective of this study was to evaluate the long-term efficacy of hepatitis B virus immunization program in Gaza strip on preventing hepatitis B virus"HBV" infection, and assesses its impact on the incidence of hepatitis B in children.

The participants in this study were 180 children; 90 males and 90 females categorized into three age groups (2 – 5 y), (6 – 8 y) and (9 –11 y).

Serum samples from each participant were tested for the quantitative determination of anti-hepatitis B virus surface antigen (anti-HBs) antibody using microparticle enzyme immunoassay (MEIA) technology as well as immunochromatographic qualitative testing for hepatitis B surface antigen (HBsAg).

The study showed that the efficacy of hepatitis B vaccine was 98.3%. HBsAg was not detected in any participant. The protective level for the (2 – 5 y) and (6 – 8 y) groups were 100% while the protective level for the (9 – 11 y) group was 95% ($P \leq 0.04$). The study also showed that the efficacy of hepatitis B vaccine was statistically significant among the three age groups ($P < 0.04$), especially between (2 – 5 y) group and (9 – 11 y) group, as well as between (2 – 5 y) group and (6 – 8 y). The results of this study revealed that there was no significant difference between males and females for hepatitis B vaccine efficacy ($P > 0.05$).

We concluded that the hepatitis B vaccine efficacy is high in immunocompetent population and a booster dose is not necessary at least in the studied age groups, due to the existence of an anamnestic response.

key words: hepatitis b vaccine, hepatitis b surface antigen (hbsag), hepatitis b surface antibody (hbs antibody), antibody titer, efficacy.

Introduction

Palestine is considered an endemic area for HBV carriers; the prevalence among blood donors is 5% - 7%. Cultural and behavioral factors contribute to the viral transmission, including low socioeconomic status, high population density especially in Gaza strip, high household crowding and vertical transmission [1].

Universal hepatitis B immunization was introduced in Palestine on January 1st,1993. Like the other types of universal immunization, it is given free of charge to all children at 0,1 and 6 months of age. The Justification for its introduction was the estimated high prevalence of HBV infection among the population and the availability of a safe vaccine (S. Jamal et al., unpublished data, 1995).

The present policy of Ministry of Health is to examine routinely blood donors and to give pre-employment examinations in order to detect HBV carriers and those with human immunodeficiency virus (HIV). Screening for HBV – Positive contacts and immunization of those who are negative is free of charge [2]. The immunization coverage in Gaza Strip since the introduction of HBV vaccination reaches 97.7% [3].

This study aims at investigating the efficacy of hepatitis B virus vaccination program which is implemented in Gaza strip. The subjects who were chosen for this study were 2 -11 years old children.

Materials and methods

The study samples were collected between April to July 2005, they were composed of randomly selected 180 children, aged between 2 - 11 years. All the children received three doses of recombinant hepatitis B vaccine at 0,1 and 6 months of age. The study sample comprised three equal groups, 60 children in each group, (30 boys and 30 girls) at different post vaccination intervals following the completion of third dose of hepatitis B vaccine. The first group was 2 - 5 years, the second group was 6 - 8 years and the third group was 9 -11 years. The participants in the first group were recruited from children who were receiving health care at Al-Naser children's hospital while the second and third group were recruited from different primary schools of Gaza city.

Reagents

Two types of reagents were used in this study:

1. SD BIOLINE HBs Ag kits manufactured by standard Diagnostics, INC, Korea. They were used to detect HBsAg.

2. IMX AUSAB kits manufactured by Abbott Laboratories, diagnostics Division, Germany. They were used for the determination of HBsAg antibody titer.

Blood sampling and serological analysis

About 3 ml of venous blood were collected from each child. The sera were removed from the tubes after clotting and centrifuged. Serum samples were divided into three labelled sterile tubes to avoid repeated freezing and thawing. Serum samples were stored frozen at - 20°C in the Islamic University laboratory before being analyzed.

Data analysis

Data were analyzed using SPSS (statistical package for social science) program. The arithmetic mean of absolute values of anti-HBs titers were calculated for age groups and sex. The percentage of children who had antibodies titer less or more than 10 mlU/mL was calculated. Statistical significance was analyzed using one way analysis of variance (ANOVA) test for age group followed by Tukey's procedure to find the difference among pairwise contrast groups. Statistical significance between male and female was calculated by chi-sequare. Also the percentage was used to express the results of HBs Ag. The significance level that equals or less than (0.05) was considered.

Results

The study samples were divided into three age groups: 2 - 5Y, 6 - 8Yand 9-11Y as shown in tables (1) and (2). Blood samples were analyzed for HBsAg and anti-HBs antibodies. The results showed that three cases from 180 children had anti-HBs antibody titer less than 10 mlU/mL, those composed 1.7% from the total samples, while there were 177 children who had anti-HBs antibody titer more than 10 mlU/mL which represented 98.3% as shown in table (3). Those three children had anti-HBs antibody titer less than 10 mlU/mL, they were males and belonged to the 9 - 11 y age group. It was observed that in both age groups (2 - 5 y) and (6 - 8 y) there was similar results among males and females for the titer of anti-HBs antibodies. The percentage of 30 cases of females within 9 - 11 years was 52.6% while the percentage of 27 cases for males within the same group was 47.4% as shown in table (4).

Table (5) showed that three cases had anti-HBs antibody titer less than 10 mIU/mL (non protective), those three cases were from age group (9 - 11 y) and represents 5% in this age group, therefore the efficacy of vaccine for age

group (9 -11y) was 95%. Whereas no cases were found in age group (2 - 5 y) and (6 - 8 y) having anti-HBs antibody titer less than 10 mIU/mL (protective level). Therefore the efficacy for hepatitis B vaccine in the two groups was 100%. The efficacy of hepatitis B vaccine was also statistically significant in different age groups. {(x^2)=6.102, df=2, P≤ 0.04}.

To ensure the significant difference between the three age groups, one way analysis of variance for age groups (ANOVA) was conducted. There was statistical difference between age groups at (0.001) level of significance, where F value (6.88) at df (2,179) by using ANOVA test as shown in table (6). Pairwise comparison of means were followed up for age groups using Tukey's procedure. The pairwise comparison showed significant differences (P < 0.04) between the mean titer of age group (2 - 5 y) and mean titer for age group (6 - 8 years), there was a highly significant difference (P < 0.01) between the mean differences of age group (2-5 y) and age groups (9 -11 y). Whereas there were no significant differences between the means of age groups (6 - 8 y) and (9 -11 y) as showen in table (7). The study also revealed that there were three cases having anti-HBs antibody titer less than 10 mlU/mL. All of them were males and constituted 3.33% of the tested males. No statistical difference was found in the titer values between males and females using Chi-Square (x^2) = 3.051, df = 1,P> 0.05} as shown in table (8). The study also revealed that no cases were found positive for HBs Ag when examined for all age groups for both males and females as shown in table(9).

Table (1): Distribution of examined children according to age groups.

Age group	No.	%
2 – 5 years	60	33.3
6 – 8 years	60	33.3
9 – 11 years	60	33.3
Total	180	100

Table (2): Distribution of the examined children according to sex.

Sex	No.	%
Males	90	50
Female	90	50
Total	180	100

Table (3): Number of examined children for anti-HBs antibody titers less or more than 10 mlU/mL.

Titer (mlU/mL)	No.	%
1 – 9.9*	3	1.7
10 – 9999**	177	98.3
Total	180	100
* Non Protective group ** protective group		

Table (4): Distribution of both sexes for anti-HBs antibody less or more than 10 mlU/mL according to the age groups

Titer	Age group	Sex			
		Males		Females	
		No.	%	No.	%
Less than 10 mlU/mL	9 – 11 years (n = 3)	3	100	-	-
More than 10 mlU/mL	2 – 5 years (n = 60)	30	50	30	50
	6 – 8 years (n = 60)	30	50	30	50
	9 – 11 years (n = 57)	27	47.4	30	52.6

$(x^2) = 0.107, df = 2$, $P \leq 0.05$

Table (5): Distribution of anti-HBs antibody titer less or more than 10 mIU/mL according to age groups

Age group	Titer (mlU/mL)			
	1-9.9 (Not Protective)		10 – 9999 (protective group)	
	No.	%	No.	%
2 – 5 years (n = 60)	-	-	60	100
6 – 8 years (n = 60)	-	-	60	100
9 – 11years (n = 60)	3	5	57	95

Table (6): One way analysis af variance for age groups

	Sum of squares	Df	Mean Square	F	Sig.
Between Groups Within Groups Total	12880777.432 165685577.4 178566354.9	2 177 179	6440388.716 936076.709	6.880	.001

Table (7): Pairwise Comparison among mean differences for age groups

Means Groups	865.9 2 – 5 years	405.7 6 – 8 years	231.9 9 – 11 years
2 – 5 years	-	460.27*	634.03**
6 – 8 years	-	-	173.75***
9 – 11 years	-	-	-
* P < 0.04	** P < 0.01	*** p > 0.05	

Table (8): Distribution of anti-HBs antibody titer according to sex

Titer (mlU/mL)	**Sex**			
	Males (n=90)		**Females (n=90)**	
	No.	%	No.	%
1-9.9*	3	3.3	-	-
10-9999**	87	96.7	90	100
* Non protective group ** protective group				

$(x^2) = 3.051$, df = 1, P > 0.05

Table (9): Percentage of HBs Ag within age group and sex

Sex	N	Male %		Female	
Age groups		Negative	Positive	Negative	Positive
2 – 5 y	60	100	0	100	0
6 – 8 y	60	100	0	100	0
9 – 11 y	60	100	0	100	0
Total	180	100%		100%	

Discussion

The efficacy of hepatitis B vaccine in this study was high (98.3%) [table (5)]. This could be attributed to good vaccination coverage in Gaza strip and providing cold chain conditions to maintain the vaccine activity.

The obtained finding is in agreement with those studies reported by Lio et al [4], El-Sawy and Mohamed [5], Hsu et al [6], and Karaglu [7]. The efficacy of hepatitis B vaccine of previous studies were 92%, 93.3%, 94.1% and 96.7%, respectively. Whereas the vaccine efficacy in other studies which were conducted by Wildgrub et al [8], Pongpithead and Assateerwait [9], Lin and Ou-xang [10], Kuhil et al [1] were 85%, 88.2%, 85.4% and 85%; respectively. Studies of Yvonne et al [11], LiL et al [12], Nedelcu et al [13], XuH et al [14] and Garcia et al [15] showed efficacy of 78.1%, 79.2%, 66.3%, 65.8% and 70.6%, respectively.

The results obtained from previous studies support the notion that the efficacy of hepatitis B vaccine is variable from one study to another, this could be attributed to many reasons including: prevalence of hepatitis B disease "endemicity", vaccination coverage, vaccination schedule [16], variability in vaccine synthesis or preparation, defect in vaccine cold chain and differences in the methods used to evaluate antibody titer.

We conclude from table (5) that the efficacy of hepatitis B vaccine in (2-5 y) and (6 – 8 y) groups was 100%, then the efficacy begin to decrease in age group (9 - 11 y) to 95%, this decline is manifested by the presence of three children who had anti-HBs antibody titer less than the protective level. Non responders represented 5% of all subjects in the age group (9 - 11 y). The decline of hepatitis B vaccine efficacy in age group (9 - 11 y), could be attributed to decrease of anti-HBs antibodies titer with increasing age [17], or it may be due to decreased immune – reactivity of those children, however, those subjects need extensive investigations to determine the real cause.

This result is consistent with the previous studies reported by LiL et al [12], which revealed that hepatitis B vaccine efficacy had decreased after six years compared with the efficacy in the first years, and Lin et al [18] who

found that the percentage of protective anti-HBs antibody in vaccinated children gradually decreased from 71.1% in 7 years to 37.4% in 12 years old.

Also the findings of Hsu et al [6] and Karaglu [7] showed that the percentage of non responders was 3.3% and 2.3%, respectively and this is in agreement to some limits with the results of this study which revealed that the rate of non responders was 5%.

However, the finding of this study is not congruent with the study conducted by Kuhil et al [1] and Dahifer [19] who found that the percentages of non responders were 14.6% and 15.6%, these results are considered very high when compared with this study.

The simple main effects analyses and Tukey's pairwise comparisons were conducted to investigate the differences between the three age groups. The results of analyses showed that there was a significant difference between age groups (2 - 5 years) and (6 - 8 years). Also a highly significant difference found between (2 - 5 years) age group and (9 - 11 years) age group as shown in table (8) and (9). This could be explained again by decreasing the titer of anti-HBs antibody by increasing the age as well as many other factors related to immune response of each individual.

These results are consistent with the previous study reported by LiH et al [20] who found that the anti-HBs antibody titer declined from 75% in age group 1 - 2 years to 52.2% in age group 3 - 4 years, 45% of 5 - 6 years age group and 43.2% of 7 years age group. The findings are also in agreement with the study conducted by El-Sawy and Mohamed [5] which revealed a high seroprotection rate(93.3%) one month after vaccination, then the study proved that anti-HBs antibodies rate declined rapidly over time, at fifth year post vaccination, it was 53.3%. Also the results from Xia et al [21] study which revealed that the titers of anti-HBs antibodies were 40 - 50% and 30 - 42% during the 9 -10 year and 13 -14 year of vaccination, respectively. This same study indicated that the titer of anti-HBs antibodies declined by 90% at age of 14 years. Whereas the finding from tables (8) and (9) are not congruent with study conducted by Karaglu [7] which showed that it did not differ from the results in the different age groups and it is not in agreement with Sallam et al [17] study which indicated that there was no significant difference in antibody titer between age groups, however Sallam study found that there was trend of decreasing antibody titer with increasing age.

As shown in table (5) there was no case which had anti-HBs antibody titer less than 10 mlU/mL in age groups (2 - 5 years) and (6 - 8 years), this indicates that those two groups do not need booster dose. Whereas there

were three cases which consist 1.7% having anti-HBs antibody titer less than 10 mlU/mL in (9 - 11 years) group. The high efficacy of the first two groups could be attributed to anamenstic responses in immune competent population.

We concluded from the tested children samples that hepatitis B vaccine is an effective one. Hence there were three cases out of 90 males of the sample who had anti-HBs titer less than 10 mlU/mL. Therefore a monitoring test of anti-HBs for this age group (9 - 11 years) should be done to identify children who need a booster dose.

These results are in accordance with previous studies; XuH et al [14] reported that the effectiveness of hepatitis B vaccine still existed after primary immunization and no booster dose needed. Gracia et al [14] study showed that a good level of anti-HBs antibodies was found until (6 - 7) years after vaccination, therefore booster dose is not required. Also the results are in agreement with Lin and Ou-xang [10] study and Ayerbe et al [22], the first study showed that the efficacy of hepatitis B vaccine was long lasting and a booster dose was not necessary at least up to 11 years. The second study revealed that in immune competent population it is not necessary to administer a booster dose 6.5 years after hepatitis B vaccination. Another study conducted by Lin et al [18] indicated that the routine booster vaccination may not be required to provide protection before age 15 years.

However, the finding of this study are not in concordance with the study reported by Kuhil et al [1] which recommended the introduction of booster fourth dose after five years of immunization. Also, El-Sawy and Mohamed [5] study is not congruent with this study, which recommended a booster inoculation for all previously vaccinated children.

As shown in table (4) there were three cases having anti-HBs antibody titer less than 10 mlU/mL. All of them were males and represented 3.3% according to the sex comparison. Also the table showed that there was no statistical difference between males and females, this could be explained on the basis that both sexes were immunologically competent.

The current result is consistent with the findings obtained from Kuhil et al [1] study which revealed that there was no significant difference of results regarding sex. Also the previous studies conducted by Karaglu [7] and Sallam et al [17] which revealed that there was no difference in results regarding the sex. While the study conducted by Wildgrub et al [8] found that there was a high anti-HBs antibody concentrations occurred significantly more frequently in females than in males.

The HBs Ag test for samples showed that all children exposed to vaccine had negative results for HBs Ag as shown in table (9). This finding supports the conclusion that a high efficacy of hepatitis B vaccine exists.

The present findings of this study are in agreement with El-Sawy and Mohamed [5] study and Lio et at [4] which showed that HBs Ag was not detected in any participants and found a high seroprotection rate. Whereas Hsu et al [6] and Xia et al [21] studies are not in agreement with this study, they found positive HBs Ag between immunized children in the rate 2.5% and 1.7%, respectively. Also the study conducted by Karaglu [7] found 0.5% positive HBs Ag. Although our findings proved the absence of HBsAg in our subjects, others noticed that presence of positive HBs Ag is variable, this could be attributed to low immuno-reactivity of children, non vaccination or incomplete vaccination and high HBs Ag carriage rate among the mother's [23].

In the literature review, there were different methods used for evaluating HBsAg and its antibodies which are Enzyme-linked immunosorbent assay (ELISA), Radio immunoassay (RIA) and (MEIA). While in this study the methods used to test blood samples were MEIA for detection of anti-HBs antibody and in-vitro immune chromatographic one step assay designed for qualitative determination of HBsAg, these two methods are characterized by accuracy and rapidity when compared with ordinary ELISA which is time consuming and less accurate. Concerning RIA method, it is considered the most accurate method, however it has disadvantages including the exposure to radioactive material and difficulty in disposing the waste. The results of this study are comparable to those obtained by El-Sawy and Mohammad [5] who used the MEIA technique.

The emergence of HBsAg mutants Which may propagate in the presence of a neutralizing immune response should be taken into consideration . In addition , HBsAg mutants may yield false-negative results in some immunoassays(24). This new area of HBV infection research needs more attention.

Conclusions and recommendations

Hepatitis B vaccine efficacy is very high (98.3%), and HBs Ag was not detected in any participant in the study, this indicates that immunization has a significant impact on hepatitis B virus transmission, therefore we recommend the continuation of hepatitis B universal immunization program and increase the vaccine coverage in Gaza strip to 100%, also we emphasize giving the first dose of hepatitis B vaccine immediately after birth and ensure cold chain preservation. Based on the findings of our study there is a trend of decreasing antibody level with increasing age especially at age of 11 years, we recommend monitoring test of anti-HBs antibody for children aged 11 years and over to identify those who need a booster dose.. This study also found that there was no sex differences in antibody level, therefore the effect of hepatitis B vaccine on both sexes was comparable. Finally future studies should be conducted to cover other groups and in particular who are exposed to occupational hazards and studies evaluating other vaccination programs also should be conducted to explore shortcomings.

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