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Assessment of Protective Immunity and Protective Capacity of Hepatitis B Virus Vaccine for Children Living in Different Regions of Jordan

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Abstract:

Hepatitis B vaccine has been integrated into the current childhood immunization program in Jordan since 1995. Since that date, no studies were made for HBV vaccine assessment on Jordanian children. Main objective in our study is to evaluate the protective immunity levels induced by HBV vaccination among a school entry age group. Risk factors to hepatitis B infection were investigated and the protective capacity of the vaccine was studied. Blood samples were obtained from the study group, ELISA tests performed for all cases for anti-HBsAg, anti-HBcAg, and HBsAg. PCR performed for subjects that exposed to different risk factors. No hepatitis B virus was detected in all the blood samples, where all of them were negative to HBcAb, and the group of risk factors has negative results of HBV DNA and HBsAg. The response to HBV vaccine (Anti-HBsAg) values were classified according to child gender (sex), age and several risk factors in order to investigate any effect of each factor on the values of the Anti-HBsAg, after giving the vaccine. 78.9% of children at school entry age had more than 10 (>10 mIU/ml) of anti-HBsAg, so most children were protected from HBV infection, and this result agree with recent studies. 35.4% of the study children were exposed to different risk factors, which increase the probability of HBV infections. Presence of sub-protecting immunity suggests giving a booster dose at the age of school entry, to prolong and increase the protection immunity of school children**.** The levels of anti-HBsAg decrease with increase age and the immunity against HBV infection will be decreased too. The infection rate and vaccine response of HBV in both sex were almost the same.

Keyword: HBV, vaccine, protecting immunity, Jordan, anti-HBsAg.

Introduction:

The major aim of hepatitis B vaccination is the prevention of chronic infection, such as cirrhosis and hepatocellular carcinoma.

Because HBV-related cirrhosis and hepatocellular carcinoma usually occur in adults who were infected with HBV as children, decades must pass before the most significant benefits of HBV vaccination are

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realized [1]. Recent studies talk about contributing HBV vaccine to reduce infection cases at different ages. During 1990-2005, the overall occurrence of reported cases of acute hepatitis B declined to a great extent. In 2006, the estimated number of new HBV infections was about 46,000, a decrease from an estimated 232 thousands new infections in 1990 [2]. Hepatitis B vaccine has been successfully integrated into routine infant immunization in China, and the prevalence of hepatitis B surface antigen has been greatly reduced among children born after 1992 [3]. In Jordan, the World Health Organization (WHO) through Expanded Program on Immunization (EPI) added HBV vaccine to the national immunization programs in 1995 [4]. EPI supplies the vaccine to the Jordanian ministry of health. Since the start of EPI program in 1995, no studies were made for HBV vaccine assessment on Jordanian children. All recent studies showed the effect of the vaccine on reduction of HBV infections, this lead to decrease chronic liver disease and liver cancer. When we talk about children at school entry age, it was found that children at this age have many of risk factors expose, such as visit dentists frequently that include using surgery tools in dental surgery room. If some these surgery tools are unsterilized, this will increase risk factors for hepatitis B virus infections. In a previous study in Jordan, Al-Omari and Al-Dwairi (2005) found that only 41.8% of dentists had sterilized their surgery tools in contrast to other studies that found higher rates of sterilization [5]. Many survey's respondents who did not sterilize their surgery tools, think that sterilization by autoclaving could damage their tools. The study shows that dental surgery operations have a high risk factor to variety of infectious transmissions. This agrees with the findings of a previous study [6]. In addition, when children, especially males, go to barbers (hair saloons), using of unsterilized shaving equipment will increase the probability of risk factors for hepatitis B virus infections. Adding to that, surgery operations and blood transfusions increase probability to HBV infections. Main objectives of this work are chosen to evaluate the protective immunity levels induced by HBV vaccination among a school entry age group. In addition, risk factors to hepatitis B infection will also be investigated and the protective capacity of the vaccine was studied.

Materials and Methods:

Sample study

Children at school entry age (5, 6, and 7 years) were chosen for this study. Samples were taken from different sources in different regions in Jordan (schools and outpatient clinics). The total number of samples (children) was 1000.

Questionnaire forms:

Each child had one questionnaire form; this form contained a number of questions about the research subject. It included approval from parents to make an interview, and to take blood sample from the child. Each child had been interviewed in the presence of his parents and was briefed about the study objectives. A few numbers of children parents disagreed to take blood from their children and these were not including in the study. Upon agreeing to participate in the study and the approval of the child to donate his blood sample, parents signed a consent form and filled a questionnaire form containing the detailed demographic data of the child in addition to his full medical history.

Blood treatment

Venous blood samples (3-5ml) were obtained by vacutainor into plane tubes (non-oxalate tube). Blood samples were placed in an ice box to transfer to the laboratory and serum separation was done by centrifugation at 4000-5000 rpm/min for 5 minutes. Serum samples were frozen at -80C^{o} until performing the immunological assays.

Immunological assay

Detection and quantification of antibodies to Hepatitis B surface antigen (anti-HBs) in human serum by ELISA. Detection of total

antibodies to hepatitis B core antigen (anti-HBc) in human serum or plasma by ELISA. Detection of hepatitis B surface antigen (HBsAg) in human serum by ELISA.

Detection of Hepatitis B virus DNA using Polymerase Chain Reaction (PCR)
Quantitative PCR tests particularly based on real time PCR have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. Quantitative data is used to assessing a person's infection status, to support therapeutic decisions and to monitor treatment.

Data analysis and biostatistics
SPSS (version 15.0) program were used for applying the chi-square test for association as well as other measure of association called the Odds Ratio (OR) whenever it is applicable.

Results:

Study of population:
All cases were interviewed for the various risk factors to find out the possibility for HBV infection in their communication. The risk factors distributions were dental procedures, shaving from community barber, past surgical operations and blood transfusion. The percentage of children who have risk factors to HBV infection was 35.4%, some of them are going to dental surgery periodically (26.7 %), and some of them are using sharp blade in hair salons (21.8 %), others have some chronic diseases that need blood transfusion (thalassemia, hemolytic anemia, renal failure) (3.1 %) and finally there is a group of cases under risk exposed to surgical operations (5%). Table (1) shows the characteristics of samples.

Table 1: characteristics of the sample (*n*=1000).

Subject	No. of samples (*n*)	Frequency (%)
Age (years)		
5 years	255	25.5
6 years	589	58.9
7 years	156	15.6
Sex		
Male	543	54.3
Female	457	45.7
Risk of HBV infection		
Group not under risk	646	64.6
Group Under risk	354	35.4
1- Blood transfusion	31	3.1
2- Surgery operation	50	5.0
3- Dental procedure	267	26.7
4- Shaving (sharp blade in hair salons)	218	21.8

Immunodiagnostic assays:
For studding if any of our cases have active infection with HBV, HBsAg are measured by ELISA. The result shows that HBsAg was negative for all cases. HBV-PCR confirms the result of ELISA.
HBcAb wase measured for all subjects enrolled in the study to measure past infection with HBV. All cases were negative to HBcAb. Table (2) shows different assays for HBV immunodiagnostic.

Immune protection levels:
The current study shows that 78.9% of children have protective level against HBV according to the response to HBV vaccine.
In the following results, the response to HBV vaccine (Anti-HBsAg) values were classified according to child gender, age and several risk factors in order to investigate any effect of each factor on the values of the Anti-HBsAg (immunoprotection) after giving the vaccine (efficacy of vaccine on long term).

Anti-HBsAg titer levels according children sex (gender):
Data showed that 78.6% of female hase level of Anti-HBs antibody higher than 10 mIU/ml, while 79.2% of male hase the same results. 21.4 % of female shows no protection level against HBV (level of anti-HBs less than 10 mIU/ml) and that is not significantly difference than male (20.8%)
The value of $\chi 2 = 0.06$ compared with the tabulated value (3.841) indicates no significant association between gender and Anti-HBsAg levels at significant level of $\alpha = 0.05$. Table (3) appears that.

Anti-HBsAg titer levels according children age:
Table (4) shows that protection level (> 10 mIU/ml) of antibodies against HBsAg is 78.8% for 5 years old children , 79.5% for 6 old year's children and 76.9% for 7 years old group. $\chi^2 = 0.477$ compared with the tabulated value of (3.841) indicates no significant association between age and Anti-HBs levels at significant level of $\alpha = 0.05$.
However the data means that there is no strong relationship between result of protection and age, but it also shows that level of protection decrease in children at age 7 than other younger children. Table (5) shows that this decrease is clearer at level of protection higher than 100 mIU/ml.

Anti-HBsAg titer levels according Risk factors:

Rate of anti-HBs in children that have blood transfusion risk factor:
Table (6) shows slight difference between the 2 groups of the children (those with risk of blood transfusion and those without) in terms of the % of high (>10) Anti-HBs which were 74% and 79% respectively. Children with risk of blood transfusion showed lower proportion of anti-HBs.
Also the value of $\chi^2 = 0.426$ compared with the tabulated value of (3.841) indicates no significant association between risk of blood transfusion and Anti-HBs levels at significant level of $\alpha = 0.05$
The value of Odds Ratio (OR=1.32) indicates a low association between risk of blood transfusion and Anti-HBs levels. It indicates that each 100 cases of Anti-HBs >10 among children with blood transfusion, it is expected that this number to be increased up to 132 cases among children free of such risk.

Rate of anti-HBs in children that have surgery operations risk factor:
Table (7) shows almost no difference between the 2 groups of the children (those with risk of surgery operation and those without) in terms of the % of high (>10) Anti-HBs which were 78% and 79% respectively. Children with risk of surgery operation showed lower proportion of Anti-HBs.
Also the value of $\chi^2 = 0.026$ compared with the tabulated value of (3.841) indicates no significant association between risk of blood

transfusion and Anti-HBs levels at significant level of $\alpha = 0.05$

The value of Odds Ratio (OR=1.06) indicates almost no association between risk of surgery operation and Anti-HBs levels. It indicates each 100 cases of Anti-HBs >10 among children with surgery operation, it is expected that this number to be increased up to 106 cases among children free of such risk.

Table (2): HBV immunodiagnostics.

Assay	**Samples**	**Results**
HBsAg	1000	Negative
HBcAb	1000	Negative
HBV-PCR	354*	Negative

* Cases with risk factors for HBV infection.

Table (3): Rate of Immunoprotection (Anti-HBs) according to Gender.

Gender	Anti – HBsAg (mIU/ml)				Total	
	0.0 – 10*		> 10**			
	n	%	*n*	%	*n*	%
Female	98	(21.4)	359	(78.6)	457	(100)
Male	113	(20.8)	430	(79.2)	543	(100)
Total	211	(21.1)	789	(78.9)	1000	(100)

$\chi^2 = 0.06$ *= Non Protective level **= Protective level

Table (4): Rate of Immunoprotection (Anti-HBs) according to Age.

Age	Anti – HBsAg (mIU/ml)				Total	
	0.0 – 10		> 10			
	n	%	*n*	%	*n*	%
5	54	(21.2)	201	(78.8)	255	(100)
6	121	(20.5)	468	(79.5)	589	(100)
7	36	(23.1)	120	(76.9)	156	(100)
Total	211	(21.1)	789	(78.9)	1000	(100)

$\chi^2 = 0.477$

Table (5): different level of Immunoprotection (Anti-HBs) in different Age.

Age	Anti – HBsAg (mIU/ml)						Total	
	0.0 – 10		11 – 100		> 100			
	n	%	*n*	%	*n*	%	*n*	%
5	54	(21.2)	119	(46.6)	82	(32.2)	255	(100)
6	121	(20.5)	307	(52)	161	(27.5)	589	(100)
7	36	(23)	89	(57)	31	(20)	156	(100)
Total	211	(21.1)	515	(51.5)	274	(27.4)	1000	(100)

$\chi^2 = 7.844$

Table (6): Rate of immune protection (Anti-HBs) in cases exposed to Blood Transfusion.

Blood Trans. Risk	Anti – HBsAg (mIU/ml)				Total	
	0.0 – 10		> 10			
	n	%	*n*	%	*n*	%
No	203	(21)	766	(79)	969	(100)
Yes	8	(26)	23	(74)	31	(100)
Total	211	(21.1)	789	(78.9)	1000	(100)

$\chi^2 = 0.426$ and OR = 1.32

Table (7): Rate of immunoprotection (Anti-HBs) in cases exposed to surgical operation.

Surg. Risk	Anti – HBsAg (mIU/ml)				Total	
	0.0 – 10		> 10			
	n	%	*n*	%	*n*	%
No	200	(21)	750	(79)	950	(100)
Yes	11	(22)	39	(78)	50	(100)
Total	211	(21.1)	789	(78.9)	1000	(100)

$\chi^2 = 0.026$ and OR = 1.06

Rate of anti-HBs in children that have dentist visit risk factor:

Table (8) shows almost no difference between the 2 groups of the children (those with risk of dentist visit and those without) in terms of the % of high (>10) Anti-HBs which were 78.7% and 79% respectively. Children with risk of dentist visit showed lower proportion of Anti-HBs.

Also the value of $\chi^2 = 0.013$ compared with the tabulated value of (3.841) indicates no significant association between risk of dentist visit and Anti-HBs levels at significant level of $\alpha = 0.05$.

The value of Odds Ratio (OR=1.02) indicates almost no association between risk of dentist visit and Anti-HBs levels. It indicates that each 100 cases of Anti-HBs >10 among children with dentist visit, it is expected that this number to be increased up to 102 cases among children free of such risk.

Rate of anti-HBs in children that have blade use in hair salons risk factor:

Table (9) shows some difference between the 2 groups of the children (those with risk of blade use in hair salons and those without) in terms of the % of high (>10) Anti-HBs which were 76.1% and 79.7% respectively. The value of $\chi^2 = 1.27$ compared with the tabulated value of (3.841) indicates no significant association between risk of blade use and Anti-HBs levels at significant level of $\alpha = 0.05$.

The value of Odds Ratio (OR=1.23) indicates some association between risk of blade use in hair salons and Anti-HBs levels. It indicates that each 100 cases of Anti-HBs >10 among children with blade use, it is expected that this number to be increased up to 123 cases among children free of such risk.

Table (8): Rate of immunoprotection in cases exposed Dental procedure.

Dental Risk	Anti – HBsAg (mIU/ml)				Total	
	0.0 – 10		> 10			
	n	%	*n*	%	*n*	%
No	154	(21)	579	(79)	733	(100)
Yes	57	(21.3)	210	(78.7)	267	(100)
Total	211	(21.1)	789	(78.9)	1000	(100)

$\chi^2 = 0.013$ and OR = 1.02

Table (9): Rate of immune protection (Anti-HBs) in cases exposed to shaving tools (as blade).

Blade use Risk	Anti – HBsAg (mIU/ml)				Total	
	0.0 – 10		> 10			
	n	%	*n*	%	*n*	%
No	159	(20.3)	623	(79.7)	782	(100)
Yes	52	(23.9)	166	(76.1)	218	(100)
Total	211	(21.1)	789	(78.9)	1000	(100)

$\chi^2 = 1.27$ and OR = 1.23

Discussion:

A safe and effective vaccine against HBV infection has been available for nearly 30 years. HBV vaccine is effective in preventing HBV infections when it is given either before exposure or shortly after exposure. At least 85%-90% of HBV-associated deaths are vaccine-preventable [7].

Jordan is a country with intermediate endemicity for hepatitis B virus (HBV) infection [8] where early childhood horizontal transmission was considered the predominant method by which HBV infection endemicity is maintained [9]. Hepatitis B vaccine has been integrated into the current childhood immunization program in Jordan since 1995. It is expected that there is a shift away from childhood with the integration of HBV vaccines into the Jordanian Expanded Program on Immunization.

Ayerbe et al., (2001) studied the assessment of long term efficacy of HBV vaccine. He carried out a follow up for 462 subjects in which HBV vaccine administered between 1990 and 1992 to determine the duration of protection. He concluded that the protection level is stable at 6.5 years after vaccination and no need for booster dose [10]. The two-dose schedule of hepatitis B vaccine confers long-term immunogenicity and shows evidence of immune memory for at least five years following vaccination [11]. No previous studies were made for HBV vaccine assessment on Jordanian children.

In current study, we chosen children from school entry age (5-7 years) to test persistence of antibodies and immune memories against HBV by measuring the blood levels of anti-HBsAg antibodies that is induced by the HBV vaccination. Response to HBV vaccine (Anti-HBsAg) values were classified according to child gender, age and several risk factors in order to investigate any effect of each factor on the values of the Anti-HBsAg after giving the vaccine .

Immunodiagnosis of all cases enrolled in our study showed that all vaccinated children have neither HBsAg nor anti-HBcAb in their blood samples. That support fact HBV vaccine ability to protection the body from infection by adequate level of anti-HBsAg antibodies. These results agreed with several previous studies that were carried out to estimate the efficiency of HBV vaccine in infection prevention. In 2006, the estimated number of new HBV infections was about 46,000, in compared with 232,000 new infections in 1990 [2]. HBsAg seroprevalence among Taiwanese children decreased from 9.8 % in 1984, the year when universal infant immunization began, to 0.7 % in 1999 [12]. In Malaysia, which introduced universal infant vaccination in 1990, HBsAg seroprevalence among schoolchildren (ages 7–12 years) decreased from 1.6 % in 1997 to 0.3 % in 2003 [13].

The results of this study showed no apparent sex differences in immune response for HBV vaccination. Males and females showed no significant statistical differences in the anti-HBsAg levels. While in a previous study (wasley et al., 2008) in USA, the data showed that the infection percentage in males was more than females, for males it was 1.9 % per 100,000, and 1.2 % per 100,000 in

females. But in a chronicle revision (between1990-2007), in the same study, the infection rates in both sexes were almost the same In contrast to that [14]. Yvonnet et al., (1987) results confirm that there is better immune response to HBV vaccine in females than in males [15].

This study also showed a little difference (but not significant) in the decreasing of immunoprotection with age. It showed that children at the age of five years and six years were almost having the same concentrations of anti-HBsAg, but compared to seven years children, the levels were decreased. This implies that levels of the antibodies will be decreased with age, and the immunity against HBV infection will be decreased too. Previous studies showed that the prevalence of anti-HBs alone decreased as the subjects' age increased [16].

The immunity after ten years of the last dose decreased to 64%, with more than 10 (>10 mIU/ml) [17]. Previous Cross-sectional studies were conducted over a decade among school children in an endemic area in Egypt for screening of hepatitis B (HB) virus markers. The persistence of protective titer of anti-HB surface among 54% of vaccinated children ages 6-10 years was encouraging [18].

Khan et al. (2011) [19] cited that important factors contributing to HBV spread (risk factors) include unsafe use of therapeutic injections [20], blood transfusion [21], tattooing [22], mother to child transmission [23] and unsafe sexual practices [24, 25].

In our study, we randomly interviewed for the various risk factors to find out the possible way of HBV infection of school children. Results showed that there was 354 children (35.4%) had risk factors for HBV infection, such as going to dentists, exposed to sharp tools in the hair cut saloons, and some had blood transfer. Samples have dentist visit risk factor which the most of all risk factors in our study. This category of children goes regularly to dentists, so they are exposed to sharp tools, and there is probability for insufficient sterility of these tools, which represent an implicit source of viral infections, including HBV infection. In our study, 26.7% (267 out of 1000) of the chosen children were going regularly to dentists. All of these children took the HBV vaccine, and all were uninfected with HBV, and this indicates that the vaccine was efficient and had prevented the HBV infection. Recent studies in Jordan, Hayajneh et al., (2010) identified unhygienic dental care as an independent risk factor for acquisition of HBV infection [26]. This finding was not unexpected since several recent studies from Jordan [27], and previously elsewhere [28] have shown significant lack of compliance with infection control practices by dental personnel. One of these studies [5] showed that only 60% of private dental clinics use autoclaves for disinfection and only 50% change hand pieces or use plastic wrappings for sterilized instruments [29]. That is appeared the transmission of HBV in the dental clinic is a potential hazard.

The results of this study reinforce the fact that HBV vaccine prevents the infection with the HBV virus by preventing the completion of the virus life cycle, using the anti-HBsAg antibodies, where it had a large contribution in this prevention. Recent study appears that the most effective approach to preventing HCC is to prevent HBV infection through vaccination. Indeed HBV vaccine is the first vaccine demonstrated to prevent cancers [30]. Another study shows that the incidence of HBV notifications in children born after the introduction of targeted childhood HBV vaccinations is lower compared with the incidence in children born before the start of this vaccination programme. Although this is consistent with a good HBV vaccine coverage, the interpretation is hampered by a change in case definition for notification in 1999 [31].

In addition, because this age was chosen due to its importance and because they are exposed to a number of risk factors that will increase the infection rate with HBV. In this age category (5-7 years), it is supposed that the vaccine is in its late efficiency period, where literature states that the vaccine should

be active 5-7 years after the last vaccine dose [32, 33]. A booster dose of HBV vaccine every 5-6 years should provide adequate protective anti-HBs levels [15]. So, it is suggested to give a boosting dose at the age of school entry, to prolong the protection period against HBV infections for five more years.

How long protection lasts for children who are vaccinated in infancy has not been established [34]. At the present time, there are no assays to measure correlates of protection against HB infection directly [35]. It is therefore possible that immunological memory itself depends on the development of a protective level of antibodies after vaccination [36].

The strong anamnestic response following the challenge dose regardless of the priming schedules provides the evidence of strong immunological memory for at least five years following vaccination [11]. A previous study on Austrian children showed the Austrian Advisory Committee on Immunization Practices ACIP recommends a booster vaccination against hepatitis B at the age of 7–13 years [37]. Considering the high percentage of children with questionable immunity against Hepatitis B in study this recommendation seems to be justified [38]. The recommendation is also supported by a recent study by Zanetti et al.: in children 5 years after primary immunization with Hexavac® and antibody levels below 10 mIU/mL in 62%, satisfactory antibody responses to a hepatitis B booster dose of at least 10 mIU/mL were detected in 92% [39].

This study with the current variables is the first in this field in Jordan; no previous studies were done on the HBV vaccination and children at school entry age.

Conclusion:

In conclusion, the data of present study found that, 78.9% of children at school entry age contained more than 10 (>10 mIU/ml) of anti-HBs antibodies, so most children were protected from HBV infection. 35.4% of the study children were exposed to different risk factors, which increase the probability of viral infections, such as HBV infections. The HBV vaccine was efficient and had prevented the HBV infection by preventing the completion of the virus life cycle, using the anti-HBsAg antibodies.

Presence of sub-protecting immunity suggests giving a booster dose at the age of school entry, to prolong and increase the protection immunity of school children. The levels of anti HBs antibodies decrease with increase age, and the immunity against HBV infection will be decreased too. The infection rate and vaccine response of HBV in both sex were almost the same.

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