Evaluation of Hepatitis B Virus Vaccination among Children in Al-Diawynia City.

-Amina N. Al-Thwani, Head of Genetic Engineering and Biotechnology Institute for Postgraduate Studies, Baghdad University.
-Nihad A.M. Al-Rashedi, Biology Department, Science College, Al-Muthana University.
-Ali R. Omer, Public Health Laboratory, Baghdad Health Cycle.

الخلاصة

هدفت الدراسة تحديد المستوى الواقي لمضادات التهاب الكبد البائي عند الاطفال في مدينة الديوانية الذين تتراوح أعمارهم ١ - ١٠ سنة مجموع عينة وفحصت لكشف المؤشرات المصلية لفايرس الكبد البائي السطحي ومضاد التهاب الكبد البائي السطحي ومضاد التهاب الكبد البائي اللبي(تمثل مائة وخمسون من الاطفال مجموعة الملقحين وسبعون من الاطفال غير الملقحين)لا توجد نسبة انتشار لمولد مضاد التهاب الكبد البائي السطحي ضمن الاطفال وجد انتشار اعلى لمضاد التهاب الكبد البائي السطحي لمجموعة الملقحين (٦٧٠٣٪)من مجموعة غير الملقحين ٤,٢٪ تسجل نسبة انتشار مضاد التهاب الكبد اللبي في مجموعة الملقحين،بينما كانت ٥,٧٪ في مجموعة غير الملقحين.

أوضحت الدراسة قياس المستوى الواقي لمضاد التهاب الكبد بمستويات أقل من ١٠ و ١٠ – ١٠٠ و ١٠٠ – أكثر من ٥٠٠ ملي وحدة دولية/بين المجاميع المدروسة والتي أعطت ١٤ (٩,٣٪) و ٣٤ (٢٢,٦٪) عند ٥٣(٣٥,٣٪) الملقحين و ٠,٠٪ و ١(١,٤٪) و عند(غير الملقحين كما سجل مضاد التهاب الكبد البائي السطحي في ٦٪ للاطفال الملقحين الذين أكملوا التلقيح بثلاث جرعات هذا يوضح تأثير اكمال السير الاولي للقاح على مستوى مضاد التهاب الكبد البائي السطحي.

Abstract

This study attempts to determine the protective level of antibodies against hepatitis B surface antigen among vaccinated and unvaccinated children in Al-Diawynia city, age ranging 1- 10 year. A total of 220 blood samples have been collected and tested for detection of serological markers of hepatitis B virus (HBsAg, anti-HBs and anti-HBc). They included 150 children represented the vaccinated group and 70 children represented the unvaccinated group. Prevalence of anti-HBs was higher in vaccinated children 67.3% than in unvaccinated children 3.3%. Anti-HBc was not found in vaccinated children, while 4.2% in unvaccinated children. The distribution of protective level is divided to less than 10; 10-100; and 100-more than 500 mIU/ml among studies groups that have anti-HBs 14(9.3%); 34(22.6%); and 53(53.3%) of vaccinated and (0.0%); (1.4%); and 2(2.8%) of unvaccinated group. Finally, prevalence of protective anti-

HBs level according to the doses received by vaccinated group has revealed that 66% of those who received the three primary doses.

Introduction

Viral hepatitis B caused by hepatitis B virus (HBV) constitutes a major economic and public health problem throughout the world, particularly in the developing countries including the Middle East, where it is responsible for considerable morbidity and mortality and the development of a chronic carrier state which may end in chronic hepatitis, liver cirrhosis, and in some cases may give rise to hepatocellular carcinoma (1). Infection in infancy and early childhood is strongly associated with progression to chronic HBV infection, contributing to a significant proportion of chronic liver diseases (3). It has been estimated that more than one third of the World's population has been infected with HBV leading to the deaths of 1 – 2 million people every year, and there are nearly more than 350 million chronic carriers of hepatitis B (4, 5).

The acute and chronic of HBV infections are a serious health problem in Iraq .The reported prevalence of the disease is 3-4 % among normal Iraqi population, which is among the countries of intermediate hepatitis B endemicity (6). Countries with intermediate or high endemicity must have effective immunization programme for all infants at birth (7, 8). Hepatitis B vaccines have been available since 1982, and well in excess of 1 billion doses have been used. Hepatitis B vaccine is safe and effective that could prevent most of this infection. In 1992, the World Health Assembly recommended that hepatitis B vaccines should be integrated into national immunization programs (9,10). The newborn infants are now routinely vaccinated for HBV. The series of immunizations consists of three injections of the hepatitis B antigen given over a period of 6 months. Immunity is conferred after vaccination and proven by finding a titer greater than 10 mIU/ml of surface antibody in the vaccine recipient (11, 12). In Iraq, although several studies have been carried out on prevalence of HBV markers few studies have been done to determine the protective antibody level after HBV vaccination (6, 13, 14).

Materials and Methods

Blood sampling: Blood samples were obtained from each subject through a venopuncture using 5 ml syringes with needle gauge 23 transported to unhaprized tube. The blood was left for a while at room temperature to clot. The serum was separated from collected blood by

centrifugation at 3000 RPM for 5 minutes and transferred into labeled tube and stored at 4° C for one week, if not used immediately.

Determination of HBsAg: The HBsAg kit used two highly specific monoclonal antibodies, directed to different and sterically distant epitopes of the common "a" antigen that could be found on the surface antigen of HBV .One of the two monoclonal antibodies is bound to microplate while the second one is conjugated with peroxidase (HRP). The two antibodies form a "sandwich" complex with the antigen when present in the sample by means of a two steps analytical system. After the final washing step, the enzymatic activity is detected by incubation with the mixed specific chromogen / substrate (TMB-peroxide). The intensity of the generated color is proportional to the amount of antigen in the specimen.

Determination of anti-HBs: An enzyme linked fluorescent immunoassay (ELFA) was performed to detect Anti-HBs by using Vitek Immunodiagnostic Assay system (VIDAS). All of the assay steps are performed automatically by VIDAS instrument. Once the assay is completed, the computer analysis results automatically. A titer is greater than 10 mIU / ml indicates that antibody is protective.

Determination of anti-HBc: The assay was based on a competition between human antibodies present in the sample and rabbit IgG anti-HBc conjugated to peroxidase (HRP) when they were simultaneously incubated in a well coated with recombinant HBcAg. After incubation and washing to remove unbound material an enzyme substrate solution containing a chromogen was added. This solution will develop a blue colour if the sample is negative. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The presence of anti-HBc in the sample will reduce the development of colour proportionally to its concentration.

Results and Discussions

HBV vaccination introduced in Iraq in 1986 for risk groups as part of Expanded Program of Immunization (EPI) .The provision of routine vaccination of risk groups and all infants together with rigorous screening of blood donors for HBsAg which was introduced routinely in 1980, in addition to the use of disposable syringes and transfusion sets with general hygienic measures, were responsible for the decline of HBsAg prevalence in the country as whole (15).

The main goal of our research aimed to estimated the prevalence of HBsAg as a serological marker, the protective level of anti-HBs and anti-HBc level among 220 serum samples collected from vaccinated and unvaccinated children at 1-10 years age. Two hundred and twenty

samples, there taken from Al-Diawynia city, which included 150 vaccinated with and 70 unvaccinated children. Of the 150 vaccinated children 95 (63.3 %) were males and 55 (36.6 %) were females. While of the 70 unvaccinated children 20 (28.5 %) were males and 50(71.4 %) were females. Result presented in this study, showed that prevalence of HBsAg was not present in vaccinated and unvaccinated groups (0.0 %). The difference between the two groups appeared clearly due to the presence anti-HBs in vaccinated and unvaccinated sera which found in 101(67.3%)-(t.test=0.93, p=0.45) and 3(4.2%) ,respectively. Anti-HBc was not presented (0.0%) in vaccinated children and only 4(5.7%) of unvaccinated children had these antibodies in their sera (Table 1).

Table 1: Prevalence of HBsAg, anti-HBs and anti-HBc among the Studies groups.

Groups	No. Tested	HBsAg		Anti-HBs		Anti-HBc	
		No.	(%)	No.	(%)	No.	(%)
Vaccinated children	150	Nil	(0.0)	101	(67.3)	Nil	(0.0)
Unvaccinated children	70	Nil	(0.0)	3	(4.2)	4	(5.7)

The prevalence rate of HBV carriers in neighbour countries of Iraq are clerafied the moderate endemicity of hepatitis B infection in these countries . Also the researchs in Iraq indicated that Iraq is among countries of intermediate hepatitis B endemicity. The latter researchers mentioned that the highest prevalence of HBsAg is seen in 15-20 year of age and varies with very low ratio of HBsAg positive in infants (6). Most HBV infections among children under 16 year of age in U.S.A.(16). The effectiveness of universal HBV immunization programme has been demonstrated in many countries, Taiwan was one of the first areas to introduce mass HBV immunization, in July 1984, and over the following 10 year the prevalence of HBsAg antigenemia dropped from 9.8% to 1.3%, anti-HBc prevalence fell from 26% to 4%, and 79% of the population had developed protective anti-HBs antibodies(17). Similarly, in China the prevalence of HBsAg dropped from 8.2% to 0.3% over a 15-year period (17); and in Saudi Arabia it

dropped from 6.7% to 0.3% over the period 1989—1997 (18). The impact of universal childhood HBV vaccination programme in South Africa to reducing HBsAg carriage in the first five years (1995-1999) . Among of 598 babies (mean age =23.3 months) who received 3 doses , the overall seroprotection rate was 86.8 % in vaccinated babies .It can be concluded that the HBV vaccine is highly effective with in the framework of the South Africa and already shows a positive impact in the elimination of HBsAg carrier rate in children < 5 years (19) .

This study shed light on the connection or relationship between serological markers and age of children. The results revealed that the age groups, 1 – less than 2 , 2 – 4 , 5 – 7 , 8 – 10 years of HBsAg and anti-HBc (0.0%) for all of them, while the anti-HBs were 43(71.6%); 27(67.5%); 19(63.3%) and 12(60%) of vaccinated groups. It seemed that generally level of anti-HBs remain stable range until 8-10 years, then started with the decline as shown in Table (2).

The protection against HBV of vaccinated children in believed to persist for at least 15 years after successful immunization (20). The relation between anti-HBs level and age among vaccinated group decline with over 5 yeas of their life(21). In another study indicated seroprotection rates and geometric mean titers decreased significantly with increasing age possibly reflecting waning anti-HBs titer over timer(22) , but in some cases such as no completed course of three vaccine doses can be possible to believe no persist more than 5 years(23).

Table 2: Age distribution of the prevalence of HBsAg, anti-HBs and anti- HBc among vaccinated groups.

Age group	No.	HBsAg		Anti-HBs		Anti-HBc	
		No.	(%)	No.	(%)	No.	(%)
1–less than2 years	60	Nil	(0.0)	43	(71.6)	Nil	(0.0)
2– 4 years	40	Nil	(0.0)	27	(67.5)	Nil	(0.0)
5 – 7 years	30	Nil	(0.0)	19	(63.3)	Nil	(0.0)
8 –10 years	20	Nil	(0.0)	12	(60)	Nil	(0.0)
Total	150	Nil	(0.0)	101	(67.3)	Nil	(0.0)

According to this study the result of vaccinated group showed 62(65.2%) males (t.test=1.067 d.f.=62) and 39(70.9%) females (t.test=1.053, d.f=38) had anti-HBs, while both of them were anti-HBc negative 0.0%, anti-HBs was observed in two males (10%) and one females 2% and anti-HBc in three males (15%) and one females 2% of

unvaccinated group, as shown in table -3 . The female showed higher level of anti-HBs than males which were (70.9%) and (65.2%), respectively. The finding of one case (25%) of ant-HBc in absence anti-HBs of unvaccinated group(as shown in table -3), might be pointed to undetectable infection with HBV.

In previous study confirmed that the presence of serum anti-HBc, in the absence of HBsAg and anti-HBs considered a marker of "silent" HBV infection. Most of the epidemiological studies have shown higher carrier rates among males than females (24), as in the countries of Middle East, like Iran, the prevalence rate of HBsAg was (1.9%) among men and (1.5%) among women(25).

Table 3: Prevalence of anti-HBs and anti-HBc among both sexes of the studies groups.

Group	No. tested		Anti-HBs		Anti-HBc	
			Male	Female	Male	Female
	Male	Female	No. (%)	No. (%)	No. (%)	No. (%)
Vaccinated group	95	55	62 (65.2)	39 (70.9)	Nil (0.0)	Nil (0.0)
Unvaccinated group	20	50	2 (10)	1 (2)	3 (15)	1 (2)

The result of this study revealed that the distribution of anti-HBs level divided to less than 10, 10-100 and 100-more than 500 m.i.u/ml among studied groups that have anti-HBs were 13(6.8%); 21(11%) and 99(52.1%) of vaccinated and 0.0% ; 0.0% and 2(3.3%) of unvaccinated groups, significant difference was detected between the two groups (Table 4).

Immunity is conferred after vaccination in 85-90% of persons and is proven by finding a titer greater than 10 m.i.u./ml of surface antibody in the vaccine recipient. Hepatitis B vaccine induced protective levels of antibody to HBsAg in >95% of vaccinated children and in >90% of healthy young adults one year after receiving three doses of hepatitis B vaccine(2,26).

Table 4: Distribution of the level of anti-HBs (less than 10, 10 -100 and 100- 500 mIU. /ml) among the studied groups.

Groups	Total No.	Anti-HBs		
		Less than 10 m.I.U/ml	10–100 m.I.U./ml	greater than 100–500 m.I.U./ml
		No. (%)	No. (%)	No. (%)
Vaccinated	150	14 (9.3)	34 (22.6)	53 (35.3)
Unvaccinated	70	Nil (0.0)	1 (1.4)	2 (2.8)

The finding that prevalence of protective anti-HBs level according to the doses received by vaccinated group, revealed that (66%) of those who received the three primary doses had protection (Table- 5). When we compared the percentage of number of children had gained protective anti-HBs level according to number of doses received, it seemed clearly that the levels of protection are increase significantly with the number of doses, so, we found that levels increased gradually 32%, 52%, and 66%, respectively as shown in Table-5.

Table 5: Prevalence of the protective anti-HBs level according to the doses of vaccination.

No. of doses received	Total no.	Protective anti-HBs level
		No.(%)
1	25 (16.6)	8 (32)
2	25 (16.6)	13 (52)
3	100 (66.6)	66 (66)
Total	150	87 (58)

The low percentage of anti-HBs level of vaccinated children which reached to 33.3% and 50% could be affected by no completed doses course of vaccination (1 and 2 doses) or may be due to improper handling of the vaccine .

In previous studies protective levels of anti-HBs of healthy people were 86.8%, 91%, 96% and 98% among vaccinated group(7,27). More than 116 countries have included HB vaccine as part of their routine infant or adolescent immunization programs and in case babies of mothers who are HBeAg positive received both hepatitis B vaccination and HBIg at birth , as opposed to only hepatitis B vaccination for babies of mothers who are anti-HBe negative(28). Several studies details the long-term benefits of preventing chronic liver disease make routine prenatal HBsAg testing as cost-effective as other widely implemented prenatal and blood donor screening practices(29,30,21). Despite current recommendations for universal vaccination of newborns against HBV screening all pregnant women for HBV infection is recommended as an effective intervention because the vaccine alone appears to be less efficacious than the recombination of vaccine and HBIg in preventing HBV infection of infants exposed to HBsAg-positive mothers.

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