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Original Article

Anti-Hepatitis B Surface Antibody Response and Levels in Hepatitis B Vaccinated Children in Diyala Province, Iraq

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Abstract

Background: Hepatitis B virus (HBV) vaccination was one of the success stories of the 20th century. It is highly effective and safe and was incorporated into national immunization programs of countries worldwide. **Objectives:** The objective of the study as to explore the response rate and titers of anti-HBs antibodies among children 1–14 years old immunized with HB vaccine in Baquba city Diyala province. **Vaccinees and Methods:** This cross-sectional study was conducted in Baquba City, the center of Diyala province, Iraq for the period July 20, 2016–March 20, 2017. A total of 452 children were enrolled, 247 (54.6%) were males and 205 (45.4%) were females, with an age range of 1–14 years. All were previously immunized with genetically engineered HBV vaccine (Euvax B, Korea), with a dose of 10 μg of HBsAg/0.5 ml intramuscularly. Venous blood samples were collected aseptically; sera were separated and subjected for the detection of HBsAg, anti-HBs antibody titer, HBe Ag, and total antibody and anti-HBc total antibody using Enzyme-Linked Immunosorbant assays (ACON, Foresight-USA). A preconstructed questionnaire form was prepared including information of sociodemographic, health, and vaccination status. Statistical analysis was done using the Statistical Package for the Social Science (SPSS), Version 23. $P < 0.05$ was considered statistically significant. **Results:** The overall anti-HBs Ab positivity rate was 75.66% (95% confidence interval was 71.5%–79.6%), with a mean anti-HBs Ab titers range (10.1–673.4 mIU/ml). However, the titer was failed to reach the protective level in 110 (24.34%) vaccinees. The highest anti-HBs Ab positivity rate among children received 1 or 2 vaccine dose is significantly higher among those after <1-year duration (80.0%, $P = 0.043$), while the mean anti-HBs Ab titer is insignificantly highest among the same group of children (43.7 mIU/ml, $P = 0.24$). The highest anti-HBs Ab positivity rate among children received three vaccine doses is significantly higher among those after <1-year duration (91.3%, $P = 0.003$), and similarly the highest mean of anti-HBs Ab titer is insignificant higher among the same children group (81.3 IU/ml, $P = 0.2$). Regarding those children received four vaccine doses, the anti-HBs Ab positivity rate and the mean anti-HBs Ab titer are significantly higher among children after <1 year (89.9%, $P = 0.004$ and 97.7 IU/ml, $P < 0.001$), respectively. Finally, the anti-HBs Ab positivity rate after five vaccine doses was insignificantly higher after 1–3-year duration (72.4%, $P = 0.15$), while the highest mean anti-HBs Ab titer was significantly higher among those with <1-year duration (55.0 IU/ml, $P < 0.041$). **Conclusion:** Universal hepatitis B vaccination is generally produce high rate of protection among children and the highest anti-HBs antibody titer was achieved through 3 or 4 doses after <1-year postvaccination.

Keywords: Anti-HBs Ab, hepatitis B virus infection, hepatitis B virus vaccine

Introduction

Like many other countries, Iraq was adopted the WHO's recommendation and introduced the recombinant Hepatitis B virus (HBV) vaccine for newly born in the Iraqi Expanded Program of Immunization (IEPI) since 1994. HBV infection is a worldwide leading cause of a wide spectrum of liver disease ranging from acute to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[1,2] Infection with HBV in infancy or early childhood may lead to a high rate of chronic carriers (25%–90%), while it is markedly decreased during adulthood (5%–10%). In most endemic areas, infection occurs mainly during early childhood and mother-to-infant transmission accounts for approximately 50% of the chronic infection.[3,4] Effective vaccines have been available for over 20 years, and vaccination has proved highly successful

Access this article online

Quick Response Code:

Website: www.journalijcm.org

DOI: 10.4103/IRJCM.IRJCM_5_20

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Submitted: 08-Mar-2019 **Revised:** 12-Mar-2019
Accepted: 21-Jul-2019 **Published:** 29-Jun-2021

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How to cite this article: H. Hasan AS, Mustaf MM. Anti-Hepatitis B surface antibody response and levels in hepatitis B vaccinated children in diyala province, Iraq. IRAQI J COMMUNITY MED 2020;33:15-9.

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in reducing the disease burden, the development of the carrier state, and the HB-related morbidity and mortality in the countries where vaccination has been implemented.[5,6]

In Diyala province, an early seroepidemiological study of HBV infection conducted for the period from 1989 to 2002 found that the grand prevalence of HBsAg positivity rate among blood donors was 1.5%, and the highest positivity rate was among hemophilia patients (42.5%).[7] Subsequent seroprevalence study during the period from 2003 to 2008 found a similar high HBsAg positivity rate with a significantly higher infection rate among male and the age group of 20–29 years.[8] In a clinicoserological study on acute icteric patients, it was found that 75.8% of these patients were positive for different viral markers including 20.9% were positive for HBsAg, and 38.4% and 7.7% of those HBsAg-positive patients were also positive for anti-HBc IgM and anti-HDV antibody, respectively.[9] Intrafamilial clustering was seems to be one of unique features of HBV infection in Diyala, as the overall prevalence of HBsAg among household contacts was 14.2%, suggesting that it may contribute for maintaining the endemicity of the disease in Diyala community.[10] Other fascinating results were those reported the presence of a relatively high rate of occult HBV infection incriminating the occult HBV infection, at least in part, for the perpetuation of infection in the community.[11] Surprisingly, even an outbreak of HBV infection was occurred in a rural district in 2004 which was linked to the exposure-prone practices carried out by private HCWs.[12] Finally, a population-based survey for detection of HBV infection in 2013 found that the overall HBsAg positivity rate among general population was 0.65, while the anti-HBc IgM and total antibody positivity rates were 3.67% and 9.56%, respectively.[13]

Different studies conducted to assess the efficacy of HBV vaccine through determination of anti-HBsAb titers had yielded variable protection rates.[14-17] In a previous study in Baghdad to evaluate HBV vaccine among children 1–10 years found that 77.2% of children received three primary vaccine doses had protective level of anti-HBs Ab.[18] Finally, in a recent study in Diyala province, the HBV vaccine was proven to be efficacious in the majority of vaccinees[19] Therefore, this study was designed to explore the response rate and titers of anti-HBs antibodies among children 1–14 years old immunized with HB vaccine in Baquba city Diyala province.

Vaccinees and Methods

This cross-sectional study was conducted in Baquba City, the center of Diyala province for the period July 20, 2016–March 20, 2017. A total of 452 HBV vaccinated children were enrolled randomly from those attending primary health-care centers. 247 (54.6%) were males and 205 (45.4%) were females, with an age range of 1–14 years. All were previously immunized with genetically engineered HBV vaccine (Euvax B, Korea), with a dose of 10 μg of HBsAg/0.5 ml (children vial) intramuscularly.

The durations (period of time in years elapsed since the last vaccine dose up to the time of sample collection) were extended from <1 to >4 years. Venous blood samples were collected aseptically; sera were separated and subjected for the detection of HBsAg, anti-HBs antibody titer, HBe Ag, and total antibody and anti-HBc total antibody using enzyme-linked immunosorbant assays (ACON, Foresight-USA). Further inclusion criteria were that the HBsAg, HBeAg, and anti-HBc total antibodies should be negative. According to the WHO criteria, HBV vaccinees were considered positive, only if they had ≥10.0 mIU of anti-HBs Ab/mL of their sera. A preconstructed questionnaire form was prepared including information of sociodemographic, health, and vaccination status.

The human privacy was respected by taken the participants or their parent's consent. Furthermore, this study was approved by the Ethical Committee in the College of Medicine-Diyala University. Statistical analysis was done using the Statistical Package for the Social Science (SPSS), version 23 (It is international software for statistical analysis, USA). $P < 0.05$ was considered statistically significant.

Results

The results showed that 342 of the vaccinated children were anti-HBs Ab positive with an overall positivity rate 75.66% (95% confidence interval for prevalence of anti-HBs Ab 71.5%–79.6%). They were grouped as positive according to the WHO criteria (HBV vaccinees were considered positive, only if they had ≥10.0 mIU of anti-HBs Ab/mL of their sera) the range of anti-HBs Ab titers among current participants was 10.1–673.4 mIU/ml, Table 1.

The anti-HBs Ab positivity rate by duration since last dose of the vaccine stratified by the number of vaccine doses was shown in Table 2. The highest anti-HBs Ab positivity rate among 41 children administered 1 or 2 vaccine is among those with <1-year duration (80.0%), and the lowest positivity rate is among those with 4 years or more duration (33.3%). The difference is statistically significant ($P = 0.043$). Similarly, the mean anti-HBs Ab titer is highest among those children with <1-year duration (43.7 mIU/ml), while the least mean titer is among those with 4 years or more duration (16.6 mIU/ml). Nevertheless, the difference is statistically insignificant ($P = 0.24$). In addition, the means of anti-HBs Ab titer among the three categories are above the protective level (≥10 IU/ml), and that the overall anti-HBs Ab positivity rate (those with ≥10 IU/ml Ab titer) is 58.5%.

The highest anti-HBs Ab positivity rate among children administered three vaccine doses is among those after <1-year duration (91.3%), and the lowest positivity rate is among those with 4 years or more duration (56.3%). The difference is statistically significant ($P = 0.003$). However, the positivity rates of anti-HBs Ab among different categories after three vaccine doses are generally higher than those after one or two vaccine doses[Table 3]. The overall anti-HBs Ab positivity

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Table 1: Distribution of anti-HBs Ab positivity rate and titer

Anti-HBs Ab	Log serum HBs Ab (mIU/ml)				Anti-log values (mIU/ml)	
	Mean	SD	SE	*n* (%)	Range	Mean
Subjects with positive Ab (≥10 mIU/ml)	1.88	0.53	0.03	342 (75.66)	10.1-673.4	75.3

SD: Standard deviation, SE: Standard error

Table 2: Anti-HBs Ab positivity rate and titer after 1-2 vaccine doses by duration

Duration (years)	Total number	Positive anti-HBs Ab, *n* (%)	Log serum HBs Ab (mIU/ml)			Anti-log values (mIU/ml)	
			Mean	SD	SE	Mean	Range
<1 year	5	4 (80.0)	1.64	0.57	0.25	43.7	5.2-158.5
1-3 years	21	15 (71.4)	1.47	0.61	0.13	29.5	3.4-275.4
4 and more	15	5 (33.3)	1.22	0.81	0.21	16.6	4.3-660.7

χ^2=6.31; *P*=0.043 (S), *F* ratio=1.411; *P*=0.24 (NS). S: Significant, NS: Not significant, SD: Standard deviation, SE: Standard error

Table 3: Anti-HBs Ab positivity rate and titer after 3 vaccine doses by duration

Duration (years)	Total number	Positive anti-HBs Ab, *n* (%)	Log serum HBs Ab (mIU/ml)			Anti-log values (mIU/ml)	
			Mean	SD	SE	Mean	Range
<1 year	69	63 (91.3)	1.91	0.63	0.08	81.3	4.3-112,201.8
1-3 years	61	50 (82.0)	1.57	0.56	0.07	37.2	3.9-371.5
4 and more	16	9 (56.3)	1.66	1.21	0.30	45.7	4.8-165.958.7

χ^2=11.81; *P*=0.003 (S), *F* ratio=1.691; *P*=0.2 (NS). S: Significant, NS: Not significant, SD: Standard deviation, SE: Standard error

Table 4: Anti-HBs Ab positivity rate and titer after 4 vaccine doses by duration

Duration (years)	Total number	Positive anti-HBs Ab, *n* (%)	Log serum HBs Ab (mIU/ml)			Anti-log values (mIU/ml)	
			Mean	SD	SE	Mean	Range
<1 year	69	62 (89.9)	1.99	0.54	0.06	97.7	5-426.6
1-3 years	106	73 (86.9)	1.46	0.64	0.06	28.8	3-14791.1
4 and more	25	17 (68.0)	1.25	0.38	0.08	17.8	4.4-95.5

χ^2=11.09; *P*=0.004 (S), *F* ratio=29.53; *P*<0.001 (S). S: Significant, SD: Standard deviation, SE: Standard error

rate in this group of participants is 83.6% . The highest mean of anti-HBs Ab titer is among those children with <1-year duration (81.3 IU/ml), whereas the lowest mean titer is among those with 1–3-year duration (37.2 IU/ml). The difference is statistically insignificant ($P = 0.2$). Of note, the means of anti-HBs Ab titer among the three categories are above the protective level (≥10 IU/ml). It is also clear that the mean titers in the three categories are generally higher than its counterparts of those children administered one or two vaccine doses.

The highest anti-HBs Ab positivity rate among children administered four vaccine is among those children after <1 year (89.9%), and the lowest positivity rate is among those children after 4 years or more duration (86.0%). The difference is statistically significant ($P = 0.004$). It is also clear that the positivity rates among those children after 3 and 4 years or more are becoming higher compared to their counterpart after three vaccine doses. The overall anti-HBs Ab positivity rate in this group of children is 76.0% [Table 4]. On the other side, the highest mean anti-HBs Ab titer is among those children with <1-year duration (97.7 IU/ml), while the lowest titer is among those with 4 years and more (17.8 IU/ml). The difference is statistically significant ($P < 0.001$). Another worthy interpretation is that comparing to those received three vaccine doses [Table 4], the mean anti-HBs Ab titers are falling down rapidly after 1–3 years and 4 years and more (28.8 IU/ml and 17.8 IU/ml), respectively.

In Table 5, although the anti-HBs Ab positivity rate among those children after 1–3 year duration is higher than those after <1 year and after 4 years and more duration (72.4%, 71.4% and 37.5%, respectively), the difference among the three categories is statistically insignificant ($P = 0.15$). Moreover, the positivity rates in the three categories after five vaccine doses are generally lower than that after three [Table 4] or four vaccine doses [Table 4]. Of note, the overall anti-HBs Ab positivity rate in this group of children is 67.7% [Table 4]. Similarly, the highest mean anti-HBs Ab titer is among those children with <1-year duration (55.0 IU/ml), while the lowest titer is among those with 4 years and more (12.3 IU/ml). The difference is statistically significant ($P < 0.041$). In this group of children, the overall mean anti-HBs Ab titers in the three categories are generally lower than those received three and four vaccine doses [Table 4], respectively.

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Table 5: Anti-HBs Ab positivity rate and titer after 5 vaccine doses by duration

Duration (years)	Total number	Positive anti-HBs Ab, *n* (%)	Log serum HBs Ab (mIU/ml)			Anti-log values (mIU/ml)	
			Mean	SD	SE	Mean	Range
<1 year	28	20 (71.4)	1.74	0.73	0.14	55.0	4.6-9332.5
1-3 years	29	21 (72.4)	1.42	0.56	0.10	26.3	3.7-398.1
4 and more	8	3 (37.5)	1.09	0.55	0.19	12.3	4.4-166

χ^2=3.81; *P*=0.15 (NS), *F* ratio=6.361; *P*=0.014 (S). S: Significant, NS: Not significant, SD: Standard deviation, SE: Standard error

Discussion

Like many other countries, Iraq was adopted the WHO's recommendation and introduced the recombinant HBV vaccine for newly born in the IEPI since 1994. Fortunately, countries of Eastern Mediterranean Region have made notable achievements, improving coverage from only 6% in 1992 to 83% in 2014. Member states adopted a hepatitis B control target in 2009 to reduce chronic HBV infection prevalence to <1% among children aged <5 years by 2015.[20] The importance of the present study may be streaming from two roots; first, the relatively high prevalence of HBV infection, particularly the occult infection, as reported by the local epidemiological survey.[13] Second, the urgent need for evaluation of HBV vaccination program as it is universally implemented since more than 20 years ago. The study design was arranged to be comprehensive in evaluating the efficacy of HBV vaccine and more importantly, all participants were negative for HBs Ag, HBe Ag, anti-HBc total antibodies, and anti-HBe total antibodies, to exclude the breakthrough HBV infection.

Based on the WHO criteria, the study found that overall positivity rate of protected children is 75.66%. The present results are much closer to previous study conducted in Baghdad, which reported that 77.2% of children received three primary vaccine doses.[18] This result is also consistent with other studies from surrounding countries.[14-17,21] Moreover, the protection rate obtained in this study is within the range of HBV vaccine protection rate worldwide, since upon reviewing meta-analytic studies published between 1987 and 2015 assessed the seroprotection among healthy newborns, children, adolescents, and adults, the overall median seroprotection rate was 98% (range 52%–100%).[5,22] Therefore, the current study is strongly support the notion that there is a universal consensus regarding the protection rates of HBV vaccine against HBV infection.

The worldwide evidences of the efficacy of the DNA recombinant HB vaccine certainly related to its unique technology of preparation, since it is the first genetically engineered vaccine.[6,23] It is worth to mention that anti-HBs Ab-positive vaccinees in this study had a range of anti-HBs Ab titers (10.1–167,428 mIU/ml). Hence, the present study is in agreement with numerous studies worldwide.[24,25] Logically, as the protection rate increased in the community, the infection rate should be dropdown, and this is what actually evident from cumulative studies worldwide.[17,26,27]

The present study has emerged many important outcomes; first, after any number of vaccine doses, the anti-HBs Ab positivity rate was significantly higher after <1-year duration. Second, the anti-HBs Ab positivity rate was fading down as the duration increase, i.e., being the lowest after 4 years or more after any given number of doses. Third, the highest anti-HBs Ab positivity rate after 4 years or more was among those administered four vaccine doses (68% of those participants received 4 vaccine doses were still positive for anti-HBs Ab and protective against HBV infection after 14 years). Therefore, this study is highly recommended the vaccination schedule of three primary vaccine doses plus one booster dose and considered it as the ideal one. Hence, in this regard, the present results are compatible with previous study.[28] Furthermore, it is worth to mention that the present results are in agreement with other studies in Egypt and Yemen, respectively,[21,29] and strongly support the notion that time since vaccination and a low peak response were the strongest risk factors for HBV infections.[27]

The effect of duration after one or two, three, four, and five vaccine doses was found to have multioutcomes: first, regardless the number of vaccine doses, the mean anti-HBs Ab titer was higher as the duration since last vaccine doses was shorter (<1 year), and thereafter it decline gradually as the duration prolonged (4 and more years), affirming that as the duration since last vaccine dose is shorter as the antibody titer is higher and vice versa. Second, again regardless the number of vaccine doses, the mean anti-HBs Ab titers after different categories of duration were never below the protective levels (≥10 IU/ml), indicating the efficaciousness of the vaccine in provoking high and prolonged antibody titer. Third, the highest anti-HBs Ab titer was after <1-year duration among those administered 4 vaccine doses (97.7%, $P < 0.001$). However, surprisingly, the mean titer of anti-HBs Ab was dropped down rapidly after the fifth vaccine dose, in all categories of duration, suggesting that a vaccination schedule of three primary doses at infancy (0, 2, and 4 month schedule) followed by fourth booster dose (18 months) is the perfect schedule as the titer of anti-HBs Ab is concern. Similar results have been reported by other studies.[24,28,29] More precisely, the present study is totally consistent with that of La Fauci *et al*., (2016)[30] who affirm the importance of recommend anti-HBV vaccination at a young age, ideally during childhood in accordance with the national vaccination policy. These facts are also reported by many other studies.[25,28,31] Moreover, it has been found that the percentage of memory CD4+ T-cells secreting interferon-gamma, and those secreting interleukin-4 were higher among participants with serum anti-HBs >100

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mIU/ml than those having <10 mIU/ml or absent.[21] More interestingly, in vaccinees with anti-HBs Ab titer <10 IU/ml after complete primary vaccine at infancy, the immune effect of a three dose booster revaccination is good, and the booster-induced immune response was correlated with the prebooster titer, indicating the need for a course of booster vaccination. However, there are other studies denied the requirement of booster vaccine dose for children under 15 years old[26,27,32,33] or till the age of 16 years.[34]

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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