Characteristics of Low Responder Vaccinees after Hepatitis B Vaccination in Diyala Province-Iraq

Abdulrazak SH. Hasan **Abdulqader Yahya Al-Azzawi**

Abstract:

Background: Hepatitis B vaccine has been introduced for more than 3 decades. The duration and protective efficacy in early infancy seems to be multifactorial. Age, vaccination schedule, dosages, vaccine type and population characteristics may potentially influencing the protection rate.

Objectives: Identification of characteristics of low responders who administered full doses of HB vaccine, and trying to figure out the reasons behind their failure to develop protective levels of anti-HBs antibodies.

Vaccinees and methods: This cross sectional study was conducted in Baquba City, the center of Diyala province for the period 20 July/ 2016 to 20 March /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 micrograms of HBsAg/ 0.5 milliliter (children vial) intramuscularly according to Iraqi vaccination schedual. 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 pre-constructed questionnaire form was prepared including information of socio-demographic, health, and vaccination status. Statistical analysis was done using the Statistical Package for Social Science (SPSS), Version 23. P values < 0.05 were considered significant.

Results: The study found that a total of 110 (24.34%) vaccinees were failed to achieve the protective levels of anti-HBs Ab (10 mIU/ml) as recommended by the WHO. The mean ± SD of age was 64-76 ± 38.4 months with an age range of 13-168 months. The mean duration of time since last vaccine dose was 41.2 ± 41.4 months (range 1-171 months). They were 53(48.2%) females and 57(51.8%) males. The mean ± SD of the anti-HBs Ab titer was 6.31 ± 1.9 mIU/ml (range 3.0- 9.92 mIU/ml). The results also showed that the anti-HBs Ab titers are insignificantly elevated as the number of vaccine doses increased (3.419, P=0.507). On the other hand, these Anti-HBs antibody titers are insignificantly declining after more than fourth year since last vaccine dose (3.841 P=0.168). Furthermore, non-regular vaccination schedule elicit insignificantly higher anti-HBs Ab titers (2.155, P=0.190). Finally, the anti-HBs Ab titers are marginally affected by other scio-demographic factors.

Conclusion: The anti-HBs antibody titers in low responder HBV vaccinees are non-remarkably affected by vaccine or vaccinees related factors, recommending that those low responders should receive a booster dose of HBV vaccine.

Keywords: HB vaccine, Anti-HBs antibody, vaccine low responder

Introduction:

Hepatitis B virus infection is a worldwide public health problem of major concern that may lead to chronic liver disease, including cirrhosis and hepatocellular carcinoma [(1).] Recombinant HB vaccination is the most effective measure to control and prevent HB and its long-term serious sequelae on global scale as manifested by substantial decrease in disease burden, chronic carrier rate and in HBV-related morbidity and mortality [(2_5)] Upon reviewing 43 studies, the overall median seroprotection was 98% (range 52% -100%). The final median seroprotection proportions did not vary appreciably by maternal HBsAg status, HBIG administration, or vaccination schedule. Higher compared to lower dosage resulted in earlier increases in anti-HBs but not in final seroprotection proportions. Infants with birth weights < 2 killograms had lower median seroprotection. Median seroprotection was also lower when infants with birth weights < 2 killograms were vaccinated at 0-3 days of age compared to 1 month of age or older [(5)]

People who fail to respond (serum anti-HBs antibody level below 10 mIU/ml) should be tested to exclude current or past HBV infection, and given a repeat course of 3 vaccinations, followed by further retesting 1–4 months after the second course. Those who still do not respond to a second course of vaccination may respond to intradermal administration or to a high dose vaccine [(6)] or to a double dose of a combined hepatitis A and B vaccine. [(7).] Those who still fail to respond will require hepatitis B immunoglobulin (HBIG) if later exposed to the hepatitis B virus [(8)] Poor responses are mostly associated with being over the age of 40 years, obesity and smoking, and also in alcoholics, especially in those with advanced liver disease [(7).] Patients who are immunosuppressed or on renal dialysis may respond less well and require larger or more frequent doses of vaccine [(8).] At least one study suggests that hepatitis B vaccination is less effective in patients with HIV [(9)]

It has been found that 87.9% of the children achieved seroprotection one-month after primary vaccination with HB vaccine, and 12.1% were non-responders. Human leukocyte antigen types, DRB 111 (64.7%), B5 (52.9%), DRB 104 (52.9%) and DRB 11001 (47%) were detected at increased frequency in non-responders **(10).** The higher rate of vaccine failure in males than females requires further

investigation as it may explain the higher prevalence of HBV in the male population [11]. Maternal HBsAg-positive status was an independent risk factor for vaccination-protected children to develop HBV breakthrough infection in adulthood. Adolescent boosters might be appropriate for high-risk individuals who were born to HBsAg-positive mothers when their serum anti-HBs <10mIU/ml [12]. In another study in Yemen, it has been found that 72.2% of children below 5 years were responded to the vaccine with anti-HBs level ≥ 10 IU/L, while 27.8% had non-protective anti-HBs levels of <10 IU/L [13].

Vaccinees and methodology:

This cross sectional study was conducted in Baquba City, the center of Diyala province for the period 20 July/ 2016 to 20 March /2017. It is a part of larger study which enrolled a total of 452 children, 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 micrograms of HBsAg/ 0.5 milliliter (children vial) intramuscularly. They were randomly chosen from those attending the primary health care centers. 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 pre-constructed questionnaire form was prepared including information of socio-demographic, health, and vaccination status. The human privacy was respected by taken the participants or their parents consent. However 34 of them were refused participation. Statistical analysis was done using the Statistical Package for Social Science (SPSS), Version 23. P values < 0.05 were considered significant.

Results:

The study found that a total of 110 (24.34%) vaccinees were failed to achieve the protective levels of anti-HBs Ab (10 mIU/ml) as recommended by the WHO. The mean ± SD of age was 64-76 ± 38.4 months with an age range of 13-168 months. The mean duration of time since last vaccine dose was 41.2 ± 41.4 months (range 1-171 months). They were 53(48.2%) females and 57(51.8%) were males. The mean ± SD of the anti-HBs Ab titer was 6.31 ± 1.9 mIU/ml (range 3.0- 9.92 mIU/ml). Table (1) revealed that there was no association between anti-HBs Ab titer and age groups.

Table (1): Association of anti-HBs Ab titer and age groups.

Anti-HBs Ab titer (mIU/ml)	Age (Ys)			Total (%)	Pearson Chi-Square (p value)
	1-4	5-9	10-14		
1-4	9 (8.2%)	14 (12.7%)	9(8.2%)	32 (29.1%)	3.0 (0.223)
5-9	26 (23.6%)	41(37.3%)	11 (10.0%)	78 (70,9%)	
Total	35 (31.2%)	55 (50.0%)	20(18.2%)	110 (100%)	

Concerning the gender, the results again showed that there was no association between anti-HBs Ab titer and gender, table [2]. Table (3) showed that although the majority of individuals were with 5-9 mIU/ml titer of anti-HBs Ab compared to those with 1-4 mIU/ml; However, the difference was failed to reach the levels of statistical significance.

Although the highest titers of anrti-HBs Ab were appeared after one year of the last dose of vaccination; however, table (4) revealed that there was insignificant association with the duration since the last vaccine dose administered. Table (5) showed that the anti-HBs Ab titer was higher among those who received their vaccine doses irregularly (intermittent) compared to those received the vaccine doses regularly; However, the difference was statistically insignificant.

Based on the type of feeding during the period below 2 years, those who were fed on breast milk had higher titers of abti-HBs Ab compared to other type of feedings; However the difference was statistically insignificant, Table (6).

Table (2): Association of anti-HBs Ab titer and gender.

Anti-HBs Ab titer (mIU/ml)	Gender		Total (%)	Pearson Chi-Square (p value)
	Female	Male		
1-4	15 (13.6%)	17(15.5%)	32(29.1%)	0.031(0.861)
5-9	38 (34.5%)	40(36.4%)	78(70.9%)	
Total	53(48.2%)	57(51.8%)	110(100%)	

Table (3): Association of anti-HBs Ab titer and number of doses (According to Iraqi Expanded Program of Immunization).

No. doses	Anti-HBs Ab titer (mIU/ml)		Total (%)	Pearson Chi-Square (p value)
	1-4	5-9		
1	6 (5.5%)	6(5.5%)	12 (10.9%)	3.419(0.507)
2	2 (1.8%)	3(2.7%)	5(4.5%)	
3	6 (5.5%)	18(16.4%)	24(21.8%)	
4	12 (10.9%)	36(32.7%)	48(43.6%)	
5	6 (5.5%)	15 (13.6%)	21(19.1%)	
Total	32 (29.1%)	78(70.9%)	110(100%)	

Table (4): Association of anti-HBs Ab titer and duration since last vaccine dose.

Anti-HBs Ab titer (mIU/ml)	duration (Ys)			Total (%)	Chi-Square (p value)
	<1	1-3	4 +		
1-4	2 (1.8%)	18 (16.4%)	12 10.9%)	32 (29.1%)	3.841 (0.168)
5-9	17 (15.5%)	37 (33.6%)	24(21.8%)	78 (70.9%)	
Total	19 (17.3%)	55 (50.0%)	36 (32.7%)	110(100%)	

Table (5): Association of anti-HBs Ab titer and vaccination schedule.

Anti-HBs Ab titer (mIU/ml)	Vaccination schedule		Total (%)	Pearson Chi-Square (p value)
	Regular	Non-regular		
1-4	15 (13.6%)	17 (15.5%)	32 (29.1%)	2.155 (0.190)
5-9	25 (22.7%)	53 (48.2%)	78 (70.9%)	
Total	40 (36.4%)	70 (63.6%)	110 (100%)	

Results in table:7 revealed that participants who didn't gate blood transfusion had higher anti HBs Ab titers compared to their counterparts, but the difference was insignificant**.** The results also showed that the majority of participants with higher titers of anti-HBs Ab were free from chronic diseases compared to their counterparts; However, the difference was statistically in significant, table :8.

Table (6): Association of anti-HBs Ab titer and type of feeding.

Anti-HBs Ab titer (mIU/ml)	Type of feeding			Total (%)	Chi-Square (p value)
	Breast	Artificial	Mixed		
1-4	10 (9.1%)	7 (6.4%)	15 (18.2%)	32 (29.1%)	1.478 (.492)
5-9	34 (30.9%)	13 (11.8%)	31 (28.2%)	78 (29.1%)	
Total	44 (40.0%)	20 (18.2%)	46 (41.8%)	110 (100%)	

Table (7): Association of anti-HBs Ab titer and history of blood transfusion.

Anti-HBs Ab titer (mIU/ml)	Blood transfusion		Total (%)	Pearson Chi-Square (p value)
	Yes	No		
1-4	1 (0.9%)	31 (28.2%)	32 (29.1%)	0.027 (1.0)
5-9	2 (1.8%)	76 (69.1%)	78 (70.9%)	
Total	3 (2.7%)	107 (97.3%)	110 (100%)	

Table (8): Association of anti-HBs Ab titer and presence of chronic disease.

Anti-HBs Ab titer (mIU/ml)	presence of chronic disease		Total (%)	Pearson Chi-Square (p value)
	Yes	No		
1-4	1 (0.9%)	31 (28.2%)	32 (29.1%)	0.027 (1.0)
5-9	2 (1.8%)	76 (69.1%)	78(70.9%)	
Total	3 (2.7%)	107 (97.3%)	110(100%)	

Discussion:

The study found that 24.34% of vaccinated children failed to have a protective levels of anti-HBs Ab, and thus they were considered as low responders since their mean ± SD of anti-HBs Ab titer was 6.3 ± 1.9 mIU/ml compared to 75.66% who achieved a full protection with a range of anti-HBs Ab titers (10.1 – 673.4 mIU/ml) [(14).] These results are in agreement with Peto *et al*. (2014) [(15).] who reported that infant HB vaccination achieves substantial protection against chronic carriage in early adulthood, even though approximately a quarter of vaccinated young adults have still non-protected. Similar results have also been reported by other studies suggesting that those low responders may later on become susceptible again to HBV infection and probably may required a booster dose of the vaccine [(16_18)]

People who fail to respond properly (serum anti-HBs Ab level below10 mIU/ml) should be tested to exclude current or past HBV infection, and given a repeat course of 3 vaccine doses, followed by further retesting 1–4 months after the second course. Those who still do not respond to a second course of vaccination may respond to intradermal administration or to a high dose vaccine **(6)** or to a double dose of a combined hepatitis A and B vaccine [(7).]

Those who still fail to respond will require hepatitis B immunoglobulin (HBIG) [(8).] Poor responses are mostly associated with being over the age of 40 years, obesity and smoking, and also in alcoholics, especially those with advanced liver disease. Patients who are immunosuppressed or those on renal dialysis may require larger or more frequent doses of vaccine. Undoubtedly, the hepatitis B vaccination is less effective in patients with HIV [(7_9).]

The reason behind that may be multifactorial; In Iraq, generally speaking, the United Nations non-humanitarian economic sanction on Iraq during the 1990s and early new millennium which include medical appliances, drugs and vaccines had devastating consequences. It has been reported that the health-care services for millions of Iraqis have been drastically reduced because of abudget shortfall and that 84% of Iraq's donor-funded health programmes have either been reduced or shut down [(19).]

Furthermore, and specifically speaking about Diyala province which was one of the hot spots that witnesses military conflicts, resulting in frequent population displacement, deterioration of the health services and interruption of heath projects [(20).] Taken together, these events beside the financial difficulties certainly resulted in children malnutrition and disruption of vaccination schedule that in turn may contribute in lowering levels of anti-HBs Ab titer [(21).] Therefore, it is unsurprisingly to know that 70% of low responders in this study were irregularly administered their vaccine doses. On the other hand, it has been found that transplacental transfer of anti-HBsAb occurs and high titres of maternal anti-HBs Ab may suppress the immune response of infants to the standard HBV vaccination, suggesting that the standard vaccination schedule may not be the optimal choice of infants with anti-HBV positive mothers [(4)]

It is worthy to remember here that 27.7% of HBs Ag positive people in Diyala province are women plus the high rate of intrafamilial clustering beside the relatively high rate of occult HBV infection, suggesting the perpetuation of endemicity of HBV in Diyala province [(22_23).]

Urganci, *et al*. (2015) [(10).] found that 87.9% of the children achieved seroprotection (anti-HBs titres ≥ 10 mIU/mL) one month after primary vaccination, and there was no difference between vaccine responsiveness and age, gender, birth weight, maturity, duration of breastfeeding, exposure to HBsAg-positive family members, but a significant correlation was found between anti-HBs Ab titers and mid-upper arm circumference (p = 0.005), Human leukocyte antigen types, DRB 111, B5, DRB 104 and DRB 11001 were detected at increased frequency in non-responders, concluded that a significantly higher

anti-HBs Ab titer were found in children who breastfed for the first six months and longer and who were vaccinated concomitantly with other common vaccines. It is worth to mention that the use of HB vaccine within hexavalent combination for primary immunization of children against diphtheria, tetanus, pertussis, poliomyelitis, and *H. influenzae* b or trivalent with tetanus-diphtheria were effectively enhance protective levels of HBs Ab titers in low-responder individuals [24,25].

The duration since last vaccine dose is unlikely to be one of the causes behind the low levels of anti-HBs Ab titer, since 50% of low responder in the present study were tested after 1 – 3 years of the last vaccine dose, which is quite short period. In this regards most of studies demonstrated the presence of immune memory and protection 5-15 years after the initial course of newborn immunization with recombinant HBV vaccines [26,27]. or even in adult healthcare workers, affirming that 10 years after vaccination, anti-HBs antibody levels were significantly inversely related to age at vaccination [28]. Ultimately, the present result is totally in agreement with Dumaidi and Al-Jawabreh (2015) [29]. who recommended that low responders (below 10 mIU/mL) should receive a booster dose of vaccine to ensure absolute protection. It can be concluded that the anti-HBs antibody titers in low responder HBV vaccinees are non-remarkably affected by vaccine or vaccinees related factors, recommending that those low responders should receive a booster dose of HBV vaccine.

References:

1. Ott, J.J.; Stevens, G.A.; Groeger, J.; Wiersma, S.T. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 2012; 30(12):2212-9.
2. Zanetti, A.R.; Van Damme, P. and Shouval, D. The global impact of vaccination against hepatitis B: a historical overview. Vaccine, 2008; 26(49):6266-73.
3. Orlando, R.; Foggia, M.; Maraolo, A.E.; Mascolo, S.; Palmiero, G.; Tambsro, O. and Tosone, G. Prevention of hepatitis B virus infection: from the past to the future. Eur. J. Clin. Microbiol. Infect. Dis. 2015; 34(6):1059-70.
4. Chen, M.; Jagya, N.; Bansal, R.; Frelin, L. and Sallberg, M. Prospects and progress of DNA vaccines for treating hepatitis B. Expert Rev Vaccines 2016; 15(5):629-40.
5. Schillie, S.F. and Murphy, T.V.Seroprotection after recombinant hepatitis B vaccination among newborn infants: a review. Vaccine, 2013; 31(21):2506-16.
6. Levitz; C.B. and Regan, H. Immunization with high-dose intradermal recombinant hepatitis B vaccine in healthcare workers who failed to respond to intramuscular vaccination. Infect. Cont. Hospit. Epidemiol. 1995**;** 16 (2): 88–91.
7. Roome, A. J.; Walsh, S.; Cartter, M. and Hadler, J. Hepatitis B vaccine responsiveness in Connecticut public safety personnel. JAMA. 1993; **270** (24): 2931–2934
8. Cardell, K.; Akerlind, B.; Sallberg, M. and Fryden, A. Excellent response rate to a double dose of the combined hepatitis A and B vaccine in previous nonresponders to hepatitis B vaccine. J. Infect. Dis. 2008**;** 198 (3): 299–226.
9. Chotun, N.; Nel, E.; Cotton , M.F.; Preiser, W. and Andersson, MI. Hepatitis B virus infection in HIV-exposed infants in the Western Cape, South Africa. Vaccine, 2015; 33(36):4618-22.
10. Urganci, N.; Kalyoncu, D. and Ozer, A.S. Hepatitis B vaccination and factors related with unresponsiveness in healthy children. West Indian Med. J. 2015; 15:146.
11. Al Ghamdi, S.S.; Fallatah, H.I.; Fetyani, D.M.; Al-Mughales, J.A. and Gelaidan, A.T. Long-term efficacy of the hepatitis B vaccine in a high-risk group. J. Med. Virol. 2013; 85 (9) : 1518-22.
12. Wang ,Y.; Chen, T.; Lu, L.L.; Wang, M.; Wang, D.; Yao, H.; *et al*. Adolescent booster with hepatitis B virus vaccines decreases HBV infection in high-risk adults. Vaccine 2017; 35(7):1064-70.
13. Alssamei, F.A.; Al-Sonboli, N.A.; Alkumaim, F.A.; Alsayaad, N.S.; Al-Ahdal, M.S.; Higazi, T.B. and Elagib, A.A. Assessment of immunization to hepatitis B vaccine among children under five years in rural Areas of Taiz, Yemen. Hepat. Res. Treat. 2017;2017:2131627.
14. Hasan, A.SH. and Mustaf, M.M. The efficacy of hepatitis B vaccine among children in Baquba-Diyala province. Iraqi J. Commun. Med. 2018; 30 (3&4): 176-81.
15. Peto, T.J.; Mendy, M.E.; Lowe, Y.; Webb, E.L.; Whittle, H.C. and Hall, A.J. Efficacy and effectiveness of infant vaccination against chronic hepatitis B in the Gambia Hepatitis Intervention Study (1986-90) and in the nationwide immunisation program. BMC infect. Dis. 2014; 14:7.
16. Hammitt, L.L.; Hennessy, T.W.; Fiore, A.E.; Zanis, C.; Hummel, K.B.; Dunaway, E.; *et al*. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: a follow-up study at 15 years. Vaccine 2007; 25(39-40):6958-64.
17. Zhu, C.L.; Liu, P.; Chen, T.; Ni, Z.; Lu, L.L.; Huang, F.; *et al*. Presence of immune memory and immunity to hepatitis B virus in adults after neonatal hepatitis B vaccination. Vaccine 2011;29(44):7835-41..
18. Lu, S.; Ren, J.; Li, Q.; Jiang, Z.; Chen, Y.; Xu, K.; *et al*. Effects of hepatitis B vaccine boosters on anti-HBs-negative children after primary immunization. Hum. Vaccin immunother. 2016 Dec 1:0. [Epub ahead of print]

19. Devi, S. Iraq's health services curtailed by funding shortfall. Lancet, 2015; 386: 488.
20. van der Berghe,A. Breathless in Iraq. Lancet Respir. Dis. 2016; 4 : 14-5.
21. Ali, H.Y.M. Hepatitis B infection among Iraqi children: The impact of sanctions. East. Medit. Health J. 2004: 10(1/2): 6-11.
22. Hasan, A.SH.; Omer, A.R. and Al-Mashhadani J.I. Seroepidemiological study of hepatitis B virus infection among blood donors and risky groups in Diyala. Iraqi J. Commun. Med. 2006; 19(1): 14-8.
23. Hasan, A.SH. Intrafamilial clustering of HBV infection. Iraqi J. Commun. Med. 2005; 18(2): 134-9.
24. Zanetti, A.R.; Romano, L.; Giambi, C.; Pavan, A.; Carnelli, V.; Baitelli, G.; *et al*. Hepatitis B immune memory in children primed with hexavalent vaccines and given monovalent booster vaccines: an open-label, randomised, controlled, multicentre study. Lancet Infect. Dis. 2010 ;10(11):755-61.
25. Haghighat, A.; Moafi, M.; Sharifian, J.; Salehi, H.; Taleban, R.; Kalbasi, N.; *et al*. Effect of Tetanus-diphtheria Vaccine on Immune Response to Hepatitis B Vaccine in Low-responder Individuals. Int. J. Prev. Med. 2016;7:94.
26. Zanetti, A.; Parlato, A.; Romano, L.; Desole, M.G.; Ferrera, G.; Giurdanella, F.; *et al*. Challenge with a hepatitis B vaccine in two cohorts of 4-7-year-old children primed with hexavalent vaccines: an open-label, randomised trial in Italy. Vaccine 2012;30(39):5770-5.
27. Teoharov, P.; Kevorkyan, A.; Petrova, N.; Baltadzhiev, I. and Van Damme P. Immune memory and immune response in children from Bulgaria 5-15 years after primary hepatitis B vaccination. Pediatr. Infect. Dis. 2013; 32(1):51-3.
28. La Fauci, V.; Riso, R.; Facciolà, A.; Ceccio, C.; Lo Giudice, D.; Calimeri, S. and Squeri, R. (2016). Response to anti-HBV vaccine and 10-year follow-up of antibody levels in healthcare workers. Public Health, 139:198-202
29. Dumaidi, K. and Al-Jawabreh, A. Persistence of anti-HBs among Palestinian medical students after 18 - 22 years of vaccination: A cross-sectional study. Hepatol. Mon. 2015; 15(11):e29325.