International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 2S, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Immunity profile of hepatitis B antibodies among vaccinated healthcare workers after 10 years of last dose

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Received: 19.08.2024 Revised: 17.09.2024 Accepted: 23.10.2024

ABSTRACT
Background:Hepatitis B virus is a major public health problem causing chronic hepatitis, Health care workers are cosidered at high risk group for being infected from contaminated needles injection, vaccination is the main way to prevent infection with a high protection rate, however testing anti-HBS antibody is important to evaluate the effectiveness of the vaccine as below 10 IU/ml is not protective as the level of the antibody declines.
Methodology: This cross-sectional study is based on data collected from health care workers who have been vaccinated within 5, 10 and more than 15 years.Data were collected from January 1, 2024 to March 31,2024 from a total of 90 health care workers who were employed in Azadi teaching hospital. A blood sample was taken from each worker under strict aseptic conditions using a plain vacutainer. Blood was allowed to clot and serum was separated and stored at −20 °C until testing. HBV serum markers (HBsAg, anti-HBs and Interleukin-2) were determined using commercial immuno-enzymatic assays.
Results : The level of anti-HBS antibody in the serum of the health care workers were studied to detect weather it is protective or not .The ageranging from (25-65) from both genders.The antibody level was obviously decreased after 10 and 15 years of being vaccinated and the result was highly significant.
Conclusion: The objective of this study was to evaluate long-term immunogenicity and efficacy of HBV vaccination in two hospitals in health care workers in azadi teaching hospital. An additional objective was to identify the independent predictors of long-termimmunogenicity.

Keywords: collected, data, antibody, level

INTRODUCTION

Hepatitis B virus (HBV) infection is the main cause of acute and chronic liver disease worldwide. [1] The World Health Organization (WHO) estimates that about 257 million people were infected with HBV2 in 2015. The chronic sequelae of the infection, such as fulminant hepatitis, liver cirrhosis, and hepatocellular carcinoma led to 887,000 deaths. [2] Hepatitis B is the most important occupational infectious disease. Transmission of hepatitis B from patients to healthcare workers (HCWs) and from HCWs to patients has been described.[3] According to the WHO, 5.9% of HCWs are each year exposed to blood-borne HBV infections corresponding to about 66,000 HBV infections in HCWs worldwide.[4]
Hepatitis B virus (HBV) infection can be prevented through HBV surface antigen (HBsAg) vaccination. Vaccine efficacy studies have demonstrated protection against acute and chronic disease in immunocompetent vaccine responders.[5] The schedules for vaccination approved by most regulatory authorities are as follows:[6] 0,1 and 6 months (first dose followed by a dose 1 month and 6 months after the first) and 0, 1, 2, and 12 months (first dose followed by a dose 1 month, 2 months and 12 months after the first). The '0, 1, 2, and 12 months' schedule should be considered when rapid protection is required in high-risk groups such as HCWs.
Testing for evidence of protective immunity to HBsAg vaccination is essential, as some vaccinated HCWs do not develop sufficient levels of antibodies against HBsAg (anti-HBs).[7] An anti-HBs level of >10 mIU/ml, is considered protective against HBV infection.[8] An anti-HBs titer of <10 mIU/ml is regarded as non-responsiveness to HBsAg vaccination.[9] Anti-HB levels between 10 and 100 mIU/ml are regarded as hyporesponsiveness, and levels >100 mIU/ml are taken as a high level of 38
Immunity. Countries where hepatitis B vaccine coverage has been high for several decades have noted reductions in death rates from hepatocellular carcinoma [10].
HBV infection is the most prevalent work-related infectious disease, thus it can affect healthcare personnel and different risk areas including pediatric areas, emergency rooms, and ambulatory care facilities. HVB

transmission may be quite easy through contact with infected patients and potentially infectious biological material like blood, saliva, semen, and feces.5 Standard precautions are required for HBV prevention in healthcare workers. The use of personal protective equipment (PPE), as well as the disinfection and sterilization of medical devices, comply with the HBV prophylaxis program. [11-14].
In case of a positive test in a subject with a protective titer of anti-HBs (> 10UI/L), no hepatitis B vaccination booster dose and no further health status checks are necessary, independently of the primary vaccination cycle. On the other hand, in case of a negative test in a subject with a not-protective titer (anti-HBs <10 UI/L) a fourth dose of hepatitis B vaccine is recommended, together with a further evaluation of the antibody titer after two months [15]. Previous studies reported that the anti-HBs antibody titer ≥10 IU/L could immunize from hepatitis for up to 10 years even after 30 years from the last dose. [16,17].
According to the World Health Association, even the vaccinated subjects presenting with an absent or <10 IU/L anti-HBs antibody titerhave an immunological memory that protects them against HBV infection [18].
Healthcare workers (HCWs) are more frequently exposed to the risk of acquiring HBV infection than the general population through mucosal. Cutaneous exposure to potentially infectious blood (eyes, oral mucosa, or skin) or through percutaneous exposure to contaminated sharp objects [19].

1.2.Objectives
Evaluate the effectiveness of HBV vaccine after 5 , 10 and 15 years of being vaccinated in health care workers and assess if a booster dose is required or not?

2. Patients and methods
2.1. Study Design
This cross-sectional study was conducted in Azadi teaching hospital and Kirkuk teaching hospital. Data were collected by personal interview and using questionnaire.Participants were informed about the study, and those who agreed to participate were included.

2.2. Patients Selection
90 health care workers were included in this study from both genders. Their age ranging from 25 to 65 years. 30 of them were vaccinated sinc five years, 30 of them since 10 years and the rest since 15years. All of them were tested for HBs antigen to ensure that they are not infected and the source of the antibody is from being vaccinated.

2.3. Exclusion Criteria
1. health care workers who didn’t complete the vaccine schedule
2.Uncooperative health care workers

2.4. Questionnaire
The participants were requested to fill in a questionnaire for identifying their demographic characteristics such as age, gender, vaccination history.

2.5. Body Parameter
The following anthropometric measurements were obtained: weight, and height, BMI (body mass index). Weight was measured after calibration the scale before each weight measurement. Height was obtained with an aluminum cursor stadiometer graduated in millimeters. The subject was barefoot, with heels, back, and head in contact with the stadiometer in horizontal plane. Body mass index (BMI) had been estimated from person's weight and height; it was calculated by dividing weight (in kilograms) by height (in square meters).
BMI≤18.5 (Underweight), BMI=18.5-24.9(Normal weight), BMI=2529.9(Over weight) and BMI≥ 30(Obese) (20).

2.6. Blood Sampling
Alcohol 70% is used to sterilize the area of blood aspiration from basalic vein in the anticubital fossa. Five ml blood sample was collected from all subjects (patients and control) using disposable syringes 3 ml of blood sample collected in plane tube for the estimation of serum was allowed to clot and then centrifuged at 3000 rpm for 15 minutes and the serum was then removed to another plain tube and stored at -20℃ for further studies. Any sample showed haemolysis was discarded (21).

2.7. Methods
2.7.1. Instruments and Equipments
2.7.1.1. Sterile syringe.
2.7.1.2. Sterile plain tube.

2.7.1.3. Rack; (Meheco co., China).
2.7.1.4. Cotton; (Trog co., Germany).
2.7.1.6. Alcohol 70%.
2.7.1.8. Pipette.
2.7.1.9. Centrifuge.
2.7.1.10. HBs antigen ELISA detection kit
2.7.1.11. Anti HBs antibody ELISA detection kit
2.7.1.12.IL-2 ELISA detection kit
2.7.1.13. ELISA reader and washer

2.8. HBs antigen ELISA detection kit
The HBs antigen was detected utilizing sandwich enzyme linked immunosorbant format following the manufacturer instructions.

Figure 2.1. HBs antigen ELISA detection kit

2.8.1. ELISA Protocol:
1. 200µl of Washing Solution was added to each well. The wells were aspirated to remove liquid and the plate was washed 3 times using 300µl of washing solution per well. After the last wash, plate was inverted to remove residual solution and was blotted on paper towel.
2. 100µl of standard or sample was added to each well in duplicate. The Plate was covered with the Sealer provided and incubated at room temperature for at least 2 hours.
3. The wells were aspirated to remove liquid and the plate was washed 4 times like as step 1.
4. 100µl of the diluted detection antibody (0.25 ug/ml) was added per well. It was covered with the Plate Sealer provided. Incubated at room temperature for 2 hours.
5. The plate was aspirated and washed 4 times like as step 1.
6. 100µl of the diluted color development enzyme (1:20 dilute) was added per well and covered with the Plate Sealer provided this incubation was for 30 minutesat room temperature (or 37°C for 30 minutes).7. The plate was aspirated and washed 4 times like as step 1.8. 100µl of colour development solution was added to each well and Incubated atroom temperature for (10-20 minutes) for a proper color development. To stop the color reaction, 100µl of the stop solution was added to eachwell.
Note: awareness was taken that the colour may develop more quickly ormore slowly than the recommended incubation time depending onthe localized room temperature. The colourdevelopment was visually monitored to optimize the incubation time.
9.Using a microtiter plate reader, the plate was read at 450 nm wavelength.

2.8.2. Results
The duplicate readings were averaged from each standard, control, and Samples, The zero reading was subtracted from each averaged value above. A standard curve was created by reducing the data using ELISA reader's computer software capable of generating standard curve-fit and A standard curve was generated for each set of samples

2.9. Anti HBs antibody ELISA detection kit
Test Description ANTISURASE B-96 II (TMB) is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immuno-sorbent assay) based on the "sandwich principle". The solid phase of the microtiter plate is made of polystyrene wells coated with HBsAg (subtype Ad and Ay), and the liquid phase of peroxidase conjugated HBsAg (subtype Ad and Ay). When a serum or plasma specimen containing Anti-HBs is added to the HBsAg-coated wells together with the peroxidase conjugated HBsAg and incubated, antigen)-(Anti-HBs)-(antigen .

Peroxidase) complexes will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. A color develops in proportion to the amount of Anti-HBs bound to HBsAg. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm"

Fig 2.2. Anti HBs antibody ELISA detection kit

2.9.1. Materials Provided with the Test Kits

1. Antibody-coated microtiter wells, 96 wells per plate.
2. Reference standards, 0, 10, 50, 100, 400, and 1000 IU/ml. Liquid, ready for use.
3.HBsAg +peroxidase solution
3. Zero control and positive control
4. Enzyme Conjugate Reagent.
5. TMB Substrate.
6. Stop Solution.
7. Wash Buffer Concentrate (50X).

2.9.2. Assay Procedure

1. The desired number of coated wells were secured in the holder.
2. one well was reserved for black. 50µl of standard, specimens, and controls was dispensed into appropriate wells.
3. It was thoroughly mixed for 10 seconds until a complete mixing was obtained.
4. It was incubated at room temperature (37°C) for one hour.
5. The incubated mixture was removed by flicking plate content into a waste container.
6. The microtiter wells were rinsed and flicked 5 times with washing buffer (1X). The wells were stricken sharply onto absorbent paper or paper towels to remove all residual water droplets
7. 50µl of TMB substrate solution A first and then 50µl of TMB substrate solution B in to each well including blank .
8. It was incubated at room temperature for 30 minutes.
9. The incubation mixture was removed by flicking plate contents into sink.
10. The microtiter wells were rinsed and flicked 5 times with washing buffer (1X).
11. The wells were stricken sharply onto absorbent paper or paper towels to remove allresidual water droplets.
12. The reaction was stopped by adding 100µl of stop solution to each well.
13. It was gently mixed for 30 seconds. It was important to make sure that all the bluecolour changes to yellow colour completely.
14. Optical density was read at 450nm with a microtiter reader.

2.9.3. Results

The mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples was calculated. A standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentration in IU/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Mean absorbance values for each specimen was used to determine the corresponding concentration of HBs antigen in IU/ml from the standard curve.

2.10. Interleukin 2 (IL-2) ELISA detection kit

This sandwich kit is for the accurate quantitative detection of Human Interleukin 2 (also known as IL-2) in serum, plasma, cell culture supernates, Ascites, t1ssue homogenates or other biological fluids

2.10.1.Assay Principle

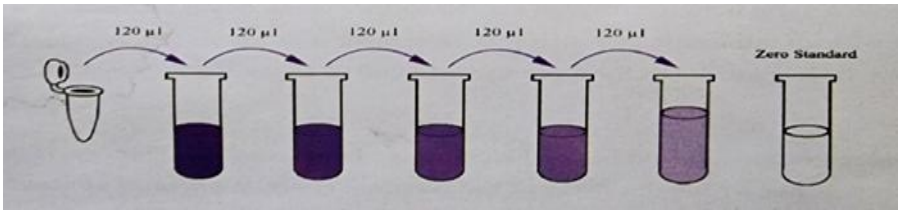
This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL-2 antibody. IL-2 present in the sample iS added and binds to antibodies coated on the wells And then biotinylated Human IL-2 Antibody is added and binds to IL-2 in the sample, Then Streptavidin-HRP is added and binds to the Biotinylated IL-2 antibody. After incubation unbound

Components	Quantity (96T
Standard Solution (2400ng/L	0.5ml *1
Pre-coated ELISA Plate	12*8 well strip
Standard Diluent	3 ml*1
Streptavidin-HRP	6 ml * 1
Stop Solution	6 ml * 1
Substrate Solution A	6 ml * 1
Substrate Solution B	6 ml * 1
Wash Buffer Concentrate (25x(	20 ml*1
Biotinylated Human IL-2 Antibody	1 ml *1
User Instruction	1
Plate Sealer	2 pcs
Zipper bag	1 ps

Fig 2.3. Interleukin 2 (IL-2) ELISA detection kit

2.10.2.Reagent Preparation

All reagents should be brought to room temperature before use.Standard Reconstitute the 120ul of the standard (2400ng/L) with 120ul of standard diluent to generate a 1200ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1200ng/L) 1:2 with standard diluent to produce 600ng/L, 300ng/L150,ng/L and 75ng/L solutions. Standard diluent serves as the zero standard(0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows;



2.10.3. ELISA protocol

1. All reagents, standard solutions and samples were prepared as instructed.
2.The number of strips required for the assay was determined.
3. 5Oul standard was added to standard wéll. Note: Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

4.40ul sample was added to sample wells and then 10ul anti-IL-2 antibody was added to sample wells.
5.50ul streptavidin-HRP was added to sample wells and standard wells (Not blank control well).
Cover the plate with a sealer, Incubate 60 minutes at 37°C.6
7.Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material..
8.Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with new sealer for 10 minutes at 37°C in the dark.
9.Stop Solution was added to each well, the blue color will change into yellow immediately.
10.Determine the optical density (OD value) of each well immediately using microplate reader set to 450 nm within 10 minutes after adding the stop solution

2.11. Statistical Analysis

Computerized statistically analysis was performed using Minitab version 11 statistic programme. Comparison was carried out using Chi-square (X^2) , ANOVA and probability (P value).

2.12. Ethical Consideration

Eathical consideration for study has was obtained from Azadi teaching hospital and Kirkuk teaching hospital. A verbal consent was taken for all participants in this study.

3. RESULTS

3.1. Age Distribution of HBV Vaccinated Health care workers of the study group :

Table 3.1. shows that the highest percentage (80%) of 5-10 yrsHBV Vaccinated Health care works of the study group was in the age group 25-35 years old and (67%) in 10-15 yrs respectively. The present data showed that the lowest percentage (0%) of the study group was in the age group 56-65 years old in 5-10 yrs and10-15 yrs. These findings were calculated at a P value less than 0.05 and there was a highly significant difference in age distribution among HBV Vaccinated Health care workers of the study group (P < 0.0001).

Table 3.1. Age Distribution of HBV Vaccinated Health care works of the study group

Age	5-10 yrs		10-15 yrs		≥15 yrs	
	No.	%	No.	%	No.	%
25-35	24	80	20	67	7	23
36-45	1	3	3	10	17	57
46-55	5	17	7	23	3	10
56-65	0	0	0	0	3	10
Total	30	100	30	100	30	100

X^2 = 38.6; DF=6 Contingency coefficient =0.548 P < 0.0001** HS

3.2. Gender Distribution of HBV Vaccinated Health care works of the study group

Table 3.2. shows that 13 (44%) of HBV Vaccinated Health care works of the study group of 5-10 yrs of being vaccinated were females and 17 (56%) were males whereas 12 (40%) HBV Vaccinated Health care works of the study group of 10-15 yrs were females and 18 (60%) were males in the same group and 9 (30%) HBV Vaccinated Health care works of the study group of ≥15 yrsyrs were females and 21 (70%) were males in the same group and revealed that there was statistically a non-significant difference (P =0.5409).

Table 3.2. Gender Distribution of HBV Vaccinated Health care works of the study group

Gender	5-10 yrs		10-15 yrs		≥15 yrs	
	No.	%	No.	%	No.	%
Male	13	44	12	40	9	30
Female	17	56	18	60	21	70
Total	30	100	30	100	30	100

X^2 = 1.22; DF=2 Contingency coefficient =0.116 P =0.5409 Non-Significant

3.3. Occupation Distribution of HBV Vaccinated Health care works of the study group

Table 3.3. shows that the highest percentage (86%), (80%) and (53%) were lab technicians in the 5-10 yrs, 10-15 yrs and ≥15 yrs of the health care workers being vaccinated respectively, There was highly significant difference (P < 0.0001).

Table 3.3 Occupation Distribution of HBV Vaccinated Health care works of the study group

Occupation	5-10 yrs		10-15 yrs		≥15 yrs	
	No.	%	No.	%	No.	%
Nurse	0	0	3	10	11	37
Doctor	2	7	2	7	2	7
Lab technician	26	86	24	80	16	53
Other	2	7	1	3	1	3
Total	30	100%	30	100%	30	100%

X^2 = 16.9; DF=6 Contingency coefficient =0.116 P = 0.0096** HS

3.4. Body Mass Index Distribution HBV Vaccinated Health care works of the study group

Table 3.4. shows that the highest percentages were (56%) normal weight, (57%) and (60%) over weight of 5-10 yrs, 10-15 yrs and ≥15 yrs of being vaccinated respectively control group. There was statisticallyhigh significant difference (P < 0.0001).

Table 3.4. Body Mass Index Distribution HBV Vaccinated Health care works of the study group

Group	5-10 yrs		10-15 yrs		≥15 yrs	
	No.	%	No.	%	No.	%
Normal weight (18.5-24.9)	17	56	5	17	6	20
Over weight (25-29.9)	13	44	17	57	18	60
Obese (≥ 30)	0	0	8	26	6	20
Total	30	100	30	100	30	100

X^2 = 17.8; DF=4 Contingency coefficient =0.116 P = 0.0013** HS

3.5. Level of anti- HBs antibody of the study group

Table 3.5. shows that the only 40% of the 5-10 years vaccinated group have antibody level **>100**, while There was statistically There was statistically high significant difference (**P < 0.0001**).

Table 3.5. Level of anti- HBs antibody of the study group

Anti- HBs antibody level IU/ML	5-10 yrs		10-15 yrs		≥15 yrs	
	No.	%	No.	%	No.	%
<10	1	16	6	18	23	24
10-100	19	44	24	22	7	34
>100	10	40	0	60	0	42
Total	30	100	30	100	30	100

X^2 = 55.76; DF=4 Contingency coefficient =0.619 P < 0.0001** HS

3.6. Mean Interleukin 2 (IL-2) level ng/L of the study group

Table 3.6 shows that the mean IL-2 level was higher in the 10-15 yrs vaccinated group followed by the 5-10 yrs vaccinated group and finally the ≥15 yr vaccinated group. There was statistically a non-significant difference among study groups

Table 3.6. mean Interleukin 2 (IL-2) of the study group

Group	5-10 yrs	10-15 yrs	≥15 yrs
Number	30	30	30
Mean	1702	1795	1585

ANOVA P = 0.804

4.DISCUSION

This study aims at detecting the effectiveness of HBV vaccine after 5, 10 and 15 years oAssessment of HBV vaccination coverage in health care setting is needed to evaluate the proportion susceptible to HBV infection [21]. Immunization programs are highly effective and clearly protect populations and individuals at risk if they are properly implemented [22].

The age of the participtants ranged from (25-65) the age was statistically significant and this result is in concurrence with The difference of anti-HBs status in gender was not statistically significant [23].

13 (44%) of HBV Vaccinated Health care works of the study group of 5-10 yrs of being vaccinated were females and 17 (56%) were males whereas 12 (40%) HBV Vaccinated Health care works of the study group of 10-15 yrs were females and 18 (60%) were males in the same group and 9 (30%) HBV Vaccinated Health care works of the study group of ≥15 yrs yrs were females and 21 (70%) were males in the same group and revealed that there was statistically a non-significant difference between these groups ($P > 0.05$). in concurrence with The difference of anti-HBs status ingender was not statistically significant[24].

This study showed that the level of Anti-HBS antibody The evaluation of HBV markers in HCWs is useful to identify and reduce the number of unprotected workers who have not been vaccinated or show a low antibody titre. The result of the study corroborates the central role of the Occupational Health Service in hospital facilities related to primary prevention and maximum protection of workers in the healthcare environment, particularly those highly exposed to the risk of exposure to body fluids and at increased risk of sharps injuries.Multivariate analysis demonstrated that, laboratory technicians and medical attendants were six and seven times more likely to be unvaccinated/incomplete vaccination as compared to intern doctors. This finding is worrisome because the risk of occupational injury and exposure to HBV is higher among nurses and intern doctors [25-28].

A total of 60 subjects had protective levels of Anti-HBs (more than 10mIU/ml) decline in antibody levels over time post-vaccination these results were in consistency with Chaudhari [29].Who found that There was decline in anti-HBs levels with time after last vaccine dose. However, even after 11 years of primary vaccination, HCWs had anti-HBs levels of more than 10 mIU/ml.

This study showed that there was statistically non-significant difference in interleukin-2 mean level among study groups.

CONCLUSION

Booster dose of HBV vaccine is not necessary in healthy HCW for at least ten years after primary vaccination. The study recommends early primary vaccination of HCW and ‘initial’ anti-HBs assay for confirmation of vaccine response.

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