

# Hepatitis B Vaccination Coverage among Healthcare Workers and Evaluation of Immune Response by Estimating Anti-HBs Antibody Titers over Time at a Tertiary Care Hospital: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Healthcare Workers (HCWs) are at a high-risk of acquiring Hepatitis B Virus (HBV) infection. However, this risk can be prevented through Hepatitis B vaccination. In some institutes, HCWs have a lower percentage of HBV vaccination, leading to a higher risk of HBV transmission. Therefore, the coverage of vaccination is an important point, along with the evaluation of protective immune status.

**Aim:** To assess the vaccination coverage and evaluate the immune response post-vaccination through Anti-Hepatitis B surface Antibodies (Anti-HBs) titre.

**Materials and Methods:** This cross-sectional study was conducted at Government Medical College, Pali, Rajasthan, India, over a period of one year from April 2021 to March 2022, following approval from the Institutional Ethics Committee (IEC). A total of 455 HCWs below 60 years of age were included in the study, and their demographic details such as age, gender, occupation, needle stick injury, blood exposure to mucous membranes and breached skin, hepatitis B vaccination status, and time duration since vaccination were noted. Additionally, their Anti-HBs titer was examined. The participants were initially classified into three groups: completely vaccinated, partially vaccinated, and non vaccinated. Among those who were vaccinated, they were further divided into two groups: vaccination  $\leq 5$  years (Group A) and  $>5$  years (Group B). Furthermore, those who received a booster dose were divided into Group I ( $\leq 1$  year) and Group II ( $>1$  year). Blood samples were collected to assess the anti-HBs levels quantitatively in the sera using Enzyme Linked Immuno

Sorbent Assay (ELISA). The data was entered into Microsoft Excel and later imported into Statistical Package for Social Sciences (SPSS) version 22.0 for statistical analysis.

**Results:** Participants had a mean age of  $29.65 \pm 9.603$  years. Among them, 43 (30.1%) were doctors, and 37 (25.9%) were medical students who were found to be completely vaccinated. On the other hand, among other HCWs, 15 (10.5%) were nurses, 16 (11.2%) were nursing students, and 32 (22.4%) were lab technicians who were vaccinated. None of the support staff were vaccinated ( $p$ -value=0.0001). A total of 143 participants were completely vaccinated, and 97.9% ( $n=140$ ) had protective immunity to Hepatitis B. The anti-HBs titre was  $567.32 \pm 434.494$  in group A and  $265.74 \pm 211.80$  in group B ( $p$ -value=0.0001). Similarly, it was  $688.34 \pm 424.617$  in group I and  $221.14 \pm 141.221$  in group II ( $p$ -value  $< 0.0001$ ). The anti-HBs titre did not significantly increase among the partially vaccinated participants ( $n=162$ ). It was found to be  $25.47 \pm 27.595$  in group A and  $14.60 \pm 19.939$  in group B ( $p$ -value=0.004). There was no significant difference in the results between males and females ( $p$ -value=0.961).

**Conclusion:** The coverage of complete vaccination among HCWs was significantly low, which is crucial for obtaining a protective Anti-HBs titre. Incomplete vaccination does not result in a sufficient level of anti-HBs titre, and there may be a significant decline in the immune response over time ( $p$ -value  $< 0.05$ ). Therefore, it is essential to estimate the titre after 1-2 months of complete vaccination to ensure that individuals are fully protected against Hepatitis B.

**Keywords:** Anti hepatitis B, Hepatitis B antibodies, Hepatitis B vaccines

## INTRODUCTION

The HBV is infectious and can cause serious health problems such as chronic active hepatitis, cirrhosis of the liver, and hepatocellular carcinoma [1]. HBV infection is a well-recognised occupational risk for HCWs. The risk of HBV infection is primarily related to the degree of contact with blood in the workplace and also to the hepatitis B e antigen (HBeAg) status of the source person [2]. HBV infection can spread from a source to a HCW due to exposure of mucous membranes and breached skin to blood [3-5]. Hepatitis B is a major global health problem. The burden of infection is highest in the World Health Organisation (WHO) Western Pacific Region and the WHO African Region, where 116 million and 81 million people, respectively, are chronically infected. Sixty million people are infected in the WHO Eastern Mediterranean Region, and 18 million in the WHO South-East Asia Region [6].

The Centers for Disease Control and Prevention (CDC) has identified the importance of vaccination as an effective means to prevent HBV and has suggested that HCWs should receive a complete course of Hepatitis B vaccination at 0, 1, and 6 months [7]. Studies conducted in India suggest that only 16-60% of HCWs have received complete HBV immunisation. Among HCWs, paramedics who have a higher risk of HBV transmission have a lower percentage of HBV vaccination compared to doctors [8,9].

Although vaccination coverage is an important point, evaluation of protective immunity is also of great concern because some individuals do not develop sufficient levels of antibodies against HBsAg (anti-HBs). According to the guidelines, individuals with an anti-HBs titre  $< 10$  mIU/mL are considered 'non responders,' levels between 10 and 100 mIU/mL are considered 'low responders,' and

levels above 100 mIU/mL are considered 'sufficient responders.' Research has shown that levels above 10 mIU/mL at any time after vaccination are considered a marker of sustained immunity, providing protection against infection [10].

HBV is highly infectious and causes serious health problems. HCWs are at high-risk for HBV infection. Vaccination is effective in protecting 90-95% of adults, but the practice of vaccination is not well-accepted in remote areas like Pali, Rajasthan, India. Therefore, the aim of the study was to assess the vaccination coverage and evaluate the immune response post-vaccination through Anti-HBs titre. The objective of the study was to examine antibody titers over time since vaccination and after partial vaccination.

## MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Pali, Rajasthan, India, over one year from April 2021 to March 2022. The study received approval from the Institutional Ethics Committee (IEC) (certificate reference number: EC/NEW/INST/2020/555) to evaluate HBV immunisation coverage and anti-HBs titers among the HCWs. Written informed consent was obtained from the participants.

**Inclusion criteria:** HCWs under the age of 60 years were included in the study and grouped according to the nature of their work at the institution. The categories included doctors from all disciplines (n=71), medical students of MBBS (n=102), nursing staff (n=138), laboratory technicians (n=50), nursing students (n=50), and support staff (n=44).

**Exclusion criteria:** Participants with hepatitis B infection (HBsAg positive), chronic liver disease, and diabetes mellitus, as well as those on prolonged steroid treatment, were excluded from the study.

A total of 468 HCW participants were initially included in the study; however, 13 of them were subsequently excluded. Out of the 13, 11 did not come for follow-up, and two were found to be positive for HBsAg.

**Sample size calculation:** The sample size for the study was calculated using the single population proportion formula, considering a prevalence of hepatitis B vaccination among HCWs of 50%, a confidence level of 95%, and a marginal error of 5%. After adjusting for a non respondent rate of 5-10%, the final sample size was determined to be 455 participants. This calculation was based on a previous study [11].

All demographic details such as age, gender, occupation, needle stick injury, blood exposure to mucous membrane and breached skin, hepatitis B vaccination status, and time duration since vaccination were noted. This titre value was defined as the "primary response" to HBV vaccination. The subjects were classified based

on the anti-HBs titer in the primary response into the following three groups: non responders (<10 mIU/mL), low responders (10-100 mIU/mL), and sufficient responders (>100 mIU/mL) [10].

Participants were classified based on HBV vaccination status, i.e., completely vaccinated group (receiving 3 doses of HBV vaccination at 0, 1, and 6 months), partially vaccinated group (receiving either a single or 2 doses), and non vaccinated group (who received no dose).

To evaluate the decrease in anti-HBs titers over time, a comparison was done between two groups. Those who were vaccinated were further divided into two groups: vaccination  $\leq$  5 years (group A) and >5 years (group B). Furthermore, those who received a booster dose were divided into group I ( $\leq$ 1 year) and group II (>1 year).

Under strict aseptic precautions, 4-5 mL of venous blood was collected from all eligible subjects in a vacutainer containing clot activator (silicone and micronised silica particles manufactured by BD). Serum separation was performed by centrifuging the blood sample at 3000 rpm for five minutes at room temperature. The separated serum was stored at -80°C until further analysis.

HBsAg testing was done by ELISA using a commercial kit (HBsAg kit manufactured by J mitra) according to the manufacturer's protocol. The quantification of serum anti-HBs level was done by the ELISA technique using a commercially available kit (HBsAg kit manufactured by J mitra), strictly adhering to the manufacturer's protocol.

## STATISTICAL ANALYSIS

Data were entered into Microsoft Excel and later imported into SPSS version 22.0 for statistical analysis. Numerical variables were measured using mean and standard deviations, while categorical variables were expressed as frequencies and percentages. Inferential statistics were applied depending on the nature of the data and variables. The Chi-square test was used to find differences in vaccination status groups (complete vaccinated, partially vaccinated, and not vaccinated) by demographic characteristics. Independent sample t-tests were performed to assess any differences in mean anti-HBs titre mIU/mL, and one-way Analysis of Variance (ANOVA) analyses were conducted to assess any differences in mean age. A p-value <0.05 were considered significant in all tests.

## RESULTS

Among the 455 HCWs, 143 (31.4%) were found to be completely vaccinated, 162 (35.6%) were partially vaccinated, and 150 (33%) were not vaccinated. It was observed that most of the partially vaccinated participants had missed their third dose, which is the booster dose. Males were more frequently vaccinated than females (p-value=0.961) [Table/Fig-1].

Variables	Total (n=455)	Vaccination status			p-value
		Complete (n1=143)	Incomplete (n2=162)	Not vaccinated (n3=150)	
Age (years) <sup>a</sup>	29.65±9.603	30.13±10.252	30.60±9.637	28.16±8.777	0.062
<b>Gender, n (%)</b>					
Female	133 (29.2)	38 (26.6)	50 (30.9)	45 (30.0)	0.961
Male	322 (70.8)	105 (73.4)	112 (69.1)	105 (70.0)	
<b>Occupation, n (%)</b>					
Doctor	71 (15.6)	43 (30.1)	23 (14.2)	5 (3.3)	0.0001
Nurse	138 (30.3)	15 (10.5)	94 (58.0)	29 (19.3)	
Medical student	102 (22.4)	37 (25.9)	41 (25.3)	24 (16.0)	
Nursing student	50 (11.0)	16 (11.2)	0	34 (22.7)	
Supporting staff	44 (9.7)	0	0	44 (29.3)	
Lab technician	50 (11.0)	32 (22.4)	4 (2.5)	14 (9.3)	

Needle stick, n (%)					
Yes	8 (1.8)	5 (3.5)	2 (1.2)	1 (0.7)	0.168
No	447 (98.2)	138 (96.5)	160 (98.8)	149 (99.3)	
Anti-HBs titre mIU/mL <sup>#</sup>	212.18±332.105	430.24±381.178	19.70±24.378	NA	0.0001

**[Table/Fig-1]:** Vaccination coverage i.e., complete, partial, not vaccinated among the participants according to age, occupation, needle stick and anti-HBs titre. one-way ANOVA-age; Independent sample t-test- Anti-HBs titre and Chi-square applied on applied on rest of the parameters; (#) Mean±SD

A total of 143 participants were completely vaccinated, and among them, 97.9% (n=140) had protective immunity to Hepatitis B [Table/Fig-2]. Among the combined categories of completely and partially vaccinated participants, a total of 305 individuals were subjected to anti-HBs titer estimation. Within the completely vaccinated group (n=143), only 3 (2%) had anti-HBs titer <10 mIU/mL, 16 (11.2%) had titers between 10-100 mIU/mL, and 124 (87%) had titers >100 mIU/mL [Table/Fig-2].

Anti-HBs titre (mIU/mL)	Complete vaccination, n=143, n (%)			Incomplete vaccination, n=162, n (%)		
	≤5 year N=78, 54.5% group A	>5 year N=65, 45.5% group B	Total	≤5 year N=76, 47% group A	>5 year N=86, 53% group B	Total
<10	2 (2.5)	1 (1.5)	3 (2.1)	31 (41)	60 (70)	91 (56)
10-100	2 (2.5)	14 (21.5)	16 (11.2)	45 (59)	26 (30)	71 (44)
>100	74 (94)	50 (77)	124 (86.7)	0	0	0
Total	78	65	143	76	86	162

**[Table/Fig-2]:** Distribution of complete and partial vaccinated participants according to anti-HBs titre range with time duration since vaccination among group A and group B.

Among the various groups of HCWs, the highest percentage of 'Complete Vaccination' was observed among Doctors, with 43 (60.6%) individuals, followed by medical students with 37 (36.3%), lab technicians with 32 (64%), nursing students with 16 (32%), and nursing staff with 15 (10.8%). There were no completely vaccinated individuals among the support staff (p-value=0.0001, significant). In the 'partial vaccination' category, the results showed that 23 (32.4%) doctors, 41 (40.2%) medical students, 94 (68.1%) nursing staff, 4 (8%) lab technicians were partially vaccinated, while none of the nursing students and support staff were partially vaccinated (p-value=0.0001, significant) [Table/Fig-3].

Total participants=455	Complete (n1=143), n (%)	Incomplete (n2=162), n (%)	Not vaccinated (n3=150), n (%)	p-value
Doctor, n=71	43 (60.6)	23 (32.4)	5 (7)	0.0001
Nurse, n=138	15 (10.8)	94 (68.1)	29 (21.1)	
Medical student, n=102	37 (36.3)	41 (40.2)	24 (23.5)	
Nursing student, n=50	16 (32)	0	34 (68)	
Supporting staff, n=44	0	0	44 (100)	
Lab technician, n=50	32 (64)	4 (8)	14 (28)	

**[Table/Fig-3]:** Participants according to occupation and vaccination status. Chi-square test applied

Parameters	Total (n=143)	Complete vaccinated group		p-value
		≤5 year, Group A (n1=78)	>5 year, Group B (n2=65)	
Anti-HBs titre mIU/mL	430.24±381.178	567.32±434.494	265.74±211.800	0.0001
Parameters	Total (n=143)	Booster dose complete vaccinated group		p-value
		≤1 year, Group 1 (n1=64)	>1 year, Group 2, (n2=79)	
Anti-HBs titre mIU/mL	430.24±381.178	688.34±424.617	221.14±141.221	<0.0001
Parameters	Total (n=162)	Incomplete vaccinated group		p-value
		≤5 year, Group A (n1=76)	>5 year, Group B (n2=86)	
Anti-HBs titre mIU/mL	19.70±24.378	25.47±27.595	14.60±19.939	0.004

**[Table/Fig-4]:** Anti-HBs titre among complete and partial vaccinated participants according to time duration since vaccination in group A and B and group I and II. Independent sample t-test was applied

Those who were vaccinated ≤5 year (group A, n=78) have protective antibody titre 567.32±434.494 mIU/mL and among them group I (n=64) had titre of 688.34±424.617 mIU/mL (p-value=0.0001). Whereas group B >5 year (n=65) and group II (n=79) participants had titre of 265.74±211.800 mIU/mL and 221.14±141.221 mIU/mL, respectively (p-value <0.0001). Furthermore, a separate assessment was conducted on 'partially vaccinated' individuals (n=162). In group A, the mean value of anti-HBs titer was found to be 25.47±27.595 mIU/mL, while in group B, it was 14.60±19.939 mIU/mL. The anti-HBs titer was significantly lower in group B compared to group A (p-value=0.004) [Table/Fig-4].

## DISCUSSION

Considering that HCWs work profile involves an anticipated risk of exposure to blood or body fluids, the Occupational Safety and Health Administration (OSHA) mandates that HCWs should receive a complete ≥3-dose Hepatitis B vaccine series within 10 days to prevent Hepatitis B [11, 12]. Reports from India indicate that only 16-60% of HCWs have received complete HBV immunisation. Notably, paramedics, who have a higher risk of HBV/HCV transmission, receive HBV vaccination less frequently compared to doctors [8,9]. In the present study, the vaccination coverage among paramedics was as follows: nursing students 16 (11.2%), nursing staff 15 (10.5%), lab technicians 32 (22.4%), and support staff 0 (0%). These results were similar to a study conducted by Batra V et al., where the figures were 4 (8.5%), 20 (41.7%), 8 (24.2%), and 0 (0%), respectively [13].

The implementation of universal precautions among HCWs in developing countries is suboptimal, and there is a lack of awareness about vaccination [12,14]. The 3-dose vaccine series, administered intramuscularly at 0, 1, and 6 months, elicits a protective antibody response in approximately 30-55% of healthy adults, with antibody levels exceeding 90% after the third dose [15-17].

In the present study, doctors were more often completely vaccinated (60.6%) compared to nurses (10.8%) and support staff (0%) [Table/Fig-3]. This difference in vaccination patterns among various groups of HCWs is likely influenced by factors such as education level, awareness, and knowledge regarding vaccination. Similar findings were reported in a study conducted by Batra V et al., [13].

Among the HCWs who were completely vaccinated in this study (97.9%, n=140), all of them had an anti-HBs titer >10 mIU/mL in

both group A and group B [Table/Fig-2]. However, the anti-HBs titre was lower in those who were vaccinated more than 5 years ago and had a booster dose after one year [Table/Fig-4]. On the other hand, among the HCWs who were incompletely vaccinated, 41% of group A and 70% of group B were unable to achieve an anti-HBs titer >10 mIU/mL (considered protective) [Table/Fig-2]. These findings indicate that partially vaccinated HCWs were at risk of acquiring HBV infection.

A study of 166 HCWs conducted in North India revealed that anti-HBs titre <10 mIU/mL was more common in participants whose vaccination was >5 years (36.8%) as compared to those ≤5 years (24.4%), which was significant [13]. In the present study, it was found that among the 64 vaccinated HCWs, those who had received a booster dose within or less than one year had a mean anti-HBs titre of 688.34±424.617 mIU/mL, while those who had received the booster dose more than a year ago had a mean anti-HBs titre of 221.14±141.221 mIU/mL (p-value <0.0001). Similar findings were observed in the study conducted by Batra V et al., where subjects who received the booster dose within one year had a mean anti-HBs titre of 1742.7 mIU/mL, while those who received it more than one year ago had a mean anti-HBs titre of 629.2 mIU/mL, and this difference was statistically significant (p-value <0.002) [13].

Another study conducted by Rao TV et al., showed that 79% of the participants achieved antibody levels >101 mIU/mL after completing the vaccination series [18]. Among successfully vaccinated individuals, 10.5% did not reach the minimal protective level of antibody (10 mIU/mL), and 10.5% had antibody levels ranging from >11-100 mIU/mL [18]. A separate study on 112 HCWs demonstrated that protective antibody levels were 99.9% one year after vaccination but decreased to 80.96% and 46.16% after 5 and 10 years, respectively, following vaccination [19]. Additionally, a study conducted by Kumar HN et al., showed that 93.8% of individuals who had only received a single dose were not protected against HBV infection [20].

The prevalence of anti-HBs titer <10 mIU/mL was higher in group B (vaccination >5 years) compared to group A (vaccination ≤5 years) (p-value=0.004). Therefore, there was a significant role in checking the anti-HBs titer after vaccination in HCWs, preferably 1-2 months after completing the vaccine series. Completely vaccinated HCWs with anti-HBs ≥10 mIU/mL are considered immune to hepatitis B and have long-term protection, thus not requiring periodic testing for anti-HBs levels. However, completely vaccinated HCWs with anti-HBs <10 mIU/mL should receive an additional series of HepB vaccine, followed by anti-HBs testing 1-2 months later (usually 6 doses total). Repeat anti-HBs testing should then be conducted 1-2 months after the last dose [21].

The European Consensus Group on Hepatitis B Immunity [22] and the Steering Committee for the Prevention and Control of Infectious Disease in Asia [23] do not recommend booster vaccination for HCWs who have documentation of receiving a complete series of hepatitis B vaccine and have anti-HBs levels ≥10 mIU/mL, as they are considered immune to hepatitis B. However, some immunodeficient individuals, such as those on haemodialysis, may require periodic booster doses of the hepatitis B vaccine. Therefore, timely prediction of insufficient anti-HBs titers among individuals at high-risk of HBV exposure improves economic efficiency by screening those who require booster vaccination.

In this study, the primary response was found to be useful in predicting anti-HBs titers after vaccination. Based on these results, authors propose changing the follow-up schedule to monitor and regulate anti-HBs titers based on the primary response of each individual.

## Limitation(s)

The limitations of this study were that the association of decreased immune response with risk factors such as smoking, alcoholism, nutritional status, chronic infections, site of vaccine administration, and genetic factors was not evaluated.

## CONCLUSION(S)

Since hepatitis B infection has serious outcomes, all HCWs should receive a complete series of vaccination. Incomplete vaccination does not result in a sufficient level of anti-HBs titre. As there is a gradual decline in the anti-HBs titer over time, estimating the anti-HBs antibody titer after vaccination is compulsory for HCWs. Hospitals should implement a policy to vaccinate all categories of HCWs at the time of recruitment, followed by post-vaccination measurement of antibody titres. This is a cost-effective measure compared to post-exposure prophylaxis with immunoglobulin, which is expensive.

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