Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Assessment of Anti-HBs Titre Among Healthcare Workers at a Tertiary Care Centre, Visnagar, Gujarat

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Cite this paper as: Dr. Bhumika Gaur, Dr. Parul D Shah, Mr. Apurba Anshuman Mishra, (2025) Assessment of Anti-HBs Titre Among Healthcare Workers at a Tertiary Care Centre, Visnagar, Gujarat. *Journal of Neonatal Surgery*, 14 (32s), 2918-2927.

ABSTRACT

Introduction: Healthcare workers (HCWs) are at increased risk of acquiring hepatitis B virus (HBV) infection through occupational exposure, but this risk can be effectively mitigated through hepatitis B vaccination.

Aims: Our goal is to determine the sero-prevalence of anti-Hepatitis B surface (anti-HBs) antibodies among healthcare workers in a tertiary care setting, in order to evaluate the level of protection for those in these high-risk environments.

Material and Methods: Serum samples from HCWs were analyzed at the Microbiology laboratory of Nootan Medical College and Nootan General Hospital in Gujarat. Aseptic collection of 3 ml of venous blood was performed using a vacutainer. The serum anti-HBs titers were measured through an Enzyme-Linked Immunoassay (ELISA).

Results: Of the 280 HCWs included, 259 were vaccinated, and 21 were unvaccinated. HCWs were grouped into four categories which comprised of doctors from all disciplines (n=37), nursing staff (n=140), technicians (n=53) and housekeeping (n=50). Among various groups of HCWs, the vaccination rate was highest among doctors and nursing staff's than the other groups. While comparing the anti-HBs titre among the unvaccinated HCWs, it has been seen that out of 21 unvaccinated HCWs, majority of them (n=18) were having titre below 10 mIU/ml while few of them (n=3) had titres above 10mIU/ml.

Conclusion: All healthcare workers should undergo regular screening for HBs antigen, receive a booster dose, and have their anti-HBs titers monitored.

Keywords: *Hepatitis B Virus, Healthcare workers, Hepatitis B Vaccination, Hepatitis B Surface Antibody Titre.*

1. INTRODUCTION

Hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the Hepadnaviridae family. Key components of HBV include the surface antigen (HBsAg), which is found on the outer membrane, and the core antigen (HBcAg), which constitutes part of the viral nucleocapsid. Additionally, the pre-core antigen (HBeAg) is a truncated form of the core protein and serves as an indirect marker of viral replication. [1]

HBV virions exist in two forms: infectious and noninfectious particles. The noninfectious particles are characterized as either spherical or tubular enveloped virions that contain embedded hepatitis B surface antigen (HBsAg).Infectious particles are of 42nm in diameter and are known as dane particles.[2]

Hepatitis B virus (HBV) is a significant global cause of both acute and chronic diseases, contributing to approximately 8,87,000 deaths due to complications such as hepatocellular carcinoma, liver cirrhosis, and fulminant hepatitis. Among healthcare workers (HCWs) and medical graduates, HBV infection poses the most substantial occupational risk. It's contagious in nature, particularly through sharp injuries like needle sticks or contact with infected blood and bodily fluids, underscores its importance. HCWs are at least four times more likely to be exposed to HBV compared to the general population, with a risk of infection ranging from 6% to 30%. According to the World Health Organization, around 5.9% of HCWs are exposed to this virus each year. One contributing factor to this high exposure rate is the inadequate use of personal protective equipment, such as goggles and gloves, as well as poor needle disposal practices.[3]

The virus is primarily transmitted from mother to child during childbirth and in early childhood. Additionally, HBV can spread through contact with blood or other bodily fluids, as well as through unsafe injection practices and exposure to sharp instruments.[4]

In addition, HBV has been demonstrated to survive in dried blood, at room temperature, on environmental surfaces, for at least one week.[5]

Hepatitis B infection is preventable through vaccination, which activates the body's immune defenses and offers protection to most individuals.[6] To effectively control HBV transmission in regions with high and intermediate endemicity, it is crucial to vaccinate vulnerable populations. The World Health Organization's strategy for managing HBV infection and its consequences includes mass vaccination of neonates and children as part of the Expanded Program on Immunization (EPI). It has been recommended that all countries incorporate the hepatitis B vaccine into their national immunization programs by 1997.[7]

HBV transmission can be effectively prevented through a timely vaccination schedule of three doses administered at 0, 1, and 6 months, achieving 95% efficacy.[4] The hepatitis B vaccine provides long-term protection, indicated by the presence of Anti-HBs titers, which serve as markers of protective levels.[8]

The immunity conferred by hepatitis B vaccination is directly linked to the production of anti-HBs antibodies. It is essential to evaluate the protective antibody levels in vaccinated healthcare workers (HCWs) because not all individuals develop adequate responses to the vaccine. HCWs who have completed the three-dose primary vaccination series and have a positive anti-hepatitis B surface antibody (HBs) level of ≥10 mIU/mL are considered immune to hepatitis B infection. Conversely, those with levels below 10 mIU/mL remain susceptible. Therefore, serological testing for anti-HBs identifies individuals with low antibody titers and non responders, informing the need for further counseling and possible revaccination. This study aimed to assess anti-HBs titers among HCWs at a tertiary care hospital in Gujarat.[9]

2. AIM AND OBJECTIVES OF THE STUDY

1. To titrate the level of protective anti-HBs antibodies among HCWs of Nootan General Hospital irrespective of their vaccination status.
2. To assess the percentage of staff that has received vaccination with respect to those unvaccinated.

Inclusion and Exclusion criteria

Inclusion criteria

1. The health care workers who had been vaccinated.
2. The health care workers who did not receive vaccination.

Exclusion criteria

1. Those who were not willing to be the part of the study were excluded.

Methodology

- **Study-design**: Interventional
- **Sampling**: Blood samples were obtained from all HCWs working in Nootan General Hospital including doctors, nursing staff, housekeeping staff etc.
- **Sample size**: 280

This study was carried out in the Department of Microbiology at Nootan Medical College and Research Centre, Visnagar, after approval from Institutional Ethics Committee. Written consent was obtained from doctors, nurses, technicians, housekeeping staff and laboratory technicians. A proforma was provided to all the health-care workers included in the study to fill up the demographic data such as age, gender, and vaccination status, etc. Once proforma was received, blood samples were obtained from all the health-care workers and subjected to quantitative ELISA to determine anti-HBs titre.

- **Procedure-**

Blood samples were collected from health-care workers. Blood was centrifuged and serum was extracted.

↓

Place 14 wells plus the number of wells required for sample titration. Leave A1 and B1 blank. Dispense 50 µl specimen diluents in all other wells.

↓

Dispense 100 µl of calibrator 1 in C1 and D1, calibrator 2 in E1 and F1, calibrator in G1 and H1, calibrator 4 in A2 and B2, calibrator 5 in C2 and D2.

↓

Add 100 µl of control serum in E2 and F2. Add 100 µl of samples in the remaining wells.

↓

Incubate the microtitre plate at 37°C for 60 min. Wash the microtitre plate 5 times. Vortex the conjugate before use

↓

Except A1 and B1, pipette 100 µl of enzyme conjugate for 60 min. Wash the microtitre plate 5 times.

↓

Pipette 100 µl of TMB/H_2O_2 in each well including the blanks. Incubate the microtitre plate at 37°C for 20 min. Do not expose to strong direct light.

↓

Pipette 100 µl of sulfuric acid to each well. Measure the color intensity at 450 nm for samples.

↓

Measure the color intensity of the blank at 620 nm for blank.

3. RESULT

Out of 280 participants, 105 were male and 175 were female. Among them 146 were in the age group of less than 30 years, 84 were between 30 to 50 years and 50 were greater than 50 years. The healthcare workers (HCWs) were categorized based on their roles in the hospital: doctors (37), nursing staff (140), technicians (53), and housekeeping staff (50) (Table 1).

Table 1.Baseline characteristics of study population

Category	**Total**	**Vaccinated**	**Unvaccinated**	**p-value**
N	**280**	**259(92.5%)**	**21 (7.5%)**	
Sex				
Male	105	93(89%)	12(11%)	0.0893
Female	175	166(95%)	9(5%)	
Age (years)				
<30	146	142(97%)	4(3%)	<0.01
30-50	84	77(92%)	7(8%)	
>50	50	40(80%)	10(20%)	
HCWs				
Doctor	37	35 (95%)	2(5%)	0.20
Nurse	140	133(95%)	7 (5%)	
Technician	53	48(90%)	5 (10%)	
Housekeeping	50	43(86%)	7 (14%)	

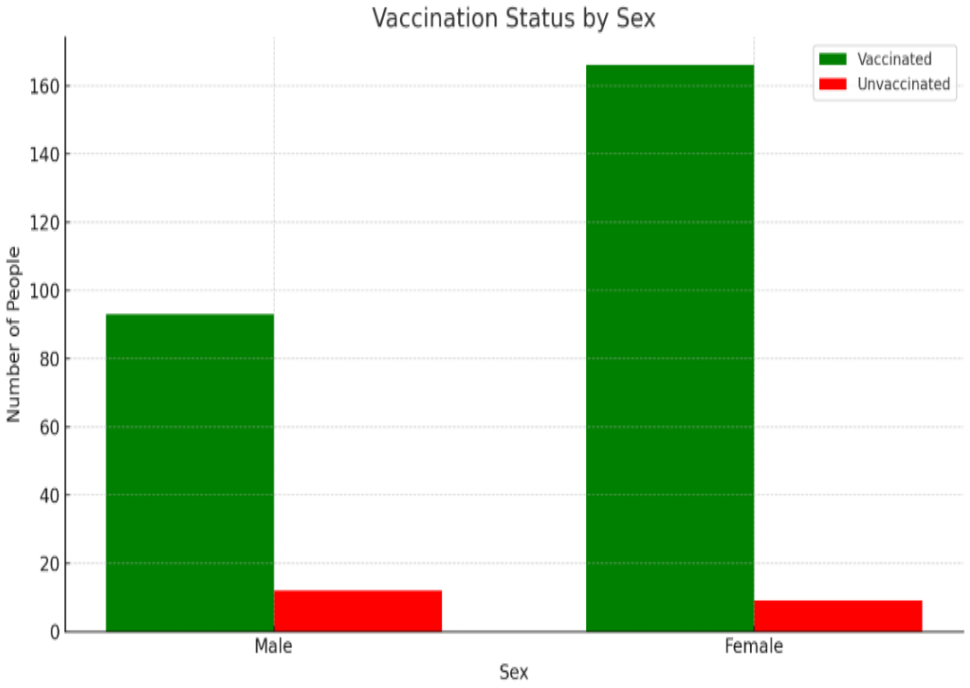


Figure 1.1 Vaccination status by sex

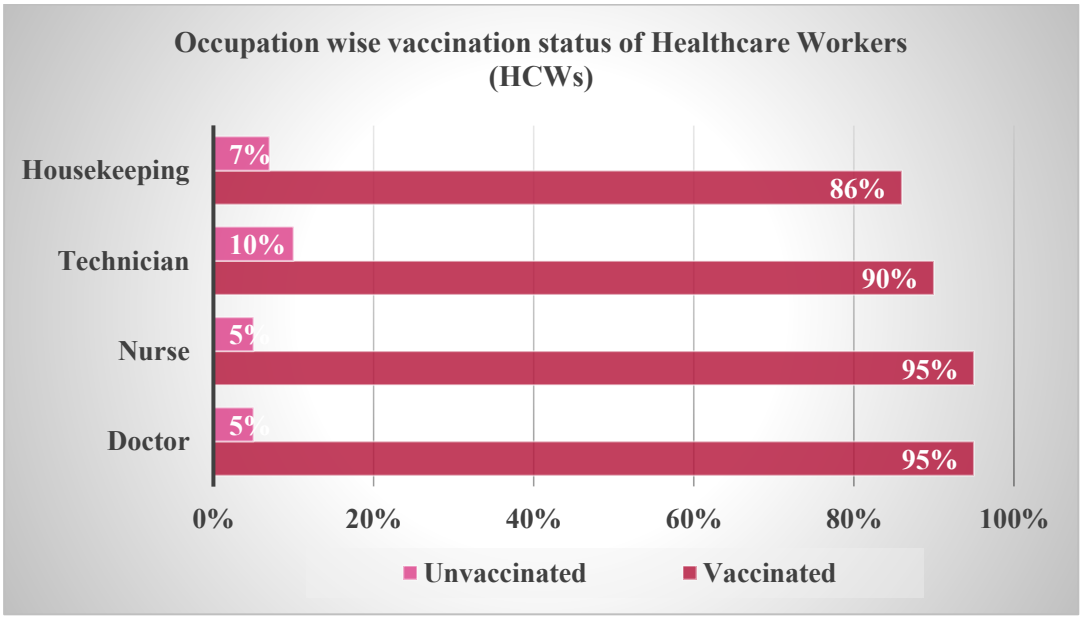


Figure 1.2 Occupation wise vaccination status of HCWs

Among the HCWs, 259 (92.5%) were vaccinated, while 21 (7.5%) were not. Out of the 259 fully vaccinated individuals, 166 were female (64.1%) and 93 were male (35.9%). The highest vaccination rates were observed among doctors and nursing staff, both at 95%, followed by technicians at 90%, and housekeeping staff at 86% (Table 1).

Table 2.Occupation wise comparison of Anti-HBs titre among the vaccinated HCWS

S. No.	Categories of HCWs	Anti-HBs Titre<10 mIU/ml N (%)	Anti-HBs Titre ≥10 mIU/ml N (%)	Total	P value
1	**Doctor**	1(3%)	34(97%)	35	0.755
2	**Nursing staff**	3(2%)	130(98%)	133	
3	**Technicians**	2(4%)	46(96%)	48	
4	**Housekeeping**	3(7%)	40(93%)	43	
5	**Total**	**9(3%)**	**250(97%)**	**259**	

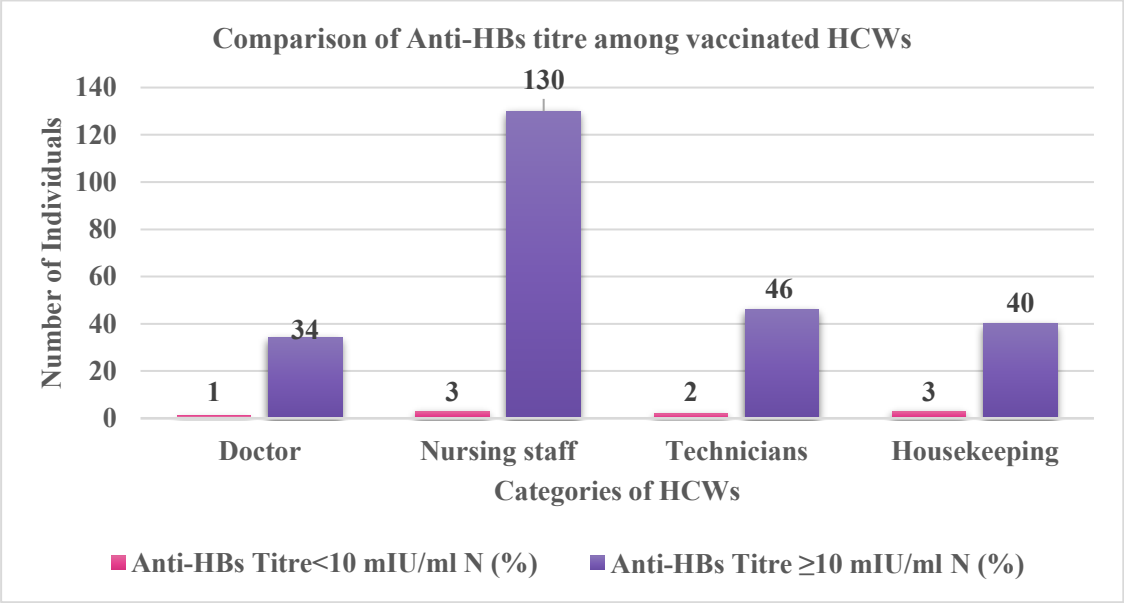


Figure 2- Occupation wise comparison of Anti-HBs titre among the vaccinated HCWS

The examination of anti-HBs titres among vaccinated health care workers by occupation revealed varying levels of immunity. Specifically, 3(7%) of housekeeping staff, 2(4%) of technicians, 1(3%) of doctors, and 3(2%) of nursing staff were found to have non-responsive titres (<10 mIU/ml), indicating that these individuals may still be at risk of hepatitis B infection. In contrast, a significant majority of the workforce had protective titres (≥10 mIU/ml), with 130(98%) of nursing staff, 34(97%) of doctors, 46(96%) of technicians, and 40(93%) of housekeeping staff achieving this level of immunity. These findings suggest that while most health care workers are well-protected, there are a few cases across all occupational groups who might require further assessment or booster vaccinations (Tables 2).

Table 3.Occupation wise comparison of Anti-HBs titre among the unvaccinated HCWS

S. No.	Categories of HCWs	Anti-HBs titre<10 mIU/ml N (%)	Anti-HBs titre ≥10 mIU/ml N (%)	Total	P value
1	**Doctor**	2(100%)	0	2	0.504
2	**Nursing staff**	6(86%)	1(14%)	7	
3	**Technicians**	5(100%)	0	5	
4	**Housekeeping**	5(71%)	2(29%)	7	
5	**Total**	**18(86%)**	**3(14%)**	**21**	

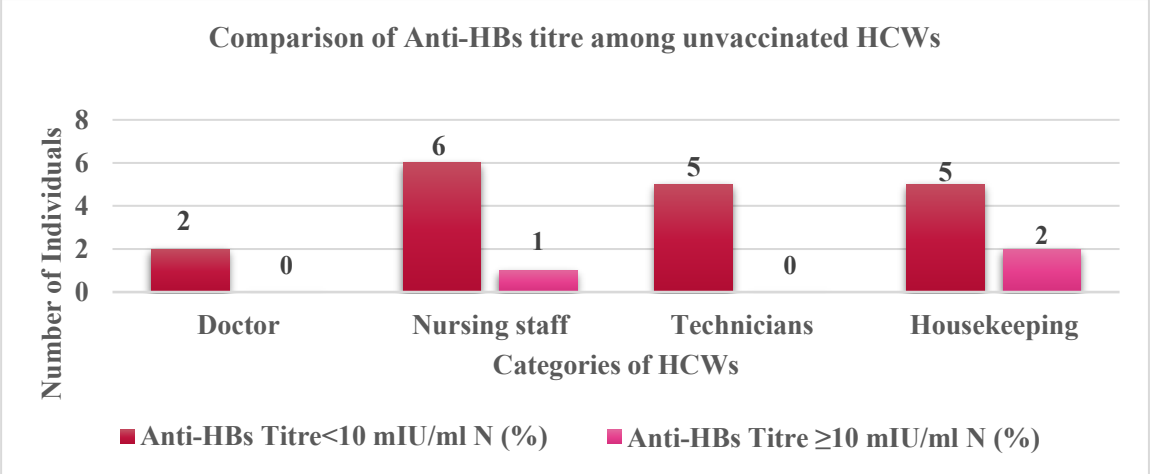


Figure 3-Occupation wise comparison of Anti-HBs titre among the unvaccinated HCWS

When comparing anti-HBs titres among unvaccinated health care workers, it was found that out of 21(7.5%) individuals, most were non-responders. However, 3 individuals, out of which 2(29%) housekeeping staff member and 1(14%) nursing staff member had titres exceeding 10 mIU/ml: despite their unvaccinated status (Table 3).

Table 4.Distribution of vaccinated HCWs with Anti-HBsAg titre ≥10 mIU/ml

S. No.	Categories of HCWs	Anti-HBs Titre 10- 100 mIU/ml N (%)	Anti-HBs titre ≥ 100 mIU/ml N (%)	Total
1	**Doctor**	9(26%)	26(74%)	35
2	**Nursing staff**	42(32%)	91(68%)	133
3	**Technicians**	15(31%)	33(69%)	48
4	**Housekeeping**	19(44%)	24(56%)	43
5	**Total**	**85(33%)**	**174(67%)**	**259**

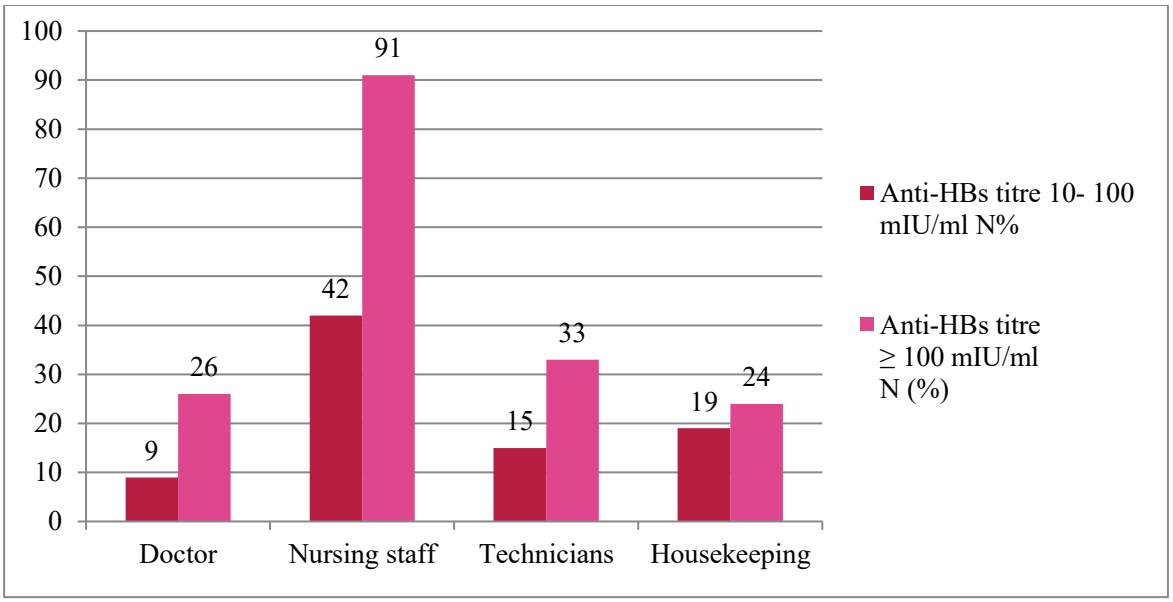


Figure 4- Distribution of vaccinated HCWs with Anti-HBsAg titre ≥10 mIU/ml

In this study, out of 280 vaccinated health care workers 259 (92.5%) exhibited anti-HBs titres exceeding 10 mIU/ml. Among these 259 individuals, 174 (67%) had titres greater than 100 mIU/ml, while 85 (33%) had titres ranging between 10 and 100 mIU/ml (Tables 4).

Table 5.Distribution of Unvaccinated HCWs with Anti-HBsAg Titre ≥10 mIU/ml

S. No.	Categories of HCWs	Anti-HBs Titre 10- 100 mIU/ml N (%)	Anti-HBs titre ≥ 100 mIU/ml N (%)	Total
1	**Doctor**	0	0	0
2	**Nursing staff**	1(100%)	0	1
3	**Technicians**	0	0	0
4	**Housekeeping**	1(50%)	1(50%)	2
5	**Total**	**2(67%)**	**1(33%)**	**3**

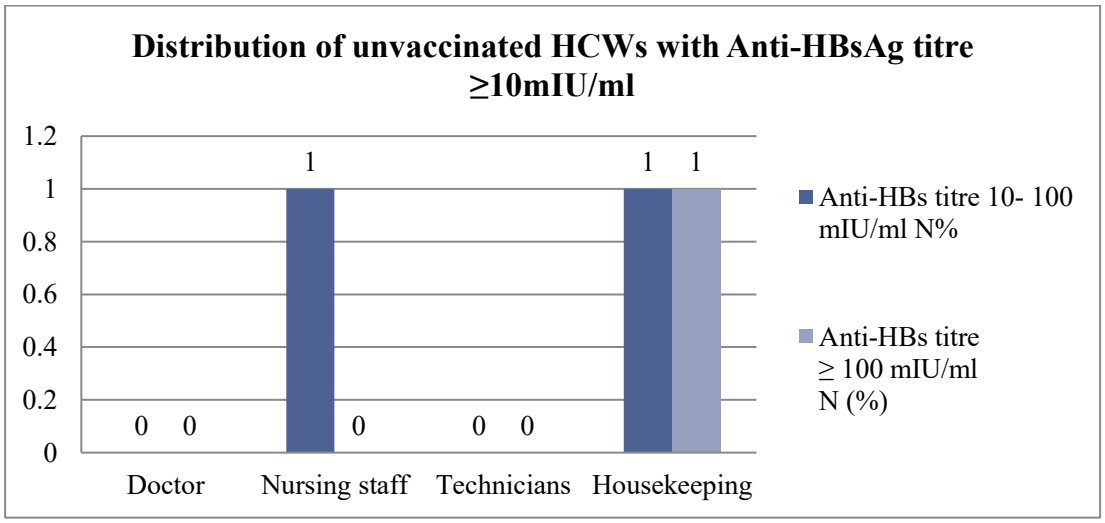
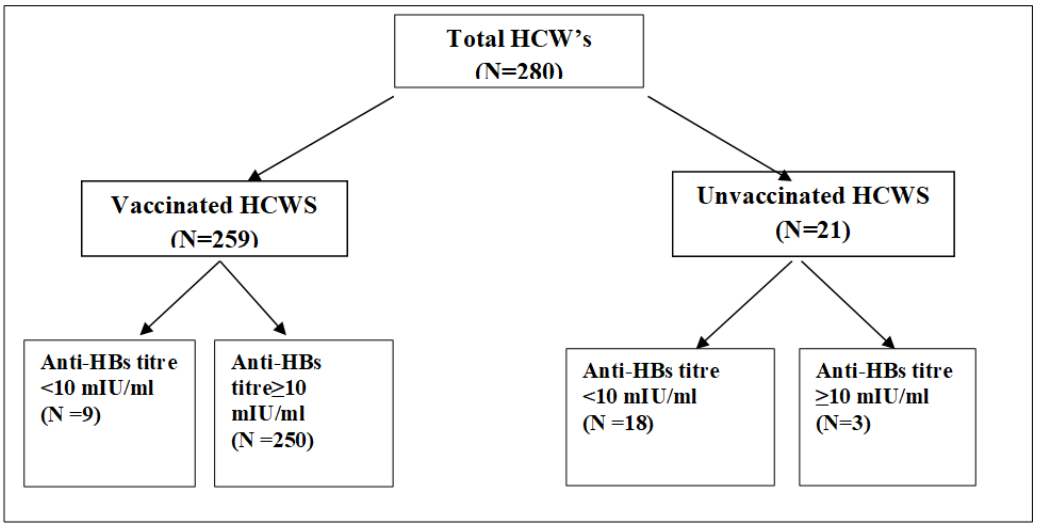


Figure 5-Distribution of unvaccinated HCWs with Anti-HBsAg titre ≥10 mIU/ml

Additionally, among the 3 individuals with titres above 10 mIU/ml, 1 (33%) had a titre greater than 100mIU/ml, while the remaining 2(67%) had titres ranging between 10 and 100 mIU/ml. (Table 5)

Figure 6.Recruitment of subjects, Hepatitis B surface antibody titre



4. DISCUSSION

Persistent hepatitis B virus (HBV) infection can result in severe health issues such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Globally, there are approximately 300 million HBV carriers, with up to 1 million deaths annually due to HBV-related complications. Although treatments like interferon-α and antiviral agents have been developed to manage persistent HBV infection, the key to eradicating HBV on a global scale lies in preventing new infections. In response to this, the World Health Organization (WHO) recommended in 1991 that HBV vaccination be integrated into national immunization programs by 1997 to help reduce the global burden of the virus.[10]

In this study, females were the predominant group among the 280 health care workers (HCWs), with 175 (63%) females compared to 105 males (37%). This finding is consistent with the results of **Randhir Kumar et al[11]**., who also reported a higher proportion of females, with 70 out of 92 participants (76%) being female. The HCWs were categorized into four groups: nursing staff (n=140), technicians (n=53), housekeeping staff (n=50), and doctors (n=37). Of the 280 HCWs, 259(92.5%) were vaccinated and 21(7.5%) were unvaccinated. Among the 259 vaccinated individuals, 166 (64%) were female and 93 (36%) were male. Similarly, **Paul M et al[12]**. found that out of 218 HCWs, 145 HCWs (66.51%) were vaccinated and 73 (33.49%) were unvaccinated.

This study revealed that some vaccinated health care workers lacked documentation of their anti-HBs titres. According to WHO guidelines, it is crucial to document these titres, and if this documentation is missing, the full three-dose vaccination series should be administered. Post-vaccination testing should be conducted 1-2 months after completing the series. Receiving additional doses of the vaccine is generally considered safe. The CDC recommends that vaccination details be entered into the hospital information system, if available, and that health care workers receive a copy of their Hepatitis B vaccination and anti-HBs testing results. They should be encouraged to retain these records with their personal health files to ensure they can be readily provided to future employers.[12]

In the current study, vaccination coverage was highest among doctors and nursing staff, reaching 95%, compared to other categories of healthcare workers (HCWs). This contrasts with findings from a study by **Batra et al[14]**, which reported vaccination rates of 92.4% among doctors, 41.7% among nursing staff, 24.2% among laboratory technicians, and none among grade 4 staff. In our study, however, 90% of technical staff and 80% of housekeeping staff were vaccinated. The variation in vaccination rates across different HCW groups may stem from differences in the roles and responsibilities of these workers and a potential lack of awareness about the importance of vaccination among those not directly interacting with patients. Socio-economic factors may also contribute to these discrepancies. [12]

In our study, the comparison of anti-HBs titres among vaccinated healthcare workers (HCWs) revealed that 130 (98%) of nursing staff, 34 (97%) of doctors, 46 (96%) of technicians, and 40 (93%) of housekeeping staff had protective titres of ≥10 mIU/ml. Conversely, 3 (7%) of housekeeping staff, 2 (4%) of technicians, 1 (3%) of doctors, and 3 (2%) of nursing staff had non-protective titres of <10 mIU/ml, indicating that these vaccinated HCWs remain at risk for HBV infection. Similarly, a study done by **Himani Bhardwaj Pandya et al[13]**. found that 5.4% of doctors and 10% of nursing staff had low titres<10 mIU/ml), while 89% of nursing staff and 95.6% of doctors had protective titres ≥10 mIU/ml. Batra et al[14] research also reported that 30% of vaccinated HCWs, primarily doctors and medical students, had anti-HBs titres<10 mIU/ml, whereas 70% had protective titres>10 mIU/ml.

Vaccination and monitoring anti-HBs titres in vaccinated healthcare workers (HCWs) are crucial for effective disease prevention, as antibody levels can diminish over time. Some individuals, known as non-responders, fail to develop adequate immunological responses despite receiving the vaccine, necessitating additional doses. Non-responders who do not achieve protective antibody levels after three doses may receive up to three additional doses to stimulate an immune response. Our protocol for managing non-responders involves administering two extra doses and then performing serological testing for seroconversion after a gap of more than two months. Research on anti-HBs response kinetics suggests that multiple booster doses may be beneficial for individuals with slow or inadequate responses [15].

In this study, out of 21 unvaccinated healthcare workers (HCWs), 18 (86%) were non-responders, while 3 (14%) were responders. In comparison, the study by **Paul M et** al[12], found that 35.61% of unvaccinated subjects had anti-HBs titres above 10 mIU/ml. This indicates a significant level of ignorance or unawareness among HCWs regarding hepatitis B vaccination, leaving them at risk of infection through exposure to blood and other body fluids. Notably, among the unvaccinated HCWs, 2 (29%) housekeeping staff and 1 (14%) nursing staff had protective titres ≥10 mIU/ml. This higher level of protection among certain unvaccinated HCWs might be attributed to increased exposure and potentially greater adherence to standard precaution and infection control practices.[12]

Prior to conducting this study, we implemented a training program to raise awareness among hospital staff about HBV infection and its vaccination, particularly targeting those at highest risk of acquiring the infection. This initiative contributed to a higher vaccination rate among healthcare workers (HCWs). It is essential for all hospital staff to be educated about the importance of completing the hepatitis B vaccination series and to have their anti-HBs titres monitored regularly, so that booster doses can be administered as needed. Hospitals should establish a policy to vaccinate all HCWs upon recruitment and subsequently measure antibody titres, as this approach is more cost-effective compared to post-exposure prophylaxis

with immunoglobulin.[16]

5. CONCLUSION

Although hepatitis B is a vaccine-preventable disease, there remains a significant gap in awareness among healthcare workers regarding the importance of vaccination, the need for periodic evaluation of antibody titres, HBsAg screening, and booster doses for those with low titres. This study emphasizes the necessity of screening for anti-HBs at regular intervals among all healthcare workers. Over time, anti-HBs titres can gradually decline in vaccinated individuals, potentially leaving even those who are fully vaccinated with insufficient protection against HBV infection. Therefore, regular monitoring and appropriate booster doses are crucial to ensure sustained immunity.[13]

6. LIMITATION-

Our study had several limitations, including the inability to assess the impact of potential risk factors such as smoking, alcoholism, nutritional status, chronic infections, vaccine administration sites, and genetic factors on decreased immune response. Additionally, regular monitoring of vaccinated individuals at 5-10 year intervals could provide valuable information on their antibody titres and highlight the need for booster doses. This approach would help ensure continued protection against hepatitis B.[16]

Source(s) of support- *None*

Conflict Of Interest

The authors declare that there is no conflict of interest.

Acknowledgements: The authors acknowledge all the nursing staff, technicians, and ward attendants for their participation and support throughout the study. The authors wish to express their gratitude for the support provided by senior technician Mrs. Nishita Prajapati. Additionally, we acknowledge Mr. Amol Mohite for his contribution to the statistical analysis using SPSS software.

Consent to Publication

All authors have reviewed the final version of the manuscript and consent to its submission and potential publication in the journal.

Author's Contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work and have approved it for publication.

Ethics Statement

The study titled ***"Assessment of Anti-HBs titre among healthcare workers at a tertiary care centre, Visnagar, Gujarat"*** was reviewed and approved by the Institutional Ethics Committee of [Nootan medical college & research centre, Mehsana, Gujarat, India,], with approval number **EC/NEW/INST/2022/GJ/0083**. All procedures are in accordance with the ethical standard guidelines.'

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