

Determination of Immunity Status of Vaccinated Health Care Workers against Hepatitis B Virus in Khartoum State, Sudan

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Abstract: Background: The vaccination of health care workers (HCWs) for the hepatitis B virus (HBV) is a crucial part of hospital infection control programs. They are considered to be a population at high risk of developing HBV infection due to the high transmissibility of the virus and the risk related to occupational injuries. The risk is particularly high in HCWs with greater exposure to accidental inoculation at work and is related to the duration of professional activity, the characteristics of the healthcare center, and the type of population served. **Objectives:** The current study was conducted to evaluate the immunity status of the vaccinated HCWs against HBV in Khartoum State. **Materials and Methods:** This cross-sectional descriptive laboratory-based study was conducted from December 2022 to February 2023. Data were collected through a structured questionnaire regarding age, gender, type of employment, length of employment, and duration of vaccination for each participant. 90 vaccinated HCWs were enrolled in this study; serum samples were collected from each participant; the Enzyme Linked Immuno Sorbent Assay technique was used to detect the anti-HBs level; and the collected data were analyzed using Statistical Package for the Social Sciences (SPSS). **Results:** This study included 90 healthcare workers, female 70 (77.8%), male 20 (22.2%) ages ranging from 20 to 52 years old, 45 (50%) of them laboratory specialists, and 23 (23.3%). Nursing 17 (18.9%), Dentists 5 (5.6%), and 2.5 (2.2%). The results of the current study reveal that 70% of the studied vaccinated HCWs have a protective level of HBs-Ab. Of these, 23.3% had an anti-HBs titer between 10 and 100 mIU/ml, and the rest, 46.7%, had an anti-HBs titer >100 mIU/ml, while 30% of them were not protective, 22.2% of them were males, and the rest, 77.8%, were females, with a mean age of 30 years. **Conclusion:** Though many HCWs had been exposed to the virus before or had received vaccinations, the majority of them were seroprotected against the hepatitis B virus. HCWs must be made aware of the prescribed immunization schedule and must be required to receive all recommended vaccinations.

Keywords: Health care workers, Immunity Status, Hepatitis B vaccine.

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INTRODUCTION

The Hepadnaviridae family of viruses includes the double-stranded, enveloped DNA virus known as the hepatitis B virus (HBV), which replicates in the liver and results in hepatic dysfunction. [1]. The currently acknowledged risk factors for infection by HBV are sexual promiscuity, intravenous drug abuse, blood and derivative transfusions, hemodialysis, and needle accidents among healthcare workers. HBV is transmitted through serum and even body fluids such as semen, saliva, sweat, tears, or breast milk [2]. One of the

most prevalent infectious diseases in the world is hepatitis B. An estimated 350 million individuals worldwide are chronic carriers, and one-third of the world's population is thought to have been infected. Every year, an estimated 620,000 to 1 million people die from HBV infection [3]. In the United States, more than 330,000 new cases of hepatitis B occur per year [4]. Hepatitis B virus infection can lead to chronic liver illnesses such as cirrhosis and hepatocellular carcinoma, which is one of the most common types of cancer worldwide. Between 1 and 10% of adults and 30 to 90% of children become chronic carriers of HBV after an

acute infection; these individuals are at risk of developing serious illnesses [5]. Chronic hepatitis B is a cause of morbidity and mortality worldwide. Healthcare workers (HCW) are at a high risk of HBV infection through occupational exposure to blood and body fluids; the incidence is estimated to be 2–4 times the level in the general population [6]. Sub-Saharan Africa is considered to be a highly endemic area for HBV, with seroprevalence among health workers in different countries ranging from 1.1% up to 17.8% [7]. Occupational safety regulations mandate that health care workers receive an HBV vaccination. Healthcare workers' HBV vaccination coverage is estimated by the WHO to be between 18% and 39% in low- and middle-income countries, and between 67% and 79% in high-income countries [6]. Three doses of the hepatitis B vaccine—the first dose, a follow-up shot one month later, and a third dose six months later—are thought to produce protective antibody concentrations in over 95% of healthy newborns, children, and teenagers as well as over 90% of healthy adults [8]. Studies conducted in Sri Lanka, Rwanda, and Northern Uganda show that the proportion of vaccinated HCWs with titers >10 mIU/ml ranged from 70–90% [9–11]. Anti-HB titer concentrations ≥ 10 mIU/ml are thought to offer protection from HBV infection. A person is classified as a hypo-responder if their antibody titer is between 10 and 100 mIU/ml, and as a hyper-responder if it is greater than 100 mIU/ml. Non-responders (<10 mIU/ml) are those healthy individuals (5–15%) who either do not produce anti-HBs or respond poorly to the surface antigen component of HBsAg. This group raises the risk of infection and transmission, particularly among HCWs. The Centers for Disease Control (CDC) advice that HCWs provide the three-dose series of vaccinations, evaluate their immune responses to the vaccine by measuring their hepatitis B surface antibody levels, and revaccinate if needed. The Hepatitis B vaccine has been linked to a number of connected factors, including chronic illnesses, obesity, smoking, and advanced age. To ascertain their HBV infection status, those who do not have protective anti-HB levels ≥ 10 mIU/ml following revaccination (6 doses) should have HBsAg and anti-HBc tests performed [9, 12–14]. Most nations, including Sudan, have been vaccinating against HBV for a number of years. On the other hand, nothing is known about the vaccination rate and level of seroprotection against HBV, particularly among Sudanese healthcare workers. This is partially explained by the high expense of immunological testing and the absence of a policy directive requiring HCWs to be fully vaccinated. Therefore, the purpose of this study was to evaluate the seroprotection and hepatitis B vaccination status of healthcare workers (HCW) in Khartoum State, Sudan.

MATERIALS AND METHODS

Study Design: The current is a descriptive, cross-sectional, laboratory-based study.

Study Area: The study was conducted in different Khartoum State hospitals in Sudan,

Study Duration: During the period from December 2022 to February 2023

Study Population: Health care workers.

Inclusion Criteria: Vaccinated HCWs who completed the HBV vaccine doses.

Exclusion Criteria: Vaccinated HCWs with positive HBs-Ag and noncomplete vaccine doses.

Data Collection:

Data were collected through a structured questionnaire; information regarding age, gender, type of employment, length of employment, and duration of vaccination were recorded for each participant. Serum was collected from each participant and tested with an ELISA titer for the detection of immunization level.

Specimen Collection:

About 3 ml of whole blood samples were collected from each participant under hygienic conditions. Serum was obtained and stored at -20°C before testing.

Detection of HBsAg: HBs-Ag was detected using ICT according to the manufacturer's protocol.

Measure Anti -HBs level using the ELISA Technique:

For detection of antibodies, this kit uses antigen Sandwich ELISA (Precheck, United Kingdom), where polystyrene microwell strip precoated with recombinant HBs-Ag and patient serum is added to the microwell together with second HBs-Ag conjugated to horseradish peroxidase, in case of the present of HBs-Abs in the sample, the pre-coated and conjugated antigens will bound to the two variants domain of antibody and during incubation, the immune complex formed is captured in the solid phase after washing to remove unbound. HRP conjugates, chromogen is added to wells, and in the presence of an antigen-antibody complex, and the colorless chromogen hydrolyzes to a blue-colored product that then turns yellow after adding stop solution sulfuric acid. Wells containing samples negative for anti-HBs remain colorless.

Assay Procedure:

All reagents and samples were allowed to reach room temperature, 50 ul of samples, calibration curve standard wells, were transferred into their respective wells, and 50 ul of HRP conjugated to each well except the blank well was incubated for 60 min at 37°C . The reagent wells were washed five times with diluted washing buffer, and the wash buffer in each well was left for 30 to 60 seconds per washing. After washing, all liquid from the microplate was disposed of by tapping it on absorbent paper with the openings facing downwards

to remove all the residual washing buffers. 50 ul of substrates A and B were pipetted to each well and incubated for 10 min at 37 °C (protect from direct sunlight).

50 ul of stop solution was pipetted into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution introduced. After that, the photometric measurement of the color intensity was made at a wavelength of 450 nm within 30 minutes of adding the stop solution. And the microplate was slightly shaken to ensure a homogeneous distribution of the solution.

Ethical Consideration:

The study was endorsed by the ethical review committee of Shendi University (Faculty of Medical Laboratory Sciences). Verbal consent was obtained from every participant before collecting data and clinical specimens. All protocols in this study were done according to the Declaration of Helsinki (1964).

Data Analysis:

The data was analyzed using SPSS. Descriptive statistics in terms of frequency tables with percentages and graphs. Tables presented with relevant graphical representations for quantitative data chi-square tests were used for statistical associations (*P value* ≤ 0.05 was considered significant).

RESULTS

The current descriptive cross-sectional laboratory-based study recruited ninety HCWs from different Khartoum state medical centers and hospitals during the period from December 2022 to February 2023 to estimate HBs-Ab levels in their plasma using an ELISA test. Besides, 22.2% of them were males, while the rest (77.8%) were females. The age of HCWs that participate in these studies ranges from 25 to 52 years old; the mean age of them is 30 years. According to the type of job, HCWs are divided into five groups: 24.4% of them are doctors, 48.9% are lab technologists, 18.9% are nurses, 2.2% are midwives, and 5.6% are dentists (Table 1). Most of the HCW employment duration was

less than 5 years 54.5%, 23.3% of the length working period was 5-10 years, 14.4% of the length working period was 11-20 years, 5.6% of the length working period was 21-30 years and 2.2% of the length working period were more than 30 years (Table 2). Generally, the study found that 70% of study populations have a protective level of HBs-Ab, while 30% of them do not have a protective level of HBs-Abs. the association between immunization level and gender among 90 HCWs, in which females show a higher level of protection against HBV than males, with no statistical difference (Table 3). The association between immunization level and age groups among 90 HCWs, which was categorized into three ranges, showed that most of the protective group were young and less than 35 years old, with no significant statistical difference between age and protective level (Table 4). The association between immunization level and employment duration length among HCWs categorized into five ranges showed a higher percentage level against HBV in groups of less than 5 years with no significant statistical difference (Table 5). The association between immunization level and vaccination duration among 90 HCWs was categorized into four ranges; most of the protective level was located in groups of less than 5 years, with no significant statistical difference (Table 6).

Table-1: Distribution of HCWs according to their jobs

Jobs	Distribution
Lab	48.9%
Doctors	24.4%
Nurses	18.9%
Dentists	5.6%
Midwives	2.2%
Total	100%

Table-2: Employment duration distribution among 90 HCWs

Employment duration	Distribution
Less than 5 years	54.5%
6-10 years	23.3%
11-20 years	14.4%
21-30 years	5.6%
More than 31 years	2.2%
Total	100%

Table-3: Association between immunization level and gender

Variables		Vaccination Level		Total
		Protective	Non Protective	
Gender	Male	16(17.8)	04(04.4)	20(22.2)
	Female	47(52.2)	23(25.6)	70(77.8)
Total		63(70.0)	27(30.0)	90(100)

P. value:0.268

Table-4: Association between immunization level and age groups

Variables		Vaccination Level		Total
		Protective	Non-Protective	
Age groups (Years)	< 35	50 (55.6)	21 (23.4)	71(78.9)
	35-50	10 (11.1)	03 (03.3)	13(14.4)
	> 50	03 (03.3)	03 (03.3)	06(06.7)
Total		63 (70.0)	27 (30.0)	90(100)

P. value 0.485

Table-5: Association between immunization level and employment duration.

Variables		Vaccination Level		Total
		Protective	Non-Protective	
Employment duration (Years)	<5	40.0% (36)	14.4% (13)	54.4% (49)
	5-10	14.4% (13)	8.9% (8)	23.3% (21)
	11-20	11.1% (10)	4.4% (4)	15.6% (14)
	21-31	3.3% (3)	1.1% (1)	4.4% (4)
	> 30	1.1% (1)	1.1% (1)	2.2% (2)
Total		70 (63)	30 (27)	100 (90)

P. value 0.848

Table-6: Association between immunization level and vaccination period.

Variables		Vaccination Level		Total
		Protective	Non-Protective	
Vaccination duration (Years)	1	12(13.3%)	4(4.4%)	16(17.7%)
	2	6(6.7%)	3(3.3%)	9(10.0%)
	3	5(5.6%)	3(3.3%)	8(8.9%)
	4	11(12.2%)	4(4.4%)	15(16.6%)
	5	5(5.6%)	3(3.3%)	8(8.9%)
	6-9	17(18.9%)	5(5.6%)	22(24.5%)
	10-16	7(7.8%)	5(5.6%)	12(13.4%)
Total		70.0% (63)	30.0% (27)	100.0% (90)

P. value 0.840

DISCUSSION

The results of the current study reveal that 70% of the studied vaccinated HCWs have a protective level of HBs-Ab. Of these, 23.3% had an anti-HBs titer between 10 and 100 mIU/ml, and the rest, 46.7%, had an anti-HBs titer >100 mIU/ml, while 30% of them were not protective, with 22.2% of them being males and the rest 77.8% being females, with a mean age of 30 years, which is lower in comparison with a study conducted by Basireddy and his colleagues, 2018 [15]. The age range of the study participants was 20–55 years. The mean months after the last dose of vaccination were 60.36. Among the 96.5% who have protective immunity to hepatitis B, the anti-HB response was similar in both males and females. There was a decline in immune response as age increased; they found that 96.5% of the vaccinated HCWs developed protective immunity to hepatitis B. Of these, 20.0% had an anti-HBs titer between 10 and 100 mIU/ml, and the rest (76.5%) had an anti-HBs titer >100 mIU/ml. 3.5% of the vaccinated HCWs did not develop a sufficient anti-HB response. And lower than others done by Yeboah *et al.*, 2019 [16]. 91.8% of study participants showed evidence of post-vaccination immunity measured as anti-HBs titer levels >10 IU/ml, while 8.2% of HCWs had anti-HBs titer levels <10 IU/ml. The median age of participants was 29 years. The majority of participants were in the nurse/midwife/health assistant category, and 64% have worked for <5 years. Most participants took their HBV vaccinations from government facilities. And 90% had detectable anti-HBs, while 10% did not have detectable hepatitis B antibodies (HBs-Ab). Most participants were females (52%), aged between 26 and 35 years (43.3%), and were either nurses or midwives (45.5%), which was

reported by Ocan *et al.*, 2022 [11]. Another study in Sudan found that 88.5% of people responded to the vaccine, while 11.5% did not. The participants were categorized into two age groups: among age groups 21–27, 45/49 (91.8%) responded to the vaccine, and among the age group 27–34, 9/11 (81.8%) responded to the vaccine with insignificant difference. Out of 88.5 respondents, 22.2% were males and 93.3% were females. Out of 11.5 non-responders, 71.4% were males and 28.6% were females, with a significant difference between the two genders. Elsir and others in Bosnia and Herzegovina have shown that the persistence of anti-HBs after hepatitis B vaccination >10 IU/l stands at 82.53% on average [17]. The largest incidence of anti-HBs >10 IU/l level was registered among the population of children vaccinated against viral hepatitis B at a rate of 88.89%, followed by health workers at a rate of 79.41%, whilst intravenous drug users show the lowest rate at 64.28% reported by Puvacić, *et al.*, (2005) [18]. and lower than the study conducted in Italy, a sufficiently high HBs-Ab titer level was maintained in about 80% of the study with only 20% of individuals resulting in an anti-HBs antibody titer < 10 IU/L, with a slight prevalence of female workers, men 54%, women 46%, and at 21 years ($SD \pm 4.9$), the largest group of HCWs were nurses 53.5%, whereas medical doctors were 30% and other HCWs were 16.5%, showed that female nurses had higher Abs titers compared to men (73.6% vs. 26.4%) [19]. In another study done in India, 80% were protective responders and 20% were nonprotective responders post-immunization. Although the percentage of female protective responders was greater (85.8%) compared to male protective responders (75.9%), the association between anti-HBs status and gender was not statistically significant. An increase in age leads to the

decline of anti-HB protective responders. The lowest number of non-protective responders (03/40) is seen in the age group less than 20 years. Whereas, the highest number of non-protective responders (18/40) is seen in the age group of more than 60 years. Badave *et al.*, 2019 the result of this study is closely similar to the study done in Bosnia and Herzegovina, The median age of individuals was 50.5 ± 9.4 years. Among them, 73.07% were females, 26.93% were males, and 75.80% were fully vaccinated [20]. The eligibility criteria for the vaccination program were unmet by 24.20% of participants [21]. And higher than a study done in Sudan among Sudanese healthcare workers Just 54.5% of the participants had antibody titers above 10 IU/mL; 15.6% of them had anti-HBs titers >100 IU/mL; and HBs-Ab titers less than 10 IU/mL were detected in 45.6%. The majority of the HCW (73.3%) were within the age range of 21–30 years; 73.3% of the participants were females and 26.7% were males, with 42.2% and 54.2% of males and females, respectively, failing to develop anti-HBsAb titers >10 mIU/mL [22].

CONCLUSIONS

The literature emphasizes the significance of receiving all three doses of the vaccine schedule, and our study demonstrates a decline in immunization levels against the HB vaccination line. Furthermore, to minimize the chance of acquiring HBV following a work-related injury and to have a strong and long-lasting antibody response. In order to ensure safety and lower transmission rates, our findings emphasize the necessity for the government to implement efforts to raise awareness among HCWs and enforce mandatory complete vaccination in accordance with the suggested vaccination schedule. This should be followed by confirmation of the recipient's serological status after immunization. We also advise the government immunization programs to take into account keeping a record of the HBV immunizations administered for future use.

RECOMMENDATIONS

All HCWs should have their post-vaccination status checked on a regular basis to assure their safety and lower the risk of transmission. Give HCWs the booster dose while they are protected by anti-HBsAg. All healthcare workers and medical students should have an HBV vaccination.

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