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Digital Object Identifier:
 Received 19 August 2025
 Accepted 2 December 2025
 Available online 25 January 2026

Detection the Persistence of Antibodies of Hepatitis B Surface Antigen among Healthy Individuals Vaccinated with Hepatitis B Virus Vaccine in Mukalla-Hadhramout/Yemen

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This is an open-access article under production of [Hadhramout University Journal of Natural & Applied Science](#) with eISSN 2790-7201

Abstract: Hepatitis B virus (HBV) can lead to both short-term and long-term liver conditions, posing a significant worldwide public health concern. The HBV vaccine serves as a prime illustration of preventive measures and has proven to be effective in averting HBV transmission and related health issues. The study aimed to assess the immune response to HBV among healthy individuals in Mukalla, Hadhramout/Yemen who had been vaccinated before 15 years or more, by measuring anti-HBs levels. During January 2023, blood samples were taken from 206 healthy individuals who had received the HBV vaccine as a part of a cross-sectional and analytical study. Out of the 206 vaccinated healthy individuals with the HBV vaccine, 78(37.9%) had protective anti-HBs titers, and factors such as Rh blood factor, smoking, and the ABO blood groups were found to be significantly associated with the level of post-vaccination immunity. The samples were analyzed for ABO blood groups, HBsAg, and anti-HBs using the electrochemiluminescence (ECL) immunoassay technique, and an anti-HBs antibody titer of ≥ 10 IU/L was considered protective against HBV infection. The study found that anti-HBs levels were not significantly linked to factors such as age, gender, residence, country of vaccination, time since last dose, blood transfusion, BMI, and chronic diseases. However, the seroprevalence rate of anti-HBs decreased in individuals vaccinated in Mukalla city, Hadhramout, putting them at risk of HBV infection. Additionally, immune response was notably associated with the Rh blood factor, smoking, and ABO blood group. Therefore, booster immunization with the hepatitis B vaccine is recommended.

Keywords: Anti-HBs, Hepatitis B vaccine, Hepatitis B virus, Immunization, Prevention, Seroprotection, Vaccination.

1. Introduction

Hepatitis B virus (HBV), which belongs to the Hepadnaviridae family, is responsible for causing liver-related diseases, and its genome size is approximately 3.2 kb with a partially circular double-stranded DNA structure;

globally, HBV infection remains a significant public health concern, with over 1.5 million new preventable infections occurring annually as of 2019 [1].

Despite the availability of a vaccine to prevent new infections, approximately 240 million individuals, which

accounts for around 3.5% of the global population, suffer from chronic HBV infections, leading to an annual death toll of 887,000 due to HBV-related illnesses like fibrosis, cirrhosis, and hepatocellular carcinoma [2,3].

HBV infections vary in frequency and severity by geography and subpopulation [4]. HBV is a commonly transmitted blood-borne virus, with mother-to-child transmission being the primary cause of hepatitis B cases, while other risk factors include (IV) drug misuse, exposure to infected blood products, multiple sexual partners, and lack of awareness [5].

Numerous attempts have been made to develop a successful treatment for eliminating HBV from individuals who are infected; however, this objective has not yet been achieved. Consequently, the hepatitis B vaccine is widely recognized as the most effective approach for managing HBV transmission and reducing HBV-related illness and death [6].

The hepatitis B vaccines stimulate the initial immune reaction to generate quicker and more efficient secondary responses when naturally exposed to HBV. Adequate protection against hepatitis B infection requires a serum antibody level of at least 10 IU/L [7,8].

The prevalence of HBV-caused hepatitis makes it the leading cause of liver cancer, highlighting the significance of the hepatitis B vaccine in providing immunity against the virus. Understanding the immune response is crucial in determining the necessity of additional stimulant doses for the body [3].

In Yemen, a routine assessment of anti-HBs levels after vaccination was not conducted to detect and induce persistent antibodies, leading to the first-time study on Hadhrami individuals to evaluate the persistence of anti-HBs after primary vaccination with the recombinant hepatitis B vaccine.

This study aimed to assess the persistence of HBV vaccine antibodies among vaccinated healthy individuals in Mukalla city, Hadhramout, Yemen.

2. Materials and Methods

2.1 Study Design

A cross-sectional and analytical study was conducted during January 2023.

2.2 Study Population

The research focused on enlisting healthy young volunteers, including undergraduate students and adolescents aged 15–25, who were born in or after 1998 (the year when the hepatitis B vaccine became a part of the Yemeni childhood vaccination program) and had previously received a three-dose series of the monovalent pediatric HBV vaccine (containing 10µg of HBsAg) as part of regular clinical practice before reaching 18 months of age.

2.3 Sample Size

Non-probability simple random sampling (convenience sampling) was used, so that maximal participation could be ensured, that included 206 participants.

2.4 Inclusion Criteria

Healthy individuals who received the HBV vaccine on a vaccination card and who accepted to participate were included.

2.5 Exclusion Criteria

Individuals who were not vaccinated, lacked vaccination cards, had compromised immune systems, were currently

infected with respiratory, urinary, or gastrointestinal tract illnesses, had hepatitis B, had recently received a booster dose, or declined to take part in the study were not included.

2.6 Data Collection Tool

Data were gathered from participants through a structured questionnaire following the receipt of written informed consent, with the questionnaire comprising several sections:

- Sociodemographic characteristics such as age, sex, and residence.
- Vaccine history is shown on their vaccination cards, such as the date of receiving the HBV vaccine, and the country where the vaccine was taken.
- Exploring elements linked to immunity after vaccination, such as ABO and Rhesus blood groups, cigarette smoking, past history of the blood transfusion, past chronic diseases such as diabetes, hypertension, kidney disease, and asthma. And the body mass index (BMI) that represents the amount of body fat, and it's derived from a person's weight in kilograms or pounds divided by their height in meters or inches squared [9].

2.7 Pre-test (Pilot of Study)

Prior to collecting data, the questionnaire underwent testing with 20 participants to ensure understanding and applicability. Following the results, adjustments were made to the questionnaire.

2.8 Collection of Blood Samples

5 ml of venous blood were collected from each participant and then divided into two tubes:

- One without anticoagulant (serum) for HBsAg and anti-HBs investigations, and the other with anticoagulant, ethylenediaminetetraacetic acid (EDTA) for the ABO blood groups.
- The samples without anticoagulant were allowed to coagulate for 30 minutes at room temperature (20-25°C).
- The samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was then transferred to another tube and stored at -20°C before being transported for HBV marker testing.

2.9 Laboratory Tests

The blood group testing for ABO and Rh was conducted with anti-A, anti-B, and anti-D reagents, while the detection of HBsAg and anti-HBs utilized the electrochemiluminescence (ECL) immunoassay technique using commercial kits of the Cobas e411, a fully automated analyzer by Roch company, Germany.

2.10 Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 25 for descriptive statistics, binary and multiple logistic regression analysis to identify independent predictors of factors influencing HBV vaccine uptake among vaccinated healthy individuals. The results were presented using Excel for Windows Microsoft version 10, and the relationship between variables was examined using the Pearson correlation (r) test with a significance level set at P-value < 0.05. Odds ratios (OR), adjusted odds ratio (AOR), and 95% confidence intervals (CI) were calculated and presented for the variables.

2.11 Ethical Consideration

The study received ethical approval from the Faculty of Science at Hadhramout University, and the participants

provided verbal informed consent with the assurance of confidentiality for their results.

3. Results and Discussion

3.1 Demographic and other characteristics of participants

Among 206 vaccinated with HBV vaccine, majority of the study participant were males 132 (64.1%) and 74 (35.9%) were females. The age was distributed into groups 15-17 years 45 (21.8%), 18-20 years 85 (41.3%) and 21-25 years 76 (36.9%). A total of 179 (86.9%) of the study participant were urban residence and 27 (13.1%) rural residence. It has been 20 years or more since 130 (63.1%) people received the last vaccine, and less than 20 years for 76 (36.9%). A total of 191 (92.7%) vaccinated in Yemen and 15 (7.3%) outside Yemen. The distribution of blood groups was A+ 50 (24.2%), A- 3 (1.5%), B+ 17 (8.3%), B- 1 (0.5%), AB+ 11 (5.3%), O+ 109 (52.9%), and O- 15 (7.3%). Rh blood factor was positive in 187 (90.8%) and negative 19 (9.2%). A total of 110 (53.4%) had a normal BMI, 51 (24.8%) underweight and 45 (21.8%) overweight, 25 (12.1%) smokers and 181 (87.9%) non-smokers. 39 (18.9%) they transfusion blood and 167 (81.1%) didn't transfusion blood. 7 (3.4%) had chronic disease and 199 (96.6%) did not.

3.2 Prevalence of anti-HBs to socio-demographic characteristics and vaccine history of study participants

The total seroprevalence of antibodies to HBsAg among healthy individuals vaccinated with HBV vaccine (≥ 10 IU/L) was 78 (37.9%) which indicates that they have serological protection from HBV infection, while the remaining 128 (62.1%) don't have anti-HBs levels (< 10 IU/L), and are at risk of contracting HBV.

The statistical analysis of the socio-demographic characteristics and vaccine history of study participants (table 1) found that gender, age groups, residence, and the country where the vaccine was taken did not show a statistically significant association with antibodies tests and the presence of immune response ($p > 0.05$).

Table 1. Prevalence of Anti-HBs to Socio-Demographic Characteristics and Vaccine History of Study Participants

Variable	Positive anti-HBs		OR	CI (95%)	P-value
	No.	%			
Gender					
Male	49	23.8	0.084	0.510-1.645	0.769
Female	29	14.1	1	1	-
Age groups (years)					
15-17	17	8.3	1	1	-
18-20	35	17.0	1.153	0.549-2.420	0.707
21-25	26	12.6	0.144	0.398-1.844	0.692
Residence					
Rural	11	5.3	1	1	-
Urban	67	32.5	0.13	0.381-1.986	0.741
The country where vaccine taken					
Out Yemen	5	2.4	0.192	0.266-2.459	0.708
Yemen	73	35.4	1	1	-

3.3 Prevalence of anti-HBs and associated factors with post-vaccination immunity of study participants

The statistical analysis of factors associated with post-vaccination immunity showed that blood transfusion and

chronic diseases were not significantly linked to antibody tests and immune response presence among study participants ($p > 0.05$). There was a statistically significant association between positive anti-HBs and blood groups (OR = 4.074, P-value = 0.037), Rh blood factor (OR = 1.273, P-value = 0.049), and smoking (OR = 3.631, P-value = 0.023) as presented in Table 2.

Table 2. Prevalence of Anti-HBs and Associated Factors with Post-Vaccination Immunity of Study Participants

Variable	Positive anti-HBs		OR	CI (95%)	P-value
	No.	%			
Blood groups					
A+	15	7.3	1.714	0.422-6.968	0.451
A-	0	0.0	0.000	0.000	0.999
B+	5	2.4	1.667	0.323-8.590	0.541
B-	0	0.0	0.000	0.000	1.000
AB+	0	0.0	0.000	0.000	0.999
O+	55	26.7	4.074	1.089-15.246	0.037*
O-	3	1.5	1	1	-
Rh Blood Factor					
Positive	75	36.4	1.273	1.006-12.683	0.049*
Negative	3	1.5	1	1	-
Smoking					
No	74	35.9	3.631	1.197-11.012	0.023*
Yes	4	1.9	1	1	-
Blood transfusion					
No	66	32.0	1.470	0.696-3.105	0.312
Yes	12	5.8	1	1	-
Chronic diseases					
No	75	36.4	0.194	0.176-3.703	0.782
Yes	3	1.5	1	1	-

For BMI and number of years since last dose there was no significantly association of anti-HBs with BMI and years since last dose, as shown in Table 3. A full three-dose vaccination regimen can provide lasting protection for over 30 years, although the precise duration of effective immunologic cellular memory remains uncertain [10-12]. This research is the inaugural exploration of anti-HBs levels in vaccinated healthy individuals in Hadhramout/ Yemen. After completing the original HBV vaccine series, our research discovered that 37.9% of participants had a protective level of anti-HBs (≥ 10 IU/L). Our findings were similar to a study conducted in South Carolina among nursing students, where 33% showed immunity with anti-HBs (≥ 10 IU/L) [13].

Table 3. The correlation between anti-HBs and BMI and years since last dose

	Anti-HBs	P-value	Pearson correlation (r)
BMI	78	0.485	- 0.080
Number of years since last dose	78	0.601	- 0.060

Other studies showed a high seroprevalence of anti-HBs vaccine (≥ 10 IU/L) in Morocco 92.7% [14], Turkey 87.5% [15], Sri Lanka 85% [16], South Korea 75.3% [17], Egypt 86% [18], Germany 60% [19], Italy 72.6% [20], and Botswana 98.9% [21]. While a local study in Thamar/Yemen showed a lower seroprotection rate than ours with a percentage of 22.4% anti-HBs (≥ 10 IU/L) [22], other study carried out in Najran/Saudi Arabia, showed a

result with a percentage of 18.3% anti-HBs (≥ 10 IU/L) [23]. The differences in seroprotection rates may be attributed to various factors, such as the genetic makeup of participants, type and dosage of the vaccine, storage conditions, economic disparities, and geographical and regional variations [22].

In the current study, anti-HBs was detected at a higher rate in males than females (23.8% and 14.1%, respectively). This is likely because the number of males is higher than females in our study, but this variation was not significant. Similar to these findings in Egypt, males showed a higher seroprotection rate than females (59.3%, 48.4%, respectively) [24]. Another study conducted in Alaska showed anti-HBs for males and females at 52.1% and 50.7%, respectively [10]. Unlike our results, local results in Tamar showed anti-HBs was detected at a higher rate in females than in males (25.5% and 19.4%, respectively) [22]. Other study in carried out in Taiwan showed anti-HBs for females and males at 36.2% and 33.3%, respectively [25]. Another study in Madinah, Saudi Arabia, showed anti-HBs had a higher rate in females than males (55.5% and 31.1%, respectively) [26]. Also, a study conducted in Makkah showed anti-HBs for females and males were 29.4% and 20%, respectively [27]. In Italy, anti-HBs was detected at a higher rate in females than in males (62.1% and 37.9%, respectively) [28]. In China, anti-HBs in females was 72.3% and 65.2% in males [29].

The research revealed that individuals aged 18-20 who received the HBV vaccine had the highest anti-HBs rate at 17%, with no statistically significant connection. Maybe the explanation for this is that they outnumber the other categories. In Uganda, a study revealed that the age group of 26-35 years had the highest percentage (41.4%) of positive anti-HBs results, and there were no significant differences [30]. In a study conducted in Alaska, it was found that the highest occurrence of anti-HBs was 61.2% among individuals aged 45 to 55 years [31].

In a study conducted in Turkey, the age group of 31-50 years had the highest anti-HBs rate at 52.6% [18], and in another study, it was 94.8% in age group (≤ 20) years [32,33] noted that the highest rate of anti-HBs in Iran was 61.7% in the age group (≥ 21) years. In the age group (3-4) years, the western Amazon had the highest anti-HBs rate at 71.1% [34]. The different age groups studied may be the reason for the different results of these studies. The findings of a nearby study mirrored our own, showing no significant correlation between anti-HBs titer levels and place of residence [22]. Also in Morocco, according to [17] The levels of anti-HBs titer were not significantly associated with the place of residence, unlike in Mongolia where urban areas exhibited higher anti-HBs rates compared to rural areas [35].

The study findings revealed that the level of anti-HBs did not show a statistically significant correlation with the number of years since the last dose was taken, as the limited range did not indicate any statistical difference. But the result does not agree with other studies in the Chinese rural community, Japan, Italy, and India, where their results showed the positive anti-HBs titer induced by the HB vaccine gradually declines over time [36,37,38]. The lack of statistical significance could be attributed to the small number of participants in the study, as opposed to studies

that contradict our findings, which included a higher number of participants.

As far as we know, this is the first time, we studied the country where the vaccine was taken to know the efficiency and quality of the vaccine used in our country and if its effect differs from the effect of vaccines in other countries. The variable did not exhibit a significant relationship in the findings, likely due to heightened public health ministry response, increased population awareness, educational initiatives, and more efficient vaccination campaigns.

The study demonstrated a significant correlation between ABO/Rh blood types and anti-HBs levels, with O+ (26.7%) having the highest proportion of participants with sufficient anti-HBs protection (≥ 10 IU/L). According to a study in Saudi Arabia, the highest percentage of participants with inadequate protection of anti-HBs (< 10 IU/L) was found among those with blood type O+ (38.8%) [23]. It is possible that the reason for the difference is that blood type O+ is the most prevalent in our country. Based on a study that examined the occurrence of ABO blood types in Yemen, the distribution was found to be O > A > B > AB, which aligns with our own findings [39].

In our study, we found no notable disparities between the proportion of participants with protective antibody levels and BMI, aligning with research conducted in China, Maryland, India, Iran, and Alaska [10,33,37,40-41]. Our research revealed a notable link between smoking and the decline of anti-HBs; just 59.1% of non-smokers experienced a loss of anti-HBs, while 84% of smokers showed a negative presence of anti-HBs. A study conducted in China found that the reduced effectiveness of the hepatitis B vaccine is associated with smoking, similar to our study findings. This is because smoking affects cells and immune responses in individuals, with nicotine inhibiting the production of antibodies by disrupting the antigen-mediated pathway in T cells and the intracellular calcium response [25]. Another study in Denmark revealed that the group of non-/low-responders were smokers [42].

Also, in China, similar to this study, which showed a significant association between smoking and anti-HBs, the ratio of anti-HBs for smokers and non-smokers was 16.4% and 30.1%, respectively [43]. In various studies conducted in Tamar governorate, Alaska, and Egypt, it was found that there was no significant correlation between smoking and anti-HBs titer [10,22,44].

Our research indicated that there was no significant correlation between anti-HBs titers and blood transfusion. Similarly, a study in Iran also found no significant link between anti-HBs titer levels and blood transfusion, which may be attributed to the limited number of samples for this variable [45]. In a study conducted in Assam, northeastern India, blood transfusions were not found to be significantly linked to low anti-HBs titers [46]. In our research, we found no correlation between the anti-HBs level and chronic disease. Perhaps this is because the people in the study were young, healthy and did not have any common chronic conditions. Similarly, a study conducted in Iran showed that no association between anti-HBs titer and chronic disease [45]. In contrast to a study carried out in India, which indicated a notable decrease in anti-HBs level and chronic disease [47].

In conclusion, the prevalence of anti-HBs is relatively low in individuals who received the HBV vaccine in

Mukalla city, Hadhrumout, posing a risk of infection for those who have lost immune protection. Factors such as age, gender, residence, years since last dose, BMI, and chronic diseases did not show a significant correlation with anti-HBs levels receive a booster immunization with the hepatitis B vaccine.

3.4 Limitations of the Study

The small sample size in this study is due to the cross-sectional study design, so we expect that future research will employ longitudinal or survey study designs over longer periods of time. Additionally, there is a need for more advanced diagnostic techniques, such as polymerase chain reaction (PCR) for HBsAg detection.

4. Conclusions

The prevalence of anti-HBs is low among individuals vaccinated against HBV in Mukalla city, Hadhrumout. A significant number of people with undetectable immune protection remain vulnerable to infection. No significant correlation was found between age, gender, residence, years since last dose, blood transfusion, vaccination country, BMI, chronic diseases, and anti-HBs levels among participants. However, a significant association was observed between Rh blood factor, smoking, ABO blood group, and protective anti-HBs titers against HBV infection.

Acknowledgement

The authors would like to thank Hadhrumout University for providing support for this research.

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الكشف عن استمرار وجود الأجسام المضادة لمستضد سطح التهاب الكبد نوع B لدى الأفراد الأصحاء الذين تم تطعيمهم بلقاح فيروس التهاب الكبد نوع B في المكلا - حضرموت / اليمن

الملخص: يمكن أن يؤدي فيروس التهاب الكبد الوبائي بي إلى أمراض الكبد قصيرة وطويلة الأمد، مما يشكل مصدر قلق كبير على الصحة العامة في جميع أنحاء العالم. يُعد لقاح التهاب الكبد الوبائي بي مثلاً رئيسياً على التدابير الوقائية وقد أثبتت فعاليته في تجنب انتقال الفيروس والمشاكل الصحية ذات الصلة. هدفت الدراسة إلى تقييم الاستجابة المناعية لفيروس التهاب الكبد الوبائي بي بين الأفراد الأصحاء في المكلا، حضرموت / اليمن الذين تم تطعيمهم قبل 15 عاماً أو أكثر، من خلال قياس مستويات الأجسام المضادة لفيروس التهاب الكبد بي. خلال شهر يناير 2023، تم أخذ عينات دم من 206 أفراد أصحاء تلقوا لقاح فيروس التهاب الكبد بي في دراسة مقطعية وتحليلية. من بين 206 أفراد أصحاء تم تطعيمهم بلقاح فيروس التهاب الكبد بي، كان لدى 78 (37.9%) مستويات واقية من الأجسام المضادة لفيروس التهاب الكبد بي، كما وُجد أن عوامل مثل عامل الدم ريزوس والتدخين وفصائل الدم مرتبطة بشكل كبير بمستوى المناعة بعد التطعيم. تم تحليل العينات لتحديد فصائل الدم، والمستضد السطحي لفيروس التهاب الكبد الوبائي بي، والأجسام المضادة لمستضد السطح باستخدام تقنية المقايسة المناعية الكهروكيميائية، واعتُبر مستوى عيارية الأجسام المضادة لمستضد السطح ≥ 10 وحدة دولية / لتر واقياً من عدوى فيروس التهاب الكبد الوبائي بي. وخلصت الدراسة إلى أن مستويات الأجسام المضادة لمستضد السطح لم ترتبط ارتباطاً وثيقاً بعوامل مثل العمر، والجنس، ومكان الإقامة، وبلد التطعيم، والوقت المنقضي منذ آخر جرعة، ونقل الدم، ومؤشر كتلة الجسم، والأمراض المزمنة. ومع ذلك، انخفض معدل الانتشار المصلي للأجسام المضادة لمستضد السطح لدى الأفراد الذين تم تطعيمهم في مدينة المكلا بحضرموت، مما يعرضهم لخطر الإصابة بعدوى فيروس التهاب الكبد الوبائي بي. إضافة إلى ذلك، ارتبطت الاستجابة المناعية بشكل ملحوظ بعامل الدم ريزوس، والتدخين، وفصائل الدم. لذلك، يُنصح بالتطعيم المعزز بلقاح التهاب الكبد الوبائي بي.

الكلمات المفتاحية: الأجسام المضادة للمستضد السطحي، لقاح فيروس التهاب الكبد الوبائي بي، فيروس التهاب الكبد الوبائي بي، التحصين، الوقاية، الحماية المصلية، التطعيم