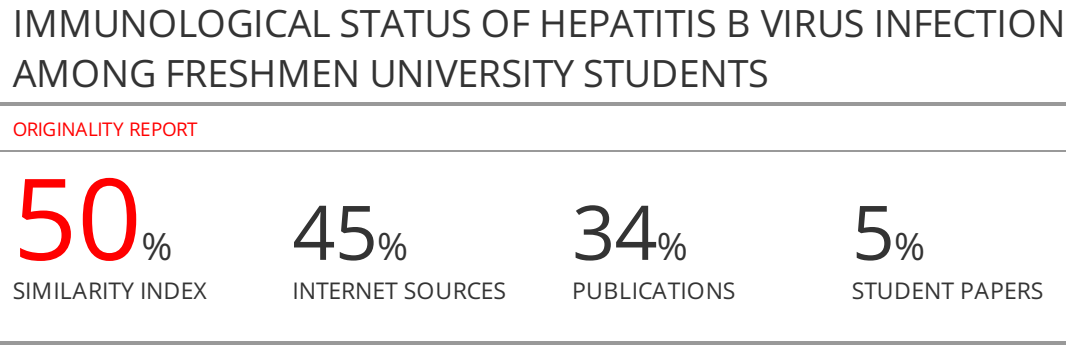
**Reviewer’s Comments**



**IMMUNOLOGICAL STATUS OF HEPATITIS B VIRUS INFECTION AMONG FRESHMEN UNIVERSITY STUDENTS**

**ABSTRACT**

**Background:** Hepatitis B virus (HBV) is a major health problem, it's a worldwide pandemic. In Yemen, previous surveys conducted showed a high prevalence of hepatitis B infection. Hepatitis B vaccination is the most effective way to prevent hepatitis B virus infection and its consequences (such as cirrhosis, hepatocellular carcinoma, and liver failure).

**Aim**: To assess the immune status of the hepatitis B virus among first-year students at the faculties of Thamar University during the academic year 2021-2022.

**Methods**: A total 196 of first-year students participated in this cross-sectional study and tested for anti-HBsAg and the antibody to HBV core antigen (anti-HBc) and HBsAg by using ELISA during the period from January to May 2022 at Thamar University. An anti-HBs antibody titer ≥10 mIU/mL was regarded as being protective against HBV infection.

**Results**: In this study, 22.4% of the students had protective levels against hepatitis B, while 77.6% had a non-protective level. HBV markers showed that 19.90%, 2.55%, and 75.51% of the students had been vaccinated and immunized due to a previous infection and exposed to HBV infection respectively and only 2.04% of them were non-obvious cases. The prevalence of anti-HBc was 4.6%. Risk factors that showed statistical significance were found between positive anti-HBc and a family history of HBV (P = 0.01).

**Conclusion**: The low prevalence of hepatitis B protection levels amongThamar University medical students needs further research and necessitates the implementation of a screening and vaccination program for all non-immunized healthcare students.

**Keywords**: Antibodies, Hepatitis B virus, Immunization in Yemen, Low immunity

**INTRODUCTION**

One of the global public health problems is Hepatitis B virus (HBV) infection. HBV belongs to the Hepadnaviridae family with a partially double-stranded DNA1-3. It has been expected that about two billion people globallyhave proof of past or present infection with HBV and more than 358 million people have a chronic lifelong infection and about 887 000 people die every year due to the outcomes of hepatitis B1-3. The endemicity of hepatitis B virus was expected in Yemen, where the prevalence of positive HBsAg in the general population and HCWs ranged from 8% to 20%, among infants, it was 4.1%, and up to 50% of health workers and populations usually had prior serological evidence of Hepatitis B virus infection in old reports4-8. On the other hand, recent studies indicated that the rate of HBsAg, which ranges from 0.7-2% among the general population and to 4% among risk groups such as HCWs, renal dialysis patients as well as HBV, decreased more among children9-15.

It is well known that hepatitis B vaccine is the mainstay of the hepatitis B prevention. To achieve this, the vaccine must trigger an immune response thatwould produce protective hepatitis B surface antibody )anti-HBs( at a concentration of ≥10 mIU/mL at least 1 month and at most 2 months after the 3 dose6,this occurs in more than 95% of infants, children and young adults. But the persistence of anti-HBs and thus the protection against infection and carrier state depends on the peak anti-HBs concentration achieved after primary vaccination. However, anti-HBs decay exponentially with the length of time since vaccination6, 16.Factors associated with a decreased immune response to the HBV vaccine include increasing age, gender, obesity, nutritional status, smoking, and genetic factors. Poverty, socioeconomic status, low level of education, and weak health systems in Yemen are interrelated factors that influence the nutritional status of people, which in turn affect their immune system6.

A study carried out in Saudi Arabia for students of Taibah University elucidated that the hepatitis B markers showed that only 15.2% of students had protective levels against the disease, while the rest showed negative markers17. A similar study conducted in Iran for first-year medical students demonstrated that 36.2% showed a non-protective anti-HBs response (anti-HBs < 10 mIU/mL) and 164/257 individuals (63.8%) showed a protective anti-HBs response (anti-HBs≥ 10 mIU/mL) 18.

To our knowledge, there are no published studies on the immunological status of the hepatitis B virus among university students in Yemen. Therefore, the aim of the current study is to assess the immunological status of the hepatitis B virus among first-year students inThamar University, during the academic year 2021-2022.

**SUBJECTS AND METHODS**

**Study area:** This study was carried out at Thamar University located in Dhamarcity;Dhamargovernorate (15°40’N 43°56’E) is located at the central area of the western highlands region of Yemen1600–3200 meters above sea level.

**Study design and sample:** A cross-sectional study was conducted from January to May 2022 at Thamar University, Dhamar Governorate, Yemen. The study targeted first-year students at all faculties of Thamar University. The total number of students enrolled at the time of the study at Thamar University in their first-year was 1724 students and the study was conducted on 196 respondent students for the assessment of their immune status by conducting Anti-HBs, Anti-HBc, and HBsAg tests.

**Inclusion Criteria:** First year students from all faculties of Thamar University who were present during the sample collection and who signed a consent form to participate in the study.

**Exclusion Criteria:**First-year students who are not available at the time of data collection due to different reasons (absence, sick leave, maternity leave…etc) and students who declined to offer consent to the study.

**Sample Size Determination:** Sample size was calculated by EPi Info 7™ using the STATCALC utility based on a 15.2% predicted frequency17, a 95% confidence level, 5% confidence limits, and a 90% response rate. Therefore, the sample size was planned to be 196 students among all first-year students in the faculties of ThamarUniversity (total = 1724).

**Sampling Method:** The choosing for them by systematic random sample method among all Thamar University first-year students from all faculties (196 students out of 1724) and according to gender was included within two groups [50% females and 50% males].

**Data Collection:** Data was collected by a pretested structured questionnaire. The study variables include Socio-economic factors (monthly income, Fathers and mothers education level etc.), demographic factors (residence, age, sex etc.) and potential risk factors of HBV infections (Previous history of surgical operations, a history of infected family, share of personal objects and blood transfusion etc.).The sesultsof HBV markers that weredetected, they wereadded to questionnaire chart. The potential risk factors were used as the independent variables, while the positive results of HBV markers were considered as the study outcome (dependent variables).

**Blood sample collection:** From each student; five mL of whole blood aseptically by venipuncture was collected. After clotting the blood serum was separated by centrifugation. At –20°C sera specimens were kept until tested for the HBV markers.

**Laboratory test:** All samples were tested for Anti-HBs, Anti-HBc and HBsAg. Serological assays of the HBsAg, Anti-HBsAg and total Anti-HBcAg serological markers were performed on the ELISA System (Roche Cobas e 411 analyzer) using the electrochemiluminescence immunoassay “ECLIA”.

**Statistical analysis:** The data were analysed byperforming Epi Info statistical program version 6 (CDC, Atlanta, USA). Conveying the quantitative data like mean values, and standard deviation (SD), as the data were normally distributed. The qualitative data were expressed as percentages; for the comparison of two variables to determine the *p-*value, the Chi-square test(χ2) was used. Odd ratio (OR) was used with a 99% confidence interval. *P-*value <0.05 was regarded as statistically significant.

**Ethical consideration:** Consents were taken from all students and they were informed that participation is voluntary and that they can refuse without giving any reason.

**RESULTS**

A total of 196 students participated in this study in their first academic year at Thamar University with anage range between 18 and 22 years and amean age (±SD) age of 20.14 (±1.09) years. Half 50% of the participants were male and nearly two-thirds 76% of them livein urban areas.

**Distribution of Anti-HBs level by gender:** 22.4% of students had a protective level against HBV, the percentage of males and females with low immunity were the same at 6.1%, while, the percentage of females who had adequate immunity and high immunity were 6.1 and 0.5 versus 2 and 1.5 of males respectively. There was no statistically significant variation between both sexes (Table 2).

**Distribution of Anti-HBs level by Age:** Table 3shows that no correlation was found between age and anti-HBs level \*=Kruskal Wallis test; χ2=3.48, P= 0.32. About 77.6% of students had anti-HBs titer < 10 mIU/mL, 12.2% had between 10-100 mIU/mL, 8.2% had between 100-1000 mIU/mL and only 2% above 1000 mIU/mL.There was no significant association between anti-HBc positive status and risk factors, except**,**for family history of HBV infection χ2= 9.74, (95% CI 1.74 –8.62); *p* = 0.01. Being cupping showed a trend but not a statistically significant difference χ2= 1.28 (95% CI 1.28–42.00) *p*=0.057 (Table 4).

**DISCUSSION**

The current study showed that a high proportion of students had a non-protective (anti-HBs <10mIU/mL) titer of 77.6% against hepatitis B virus, while only 22.4% of the students had a protective titer (anti-HBs ≥10 mIU/mL). The present findings of non-protective titer are consistent with those of Mosaad*et al.*17 who reported that more than 84.80%, of medical students had no protection anti-HBs levels. In contrast to the present findings, two Iranian studies among dental hygienists and medical students showed that most of the students had a protective titer against hepatitis B virus of 93.6% and 95.1%, respectively 19,20.The difference in the rate of protection against hepatitis B virus can result from the difference in coverage of HBV vaccine among the target groups in the studies, the difference in the economic level, the geographical and regional differences, the consideration of the cold chain for vaccine storage, the vaccination periods, also from the number of vaccines injected, and the type of combination applied to assess the titer of HBs, the genetic variation of the participants, the sex, the greater obesity, the age, the place and method of injection as well as the nutritional status6,21.

According to sero-analysis of hepatitis B markers, the present study showed that 19.9%, 75.51% of the students were vaccinated and susceptible, respectively. The overall prevalence of anti-HBc in this study was 4.6%. Similar rates have been reported in Iran 4.9% 22 and Jordan (2.0-4.1%) 23. Higher rates of anti-HBc positivity have been reported in studies conducted in Syria (10.3%) 24. Of the 4.9% of current tested students with positive anti-HBc, 2.04% had unexplained cases (positive anti-HBc and negative anti-HBs). Pattern of infection with hepatitis B virus, complete recovery from acute and chronic hepatitis B correlated with loss of HBsAg and appearance of anti-HBs in serum. Thus, anti-HBc is usually accompanied by HBsAg or anti-HBs. However, the detection of “anti-HBc alone" is not an uncommon serological pattern. On the other hand, this pattern is one of the more confusing HBV results and can have several possible interpretations such as resolving acute HBV infection, i.e., in the period between HBsAg loss and detectable anti-HBs development, false-positive results, in chronic and past infections as well as anti-HBc alone is the most common seromarker in Occult hepatitis B virus infection (OBI) individuals. The incidence of OBI in anti-HBc (+) but anti-HBs (−) blood donors has been reported to be as high as 7–15% making this an important clinical issue, therefore, screening for anti-HBc can help to identify OBI25-27. As well, in this study family history of HBV infection was significantly associated with anti-HBc positive status. This finding is consistent with recent study conducted in Ethiopia28**.** However, Risk factors such as male gender, surgical operations, dental procedures, blood transfusion and sharing shaving instruments were not significantly associated with anti-HBc positivity (Table 4).

Serological tests for hepatitis B markers also showed that only 11/54 of the students who said they had received the HBV vaccine were vaccinated, whereas the majority of them were susceptible 39/54. A possible interpretation is that the antibody titer drastically decreased with time. In addition, 3–20% known vaccinated failure rate, which can be attributed to vaccine factors (e.g. type, dose, schedule and injection site) or host factors (e.g. male sex, smoking, and chronic illness)6,23. However, the cases where the subjects were vaccinated and had low or undetected titer cannot be interpreted as having vaccine failure for two main reasons: first, to indicate vaccine failure, post-vaccination testing must be performed within 1-2 months after the third dose of the vaccine has been administered; second, the current study did not explore their memory cells to show if they still had anti-HBs antibody-secreting cells29, 30**.**

The level of anti-HBs among the study subjects was not significantly affected by age, as the range was narrow enough, 18-22 years, not to show any statistical difference. These results are in agreement with the results of AL-Shamahy*et al*., 6,7 and not agreement with results of many studies that showed proven antibody levels decrease over time and increasing age31,32. Decline in serum anti-HBs level essentially indicates reduced protection and the need for a booster dose of the vaccine**.** According to WHO recommendations, booster immunization for HBV is not recommended and the protection lasts at least 20 years, possibly lifelong. However, several studies highlight the importance of booster doses to trigger the memory immune system and maintain a higher protective rate of anti-HBs. Boosters will elicit an immune memory and offer reassurance of protective immunity against breakthrough infection33, 34**.**

The present study showed a slightly higher protective rate of anti-HBs antibody in females (25%) compared to males (19.4%) but this variation was not significant (*p*=0.30). The same findings were reported in previous studies conducted in Yemen6, and other countries 35, 36; Gender differences might be due to the opposite effects of sex hormone androgen and estrogen. Moreover,numerous immunological genes are appearing on the X chromosome while few ones are mapped on the Y chromosome. Estrogen activates monocyte to secrete IL-10, which induces Immunoglobulin G (IgG) and Immunoglobulin M (IgM) secretion through B-cells in turn, while testosterone damages the production of IgG and IgM from B-lymphocytes, as well as restrains producing IL-6 from monocyte37**.**

The results of this study showed that there is a statistical significance between the immunological status of the students and the education of the mother (p = 0.04) (Table 1), and most of the mothers of unprotected students had a level of education less than secondary (82%). A study previously conducted in Yemen showed that the social and economic status and lack of education, in many regions of Yemen are interrelated factors that affect the growth of children, which in turn affects their immune systems6.In this study, we did not find a significant correlation between levels of Anti.HBs and smoking. This result was in agreement with a study conducted by Peces*et al*.38, and it was not in agreement with a similar study conducted by Alavian*et al*.20 which showed that there is a relationship between smoking and decreased immune response to the HBV vaccine. Chronic diseases such as autoimmune hepatitis and kidney failure are risk factors for vaccine non-response and reduced body immunity 39, 40. However, in the current study there is no significant relationship between the history of chronic diseases and the immunological status of the students. Perhaps this is because the subjects who took part in the study were young, and there were no common chronic diseases among them.

**LIMITATIONS OF THE STUDY**

The main limitations of this study included a small sample size, and potential self-reporting errors in the questionnaire. Nevertheless, this study could serve as a bridgehead for further studies with larger sample sizes to test the findings discussed.

**CONCLUSIONS**

The low prevalence of hepatitis B protection levels amongThamar University medical students needs further research and necessitates the implementation of a screening and vaccination program for all non-immunized healthcare students.

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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**AUTHOR CONTRIBUTIONS**

This research is part of a master's degree in the Biology Department, Faculty of Applied Sciences, Thamar University, second author Najlaa Abdullah Mohammed Al-Mutaa, who conducted field work, and who did laboratory work and other authors contributed to data analysis, drafting, and review of the paper, and gave final approval to the research.

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Table 1 Association between immune status against HBV and socio demographics characteristics of study subjects

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***P*** | **95% CI**  **(low-upper)** | **χ2** | **Non- Protective level**  **(No=152)** | **Protective level**  **(No=44)** | **Total**  **(No=196)** | **Variable** | |
| **No (%)** | **No (%)** | **No (%)** |
| 0.38 |  |  | 20.17±1.066 | 20.02 ±1.17 |  | Age: Mean ± SD | |
| 0.30 | (0.35-1.38) | 1.05 | 79 (80.6) | 19 (19.4) | 98 (50.0) | Male | Gender |
| 73 (74.5) | 25 (25.5) | 98 (50.0) | Female |
| 0.53 | (0.57-2.94) | 0.38 | 114 (76.5) | 35 (23.5) | 149(76.0) | Urban | Residence |
| 38 (80.9) | 9 (19.1) | 47 (24.0) | Ruler |
| \*1 | (0.19–4.9) | 0.03 | 142 (77.6) | 41 (22.4) | 183(93.4) | Single | Marital status |
| 10 (76.9) | 3 (23.1) | 13 (6.6) | Married |
| 0.22 | (0.70-4.41) | 1.50 | 17 (68.0) | 8 (32.0) | 25 (12.8) | Yes | Smoker |
| 135 (78.9) | 36 (21.1) | 171(87.2) | No |
| 0.49 | (0.31-1.74) | 0.46 | 35 (81.4) | 8 (18.6) | 43 (21.9) | Yes | Practice exercise |
| 117 (76.5) | 36 (23.5) | 153(78.1) | No |
| 0.65 | (0.58-2.35) | 0.20 | 91 (76.5) | 28 (23.5) | 119(60.7) | ≤ 150 $ | Monthly income |
| 61 (79.2) | 16 (20.8) | 77 (39.3) | >150 $ |
| 0.23 | (0.73-3.34) | 1.39 | 32 (71.1) | 13 (28.9) | 45 (23.0) | <Secondary level | Father's education |
| 120 (79.5) | 31 (20.5) | 151 (77.0) | ≥Secondary level |
| \*\*0.04 | (1.01-5.1) | 3.88 | 100 (82.0) | 22 (18.0) | 122 (62.2) | <Secondary level | Mother's education |
| 52 (70.3) | 22 (29.7) | 74 (37.8) | ≥Secondary level |
| Protective anti-HBs= ≥10 mIU/ml; Non-protective anti-HBs = <10 mIU/ml. ; Chi-square (χ2) ≥ 3.84; \* = Fishers Exact Test; \*\* Multinomial logistic regression; *P* (Probability value) <0.05= (Statistically significant). CI= Confidence Interval ≥1. | | | | | | | |
|  |  |

Table 2: Distribution of Anti-HBs level by gender of study subjects (n= 196)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **χ2** | **p** | **Female**  **(N=98)** | **Male**  **(N=98)** | **Total**  **(N=196)** | **Anti-HBs level** |
| **No (%)** | **No (%)** | **No (%)** |
| **1.05** | **0.30** | **73 (37.2)** | **79 (40.3)** | **152 (77.6)** | Non-Immune (<10 mIU/ml) |
| **1** | **1** | **12 (6.1)** | **12 (6.1)** | **24 (12.2)** | Low-Immune (10–100 mIU/ml ) |
| **3.33\*** | **0.06** | **12 (6.1)** | **4 (2.0)** | **16 (8.2)** | Adequate - Immune (101–1000 mIU/ml ) |
| **0.25\*** | **0.62** | **1 (0.5)** | **3 (1.5)** | **4 (2.0)** | High- Immune (>1000 mIU/ml ) |
| Chi-square (χ2) ≥ 3.84; \* = Fishers Exact Test*; P* (Probability value) <0.05= (Statistically significant). | | | | | |

Table 3: Distribution of Anti-HBs level by Age of study subjects (n= 196)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***P*** | **\*Kruskal Wallis Test** | **Age / Year** | | | | | **Total**  **(N=196)** | **Anti-HBs level** |
| **22** | **21** | **20** | **19** | **18** |
| **No (%)** | **No (%)** | **No (%)** | **No (%)** | **No (%)** | **No (%)** |
| **0.32** | **3.48** | **23 (11.7)** | **25 (12.8)** | **62 (31.6)** | **38 (19.4)** | **4**  **(2.0)** | **152 (77.6)** | **Non-Immune (<10 mIU/ml)** |
| **2 (1.0)** | **2 (1.0)** | **12(6.1)** | **5 (2.6)** | **3(1.5)** | **24 (12.2)** | **Low-Immune (10–100 mIU/ml)** |
| **4 (2.0)** | **2 (1.0)** | **4 (2.0)** | **5 (2.6)** | **1(0.5)** | **16 (8.2)** | **Adequate-Immune (101–1000 mIU/ml )** |
| **1 (0.5)** | **1 (0.5)** | **2 (1.0)** | **0 (0.0)** | **0 (0.0)** | **4 (2.0)** | **High- Immune (>1000 mIU/ml )** |
| *P* (Probability value) <0.05= (Statistically significant) *;* \*Kruskalwallis test | | | | | | | | |

Table 4. Association between risk factors and anti-HBc status in study

subjects.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***P*** | **95% CI**  **(low-upper)** | **χ2** | **Anti-HBc (+)No=9 (4.6%)** | **Frequencies**  **(No=196)** | **Variable** | |
| **No (%)** | **N0 (%)** |
| \*1 | (0.20 –3.04) | 0.11 | 4 (4.1) | 96 (50.0) | Male | Sex |
| 5.1))5 | 96 (50.0) | Female |
| \*1 | (0.03-1.11) | 0.67 | 9 (4.9) | 183 (93.4) | Single | Marital status |
| 0 (0.0) | 13 (6.6) | Married |
| \*0.11 | (0.07- 1.10) | 3.70 | 5 (9.3) | 54 (27.5) | Yes | Receiving HBV vaccine before |
| 4 (2.8) | 142 (72.5) | No |
| \*0.45 | (0.04 –2.97) | 0.97 | 1 (2.0) | 49 (25.0) | Yes | Surgical operations |
| 8 (5.4) | 147 (75.0) | No |
| \*1 | (0.16 -4.12) | 0.05 | 2 (4.0) | 50 (25.5) | Yes | History of hospitalization |
| 7 (4.8) | 146 (74.5) | No |
| \*0.31 | (0.10-1.79) | 1.37 | 3 (2.9) | 103 (52.6) | Yes | Dental procedures |
| 6 (6.5) | 93 (47.4) | No |
| \*\*0.46 | (0.25-20.64) | 0.45 | 3 (21.4) | 14 (7.1) | Yes | Blood transfusion |
| 6 (3.3) | 182 (92.9) | No |
| \*1 | (0.24- 3.05) | 0.01 | 5 (4.5) | 112 (57.1) | Yes | Sharing shaving instruments |
| 4 (4.8) | 84 (42.9) | No |
| \*\*0.05 | (1.28 -4.00) | 3.9 | 2 (22.2) | 9 (4.6) | Yes | Cupping |
| 7 (3.7) | 187(95.4) | No |
| \*\*0.01 | (1.74 8.62) | 9.74 | 4 (17.4) | 23 (11.7) | Yes | Family history of HBV infection |
| 5 (2.9) | 173 (88.3 | No |
| Protective anti-HBs= ≥10 mIU/ml; Non-protective anti-HBs = <10 mIU/ml. ; Chi-square (χ2) ≥ 3.84; \* = Fishers Exact Test;\*\* Multinomial logistic regression; *P* (Probability value) <0.05= (Statistically significant). CI= Confidence Interval ≥1. | | | | | | |