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RESEARCH ARTICLE

IMMUNOLOGICAL STATUS OF HEPATITIS B VIRUS INFECTION AMONG FRESHMEN UNIVERSITY STUDENTS IN YEMEN

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Abstract

Background: Hepatitis B virus (HBV) is a most important health problem, it's a global pandemic. Previous surveys showed a high prevalence of hepatitis B infection in Yemen. Hepatitis B vaccination is the most efficient way to prevent hepatitis B virus infection and its outcomes (such as hepatocellular carcinoma, cirrhosis, and liver failure).
Aim: To measure the immune status of the hepatitis B virus amongst 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 family history of HBV (p=0.01).
Conclusion: The low prevalence of hepatitis B protection levels among Thamar 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

Of the global public health problems, hepatitis B infection is one of the most important. HBV belongs to the family Hepadnaviridae with partially double-stranded DNA[1-3]. Approximately 2 billion people worldwide have evidence of past or current infection with hepatitis B virus, 358 million or more people suffer from chronic lifelong infection, and about 887,000 people die each year from hepatitis B outcomes[1-3]. Hepatitis B virus is endemic in Yemen, the prevalence of HBsAg-positive virus in the general population and HCWs ranges from 8% to 20%, among infants, it was 4.1%, and up to 50% of health workers and residents have previous serologic evidence of HBV infection and this is all in the old reports[4-8]. On the other hand, recent studies have revealed that the incidence of HBsAg, is 0.7-2% among the general population and 4% among high-risk groups such as HCWs and hemodialysis patients and HBV also decreased further among children[9-15].
It is well known that HBV vaccine is the foundation of the hepatitis B infection prevention. To reach this, the vaccine must activate an immune response which would create 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 dose[6], this happens in more than 95% of infants, children and

young adults. But the persistence of anti-HBs and thus protection against infection and the carrier state depends on the peak concentration of anti-HBs reached after the initial vaccination. One problem with this is that anti-HBs decay exponentially with the length of time since vaccination and so a booster shot is required[6,17]. Factors connected with reduced immune response to HBV vaccine comprise increasing age, nutritional status, gender, obesity, genetic factors, and smoking. Poverty, socioeconomic status, low of education, and weak health systems in Yemen are interrelated factors that influence nutritional status of people, which in turn affect their immune system[6].

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 markers[17]. 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 hepatitis B virus among first-year students in Thamar University, during the academic year 2021-2022.

SUBJECTS AND METHODS

Study area: This study was carried out at Thamar University located in Dhamar city; Dhamar governorate (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 (the time allowed for fieldwork for a master's thesis) at Thamar University, Dhamar Governorate, Yemen. The study targeted the 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 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 frequency[17], 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 Thamar University (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 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 results of HBV markers that were detected, they were added to questionnaire chart. The potential risk factors were used as the independent variables, while the positive results of HBV markers was considered as the study outcome (dependent variables).

Collection of Blood sample: From each student; five mL of whole blood aseptically by venipuncture was collected. After clotting of 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 for 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 by performing 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 comparison of two variables to determine the *p* value, the Chi square test was used. Odd ratio (OR) was used with 99% confidence interval. *p* value <0.05 was regarded as statistically significant.

Ethical consideration: From all students **c**onsents were taken 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 the age range between 18 and 22 years and a mean age (±SD) age of 20.14 (±1.09) years. Half 50% of the participants were male and nearly two-thirds 76% of them lives in urban areas.

Distribution of Anti-HBs level by gender: 22.4% of students had 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.

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

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

Protective anti-HBs=≥10 mIU/ml; Non-protective anti-HBs =<10 mIU/ml; Chi-square (χ^2) ≥ 3.84; * =Fishers Exact Test; ** Multinomial logistic regression; *p* (Probability value) <0.05=(Statistically significant). CI=Confidence Interval ≥1.

There was no statistically significant variation between both sexes (Table 2).

Distribution of Anti-HBs level by Age: Table 3 shows that no correlation was found between age and anti-HBs level *=Kruskal Wallis test; χ^2=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 χ^2=9.74, (95% CI 1.74 –8.62); *p*=0.01. Being cupping showed a trend but not a statistically significant difference χ^2=1.28 (95% CI 1.28–42.00) *p*=0.057 (Table 4).

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

Anti-HBs level	Total (N=196)	Male (N=98)	Female (N=98)	*p*	χ^2
	No (%)	No (%)	No (%)		
Non-Immune (<10 mIU/ml)	152 (77.6)	79 (40.3)	73 (37.2)	0.30	1.05
Low-Immune (10–100 mIU/ml)	24 (12.2)	12 (6.1)	12 (6.1)	1	1
Adequate - Immune (101–1000 mIU/ml)	16 (8.2)	4 (2.0)	12 (6.1)	0.06	3.33*
High- Immune (>1000 mIU/ml)	4 (2.0)	3 (1.5)	1 (0.5)	0.62	0.25*

Chi-square (χ^2) ≥ 3.84; * =Fishers Exact Test*;* *p* (Probability value) <0.05=(Statistically significant).

DISCUSSION

The present 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, and 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 status[6,21].

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).

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

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

p (Probability value) <0.05=(Statistically significant), *Kruskal wallis test

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. 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 OBI[25-27]. 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 Ethiopia[28]. 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).

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

Protective anti-HBs=≥10 mIU/ml; Non-protective anti-HBs =<10 mIU/ml; Chi-square (χ^2) ≥ 3.84; * =Fishers Exact Test;** Multinomial logistic

Variable		Frequencies (No=196)	Anti-HBc (+) No=9 (4.6%)	χ^2	95% CI (low-upper)	*p*
		No (%)	No (%)			
Sex	Male	96 (50.0)	4 (4.1)	0.11	(0.20–3.04)	*1
	Female	96 (50.0)	5.1(5)			
Marital status	Single	183 (93.4)	9 (4.9)	0.67	(0.03-1.11)	*1
	Married	13 (6.6)	0 (0.0)			
Receiving HBV vaccine before	Yes	54 (27.5)	5 (9.3)	3.70	(0.07- 1.10)	*0.11
	No	142 (72.5)	4 (2.8)			
Surgical operations	Yes	49 (25.0)	1 (2.0)	0.97	(0.04 –2.97)	*0.45
	No	147 (75.0)	8 (5.4)			
History of hospitalization	Yes	50 (25.5)	2 (4.0)	0.05	(0.16 -4.12)	*1
	No	146 (74.5)	7 (4.8)			
Dental procedures	Yes	103 (52.6)	3 (2.9)	1.37	(0.10-1.79)	*0.31
	No	93 (47.4)	6 (6.5)			
Blood transfusion	Yes	14 (7.1)	3 (21.4)	0.45	(0.25-20.64)	**0.46
	No	182 (92.9)	6 (3.3)			
Sharing shaving instruments	Yes	112 (57.1)	5 (4.5)	0.01	(0.24- 3.05)	*1
	No	84 (42.9)	4 (4.8)			
Cupping	Yes	9 (4.6)	2 (22.2)	3.9	(1.28 -4.00)	**0.05
	No	187(95.4)	7 (3.7)			
Family history of HBV infection	Yes	23 (11.7)	4 (17.4)	9.74	(1.74 8.62)	**0.01
	No	173 (88.3	5 (2.9)			

regression; *p* (Probability value) <0.05=(Statistically significant). CI=Confidence Interval ≥1.

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 cells[29,30]. 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 had proven antibody levels decrease over time and increasing age[31,32]. A decrease in the level of anti-HB in the blood mainly indicates a decrease in protection and the necessitate for a booster dose of the hepatitis B vaccine. As per WHO recommendations, booster immunization for hepatitis B virus is not suggested and protection lasts for at least 20 years, and probably lifelong. Nevertheless, numerous studies emphasize the importance of booster doses for stimulating the memory immune system and maintaining a higher protective rate of anti-HBs. Boosters will activate immune memory and provide reassurance of protective immunity against super-infection[33,34]. The present study showed 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 Yemen[6], and other countries[35,36]; Gender differences might be due to the opposite effects of sex hormone androgen and estrogen. Moreover, 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 monocyte[37].

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[6]. In this study. This result was in agreement with 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.

Limitation of the study

The main limitations of this study included 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.

CONCLUSION

The low prevalence of hepatitis B protection levels among Thamar 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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AUTHOR'S CONTRIBUTIONS

This research is part of a master's degree in the Biology Department, Faculty of Applied Sciences, Thamar University. **Al Makdad ASM:** writing original draft, methodology. **Al-Mutaa NAM:** field work, lab work. **Al-Haifi AY:** statistical analysis, conceptualization. **Al-Shamahy HA:** editing, methodology, supervision. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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