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Risk Factors and Seroprevalence of Hepatitis B Virus Antigen among University Students in the Sana'a City, Yemen

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Abstract:

Background: Hepatitis B virus (HBV) is a serious global public health problem that causes morbidity and mortality, particularly in developing countries. However, no prevalence data were published among Yemeni university students. **Aims:** Therefore, the objective of this study was to find out the risk factors and prevalence of HBV among Yemeni university students in the Sana'a capital of Yemen. **Methods:** This study was conducted on bachelor's students of six universities between January and April 2022. After obtaining the required data by using a designed questionnaire, the blood specimens were collected from each participant and the HBsAg was screened in serum by using a rapid test cassette and the positive results were confirmed by the ELISA technique. **Results:** Out of 503 blood samples, only 9(1.79%) were HBsAg positive by rapid test. The high rate of HBsAg positive was recorded in males (7; 1.96%), age group of ≥ 24 years (2; 4.0%), students coming from rural areas (2.80%), unmarried students (1.98%), non-medical students (1.96%), and studying at the first years (2.61%) with statistically significant differences ($P > 0.05$). However, the majority of HBsAg was found among subjects who had a history of hospitalization, donate blood, did a dental procedure, used common shaving instruments, were exposed to sharp injury, their a family history of HBV, and used china acupuncture. Also, the ELISA technique showed only one positive (0.20%) of nine that were positive by the rapid cassette test. **Conclusion:** In spite of the low rate of HBsAg observed in this study, this type of virus will be remaining a threatening source for the adult. Consequently, it should extend the HBV vaccination programme to a vulnerable population who didn't receive the HBV vaccine.

Keywords: ELISA, Hepatitis B virus (HBV), Sana'a, Students, University, Yemen.

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Introduction

Hepatitis B virus (HBV) infection is one of the most infectious diseases in the world and remains a significant clinical and public health problem due to its role in causing acute hepatitis, chronic hepatitis, a carrier state, hepatocellular carcinoma and death¹. In acute infection, hepatitis B surface antigen (HBsAg) is the first serological marker to appear in the serum while the appearance of hepatitis B envelop antigen (HBeAg) in the serum usually indicates active HBV replication in the hepatocytes and infectivity².

It is estimated that more than 2 billion people globally have been infected by this type of virus and among these individuals, 296 million people, or nearly 5% of the world's population, were living with chronic hepatitis B infection in 2019³.

However, about 1.5 million new cases were infected by HBV each year. Also, it was annually estimated that about 820 thousand people die resulting mostly from cirrhosis (52%) and hepatocellular carcinoma (38%)^{3,4}. The prevalence of HBV infection varies between 2% (low) in developed countries and about 8% (endemic) in developing countries⁵.

The mode of HBV transmission is transfusion of infected blood, sexual exposure, use of contaminated equipment for medical processes, sharing of piercing tools as well as from an infected mother with HBV to her infants^{6,7}.

The risk factors for HBV infection include age, gender, geographic region, socioeconomic status, immunization status, lifestyle, sanitation, and hygiene, and their relative contributions or influence to the transmission and prevalence of HBV infection are well documented in literature^{8,9}.

Globally, the overall rate of HBV infection among university student was recorded at 7.3% and in southern Ethiopia¹⁰, 31.5% in Nigeria¹¹, 4.6% in Lomé, Togo¹², and 11.5% in eastern Ethiopia¹³.

Yemen is one of the developing countries that lack an effective health system due to war since 2015 and so on. Therefore, most diseases are spreading during this period as a consequence of increased poverty levels, inadequate personal hygiene, and availability of safe water¹⁴⁻¹⁸.

However, several reports documented in the frequency of common infectious viruses among Yemeni populations^{19,20}. The previous national reports reported that the prevalence of positive

HBsAg ranged between 8% to 20% among adults^{21,22} and 4.1% among infants²³. Recently, Al-Shamahy *et al.*²⁴ revealed that the prevalence rate of HBsAg among children was 1.8% in Sana'a city and 3.8% in Shabowah governorate.

Up to now, no study in Sana'a has so far specifically focused on the prevalence of HBsAg among university students, who could be considered a vulnerable population. The objective of the present study was to determine the prevalence of HBsAg and its associated risk factors among university students in Sana'a city, Yemen.

Materials and Methods

Study design and period

This is a cross-sectional study conducted among university students between January and April 2022. The subject participants were attending six universities including; Al-Razi University, Yemen University, Azal University for Human Development, University of Azal for Sciences and Technology, Al-Hikma University, and National University which are located in Sana'a City, Yemen.

Sample size

Five hundred and three 503 blood specimens were randomly collected from Universities students aged between 17-35 years old attending six universities in Sana'a city.

Data collection

A questionnaire was subjected to collect the required information from all participants. In the questionnaire, questions such as gender, age, residence, marital state, faculty of study, year group, and family history of HBV were asked. Also, medical and surgical risks include a history of hospitalization, blood transfusion, blood donation, surgical operations, dental procedures, using common shaving instruments, needle prick, sharp injury, chronic disease, cupping, and being vaccinated with HBV were gathered by interview face to face.

Inclusion and exclusion criteria

Inclusion criteria were to be a student who signed a consent form to participate in the study. The criteria of exclusion were students who declined to offer consent to the study.

Sample collection

About 5-ml of venous blood samples were collected in a plain vacutainer tube under aseptic procedures and allowed to stand for about 20 min for clotting blood before centrifuging.

Serological assay

Hepatitis B surface antigen was detected using a qualitative rapid test cassette (SD Rapid test Cassette HBsAg, Korea). Then, the positive results for HBsAg detection were confirmed by using an enzyme-linked immunosorbent assay (ELISA) (Labsystem Reader) using a commercially available kit (Biokit, Spain) at the AULAQI Specialized Med. Lab.

Ethical statement

The protocol of the present study was approved by the Al-Razi University, Yemen, Ethics Review Committee and permitted by the Universities' administration. Further, participation was voluntary and participants completed a consent form by themselves or investigator teams.

Statistical analysis

The obtained data were statistically analyzed by using the SPSS program (version 20.0). A significant difference between the proportions and the variables associated with HBV infections was determined. A P-value (< 0.05) was considered statistically significant.

Results

Socio-demographic characterization

Table 1 shows that the most rate of blood specimens was sampled from male students (71.17%), aged between 21-23 years (54.67%), living in urban areas (78.73%), single (90.46%) who study at the faculty of medical college (59.44%) at the second year of education level (38.37%).

Table 1: Socio-demographic characterization of subject participants

Variables		Examined No. (%)
Gender	Male	358 (71.17)
	Female	145 (28.83)
Age group (in years)	18-20	178 (35.39)
	21-23	275 (54.67)
	≥24	50 (9.94)
Residence	Urban	396 (78.73)
	Rural	107 (21.27)
Marital State	Married	48 (9.54)
	Single	455 (90.46)
Faculty of study	Medical	299 (59.44)
	Non-medical	204 (40.66)
Educational level	First year	153 (30.42)
	Second year	193 (38.37)
	Third year	79 (15.71)
	Fourth year	54 (10.73)
	Fifth year	24 (4.77)

Table 2 shows the distribution of specimen collection from enrolled university students having risk factors that may contribute to the prevalence of the HB virus.

Table 2: Risk factors associated with HBsAg prevalent among university students

Variables		Examined No. (%)	Variables		Examined No. (%)
Hospitalization	Yes	173 (34.40)	Sharp injury	Yes	275 (54.67)
	No	330 (65.60)		No	228 (45.33)
Blood transfusion	Yes	24 (4.77)	Chronic disease	Yes	92 (18.29)
	No	479 (95.23)		No	411 (81.71)
Blood donation	Yes	94 (18.69)	Family history of HBV	Yes	56 (11.13)
	No	409 (81.31)		No	447 (88.87)
Surgical operations	Yes	152 (30.22)	Cupping	Yes	37 (7.36)
	No	351 (69.78)		No	466 (92.64)
Dental procedures	Yes	257 (51.09)	China acupuncture	Yes	2 (0.40)
	No	246 (48.91)		No	501 (99.60)
Using common shaving instruments	Yes	298 (59.24)	Vaccinated for hepatitis B	Yes	46 (9.15)
	No	205 (40.75)		No	457 (90.85)
Needle prick	Yes	279 (55.47)			
	No	224 (44.53)			

Seroprevalence of HBV infection

This result found that the seroprevalence rate of HBsAg positively was 9(1.79%) while 494(98.21%) study subjects were showed

negatively for the HBsAg test as figured in Fig. (1).

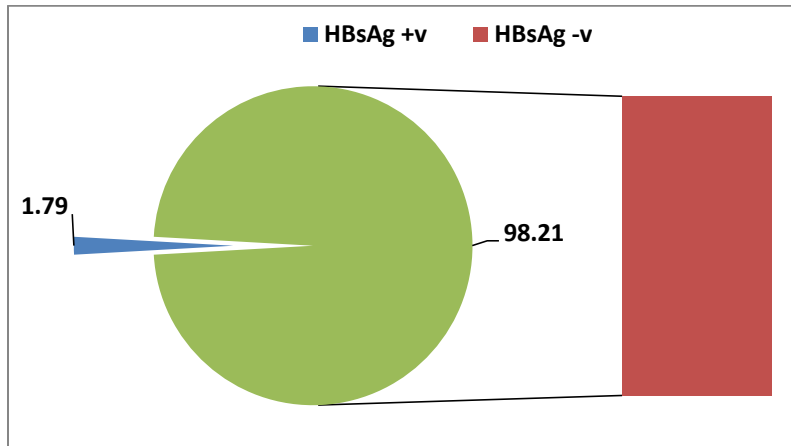


Figure 1. Seroprevalence of HBsAg among participants

In the current result, it was reported that the higher rate of HBsAg was among male students at 1.96% compared to female students at 1.38%. Also, the age group of ≥ 24 was found to be had a higher rate of HBsAg (4.0%) compared to another age group. The students coming from rural areas had a higher rate of HBsAg at 3(2.80%) and only single students were positively showed for HB surface antigen

(1.98%) whereas all married students were free of HBsAg (Table 3).

However, it was found that the non-medical students were more exposed to HB virus infection than medical students. Also, the highest rate of HBsAg was recorded among students who study in the first year (2.61%) (Table 3).

Table 3: Frequency of HBsAg according to socio-demographic factors

Variables		Examined No. (%)	HBsAg +v No. (%)	HBsAg -v No. (%)	P value
Gender	Male	358 (71.17)	7 (1.96)	351 (98.04)	0.660
	Female	145 (28.83)	2 (1.38)	143 (98.62)	
Age group (in years)	18-20	178 (35.39)	2 (1.12)	175 (98.88)	0.819
	21-23	275 (54.67)	5 (1.82)	270 (98.18)	
	≥ 24	50 (9.94)	2 (4.0)	48 (96.0)	
Residence	Urban	396 (78.73)	6 (1.52)	390 (98.48)	0.373
	Rural	107 (21.27)	3 (2.80)	104 (97.20)	
Marital state	Married	48 (9.54)	0 (0)	48 (100)	0.326
	Single	455 (90.46)	9 (1.98)	446 (98.02)	
Faculty of study	Medical	299 (59.44)	5 (1.67)	294 (98.33)	0.811
	Non-medical	204 (40.66)	4 (1.96)	200 (98.04)	
Educational level	First	153 (30.42)	4 (2.61)	151 (97.39)	0.877
	Second	193 (38.37)	3 (1.55)	190 (98.45)	
	Third	79 (15.71)	1 (1.27)	78 (98.73)	
	Fourth	54 (10.73)	1 (1.85)	53 (98.15)	
	Fifth	24 (4.77)	0 (0)	24 (100)	

Significant statistics at p -value < 0.05 .

The present result showed that the highest rate of positive HBsAg was observed among study subjects who had a history of hospitalization (2.31%), didn't receive a blood transfusion (1.88%), donate blood (2.13%), and did dental procedures (1.95%). Also, a higher rate of HBsAg was found among study participants who used common shaving instruments (2.35%),

didn't expose to needle prick (2.35%), exposed to sharp injury (1.82%), free of chronic disease (1.95%), their family history of HBV (14.29%), didn't cupping (1.93%) and used china acupuncture (1.60%), and didn't vaccinate for hepatitis B (1.97%) (Table 4).

Table 4: Risk factors of associated with HBV infection among universities students

Variables		Examined No. (%)	HBsAg +v No. (%)	HBsAg -v No. (%)	P value
Hospitalization	Yes	173 (34.40)	4 (2.31)	169 (97.69)	0.523
	No	330 (65.60)	5 (1.52)	325 (98.48)	
Blood transfusion	Yes	24 (4.77)	0 (0)	24 (100)	0.499
	No	479 (95.23)	9 (1.88)	470 (98.12)	
Blood donation	Yes	94 (18.69)	2 (2.13)	92 (97.87)	0.147
	No	409 (81.31)	7 (1.71)	402 (98.29)	
Surgical operations	Yes	152 (30.22)	2 (1.32)	150 (98.68)	0.599
	No	351 (69.78)	7 (1.99)	344 (98.01)	
Dental procedures	Yes	257 (51.09)	5 (1.95)	252 (98.05)	0.792
	No	246 (48.91)	4 (1.63)	242 (98.37)	
Using common shaving instruments	Yes	298 (59.24)	7 (2.35)	291 (97.65)	0.252
	No	205 (40.75)	2 (0.98)	203 (99.02)	
Needle prick	Yes	279 (55.47)	4 (1.43)	295 (98.57)	0.503
	No	224 (44.53)	5 (2.23)	219 (97.77)	
Sharp injury	Yes	275 (54.67)	5 (1.82)	270 (98.18)	0.963
	No	228 (45.33)	4 (1.75)	224 (98.25)	
Chronic disease	Yes	92 (18.29)	1 (1.09)	91 (98.91)	0.837
	No	411 (81.71)	8 (1.95)	403 (98.05)	
Family history of HBV	Yes	56 (11.13)	8 (14.29)	48 (85.71)	0.998
	No	447 (88.87)	1 (0.22)	446 (99.78)	
Cupping	Yes	37 (7.36)	0 (0)	37 (100)	0.395
	No	466 (92.64)	9 (1.93)	457 (98.07)	
China acupuncture	Yes	2 (0.40)	1 (50.0)	1 (50.0)	0.000
	No	501 (99.60)	8 (1.60)	493 (98.40)	
Vaccinated for hepatitis B	Yes	46 (9.15)	0(0.0)	46 (100)	0.338
	No	457 (90.85)	9 (1.97)	448 (98.03)	

Significant statistics at p -value <0.05.

Comparison between the Cassette and ELISA techniques

In this finding, it was found that the HBsAg was detected in 9(1.79%) specimens by using a rapid Cassette technique. In contrast, the ELISA technique revealed that the HBsAg was found only one of positively nine by rapid cassette test (Table 5).

Discussion

The prevalence of hepatitis B virus is considered high in Yemen ranged from 8% to 20%, and up to 50% of the general populations has serological evidence of previous HBV infection²³. Recently, a decrease in HBV prevalence was noticed among

Table 5. Comparison between the Cassette and ELISA techniques

Cassatt technique		ELISA technique	
HBsAg +v No. (%)	HBsAg -v No. (%)	HBsAg +v No. (%)	HBsAg -v No. (%)
9 (1.79)	494 (98.21)	1 (0.20)	502 (88.89)

the population after the HB vaccine was implemented in Yemen^{25,26}.

The overall rate of HBsAg seropositivity in this study was 1.79% recorded among university students. This result is in agreement with a study conducted at the University of Bangui among 801 students and found a prevalence of HBsAg positivity of 1.3%²⁷.

This finding is lower than studies conducted in Nigeria (31.5%)¹¹, eastern Ethiopia (11.5%)¹³, Lomé, Togo (4.6%)¹², Uganda (8.1%)²⁸, Tanzania (7.0%)²⁹, and southern Ethiopia (7.3%)¹⁰.

However, this result is higher than in Saudi Arabia (0.41%)³⁰. The lower seroprevalence in this report might be related to the different methods in sample collection, sample size, and population studied. Also, in Yemen, the population adheres to the value and teachings of the Islamic religion which forbids multiple sexual partners. These contribute significantly to reducing the spread of sexually transmitted diseases such as the HB virus.

This finding showed that the highest rate of HBsAg was 1.96% observed among male students with non-statistical significant differences ($P= 0.660$). This result is relatively consistent with studies carried out in a different area that high rate of HBsAg was found among males at 43% by Tula and Iyoha¹¹, 6% by Imarenezor *et al.*³¹, 13% by Chikwendu *et al.*³², 7.19% by Tawiah *et al.*³³, 26.34% by Mirambo *et al.*³⁴.

The reason that could simply be adduced for this observation was that males indulge in more of the risk factors that predispose them to HBV infection than females. Besides risk factors, studies have shown that the reason for gender differences in response to HBsAg, is that, females are more likely than males, to produce antibodies known as anti-HBs against HBsAg³⁵.

This work found that the age group ≥ 24 years had the highest prevalence rate of HBsAg at 4% with non-statistical significant differences ($P= 0.819$). In similar study documented that the high rate of HBsAg was 9.7% recorded among the age group of 21-25 years³⁶, 4.8% within age 11 to 30 years³¹, 48.7% within 18-20 years¹², and 9.68% within 25-29 years³³. The higher rate of hepatitis B virus among the older group in this study may be these group who were born before the introduction vaccination program. A vaccination program that Yemen introduced universal immunization against HBV for infants and high-risk groups early²³.

According to residency area, this result found that the students coming from the rural area had the highest rate of HBsAg at 2.80% compared to students coming from the urban area at 1.52% and there are non-statistical significant differences ($P= 0.373$). This result is in agreement with a previous study by Ekouevi *et al.*¹² who revealed that the

HBsAg was found to be more among urban students (51.4%) than rural students. In this result, the higher rate of HBsAg-positive cases was from rural areas signifying poorer vaccination coverage of the rural population and a decrease on awareness among people living in the rural areas.

As regards the marital state in this study, only single students showed positively for HB surface antigen at 1.98% and all married students were free from HBsAg, although the difference was not statistically significant ($P= 0.326$). This result confirms what was observed in previous studies recorded among single students at 97.3% by Ekouevi *et al.*¹², and 5.08% by Tadongfack *et al.*³⁷.

However, this result revealed that non-medical students were more infected by the HB virus (1.96%) than medical students (1.67%) with no statistically significant differences ($P= 0.811$). In contrast, a study by Pido and Kagimu³⁸ showed that the clinical student was more exposed to hepatitis B virus infection without statistically significant differences.

The present result revealed that the highest rate of HBsAg was found among students who study at the first level at 2.61% and no statistically significant differences ($P= 0.877$). Similar study by Tawiah *et al.*³³ found that the student who studied in the fourth year had a higher rate of HBsAg at 17.39% without statistically significant differences.

This result showed that there was no significant association between HBsAg positivity and history of hospitalization, blood transfusion, blood donation, dental procedures, chronic disease, their family history of HBV, cupping, and vaccinated for hepatitis B. While there was found a significant association between HBsAg positivity and the history of china acupuncture.

A previous study in Yemen that the patients had a varied history of exposure to HBV risk factors such as major/dental surgery or blood transfusion³⁹. The high rate of HBsAg was among study subjects who had a history of hospitalization at 2.31%. Moreover, the participant who had a history of blood donation was more exposed to hepatitis B virus infection (2.13%). Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood⁴⁰.

In Yemen, most hospitals are generally lacking to the standard guideline for instruments sterilization,

disinfection, and general standards for training the healthcare staff to protect the admitted patients⁴¹⁻⁴⁶. Previous studies showed HBV transmissions mainly resulted from non-compliance with aseptic techniques such as the use of inadequately sterilized needles and medical instruments and the reuse of disposable needles and syringes^{47,48}. However, in this study, the HBsAg positivity was the commonest recorded among students who didn't expose to needle prick and who were exposed to sharp injury. Previous studies showed HBV transmissions mainly resulted from non-compliance with aseptic techniques such as the use of inadequately sterilized needles and medical instruments and the reuse of disposable needles and syringes^{47,48}.

The present study observed that all students who received the HB vaccine were negatively for HBsAg. This finding is consistent with a study conducted in Ethiopia that showed that the subject students with no vaccination for HBV were two times more likely to be positive for HBsAg¹³. Several other studies also indicated low immunization status against the hepatitis B virus was associated with a higher chance of hepatitis B virus (HBV) infection⁴⁹. According to a comparison between the Cassette and ELISA techniques, this result revealed that the ELISA technique had more sensitivity and specificity for HBsAg detection. This result was supported by several reports^{50,51}.

The high prevalence of infectious diseases in later years in Yemen resulted from the war since 2015 and so on. Also, these conflicts have been contributing significantly in increase poverty among the population, increasing costs the live requirements, and most families immigrating to other areas that are safe for them to live⁵²⁻⁵⁹.

Conclusion

This study showed a low prevalence of hepatitis B virus infection among university students in Sana'a city-Yemen. But, this virus can pose a serious threat of silent transmission and spread among people and also create an urge for more sensitive assays as ELISA that more sensitive than rapid cassette test. The rapid test devices can be used as a screening test for HBsAg only when the resources are limited. Moreover, there is a need to educate students attending universities on the modes of transmission and prevention, the importance of

being compliant with protective vaccination as well as the need to observe universal precaution and infection control guidelines during their training and future professional practice.

Conflict of interest

No conflict of interest is associated with this work.

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عوامل الخطر وانتشار المستضد السطحي لفيروس التهاب الكبد الوبائي ب بين طلاب الجامعات في مدينة صنعاء، اليمن.

خلفية: فيروس التهاب الكبد الوبائي ب (HBV) هو مشكلة صحية عامة خطيرة على مستوى العالم تسبب المرض والوفيات، خصوصاً في الدول النامية. ومع ذلك، لم يتم نشر بيانات الانتشار بين طلاب الجامعات اليمنيين. **أهداف الدراسة:** هدفت الدراسة إلى معرفة عوامل الخطر وانتشار فيروس التهاب الكبد الوبائي ب بين طلاب الجامعات اليمنيين في العاصمة صنعاء. **الطرق:** أجريت هذه الدراسة على طلاب البكالوريوس في ست جامعات بين فبراير ويونيو 2022. بعد الحصول على البيانات المطلوبة باستخدام استبيان مصمم، تم جمع عينات الدم من كل مشارك وتم فحص المستضد السطحي لفيروس التهاب الكبد الوبائي ب (HBsAg) في المصل باستخدام اختبار الكاسيت السريع وتم تأكيد النتائج الإيجابية بتقنية ELISA. **النتائج:** من بين 503 عينات دم، كانت 9 فقط (1.79%) إيجابية لـ HBsAg بواسطة الاختبار السريع. تم تسجيل معدل عالي من الإيجابية لـ HBsAg بين الذكور (7؛ 1.96%)، الفئة العمرية ≤ 24 سنة (2؛ 4.0%)، الطلاب القادمين من المناطق الريفية (2.80%)، الطلاب غير المتزوجين (1.98%)، الطلاب غير الطبيين (1.96%)، والطلاب في السنوات الأولى (2.61%) مع اختلافات ذات دلالة إحصائية ($P > 0.05$). ومع ذلك، وجد أن غالبية HBsAg كانت بين الأشخاص الذين لديهم تاريخ في الاستشفاء، التبرع بالدم، إجراء طبيب الأسنان، استخدام أدوات حلاقة مشتركة، التعرض لإصابة حادة، تاريخ عائلي للإصابة بـ HBV، واستخدام الإبر الصينية. كما أظهرت تقنية ELISA إيجابية واحدة فقط (0.20%) من بين التسعة التي كانت إيجابية بواسطة اختبار الكاسيت السريع. **الاستنتاج:** على الرغم من انخفاض معدل HBsAg الذي لوحظ في هذه الدراسة، فإن هذا النوع من الفيروس سيظل مصدر تهديد للبالغين. وبالتالي، ينبغي توسيع برنامج التطعيم ضد HBV ليشمل الفئات الضعيفة التي لم تتلق لقاح HBV.