Prevalence of Occult Hepatitis B Virus Infection Among Assiut University Students

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ABSTRACT

Background: Approximately one-third of the world's population is exposed to hepatitis B virus infection, where 400 million are chronically infected. Approximately 25% of those infections are at risk for mortality due to chronic liver disease or hepatocellular carcinoma (HCC). In Egypt, the population prevalence rate for HBV is 1.4% in adults aged 15-59 years old mainly higher in Upper Egypt (Aswan, Assiut, and Minya Governorates).
Objective: Our study aims to demonstrate the prevalence of occult hepatitis B virus infection (OBI) among a randomly selected sample of Assiut University students, Egypt.
Patients and Methods: A cross-sectional study was conducted in the period between April 2019 and September 2019, and included 200 students, aged 17 to 27 years old. They were randomly selected during the routine checkup and enrolled in the study for biological testing of HBV.
Results: Prevalence of occult hepatitis B infection in all students reached 1.5% (2 males and one female). There was no significant difference between the mean age of OBI students compared to the mean age of OBI negative students ($p = 0.133$). There was a highly significant difference between the mean of HBV DNA PCR +ve students and the mean of HBV DNA PCR in –ve ones ($p < 0.001$). The prevalence of the core antibody in OBI students was –ve (100%). There was a highly significant difference between the mean of ALT in OBI students and the mean of ALT of negative ones ($p < 0.001$). Risk factors associated with OBI students in this study included the use of shaving blades, barber visits, sharing shaving blades, dental visits, sharing nail clippers and, surgery.
Conclusion: The prevalence of occult hepatitis B virus infection among students at Assiut University, was 1.5%.

Keywords: HBV; OBI; Students; Assiut University

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INTRODUCTION

Hepatitis B virus (HBV) is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV)[1]. It is a major global health problem. Hepatitis B infection can cause both acute and chronic diseases. It puts people at high risk of death from cirrhosis and liver cancer. It is an important occupational hazard for health workers. It is estimated that about 780,000 people die each year due to the consequences of hepatitis B (liver cirrhosis and liver cancer).An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen-positive). Hepatitis B prevalence is highest in the WHO Western Pacific Region and the WHO African Region, where 6.2% and 6.1% respectively of the adult population is infected [2]. Modes of transmission include vertical transmission, exposure to invasive medical procedures, injecting drug use, and contact with infected household members (sharing of personal hygiene materials, such as razors or toothbrushes) [3]. Occult HBV infection (OBI) is defined as the detection of hepatitis B virus (HBV) nucleic acid in the blood or liver of hepatitis B surface antigen (HBsAg) negative patients. The cause is the long-term persistence of viral covalently-closed-circular DNA (ccc DNA) in the nuclei of the hepatocytes. The majority of the OBI cases infected with replication-competent HBV showing strong suppression of replication and gene expression. It results in very low viral load or even undetectable virus in OBI cases [4]. Hepatitis B virus (HBV) genotype D is the only detectable genotype among Egyptian occult HBV

infection (OBI) patients. Higher rates of OBI reported among Egyptian chronic HCV, hemodialysis, children with malignant disorders, and cryptogenic liver disease patients [5]. Young adults are at high risk of infection (especially between the ages of 20 to 40 years). This is consistent with the allowed age range of blood donation, employment, and the childbearing age of the females, the three most common sources of HB cases' detection[6]. College students are at high risk of acquiring HBV. Risk factors include infected family members, sharing of shaving equipment, barber visits, intravenous drug use, dental surgery, blood transfusion, and living abroad.[7]

Our study aimed to estimate the prevalence of occult hepatitis B virus infection (OBI) among a sample of Assiut University students in upper Egypt.

PATIENTS AND METHODS

A cross-sectional study was conducted on 200 students at Assiut University, Egypt randomly selected and enrolled in the study for biological testing of HBV. In the period from April 2019 to September 2019. Patients with positive hepatitis B surface antigen, chronic HCV patients, patients with malignancy, or under chemotherapy in addition to patients on antiviral therapy for HBV were excluded from the study.

All participants were subjected to the following:

History taking

The history was taken and recorded including personal history (name, sex, age) and asked about the possible risk of transmission by filling questionnaire including personal behavior and health care services-related risk factors, such as intravenous drug use, sharing shaving instruments, sharing toothbrushes, shaving at a barber, Hejama, getting piercings or tattoos, STD history, receiving blood, invasive surgery (including endoscopy procedure), hemodialysis, a history of dental visits, and a history of hospitalization in addition to living abroad, other family members infected, childhood residence.

Specimen collection:

Five ml of venous blood were withdrawn from all subjects by venipuncture using sterile plastic syringes and divided into two portions as follows, 2.0 ml was added to Ethylenediaminetetraacetic acid (EDTA) tube with good mixing by inverting the tube upside down gently several times for estimation of HBV DNA by PCR. The remaining specimen was allowed to clot at room temperature before centrifugation for 15 minutes at 4000 rpm.

The separated serum was divided into several aliquots as follows: HB surface Ag using ELISA (Enzyme-linked Immunosorbent assay) kits by Dia. Pro Diagnostic Bioprobes Srl, Italy, HCV Abs using ELISA (Enzyme-linked Immunosorbent assay) kits by Dia.Pro Diagnostic Bioprobes Srl, Italy, Alanine aminotransferase (**ALT)** using Mindray BS 380 automated chemistry analyzer, China. Specific investigations: included, HB core antibody using ELISA kits by Dia.Pro Diagnostic Bioprobes Srl, Italy, and quantitative PCR for HBV DNA by real-time PCR.

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 22.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD).

Qualitative data were expressed as frequency and percentage.

The following tests were done: Independent-samples t-test of significance was used when comparing two means.

Chi-square (x2) test of significance was used to compare proportions between qualitative parameters.

Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as Probability (P-value):

P-value <0.05 was considered significant.

P-value <0.001 was considered highly significant.

P-value >0.05 was considered insignificant.

Ethical considerations: All steps of the study had been explained clearly to the students and /or caregivers before participation. The study was approved by the ethical committee of Al-Azhar University, Assiut, Egypt. Verbal and written consent was taken from all participants and they were free to leave the study at any time.

RESULTS

The data collected was analyzed, tabulated, and shows the following results.

Parameter N=200		Frequency	Percent (%)
Sex	**Male**	129	64.5
	Female	71	35.5
HBs Ag	**Negative**	200	100.0
HBc Ab	**Negative**	200	100.0
HCV Ab	**Negative**	200	100.0
HBV DNA PCR IU/mL	**<18**	197	98.5
	>18	3	1.5

HBs Ag (hepatitis B surface antigen**)**
HBc Ab (hepatitis B core antibody)
HCV Ab (hepatitis C virus antibody)

Table (I): Frequency of sex and hepatitis marker parameters in all subjects

Regarding gender, there were (129) males with (64.5%) and (71) females with (35.5%).

All 200 subjects were negative for HBs Ag (0%). Also, subjects had negative HBc Ab (0%) and negative HCV Ab (0%). In all subjects; 197 were HBV DNA PCR negative (98.5%) and 3 were HBV DNA PCR positive (occult hepatitis B) (1.5%).

SEX	Occult HBV DNA			P-Value
	+ ve (%)	-ve (%)	Total	
Male	2 (1)	127 (63.5)	129 (64.5 %)	<0.001
Female	1 (0.5)	70(35)	71 (35.5 %)	

Table (2): Frequency of sex in HBV DNA +ve and -ve students

Among OBI cases, 2 students were males (1%) and one was a female (0.5%) as shown in table (2) with a very highly significant difference between negative

and positive occult hepatitis B DNA between males and females (p-value <0.001).

Parameters	Mean or Median	S. Deviation (SD)
Age (years)	21.20 (mean)	2.09
HBV DNA PCR(IU/mL)	8.12 (median)	913.66
ALT(IU/L)	26.22 (mean)	11.69

Table (3): Demographic and laboratory data of all subjects
In all subjects,

Demographic and Laboratory data of all subjects in **Table (3),** the mean age was (21.1 ± 2.09) years. Also, the median concentration of HBV DNA PCR was (8.12 ± 913.66) IU/mL. However, the mean concentration of ALT was (26.22 ± 11.69) IU/L.

Parameter	OBI–ve (N= 197)	OBI +ve (N= 3)	P-Value
Age (years)	21.17 ± 2.06	23 ± 3.6	0.271
HBV DNA PCR (IU/mL)	7.94 ± 2.22	7101.67 ± 2951.23	<0.003
ALT (IU/L)	25.50 ±10.01	73.67 ±18.77	<0.003

Table (4): Demographic and laboratory data of Occult HBV DNA–ve and +ve students

In our study, demographic and laboratory data of HBV DNA PCR (OBI) –ve & +ve students in **Table (4).** Regarding the age, in OBI –ve subjects, the mean age was (21.17±2.06) years, while the mean age in OBI +ve subjects was (23±3.6) years with no significant difference (p =0.271).
Also, the mean of HBV DNA PCR in OBI –ve subjects was (7.94 ± 2.22) IU/mL, while the mean HBV DNA PCR in OBI +ve subjects was (7101.67 ± 2951.23) IU/mL with highly significant difference (p <0.003).
However, the mean of ALT in OBI –ve subjects was (25.50 ±10.01) IU/L, while the mean ALT in OBI +ve subjects was (73.67 ±18.77) IU/L with highly significant difference (p <0.003).

%	Frequency	Parameter N=200	
99.0	198	**No**	**BLOOD TRANSFUSION**
1.0	2	**Yes**	
100.0	200	**Total**	
89.0	178	**No**	**HOSPITAL ADMISSION**
11.0	22	**Yes**	
100.0	200	**Total**	
84.5	169	**No**	**SURGICAL OPEATION**
15.5	31	**Yes**	
100.0	200	**Total**	
74.0	148	**No**	**DENTAL VISIT**
26.0	52	**Yes**	
100.0	200	**Total**	
97.5	195	**No**	**INFECTED RELATIVE WITH HBV**
2.5	5	**Yes**	
100.0	200	**Total**	
83.5	167	**No**	**NEEDLE STICK INJURY**
16.5	33	**Yes**	
100.0	200	**Total**	
54.5	109	**No**	**SHAVING BLADES USE**
45.5	91	**Yes**	
100.0	200	**Total**	
91.5	183	**No**	**PIERCING**
8.5	17	**Yes**	
100.0	200	**Total**	
49.0	98	**No**	**BARBER VISIT**
51.0	102	**Yes**	
100.0	200	**Total**	
89.5	179	**No**	**SHARING BLADES**
10.5	21	**Yes**	
100.0	200	**Total**	
98.0	196	**No**	**IVDU**
2.0	4	**Yes**	
100.0	200	**Total**	
99.5	199	**No**	**SHARING TOOTHBRUSH**
0.5	1	**Yes**	
100.0	200	**Total**	
77.0	154	**No**	**SHARING NAIL CLIPPER**
23.0	46	**Yes**	
100.0	200	**Total**	
99.5	199	**No**	**LIVING ABROAD**
0.5	1	**Yes**	
100.0	200	**Total**	
96.5	193	**No**	**HEPATITIS B VACCINE**
3.5	7	**Yes**	
100.0	200	**Total**	
98.5	197	**No**	**Job**
1.5	3	**Yes**	
100.0	200	**Total**	
44.0	88	**Urban**	**RESIDENCY**
56.0	112	**Rural**	
100.0	200	**Total**	

Table (5): Frequency of risk factors parameters in all subjects

Risk factors that were found to be positive in the OBI positive cases included, using shaving blades, sharing blades, sharing nail clippers, barber visits, dental visits, and history of surgical operations.

DISCUSSION

Young adults are at high risk of hepatitis B viral infection (especially between the ages of 20 to 40 years). This is consistent with the allowed age range of blood donation, employment, and the childbearing age of the females, the three most common sources of HB cases' detection [6].

Prevalence of hepatitis B infection is common among students on the campus of the university and may be due to its mode of transmission. Risk factors include were unprotected sex, mouth-to-mouth kissing, blood transfusion, reused razor blade cuts, public barbing saloon clipper cuts, manicure and pedicure cuts, needle stick injury, reused needles, syringes and lancets, and scarification

Universal vaccination with the hepatitis B vaccine has been very effective at preventing infection with the hepatitis B virus (HBV) and at reducing the development of chronic infection in young children from perinatal or early childhood exposures to HBV [9].

The reduction of OBI in immunized subjects complements the well-documented universal infant immunization-related benefit of markedly reduced overt HBV infection [10].

However, the prevalence of OBI among vaccinated children/general population was 6.7%. OBI is partly prevalent in vaccinated individuals, especially in those who born to HBsAg positive mothers [11]. Determining the prevalence of HBV infection among students is important in planning for any intervention to control this infection among them [12].

Many studies were conducted on university students to detect the prevalence of active hepatitis B infection but none of them were conducted to estimate the prevalence of OBI.

The current study was the first one conducted to assess the prevalence of occult hepatitis B virus infection in college students on 200 students at Assiut University in upper Egypt in the period between March 2019 and September 2019.

In our study, the mean age of enrolled subjects was 21.1 ± 2.09 years with a range between 17 and 27 years old. The majority of them were males (64.5%). All serologic markers for HBV in our studied subjects came negative for HBs Ag, HBc Ab, and HCV Ab.

On the other hand**,** we found that 3 students had positive HBV DNA by PCR (2 were males and one female) which represent 1.5% of all enrolled students with mean HBV DNA PCR (7101.67 ± 2951.23) IU/L while HBV DNA PCR in OBI -ve was (7.94 ± 2.22) IU/L with very highly significant difference ($p < 0.001$).

There were no statistically significant differences between the mean age in positive HBV DNA (OBI) subjects compared to the mean age of negative ones ($p = 0.133$).

The prevalence of OBI in the present study considered low compared with other study conducted on a similar group of vaccinated children/ adults/ general population conducted by

Alavian and Jazayeri [11]**,** the prevalence of occult hepatitis B infection among vaccinated children/general population was 6.7%.

In a similar study by Utsumi et al. [13] carried out on 222 vaccinated individuals, occult HBV was identified in only 5 (2.3%) individuals. Also, a study by Xu et al. [(14)] conducted on a vaccinated general population with a mean age of 19-21 years showed a 2.7% OBI frequency. Furthermore, similar results are observed in a study by Mu et al. [(15)] of 46 vaccinated children which showed that 5(10.9%) individuals were found to have OBI.

However, a lower prevalence of occult hepatitis B infection in this group of individuals was observed in other studies.

For example, in a study by Meschi et al. [16] reported only one positive case (0.35%) of occult hepatitis B infection of 277 vaccinated individuals.

Also in a similar study by Elrashidy et al. [17] conducted on 170 HB-vaccinated IDDM and healthy children and adolescents, OBI was not detected in any of them (0%).

The discrepancy in the reported incidence of occult HBV between several studies, including our study, could be due to several factors. One could be the differences in sensitivity of the methods used for detection of the virus genome (nested PCR versus quantitative real-time PCR) [18].

All previous studies on university students were conducted to detect the prevalence of infection and associated risk factors using HB surface antigen but none was done to detect OBI among them and so, this study is the first to detect OBI in college students and possible associated risk factors. For example, in a study by Aminu et al. [8] on 200 college students in a Nigerian University, 25 (12.5%) were positive for HB surface antigen.

In a similar study by Komas et al. [7] conducted on 801 university students showed that the overall prevalence of hepatitis B infection was 42.3% for antibody to hepatitis B core antigen and 15.5% for HBsAg, of which 1.3% of HBsAg alone. Additionally, another study in a university in Lome, Togo by Ekouvei et al. [19] conducted on 800 students found that the prevalence of positive HBV surface antigen was 4.6%.

In our study, we found that all study subjects had a negative HBc antibody zero marker including the 3 cases of occult hepatitis B infection (0%). It is reported by Michalak et al. [20] that between 1% and 20% of all OBI cases are seronegative for hepatitis B antibody markers. The possible explanation is that OBI persons have either progressively lost the hepatitis B antibodies (anti-HBc and anti-HBs) or have been hepatitis B antibody-negative since the beginning of the infection.

In the present study, we found that among OBI subjects compared to the negative subjects there was a highly significant difference in the mean of ALT between them ($p < 0.001$).

The level of ALT is significantly correlated with HBV DNA count by PCR with a p-value <0.001. Further assessment of liver function in a larger group study is also needed in future studies to find accurate results.

CONCLUSION

The overall prevalence of occult hepatitis B infection among college students in Assiut University in upper Egypt reached 1.5%.

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