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Prevalence of Overt and Occult Hepatitis B Virus Infection among HIV-Positive People Referring to Consultation Center for Behavioral Diseases, Kurdistan Province, Iran

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ABSTRACT

Background: Based on evidence, HIV and HBV have common transmission routes; co-infection of HBV/HIV can dramatically increase disease progression. The present study aimed to determine the prevalence of overt HBV infection and OBI in HIV-positive people. **Methods:** In this descriptive study, whole blood samples were collected from 184 HIV-positive subjects referring to the Consultation Center for Behavioral Diseases, Sanandaj, Iran, during 2014 to 2016. ELISA was used for the determination of HBV serologic markers (HBsAg and anti-HBc). To evaluate OBI, DNA was extracted only from HBsAg-negative and anti-HBc-positive samples and tested for HBV DNA by real-time PCR. Test results and patients' data were analyzed by SPSS software. **Results:** The mean age of the study population was 39.2 ± 9.4 (SD) years, of whom 140 (76%) were male. Overall, 43 (23.3%) samples were positive for HBsAg (overt HBV infection), and 50 (27.2%) for anti-HBc. Among 31 HBsAg-negative and anti-HBc-positive samples (suspected OBI), one (3.2%) sample was positive for HBV DNA (verified seropositive OBI). HBV infection was higher among males (n = 37; 86.05%), jobless people (n = 23; 53.49%), and those with an injection HIV transmission route (n = 32; 74.43%).**Conclusion:** We observed a high prevalence of overt HBV and one OBI among the study population. A serologic marker such as anti-HBc indicates resolved or past HBV infection. Molecular screening for HBV is valuable for the management of HIV-infected people. ***DOI: 10.52547/ibj.25.6.434***

Keywords: Hepatitis B virus, HBV/HIV co-infection, Human immunodeficiency virus, Occult HBV Infection

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INTRODUCTION

Chronic hepatitis B is a potential health problem worldwide. According to the WHO, more than 350 million people have chronic HBV infection in the world. Around 1.34 million people die each year from complications of chronic HBV infection, including cirrhosis and HCC. The prevalence of HBV varies significantly in different geographical areas[1-3].

Following contact with HBV, acute hepatitis develops, which might be symptomatic or asymptomatic. In non-endemic areas, most HBV infections occur among adults transmitted by sexual route are self-limited. Antibodies develop against HBV core antigen (anti-HBc) and HBsAg (anti-HBs). However, in an endemic area, the most common transmission route is mother to child, and newborns develop chronic hepatitis B, marked by the persistent

List of Abbreviations:
anti-HBc, antibodies to hepatitis B virus core antigen; **anti-HBs,** hepatitis B virus surface antigen; **cccDNA,** covalently closed circular DNA; **HBeAg,** hepatitis B e antigen; **HBsAg,** hepatitis B surface antigen; **HBV,** hepatitis B virus; **HCC,** hepatocellular carcinoma; **HCV,** hepatitis C virus ; **HIV,** human immunodeficiency virus; **OBI,** occult hepatitis B virus infection; **PBMC,** peripheral blood mononuclear cells; **WHO,** World Health Organization

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HBsAg in the serum. Based on serologic markers such as HBeAg, biochemical and histological assessments, chronic HBV infection has four progressive phases: (i) chronic infection with positive HBeAg (immune tolerant), (ii) chronic hepatitis with positive HBeAg (immune clearance), (iii) chronic infection negative HBeAg (immune control or inactive), and (iv) chronic hepatitis negative HBeAg (immune escape). HBsAg-positive people who do not have liver inflammation or even viremia are known as inactive carriers. The fifth group of people infected with HBV enters an HBsAg-negative phase[4].

HBsAg detection in the blood is commonly used for routine diagnosis of HBV infection. Some patients with persistent HBV infection have replicable HBV DNA, cccDNA in the liver or blood without detectable circulation HBsAg, which is called OBI. In most OBI viremia, the quantity of HBV DNA is very low; therefore, the detection of this type of infection requires sensitive methods. Based on the HBV serologic markers, patients with OBI might be seropositive (anti-HBc and/or anti-HBs positive) or seronegative (anti-HBc and anti-HBs negative). In people with seropositive OBI, HBsAg may become undetectable after a few months, either due to the resolution of acute HBV infection or following decades of chronic HBsAg-positive HBV infection (overt HBV). Between 1% and 20% of the patients with OBI are seronegative. In seronegative OBI patients, the HBV antibodies either have lost gradually or have been undetectable since the start of the infection. Under immunosuppression, patients with OBI have a risk for HBV reactivation, as well as for transmission by blood transfusion, hemodialysis, and transplantation. In addition, OBI can exacerbate chronic HBV infection and cause the development of HCC[5-7].

The underlying mechanisms for the development of OBI are attributed to: (i) the host immune responses (humoral and cellular) against HBV, (ii) infection with a mutant virus leading to low replication of HBV, and (iii) little or modified expression of HBsAg due to HBsAg (S) gene mutation (S-promoter, S-escape, and S-splice variants). In addition, epigenetic factors and formation of HBsAg/anti-HBs immune complexes may result in HBsAg detection failure by some HBsAg detection kits. During peripheral mononuclear cells infection, these cells can act as reservoirs for co-infections by other viruses, such as HCV or HIV. In addition, consumption of antiretroviral drugs can interfere with replication of HBV[5,7-11].

Because HIV and HBV share common transmission routes, co-infections of HBV/HIV are common. Therefore, the co-infection can dramatically increase disease progression compared to HBV mono-infection[1,3]. The clinical significance of OBI in HIV-positive people includes hepatic flares, HBV reactivation, rapid progression to liver cirrhosis, and HCC. In addition, the prevalence of OBI/HIV co-infection varies according to the sensitivity of the laboratory test for HBV DNA, HBV prevalence, and antiretroviral therapy[12]. Surveys have reported a high prevalence of OBI in HIV-positive people compared to HIV-negative populations, ranging from 0% to 68%. In HIV-positive people, OBI is often is not diagnosed due to the negative result of HBsAg[1,2,10]. WHO recommends routine screening of all HIV-positive people for HBV and also the initiation of immediate antiretroviral therapy in HIV/HBV co-infected people. The presence of anti-HBc and the absence of HBsAg are used as predictors of OBI in countries where molecular tests are unavailable[10].

The prevalence of OBI among Iranian HIV-infected people has been 3.3% in Tehran[2], 4.6% in Isfahan[9], 58% in Jahrom and Fassa[8]. A systematic review and meta-analysis showed the high occurrence of OBI among Iranian high-risk people. Prevalence of OBI was variable in different regions of Iran, from zero to 63.1% in the HIV-positive people in Fars province[13]. However, knowledge about the prevalence of HBV among HIV-infected people in Iran is less, especially in the Kurdistan province. The aim of the present study was to determine the prevalence of overt and occult HBV infections among HIV-infected people referring to the Consultation Center for Behavioral Diseases in Sanandaj, Kurdistan province, Iran.

MATERIALS AND METHODS

Study population

This descriptive and analytical study was carried out on 184 HIV-positive people referring to Consultation Center for Behavioral Diseases, Sanandaj, Kurdistan province, from 2014 through 2016. In the aforementioned Center, the laboratory tests for HIV were two screening ELISA tests (4th generation ELISA for HIV 1 and 2 Ab-Ag, Pishtazteb, Iran) and a verifying test (HIV Ab and Ag DIA.PRO, Italy). All the participants' data, including age, gender, place of residence, HIV transmission route, occupation, and count of CD4^{+} T cells, were collected.

Specimen collection

Blood samples (~5 ml) were taken from each HIV-positive individual and transferred to sterile EDTA-containing tubes. Plasma was separated from blood samples by centrifugation (at 1600 ×g; 5 minutes) and stored at -20 °C until HBV serologic and molecular tests.

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Serologic tests

HBV serological markers, such as HBsAg and anti-HBc, were detected on all samples using fourth-generation enzyme immunoassay (ELISA) kits. The detection of HBsAg in human serum and plasma was carried out by HBsAg one Version ULTRA, DIA.PRO (Italy), and competitive enzyme immunoassay was used for the detection of anti-HBc in human serum and plasma with a sensitivity of 99.7% (HBcAb, DIA.PRO, Italy). The ELISA tests were conducted according to the manufacturer's instructions.

Deoxyribonucleic acid (DNA) extraction

HBsAg-negative and anti-HBc-positive samples (suspected occult HBV infection) were chosen for molecular methods for viral DNA. DNA was extracted from the plasma samples by DNA extraction kit, (QIAamp® DSP Virus Kit, Qiagen, Germany), according to the instructions provided by the manufacturer. The extracted DNAs were stored at -20 °C.

HBV DNA detection

DNA of HBV in the plasma samples was detected by the Corbett Rotor-Gene 6000 instrument using a real-time PCR kit (artus® HBV RG PCR Kit, Qiagen, Germany), according to the manufacturer's instructions. The HBV RG/TM Master contained reagents and enzymes for the amplification of a 134-bp region of the viral DNA. The real-time PCR was performed in a total volume of 50-µl mixture, containing 30 µl of master mix and 20 µl of DNA template. The limit analytical detection of the artus HBV RG PCR Kit was 0.02 IU/µl.

Statistical analysis

The test results and patients' data were entered into the statistical software (SPSS, version 20) and analyzed. To investigate the relationship between test results and collected data, t-test and Chi-square test of two independent samples and one-way analysis of variance (if normal) were used. If the data did not follow the normal distribution, the nonparametric equivalent was utilized. *p* values less than 0.05 were considered statistically significant.

Ethical statement

The proposal and sampling were approved by Kurdistan University of Medical Sciences, Sanandaj, Iran (ethical code: IR.MUK.REC.1397.107). Written informed consents were provided by all the patients.

RESULTS

Demographic data among HIV-positive people referring to Consultation Center for Behavioral Diseasesis shown in Table 1. The mean age of the people was 39.26 ± 9.47 (SD) with a range of 7 to 65 years. Of the 184 HIV-positive people, 44 (23.9%) were female, and 140 were (76.1%) male. Sanandaj was the main place of residence (131 [71.2%]), and injection was the major HIV transmission route 113 (61.4%) out of the 184 HIV-positive people. Occupations of the people were as jobless (73 [39.7%]), self-employed (61 [33.2%]), and housewives (39 [21.2%]), as represented in Table 1.

Among the 184 samples, 43 (23.37%) were positive for HBsAg, and 141 (76.63%) were negative. Also, of the 184 samples, 50 (27.17%) were positive for anti-HBc, and 134 (72.83%) were negative. HBV serologic markers among HIV-positive samples are shown in Table 2. In total, 74 out of 184 (40.2%) people had HBV serologic markers.

Based on statistical results, there was no significant association between HBsAg and anti-HBc with respect

Table 1. Demographic data of HIV-positive people

Variables	Frequency (%)
Age (mean ± SD; y)	39.26 ± 9.47
Gender	
Women	44 (23.9)
Men	140 (76.1)
Place of residency	
Sanandaj	131 (71.2)
Kamyaran	10 (5.43)
Mariwan	5 (2.71)
Saqqez	15 (8.15)
Qorveh	7 (3.8)
Bijar	8 (4.35)
Baneh	4 (2.17)
Kermanshah	3 (1.63)
Sarvabad	1 (0.54)
HIV transmission route	
Injection	113 (61.4)
Sexual	23 (12.5)
Spouse	39 (21.2)
Mother to child	6 (3.3)
Transfusion	1 (0.5)
Unknown	2 (1.1)
Occupation	
Self employed	61 (33.2)
Jobless	73 (39.7)
Employee	6 (3.2)
Housewives	39 (21.2)
Student	5 (2.7)

Table 2. Serologic profiles of HBV in HIV-positive people

Serologic markers of HBV	HBsAg	HBcAb	Frequency (%)
HBsAg positive only	+	-	24 (13.04)
Both HBsAg and HBcAb positive	+	+	19 (10.31)
HBsAg negative and HBcAb positive	-	+	31 (16.85)
All markers negative	-	-	110 (59.8)
Total			**184 (100)**

to age (Table 3). Among 43 HBsAg-positive people, 6 (14%) were females, and 37 (86%) males, but this difference was not statistically significant (p = 0.102). Among 50 anti-HBc positive people, 5 (10%) were female, and 45 (90%) were male. Based on the Chi-square test, a statistically significant difference was observed in anti-HBc results among women and men (p = 0.004). There was no significant association of HBsAg according to the place of residence (p = 0.292), HIV transmission routes (p = 0.261), and occupation (p = 0.245; Table 3). The Chi-square test showed a significant association in the results of anti-HBc according to HIV transmission routes (p = 0.0001) and occupations (p = 0.011). However, no significant association of anti-HBc was observed, according to the place of residence (p = 0.507; Table 3).

The mean of the $CD4^+$ T cells count in the study population was 475 ± 245 cells/ml, ranged from 18 to 1276. There was no significant relationship between HBsAg and anti-HBc results and $CD4^+$ T cell count (p > 0.05). Among the 184 samples, 31 (16.84%) were HBsAg negative and anti-HBc positive (suspected seropositive OBI). Moreover, 3 (9.7%) were female, and 28 (90.3%) male. Sanandaj was the main place of

Table 3. Relationship between demographic variables and HBsAg and Anti-HBc among HIV positive people referring to Consultation Center for Behavioral Diseases

Variables	HBsAg			Anti-HBc		
	Positive 43 (23.4%)	Negative 141 (76.6%)	*p* value	Positive 50 (27.2%)	Negative 134 (72.8%)	*p* value
Age (mean ± SD) years	39.28 ± 8.36	39.26 ± 9.74	0.989	40.9 ± 8.33	38.65 ± 9.82	0.152
Gender						
Women	6 (13.95)	38 (26.95)		5 (10)	39 (29.10)	
Men	37 (86.05)	103 (73.05)		45 (90)	95 (70.90)	0.004
Place of residency						
Sanandaj	33 (76.74)	98 (69.5)		40 (80)	91 (67.91)	
Kamyaran	1 (2.32)	9 (6.38)		2 (4)	8 (5.97)	
Mariwan	2 (4.65)	3 (2.13)		1 (2)	4 (2.98)	
Saqqez	4 (9.33)	11 (7.80)	0.292	5 (10)	10 (7.46)	0.507
Qorveh	1 (1.32)	6 (4.26)		2 (4)	5 (3.74)	
Bijar	0 (0.00)	8 (5.67)		0 (0)	8 (5.97)	
Baneh	0 (0.00)	4 (2.84)		0 (0)	4 (2.98)	
Kermanshah	1 (2.32)	2 (1.42)		0 (0)	3 (2.24)	
Sarvabad	1 (2.32)	0 (0.00)		0 (0)	1 (0.76)	
HIV transmission route						
Injection	32 (74.43)	81 (57.45)		43 (86)	70 (52.24)	
Sexual	4 (9.30)	19 (13.47)		2 (4)	21 (15.67)	
Spouse	6 (13.95)	33 (23.40)	0.261	4 (8)	35 (26.12)	0.0001
Mother to child	0 (0.00)	6 (4.26)		0 (0)	6 (4.48)	
Transfusion	0 (0.00)	1 (0.71)		0 (0)	1 (0.76)	
Unknown	1 (1.32)	1 (0.71)		1 (2)	1 (0.76)	
Occupation						
Self employed	13 (30.24)	48 (34.04)		17 (34)	44 (32.83)	
Jobless	23 (53.49)	50 (35.46)	0.245	28 (56%)	45 (33.58)	0.011
Employee	1 (2.32)	5 (3.54)		1 (2)	5 (3.74)	
Housewives	6 (13.95)	33 (23.40)		4 (8)	35 (26.12)	
Student	0 (0.00)	5 (3.54)		0 (0)	5 (3.74)	

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residence (25 [80.6%]), and injection was the major HIV transmission route (29 [93.5%]). Occupation of the 31 people was as follows: jobless (15 [48.47%]), self-employed (61 [33.2%]), and 39 housewives ([21.2%]). Of these 31 HBsAg-negative and anti-HBc-positive samples, only one (3.22%) was positive for HBV DNA (confirmed seropositive OBI) by real-time PCR. HBV viral load in this sample was 37.97 IU/ml. The patient with OBI was a 40-year-old man, a farmer, who had been infected with HIV by injection and a $CD4^+$ T cell count of 353 cells/ml. Also, his HCV antibody was positive.

DISCUSSION

Among HIV-infected people in Belgian from 1998 to 2008, 65.8% had overt HBV infection[14]. In another study in Maputo City, Mozambique, East Africa in 2012 among HIV-infected people, 9.1% were HBsAg positive[10]. Another study was performed in Gabon, Central Africa, an area endemic for HIV and HBV. The prevalence of HBsAg was 8.8%[3]. A cross-sectional study was performed on Iranian HIV-infected people. The prevalence of HBsAg alone was 5.2%[2]. Another cross-sectional study evaluated the prevalence of OBI in HIV-infected people in Jahrom and Fassa, Iran; 7.7% of people were positive for HBsAg[8]. In the present study, the prevalence of HBsAg (overt HBV infection) was 23.37%, an evidence of chronic HBV infection.

Among HBsAg-negative people, 45.2% had anti-HBc in HIV-positive people in Mozambique[10]. The prevalence of anti-HBc alone was 16.9% in Iranian HIV-positive people[2]. A cross-sectional study was carried out on HIV-infected people referring to Consultation Center for Behavioral Diseases, Isfahan, Iran, in 2011; 18% were HBsAg negative and anti-HBc positive[9]. Another cross-sectional study evaluated the prevalence of OBI in HIV-infected people in Jahrom and Fassa, Iran, in 2012. In that study, 7.7% of people were positive for HBsAg and 49.5% for anti-HBc. Among HBsAg-negative people, 46.4% had anti-HBc[8]. A study assessed the significance of anti-HBc alone for the prediction of OBI in high-risk and low-risk people in Iran. Of the high-risk people, 10.13% had anti-HBc alone. In addition, 2.07% of blood donors were anti-HBc positive only. The authors conducted that the anti-HBc alone could show OBI in high-risk people, but not in low-risk people[15]. In the present study, 27.17% were positive for anti-HBc, indicating a previous infection; 40.2% of people had HBV serologic markers. There was a significant association of the anti-HBc with gender, occupation, and HIV transmission route. In other words, HBV infection was higher among males, jobless people, and those with an injection HIV transmission route. In a North American cohort, 45 HIV-infected, HBsAg-negative and anti-HBc-positive patients were tested for HBV DNA in plasma and cccDNA in PBMC. Overall, 19 (42%) samples were HBV DNA positive, especially 18 in PBMC, and three samples were cccDNA positive. However, eight (17%) patients had HBV DNA in plasma[12]. In another study, 34.7% had OBI among anti-HBc-positive HBV/HIV co-infected Belgian people[14]. A cross-sectional study was conducted to determine the prevalence of OBI among antiretroviral-naive HIV-infected people in Maputo City, Mozambique, East Africa. OBI prevalence was 8.3% among people with anti-HBc alone[10]. The prevalence of the viral DNA was 69.7% among HBsAg-positive people and 17.5% among HBsAg-negative people in Gabon, Central Africa. HBV DNA was positive in one patient who had negative results for all HBV serologic markers[3].

The prevalence of OBI was 3.3% in HBsAg-negative patients (four in plasma and five in PBMC samples) in Iran[2]. In a study conducted in Iran on 66 HIV-infected people with positive anti-HBc and negative HBsAg, 12.12% of people had OBI. The rate of OBI was 22.2% in men and 0 in women[16]. Viral DNA was detected in 25% samples of HIV-infected people referring to Consultation Center for Behavioral Diseases, Isfahan, Iran, in 2011[9]. In 2012, 63% of HIV-infected people had HBV DNA in Jahrom and Fassa, Iran[8]. Viral DNA was detected in 30% of high-risk people in Iran[15]. In a systematic and meta-analysis study in Iran, the prevalence of OBI was 0.06% among blood donors regardless of anti-HBc result and 7.90% among anti-HBc-positive donors[13]. In the present study, among 31 anti-HBc-positive and HBsAg-negative samples (suspected OBI), only one (3.22%) sample was positive for HBV DNA (confirmed seropositive OBI).
In a survey, 11.8% of people with OBI showed high HBV DNA loads. OBI was not associated with $CD4^+$ T cell count[10]. The viral DNA load was considerably higher in patients with $CD4^+$ cell counts < 200 cells/ml. OBI was found among people with isolated anti-HBc and those with HBsAg seroconversion[3]. In patients with a $CD4^+$ cell count less than 350/mL OBI was 20.1% and in people with $CD4^+$ cell count more than 350/mL OBI was 4.1%. The rate of OBI was 17.94% among IV drug users, and 3.57% in those who were not IV drug users. A significant association was found between OBI and gender[16]. In the present study, the mean of the $CD4^+$ T cells counts in the study population was 475 ± 245 cells/ml, ranged from 18 to 1276. There was no significant relationship between HBsAg and anti-HBc results and $CD4^+$ T cell count.

Prevalence of overt HBV and OBI in HIV-infected

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people observed in this study was different from those reported in the previous studies in Iran[2,8,9,15]. The reason for this discrepancy may be due to the gender of the study population, geographical region, endemicity of HBV, anti-retroviral therapy, type of specimens (serum, plasma, PBMCs, and liver), sample size, and the sensitivity of laboratory tests, as well as whether samples were tested at one or more times.

Studies have shown that the presence of HBV DNA in patients is a risk factor for the progression of liver disease[17]. Diagnosis of OBI is made by the detection of the viral DNA in the blood or liver of people whose serum is negative for HBsAg. HBV DNA detection in the liver is the golden standard for OBI diagnosis, but viral DNA detection in the blood is frequently used. The best methods for the detection of viral DNA in the blood of patients with OBI are nested PCR and real-time PCR. In real-time PCR, the oligonucleotide primers are capable of detecting viral load and genotypes of HBV. In most OBI patients, the HBV viral load was reported to be about 20 IU/ml[11]. Anti-HBc detection in the blood is a representative method for OBI screening in developing countries[7]. In our study, only one sample was positive for viral DNA (confirmed seropositive OBI). Moreover, the prevalence of OBI was low with respect to the analytical detection limit of the kit that we used. Because of budget shortage, we conducted HBV DNA detection only on HBsAg-negative and anti-HBc-positive (not on all) samples. Today, OBI seems to be very rare in HIV-positive people. The reasons for this scant infection may be implemented HBV vaccination in some regions, or the use of antiretroviral therapy in HIV-infected patients, including anti-HBV-active drugs that suppress HBV-DNA at the population level, and the prevention of most HBV transmissions[4]. We had no information about antiretroviral therapy in our population to analyze the effect of the therapy.

HBsAg may become undetectable following decades of HBsAg-positive chronic HBV infection. In addition, co-infections with other viruses, including HCV and HIV, cause the development of OBI[7]. In the present study, the patient with OBI was a 40-year-old man infected with HIV by injection, and his HCV antibody was positive. The condition of the case patient in our study is compatible with facts such as the disappearance of HBsAg decades after chronic HBV infection, common transmission route of three viruses (HBV, HCV, and HIV), and probable effects of HIV and HCV on the development of OBI.

We observed a high prevalence of overt HBV infection and one seropositive OBI among HIV-positive people. OBI can occur in people with serologic markers such as anti-HBc. For the management of HIV-positive people, screening for HBV is recommended.

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CONFLICT OF INTEREST. None declared.

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