



# Prevalence and Risk Factors for Occult Hepatitis B and HIV Infections Among HCV Infected Intravenous Drug Users, Tehran, Iran

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## Abstract

**Background:** Exposure to hepatitis B virus (HBV) or human immunodeficiency virus (HIV) infection is common among hepatitis C virus (HCV)-infected Intra venous drug users (IVDUs); however there exist only a few data about frequency and risk factors of HCV, occult HBV infection (OBI) and HIV among IVDUs.

**Objectives:** This study aimed to investigate the prevalence and associated risk factors for OBI and HIV infections among Iranian HCV infected IVDUs.

**Methods:** Serum samples were screened for the presence of hepatitis C virus antibody (HCVAb), hepatitis B core antibody (HBcAb), hepatitis B surface antigen (HBsAg), and human immunodeficiency virus antibody/antigen (HIVAb/Ag) using enzyme linked immunosorbent assay (ELISA). For detection of OBI, presence of HBV DNA among HBcAb positive/HBsAg negative subjects was determined using nested polymerase chain reaction (PCR).

**Results:** Among all subjects, 94 cases (53.5%) were positive for HCVAb from which 7% and 23.4% were positive for HCV/OBI and HCV/HIV co-infections, respectively. A significant association existed between HCVAb with type of drug, sharing syringes and needles and a history of imprisonment. In the case of HCV/OBI co-infections, the only significant correlation was between sharing a syringe and occult hepatitis B infection. Also no significant association existed between risk factors and HCV/HIV co-infections.

**Conclusions:** the present data documented an alarming prevalence of HCV and HIV as well as co-infections among IVDUs, which emphasizes the requirement for expansion of public health interventions for this at-risk population. Despite previously high prevalence reported for HCV/HBV/HIV triple infections among IVDUs, low prevalence of triple HCV/OBI/HIV infections was obtained. The reason for this difference may be related to the effect of HCV on HBV expression in case of OBI which had been suggested previously.

**Keywords:** Drug User, Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus, Iran

## 1. Background

Sexually active Intravenous drug users (IVDUs) who share needles/syringes are the most important group in the transmission of blood-borne infection diseases (1). Hepatitis C virus (HCV) infection which causes chronic liver disease and cirrhosis, with 85% possibility, is significantly prevalent in IVDUs (2). Hepatitis B infection is another prevalent infection among IVDUs which may damage liver cells and produce persistent liver disease (3). The presence of hepatitis B virus (HBV) DNA by polymerase chain reaction (PCR) in liver and/or in serum of hepatitis B surface antigen (HBsAg) negative patients (occult HBV infection) is frequently recognized in patients with chronic hepatitis C virus (HCV) (4). The consequences of occult HBV

infection (OBI) in patients with chronic liver disease by HCV are still not known (5). There are multiple evidences that co-infection may accelerate the progress to liver disease, being related to the emergence of hepatocellular carcinoma, and may adversely influence the response to HCV treatment. However, other studies have shown that occult infection does not interfere with the natural history of the disorder in this population (6). Also, HIV strengthens the progression of chronic liver diseases which are dependent on HBV or HCV, while liver diseases in relation to viral hepatitis significantly cause morbidity and mortality among HIV infected patients (7, 8). The prevalence of HBV is relatively high in HIV patients (9, 10) and OBI has been detected among 8.7% - 12% of the high risk population in different

studies (11, 12).

It has been described earlier by some researchers that about one third of patients with chronic HCV infection have OBI (4). On the other hand, due to HBV vaccination among infants and high risk population, there has been a decrease in HBV chronic cases in Iran, but hepatitis B viral factors may reflect the consequences of natural infection and OBI cases provided. Exposure to HBV or HIV infection is common among HCV-infected IVDUs; however there are few data about frequency and risk factors of HCV, HIV and occult HBV among IVDUs.

## 2. Objectives

The current study was performed to investigate the prevalence and associated risk factors for OBI and HIV infections among Iranian HCV infected IVDUs.

## 3. Methods

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of the Shahid Beheshti University of Medical Sciences.

### 3.1. Sample Collection

The current cross-sectional study was performed on 173 IVDUs referred to Loghman Hakim Hospital, Tehran, Iran from January, 2013 to January, 2014. Serum samples were collected from all participants who filled a questionnaire. 5 mL blood sample was collected from each participant and serum samples were separated by centrifuge, placed in sterile serum storage vials and finally stored at -70°C.

### 3.2. Risk Factors

In this study, several risk factors were investigated for OBI and HIV infections among HCV infected IVDUs. These risk factors included age, sex, marital status, employment status, type of drug (heroin, crack, methamphetamine, opium, cocaine), duration of drug usage, frequency of injection in a day and history of imprisonment, sharing needle equipment, tattooing, surgery, dentistry, sexual behavior, blood transfusion, and HBV vaccination.

### 3.3. Serological Tests and OBI Detection

All the serum samples were screened for the presence of HBcAb, HBsAg, HCVAb, and HIV Ab/Ag using an enzyme-linked immunosorbent assay (ELISA) commercial kit (Dipro, Milan, Italy). The presence of OBI among studied patients was identified with nested PCR on HBV surface gene by using four separate primers based on a previously described method (13). Each round of this test was performed with positive and negative controls.

### 3.4. Statistical Analysis

Statistical analysis was carried out by using Chi-square test with Statistical Package for Social Sciences (SPSS) version 22. A P value < 0.05 was considered as significant.

## 4. Results

A total of 173 IVDUs were enrolled in the current study. Almost all the participants were male (170, 98.3%) and most of them were unmarried (69.9%) and unemployed (72.8%). The mean age of the studied population was  $40 \pm 5.2$  (18 - 69 years).

Among the 173 IVDUs serum samples, 94 cases (53.5%) were positive for antibodies against HCV. All participants reported several behavioral associated risk factors of blood borne infections such as type of drug, sharing syringes and needles, tattoos, receiving blood transfusions, history of density, and a history of incarceration.

Among 94 IVDUs serum samples with HCVAb, 7 samples (7%) were OBI positive and 22 cases (23.4%) were positive for HIV infection. In this study, no significant association was identified between risk factor and HIV infection.

According to the results, no significant association was observed between HCV, OBI and HIV co-infections. Two patients (0.01%) were positive for triple infection of HBV/HCV/HIV infections.

All HCVAb, OBI and/or HIV positive cases were men with dominating ages which ranged between 30 - 40 years old. The vast majority were unemployed, with a history of imprisonment and multiple sexual partners or high risk sexual behaviors.

## 5. Discussion

IVDUs are still a high risk population who are exposed to different viral pathogens due to their hazardous behaviors such as sharing needles. In this study, prevalence of HCV exposure was seen among 94 (54.3%) cases. Some Iranian studies in different cities have reported various rate of HCV infection among IVDUs. For instance among IVDUs, 64.8% in Bandar Abbas, 34.5% in Tehran (14) and 42.4% in Kohgiluyeh and Boyerahmad (15) had HCVAb. Two meta-analysis from Iran have reported that the pooled HCV prevalence among IVDUs was 51% (34% - 69%) and 45% (37% - 54%) during 1996 - 2012 and 2001 - 2012, respectively (16, 17). The present results analysis demonstrated a significant relationship between imprisonment and sharing syringe with HCV infection ( $P = 0.001, 0.04$ , respectively). These findings are consistent with the findings of earlier

studies in which both of these factors are the most critical hazardous factors associated with HCV infection (14, 16-18). These discoveries underline the need to expand the accessibility of sterile needles through needle exchange programs, including inside and outside prisons to minimize ongoing disease transmission.

In the present study, seven (7%) cases were identified as OBI among HCVAb positive subjects. In a study from Iran on 153 Iranian injecting drug users, no OBI was identified with real time PCR, which is less assuring compared to the nested PCR method utilized in the present study. The results of the present study demonstrated no significant association between the demographic and associated risk factors with the rate of occult hepatitis B infection in participants except sharing a needle ( $P < 0.05$ ). In a few reports from Iran and different nations with regards to imprisonment, the frequency and duration of drug injection were exhibited as risk factors for HBV infection. The results of the present study on demographic and associated risk factors with the rate of occult hepatitis B infection in IVDUs are partly consistent with the study of Lin and colleagues in Taiwan (19), which demonstrated that the prevalence of occult HBV infection positively corresponded to the increase in age of individual subjects. The notable point was that older injecting drug users may have a longer history of drug use, which reflects an effect of cumulative exposure.

In the present study, a 24.5% HIV infection rate was found among HCVAb positive patients. This rate was previously reported in other countries as 15% and 0.15% among IVDUs (7, 20). In one study on 133 injecting drug users (IDUs) attending a voluntary rehabilitation centre in Shahr-e-Kord, only 1 participant (0.8%) was HIV positive (21). On the other hand 18.53% of HCVAb positive IVDUs in Ahvaz were HIVAb positive (22). In one study Iranian researchers found that 78.5% of subjects were positive for HCV/HIV infections (23). In another Iranian study, HCV-HIV co-infections were detected among 5.2% of IVDUs in a central Iranian city (24). No relationship was found between different risk factors and HIV infections in the present study. However, in one study on HIVAb positive patients, low levels of education, a history of imprisonment, and youth were the main risk factors for HCV/HIV co-infection (23). The higher rate of HCV/HIV infections in the present study may show an alarming factor with respect to HCV/HIV transmission in this high risk group.

In this study, the rate of HCV/HBV/HIV infections was 0.01% and is consistent with the prevalence rate among patients who received multiple transfusions (17). On the other hand, this rate is comparable with previous studies from China 7.1% (20) and Iran (6.5% and 36.5%) (23, 25). However, the rate of triple infection was lower than IVDUs infected in Libya (0.02%) (7). One reason for the differences

between our results with other findings could be related to the HBV detection method used in this study, which focused on OBI cases only. On the other hand it could be related to the effect of HCV infection on HBV expression. Yu et al. (26) illustrated that HCV or HCV core protein might directly influence the replication of HBV and could suppress (OBI) or eliminate HBV in the patient. Furthermore, in comparison with the general population, health care workers and street children (17), the rate of triple blood borne infections rate was increased in IVDUs, and this should be taken into consideration by health policy makers.

### 5.1. Conclusions

In conclusion, a high prevalence of HCV and HIV and significant relationship between sharing needle with HCV infection among IVDUs and low prevalence of triple infections of HCV/OBI and HIV was reported in Tehran, the capital city of Iran. The risk of viral blood borne infections will increase with lengthy drug injection among IVDUs; therefore harm reduction interventions in the urban centers and more rural areas should be one of public health priorities. These interventions must address the practice of syringe sharing both inside and outside of prison. One helpful way to reduce the cost of addiction may be to introduce rapid and inexpensive HCV and HIV tests in centers and also to educate positive cases in proper services. Also, expansion services including hepatitis B antibody testing and HBV vaccination for hepatitis B negative IVDUs should be considered.

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### References

1. Hassannejad R, Kassaian N, Ataei B, Adibi P. High risky behaviors among intravenous drug users in Isfahan, Iran: A study for hepatitis C harm reduction programs. *Int J Prev Med.* 2012;3(Suppl 1):S73-8. [PubMed: 22826773]. [PubMed Central: PMC3399296].
2. Samimi-Rad K, Nasiri Toosi M, Masoudi-Nejad A, Najafi A, Rahimnia R, Asgari F, et al. Molecular epidemiology of hepatitis C virus among injection drug users in Iran: a slight change in prevalence of HCV genotypes over time. *Arch Virol.* 2012;157(10):1959-65. doi: 10.1007/s00705-012-1369-9. [PubMed: 22695769].
3. Haussig JM, Nielsen S, Gassowski M, Bremer V, Marcus U, Wenz B, et al. A large proportion of people who inject drugs are susceptible to hepatitis B: Results from a bio-behavioural study in eight German cities. *Int J Infect Dis.* 2018;66:5-13. doi: 10.1016/j.ijid.2017.10.008. [PubMed: 29097248].

4. Makvandi M. Update on occult hepatitis B virus infection. *World J Gastroenterol.* 2016;**22**(39):8720–34. doi: [10.3748/wjg.v22.i39.8720](https://doi.org/10.3748/wjg.v22.i39.8720). [PubMed: [27818588](https://pubmed.ncbi.nlm.nih.gov/27818588/)]. [PubMed Central: [PMC5075547](https://pubmed.ncbi.nlm.nih.gov/PMC5075547/)].
5. Said ZN. An overview of occult hepatitis B virus infection. *World J Gastroenterol.* 2011;**17**(15):1927–38. doi: [10.3748/wjg.v17.i15.1927](https://doi.org/10.3748/wjg.v17.i15.1927). [PubMed: [21528070](https://pubmed.ncbi.nlm.nih.gov/21528070/)]. [PubMed Central: [PMC3082745](https://pubmed.ncbi.nlm.nih.gov/PMC3082745/)].
6. Branco F, Mattos AA, Coral GP, Vanderborgh B, Santos DE, Franca P, et al. Occult hepatitis B virus infection in patients with chronic liver disease due to hepatitis C virus and hepatocellular carcinoma in Brazil. *Arq Gastroenterol.* 2007;**44**(1):58–63. [PubMed: [17639185](https://pubmed.ncbi.nlm.nih.gov/17639185/)].
7. Daw MA, Shabash A, El-Bouzedi A, Dau AA. Association with the Libyan Study Group of Hepatitis and HIV. Seroprevalence of HBV, HCV & HIV co-infection and risk factors analysis in Tripoli-Libya. *PLoS One.* 2014;**9**(6). e98793. doi: [10.1371/journal.pone.0098793](https://doi.org/10.1371/journal.pone.0098793). [PubMed: [24936655](https://pubmed.ncbi.nlm.nih.gov/24936655/)]. [PubMed Central: [PMC4060988](https://pubmed.ncbi.nlm.nih.gov/PMC4060988/)].
8. Ramezani A, Banifazl M, Aghakhani A. Occult hepatitis B infection in chronic hemodialysis patients: Comparison of results and concepts: Occult HBV in chronic hemodialysis patients. *Hepat Mon.* 2011;**11**(2):128–9. [PubMed: [22087131](https://pubmed.ncbi.nlm.nih.gov/22087131/)]. [PubMed Central: [PMC3206674](https://pubmed.ncbi.nlm.nih.gov/PMC3206674/)].
9. Okocha EC, Oguejiofor OC, Odenigbo CU, Okonkwo UC, Asomugha L. Prevalence of hepatitis B surface antigen seropositivity among HIV-infected and non-infected individuals in Nnewi, Nigeria. *Niger Med J.* 2012;**53**(4):249–53. doi: [10.4103/0300-1652.107605](https://doi.org/10.4103/0300-1652.107605). [PubMed: [23661888](https://pubmed.ncbi.nlm.nih.gov/23661888/)]. [PubMed Central: [PMC3640249](https://pubmed.ncbi.nlm.nih.gov/PMC3640249/)].
10. Askari A, Hakimi H, Nasiri Ahmadabadi B, Hassanshahi G, Kazemi Arababadi M. Prevalence of hepatitis B co-infection among HIV positive patients: narrative review article. *Iran J Public Health.* 2014;**43**(6):705–12. [PubMed: [26110141](https://pubmed.ncbi.nlm.nih.gov/26110141/)]. [PubMed Central: [PMC4475589](https://pubmed.ncbi.nlm.nih.gov/PMC4475589/)].
11. Jahanbakhsh A, Farzinepoor Z, Mansouri F, Vaziri S, Afsharian M. Prevalence of occult hepatitis B in HIV positive patients (adolescents and adults) in Kermanshah-Iran. *Int J Pediatr.* 2017;**5**(9):5797–803.
12. Bautista-Amorocho H, Castellanos-Dominguez YZ, Rodriguez-Villamizar LA, Velandia-Cruz SA, Becerra-Pena JA, Farfan-Garcia AE. Epidemiology, risk factors and genotypes of HBV in HIV-infected patients in the northeast region of Colombia: high prevalence of occult hepatitis B and F3 subgenotype dominance. *PLoS One.* 2014;**9**(12). e114272. doi: [10.1371/journal.pone.0114272](https://doi.org/10.1371/journal.pone.0114272). [PubMed: [25462190](https://pubmed.ncbi.nlm.nih.gov/25462190/)]. [PubMed Central: [PMC4252145](https://pubmed.ncbi.nlm.nih.gov/PMC4252145/)].
13. Asli M, Kandelouei T, Rahimyan K, Davoodbeglou F, Vaezjalali M. Characterization of Occult Hepatitis B Infection Among Injecting Drug Users in Tehran, Iran. *Hepat Mon.* 2016;**16**(3). e34763. doi: [10.5812/hepatmon.34763](https://doi.org/10.5812/hepatmon.34763). [PubMed: [27226802](https://pubmed.ncbi.nlm.nih.gov/27226802/)]. [PubMed Central: [PMC4875578](https://pubmed.ncbi.nlm.nih.gov/PMC4875578/)].
14. Amin-Esmaeili M, Rahimi-Movaghar A, Razaghi EM, Baghestani AR, Jafari S. Factors correlated with hepatitis C and B virus infections among injecting drug users in Tehran, IR Iran. *Hepat Mon.* 2012;**12**(1):23–31. doi: [10.5812/kowsar.1735143X.806](https://doi.org/10.5812/kowsar.1735143X.806). [PubMed: [22451840](https://pubmed.ncbi.nlm.nih.gov/22451840/)]. [PubMed Central: [PMC3298872](https://pubmed.ncbi.nlm.nih.gov/PMC3298872/)].
15. Sarkari B, Eilami O, Khosravani A, Sharifi A, Tabatabaee M, Fararouei M. High prevalence of hepatitis C infection among high risk groups in Kohgiluyeh and Boyer-Ahmad Province, Southwest Iran. *Arch Iran Med.* 2012;**15**(5):271–4. [PubMed: [22519374](https://pubmed.ncbi.nlm.nih.gov/22519374/)].
16. Malekinejad M, Navadeh S, Lotfizadeh A, Rahimi-Movaghar A, Amin-Esmaeili M, Noroozi A. High hepatitis C virus prevalence among drug users in Iran: systematic review and meta-analysis of epidemiological evidence (2001–2012). *Int J Infect Dis.* 2015;**40**:116–30. doi: [10.1016/j.ijid.2015.09.022](https://doi.org/10.1016/j.ijid.2015.09.022). [PubMed: [26460088](https://pubmed.ncbi.nlm.nih.gov/26460088/)].
17. Bagheri Amiri F, Mostafavi E, Mirzazadeh A. HIV, HBV and HCV coinfection prevalence in Iran: A systematic review and meta-analysis. *PLoS One.* 2016;**11**(3). e0151946. doi: [10.1371/journal.pone.0151946](https://doi.org/10.1371/journal.pone.0151946). [PubMed: [2703352](https://pubmed.ncbi.nlm.nih.gov/2703352/)]. [PubMed Central: [PMC4816272](https://pubmed.ncbi.nlm.nih.gov/PMC4816272/)].
18. Merat S, Rezvan H, Nouraei M, Abolghasemi H, Jamali R, Amini-Kafiabad S, et al. Seroprevalence and risk factors of hepatitis A virus infection in Iran: a population based study. *Arch Iran Med.* 2010;**13**(2):99–104. [PubMed: [20187662](https://pubmed.ncbi.nlm.nih.gov/20187662/)].
19. Lin CL, Liu CJ, Chen PJ, Lai MY, Chen DS, Kao JH. High prevalence of occult hepatitis B virus infection in Taiwanese intravenous drug users. *J Med Virol.* 2007;**79**(11):1674–8. doi: [10.1002/jmv.20985](https://doi.org/10.1002/jmv.20985). [PubMed: [17854041](https://pubmed.ncbi.nlm.nih.gov/17854041/)].
20. Zhou YH, Yao ZH, Liu FL, Li H, Jiang L, Zhu JW, et al. High prevalence of HIV, HCV, HBV and co-infection and associated risk factors among injecting drug users in Yunnan province, China. *PLoS One.* 2012;**7**(8). e42937. doi: [10.1371/journal.pone.0042937](https://doi.org/10.1371/journal.pone.0042937). [PubMed: [22916185](https://pubmed.ncbi.nlm.nih.gov/22916185/)]. [PubMed Central: [PMC3420897](https://pubmed.ncbi.nlm.nih.gov/PMC3420897/)].
21. Imani R, Karimi A, Rouzbahani R, Rouzbahani A. Seroprevalence of HBV, HCV and HIV infection among intravenous drug users in Shahr-e-Kord, Islamic Republic of Iran. *E Mediterr Health J.* 2008;**14**(5):1136–41.
22. Nikkhooy A, Nikkhooy M, Saadati N. Determination of relative frequency of HBS Ag, HCV and HIV antibodies serum markers among admitted intravenous drug users in infectious disease ward of Razi Hospital in Ahvaz, 2004–2005. *J Health Res.* 2012;**1**(1):26–33.
23. Ataei B, Tayeri K, Kassaian N, Farajzadegan Z, Babak A. Hepatitis B and C among patients infected with human immunodeficiency virus in Isfahan, Iran: seroprevalence and associated factors. *Hepat Mon.* 2010;**10**(3):188–92. [PubMed: [22308138](https://pubmed.ncbi.nlm.nih.gov/22308138/)]. [PubMed Central: [PMC3269083](https://pubmed.ncbi.nlm.nih.gov/PMC3269083/)].
24. Sofian M, Aghakhani A, Banifazl M, Azadmanesh K, Farazi AA, McFarland W, et al. Viral hepatitis and HIV infection among injection drug users in a central Iranian City. *J Addict Med.* 2012;**6**(4):292–6. doi: [10.1097/ADM.0b013e3182659928](https://doi.org/10.1097/ADM.0b013e3182659928). [PubMed: [22895463](https://pubmed.ncbi.nlm.nih.gov/22895463/)].
25. Rahimi-Movaghar A, Razaghi EM, Sahimi-Izadian E, Amin-Esmaeili M. HIV, hepatitis C virus, and hepatitis B virus co-infections among injecting drug users in Tehran, Iran. *Int J Infect Dis.* 2010;**14**(1):e28–33. doi: [10.1016/j.ijid.2009.03.002](https://doi.org/10.1016/j.ijid.2009.03.002). [PubMed: [19464218](https://pubmed.ncbi.nlm.nih.gov/19464218/)].
26. Yu G, Chi X, Wu R, Wang X, Gao X, Kong F, et al. Replication Inhibition of Hepatitis B Virus and Hepatitis C Virus in Co-Infected Patients in Chinese Population. *PLoS One.* 2015;**10**(9). e0139015. doi: [10.1371/journal.pone.0139015](https://doi.org/10.1371/journal.pone.0139015). [PubMed: [26422607](https://pubmed.ncbi.nlm.nih.gov/26422607/)]. [PubMed Central: [PMC4589515](https://pubmed.ncbi.nlm.nih.gov/PMC4589515/)].