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Occult Hepatitis B Infection in Hepatitis C Patients with Hematological Disorders

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Abstract

Background: Occult hepatitis B infection (OBI) is determined by finding hepatitis B virus (HBV) DNA in the liver cells of the patients with negative tests for HBV surface antigen. It is more common in patients with hepatitis C virus (HCV) infection which can be transmitted by blood transfusion and is frequently seen in hemophilia and thalassemia patients.
Objectives: The aim of this study was to assess the prevalence of OBI among Iranian patients with hematological disorders (thalassemia, hemophilia and other coagulation factor deficiencies) infected with chronic hepatitis C (CHC).
Methods: In this descriptive cross-sectional study, all patients with hematological disorders (thalassemia, hemophilia or other coagulation factor deficiencies) who had simultaneous CHC infection and were referred to the Tehran hepatitis center between 2009 and 2010 were enrolled. Occult hepatitis B infection identification was based on serum HBV-DNA tests. Data analysis was performed with SPSS software.
Results: All patients were HBsAg-negative and HCV RNA-positive. Only 145 patients were evaluated for HBV DNA (126 male and 19 female patients). The mean age (SD) was 28.12 (8.6) years. Thirty-five patients had thalassemia, 95 patients had hemophilia, and 15 patients had coagulation factor deficiencies. Serum HBV-DNA was negative for all cases.
Conclusions: Based on our results, it seems that there were no cases of OBI among chronic HCV-infected patients with thalassemia and bleeding disorders, particularly hemophilia. However, to improve decisions concerning OBI screening, especially in transfusion centers, and concerning the use of comprehensive screening methods, more original studies with more precise laboratory techniques and larger sample sizes are needed.

Keywords: Hemophilia, Thalassemia, Hepatitis B, Infection, Blood Coagulation Factors

1. Background

Occult hepatitis B infection (OBI) is detected by finding HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) in patients with negative tests for HBV surface antigen (HBsAg). The gold standard for diagnosis of OBI is liver biopsy and detecting the presence of HBV DNA in hepatocytes (1-3). An alternative method for diagnosis of OBI is detecting HBV DNA in peripheral blood samples. Patients who recover from self-limited acute hepatitis B infection without any clinical or biochemical signs of liver damage may develop OBI (4-6). Occult hepatitis B infection can lead to cirrhosis and fibrosis, and it is also an important risk factor for developing hepatocellular carcinoma in HCV-infected and HCV-negative patients with chronic liver disease (7, 8).

Occult hepatitis B infection is more common in patients with HCV or HIV (9, 10) and in areas with a high prevalence of HBV (11). Its prevalence in patients with CHC varies from 30% to 50% (12-15). OBI has been also reported in hemodialysis patients with CHC (16). It has been reported that the risk of primary liver cancer caused by HCV infection may increase if there is coinfection of OBI and CHC (17). Additionally, cirrhosis (15), elevated serum alanine aminotransferase (ALT) levels, and high histological activity (13, 18) are more common in these patients. These results suggest that OBI with CHC may have synergistic effects in the development of liver disease.

One important mechanism for transmission of HBV and HCV is blood transfusions. Due to this transmission risk, all blood units and their components in blood transfusion services are screened for HBsAg. Patients with OBI can transmit HBV via blood transfusion and organ transplantation (19, 20). Occult hepatitis B infection is spread widely throughout the world and epidemiological, geographical, and ethnic factors, as well as risk factors such as CHC (13), may contribute to the prevalence of OBI (21). It is well established that OBI carriers can be the source of HBV in blood recipients. In other words, it seems that the most likely cause of post-transfusion hepatitis in patients who are on hemodialysis or have hemophilia or thalassemia is OBI (10, 22). Patients with thalassemia and hemophilia are at risk because they receive large volumes of blood and blood components (21). For this reason, some studies have evaluated the prevalence of OBI in hemophilia and thalassemia patients. The prevalence of OBI among hemophilia patients has been reported to the range from zero (23) to 51.2% (24) and in patients with thalassemia from zero (21) to 31.4% (25). Therefore, the prevalence of OBI is high in these patients. In addition, it seems that there is a lack of available information to design plans for diagnosis and treatment of OBI in these patients. As mentioned previously, one of the most important risk factors for the spread of OBI is CHC (13). Furthermore, HCV is transmitted by blood transfusion and is frequently seen in hemophilia and thalassemia patients (26).

2. Objectives

We designed this study to assess the prevalence of OBI among Iranian patients with hematological disorders (thalassemia, hemophilia and some other coagulation factor deficiencies) infected with CHC. We also evaluated the need for OBI screening in blood transfusion centers.

3. Methods

3.1. Study Design, Population and Setting

In this descriptive cross-sectional study, all patients with hematological disorders (thalassemia, hemophilia or other coagulation factor deficiencies) referred to the Tehran Hepatitis Center (TCH) between 2009 and 2010 were enrolled. Positive tests for HCV-RNA and anti-HCV and negative tests for HBsAg and human immunodeficiency virus (HIV) antibody were inclusion criteria.

3.2. Ethics Approval

This study was approved by the Baqiyatallah University of Medical Sciences (BMSU) ethics committee (IR.BMSU.REC.1388.20). Each patient signed an informed consent form when enrolled to the study.

3.3. Data Collection

A questionnaire was completed for each patient that included demographic information (age, gender), past medical history (Hepatitis B infection and other diseases), history of transfusion, and family history of hepatitis and history of vaccination against hepatitis B. Patients who were known to have hepatitis B infection were excluded from the study. Blood samples were sent to one laboratory; hepatitis B viral surface (HBS) Ab, hepatitis B viral core (HBC) Ab, HCV viral load, HCV genotyping and HBV DNA were checked for each patient.

3.4. Serology and Molecular Assessment

HBsAg, anti-HBsAb, HBc Ab were tested with ELISA (Radim SpA, Rome, Italy). OBI identification was based on serum HBV-DNA detection. For detection of HBV-DNA, plasma sample was collected from patients and stored in -80°C. HBV-DNA was extracted from sample using High Pure Viral Nucleic Acid Kit (Roche, Penzeberg, Germany) according to manufacturer's instructions.

The extracted nucleic acid was subjected to (polymerase chain reaction) using HBV FLASH PCR Detection Kit (DNA-Technology, Moscow, Russia) according to manufacturer's instructions.

3.5. Data Analysis

Data were analyzed using SPSS software v.16 for Windows (SPSS Inc., Chicago, IL, USA). The results were reported as the mean $\pm$ standard deviation (SD) for the continuous variables and frequency (percentage) for categorical variables.

4. Results

166 chronic HCV patients were evaluated (Figure 1). All of the patients were HBsAg negative and HCV RNA-positive. Only 145 patients were evaluated for HBV DNA (126 men and 19 women). The mean age was 28.12 $\pm$ 8.6 years. Thirty-five patients had thalassemia, 95 patients had hemophilia, and 15 patients had coagulation factor deficiencies. The most prevalent HCV genotype was type 1a and the mean viral load was 11.71×10^5 copies/mL. Patients are described in Table 1.

The mean age was 24.21 $\pm$ 5.1 years in patients with thalassemia and 29.12 $\pm$ 8.4 in patients with bleeding disorders. Thalassemia patients were more HBV-vaccinated in comparison with patients with bleeding disorders (88.6% vs. 50%, $P < 0.001$). The viral load was higher in patients

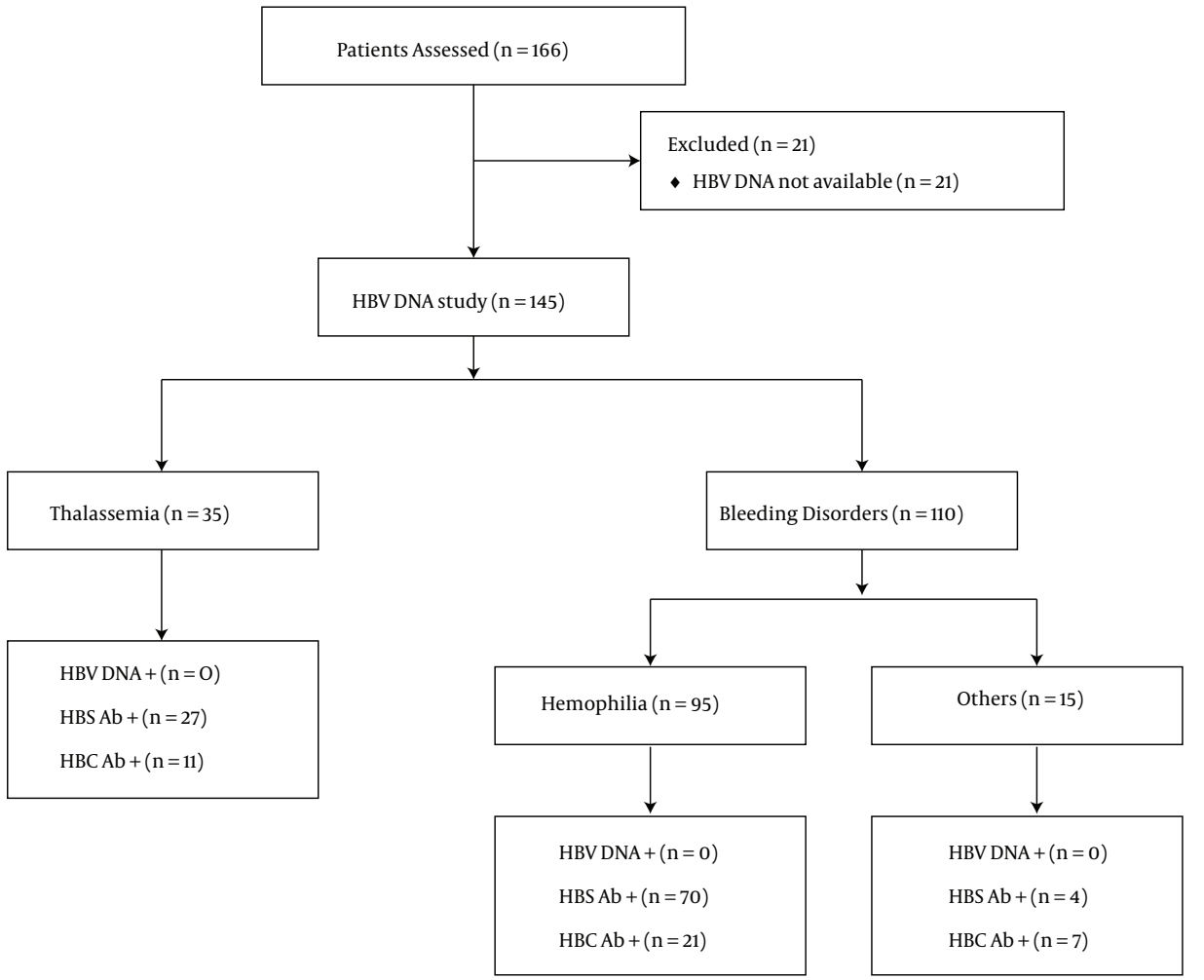

Figure 1. Study Flow Chart

with bleeding disorders (8.3×10^5 vs. 1.28×10^6 copies/mL, P = 0.003). The prevalence of HBS-Ab- and HBC-Ab-positive tests was higher in patients with thalassemia compared with patients with bleeding disorders; this difference was not statistically significant (P > 0.05). Thalassemia patients are described in Table 2 patients with bleeding disorders are described in Table 3.

5. Discussion

To the best of our knowledge, this is the first study that investigated the OBI prevalence rate among patients with hematological disorders (thalassemia, hemophilia and other coagulation factor deficiencies) who were all infected with CHC and were negative for HBs Ag. Because of the particular diagnostic tests for OBI, it is not diagnosed during common screening in blood transfusion centers and can therefore be transmitted via transfusion of infected blood or blood components and induce post transfusion hepatitis (PTH) (27, 28). These conditions should be considered among patients who need permanent transfusions of blood, as in the case of hemophilia, thalassemia and hemodialysis patients (29, 30). In addition, OBI is observed more frequently in patients with CHC infection. Among CHC patients, OBI is considered to be a risk factor for cirrhosis, HCC, and lower survival rates. OBI and HCV can be transmitted via blood (8, 31). Therefore, co-infection of OBI and HCV in thalassemic and hemophilic patients is a very important issue for blood transfusion centers and for their screening methods. Here, based on our results, we did not find any OBI cases in our patients. There are a few studies in the literature that investigate the prevalence

Table 1. Patients' Description

Description	Values[a]
Male Gender	126 (86.9)
Age, y	28.12 ± 7.6
Thalassemia	35 (24.2)
Hemophilia	95 (65.5)
Factors deficiency	15 (10.3)
HCV genotype	
Type 1a	78 (53.8)
Type 1b	9 (6.2)
Type 2a	1 (0.7)
Type 3a	49 (33.8)
Type 4a	2 (4.1)
Blood transfusion	145 (100)

[a]Values are expressed as mean ± SD or No. (%).

Table 2. Description of Patients with Thalassemia

Description	Values[a]
Male Gender	26 (74.3)
Age, y	24.21 ± 5.1
Major Thalassemia	30 (85.7)
Intermediate Thalassemia	5 (14.3)
HBV Vaccination	31 (88.6)
Splenectomy	26 (74.3)
Viral Load, $*10^5$ copy	8.3 ± 7.6
HBS Ab Positive	27 (77.1)
HBC Ab Positive	11 (31.4)
HBV DNA Positive	0 (0)

[a]Values are expressed as mean ± SD or No. (%).

Table 3. Description of Patients with Bleeding Disorders

Description	Values[a]
Male gender	105 (95.5)
Age, y	29.12 ± 8.4
Type A hemophilia	75 (68.2)
Type B hemophilia	20 (18.2)
Other bleeding disorders	15 (13.6)
Von Willberand	5 (4.5)
Deficiency factor XIII	2 (1.8)
Glanzman syndrome	4 (3.6)
Deficiency Factor XII	1 (0.9)
Deficiency Factor Xi, XIII	1 (0.9)
Deficiency Factor II	1 (0.9)
Deficiency Factor X	1 (0.9)
HBV Vaccination	55 (50)
Splenectomy	0 (0)
Viral Load, $*10^6$ copy	1.28 ± 11
HBS Ab Positive	74 (67.3)
HBC Ab Positive	28 (25.8)
HBV DNA Positive	0 (0)

[a]Values are expressed as mean ± SD or No. (%).

of OBI among patients with hematological disorders, such as hemophilia and thalassemia, particularly those infected with HCV (Tables 4 and 5). In our study, we evaluated 15 patients with coagulation factor deficiencies (Table 3). All of these patients also had CHC, but we found no cases of OBI among them. However, patients with coagulation factor deficiencies, especially with CHC, need to be more evaluated in future studies with larger sample sizes.

5.1. Occult Hepatitis B Infection and Hemophilia

In our project, we found no cases of OBI in 35 hemophilia patients infected with HCV. In contrast to our results, Toyoda et al. in 2004 evaluated 43 hemophilia patients and reported OBI in 22 of them (51.2%). It should be considered that a high prevalence of transfusion-transmitted infections had been reported in their patients. However, they did not report clinically significant implications for OBI in these patients (24). In 2004, Borhany et al. showed that the rate of OBI prevalence in patients with hemophilia was 1.73% in Pakistan. However, they found no co-infection between OBI and HCV in these patients (32). On the other hand, and similar to our results, 115 Polish hemophilia patients were investigated for OBI in 2006, and no cases of OBI were found. However, HBV DNA was found in nine subjects who were all positive for HBs Ag, and six were HCV-RNA-positive (23). As one of the advantages of our study, it should be noted that HCV infection was an inclusion criterion for our study sample. However, in these mentioned studies, some or all of their cases did not have HCV infection.

5.2. Occult Hepatitis B Infection and Thalassemia

Thalassemia patients are at risk for co-infection with HBV and HCV (35). In our study, we did not find HBV DNA in the sera of 95 evaluated thalassemia patients. In contrast to our study, Shaker and coworkers evaluated 80 Egyptian thalassemia patients and found an OBI prevalence of 32.5% (26 patients). Twenty cases of their whole study group were

Table 4. Prevalence of OBI, HCV Infection and HBV Serological Marker in Hemophilia Patients

Author	Year	Country	Sample Size	Prevalence, %					
				OBI	HCV Infection	OBI in HCV Infected Patients	Anti HBc	HBS Ag	Anti HBs
Toyoda et al. (24)	2004	Japan	43	51.2	88.37	47.36	86	0	62.8
Borhany et al. (32)	2004	Pakistan	173	1.73	51.4	0	ND a	ND	ND
Windyga, et al. (23)	2006	Poland	115	0	5.21	0	69.5	7.8	49.5

Abbreviations: HBc, Hepatitis B viral core; HBS, Hepatitis B viral surface; HCV, Hepatitis C virus; ND, not determinant; OBI, occult hepatitis B infection.

Table 5. Prevalence of OBI, HCV Infection and HBV Serological Marker in Thalassemia Patients

Author	Year	Country	Sample Size	Prevalence, %					
				OBI	HCV Infection	OBI in HCV Infected Patients	Anti HBc	HBS Ag	Anti HBs
Singh et al. (25)	2003	India	70	31.4	17.1	ND	20	5.7	75.7
Arababadi, et al. (21)	2008	Iran	60	0	45	0	33[a]	0	40.7[a]
Shaker et al. (33)	2012	Egypt	80	32.5	25	100	30	0	2.5
Sabat et al. (34)	2014	India	174	4.5	3.4	ND	21.8	0.5	30.4

Abbreviations: HBc, Hepatitis B viral core; HBS, Hepatitis B viral surface; HCV, Hepatitis C virus; ND, not determinant; OBI, occult hepatitis B infection.
[a] Prevalence of Anti HBc and Anti HBs in this study is just mentioned in HCV infected patients.

also positive for both HCV RNA and HBV DNA (33). Similar to our results, Arababad et al. found that none of 60 evaluated thalassemic patients had OBI. However, they reported that only 27 cases were positive for HCV-RNA and that anti-HBc and anti-HBs were positive in 9 and 11 subjects (out of 27), respectively (21). An Indian study in 2003 showed that the rate of OBI prevalence was 31.4% among 70 thalassemic patents (25). On the other hand, Sabat et al. in 2014 reported that the prevalence of OBI in Indian thalassemic patients was 4.5% (34). However, the rate of HCV prevalence between these two studies was different, and they did not determine the OBI prevalence in HCV-infected patients (Table 5).

5.3. Possible Reasons for Different Reported Prevalence of OBI

Differences in reported prevalence of OBI in different countries can be related to the accuracy and performance of OBI diagnostic tests. The serum level of HBV DNA in OBI patients is very low compared with HBs Ag-positive patients. Therefore, it is said that the HBV DNA test should be conducted three times to reduce the false-negative results of the test and to increase the chances of detecting HBV DNA with PCR. When two of these three tests are positive, OBI is definitively diagnosed. However, due to the fact that the HBV DNA level in the sera of OBI cases is very low, many OBI patients cannot be diagnosed with this method and in these cases, liver biopsy is more helpful (36, 37).

Another factor that may contribute to the different prevalence rates of OBI is that developed countries like Japan have more powerful surveillance systems in their blood transfusion centers and health services. This can lead to more cases of OBI being reported, which may be similar to the prevalence of OBI in hemophilia patients (30). Differences in sample size, geographical area (due to the prevalence of HBV) and diagnostic techniques are other related factors in this issue (37).

There are some unique aspects of our study that may have had an effect on the OBI prevalence rate. First, as we have said, despite other studies in which some of the patients had HCV infection, all of our cases had CHC infection. Second, our study sample was HBs Ag-negative. In other words, we selected those thalassemic and hemophilic patients with CHC infection and negative HBs Ag and evaluated them for OBI. These two important points were necessary for our project goals. A question can be raised here: is it possible that CHC can reduce the rate of identification of OBI in thalassemic and hemophilic patients? We think that using more accurate diagnostic tests, such as liver biopsies, in future studies could be helpful for answering this question.

5.4. Conclusions

In this study, we found no OBI cases in the chronic HCV-infected patients with thalassemia and bleeding disorders, especially hemophilia. However, to make better decisions about OBI screening, particularly in transfusion centers, and about the use of a comprehensive screening method, more original studies with more precise laboratory techniques and larger sample sizes are still needed.

Footnotes

Authors' Contribution: Concept: Nematollah Jonaidi-Jafari, Javad Tavallaei-Nosratabadi, Morteza Izadi, Seyed

Moayed Alavian; data acquisition: Nematollah Jonaidi-Jafari, Javad Tavallaei-Nosratabadi, Morteza Izadi, Reza Ranjbar, Seyed Moayed Alavian; data analysis: Mohammad Saeid Rezaee-Zavareh, Reza Ajudani, Mahdi Ramezani-Binabaj, Seyyed Mohammad Miri, Hamidreza Karimi-Sari; drafting the manuscript: Mohammad Saeid Rezaee-Zavareh, Reza Ajudani, Mahdi Ramezani-Binabaj, Seyyed Mohammad Miri, Hamidreza Karimi-Sari; critical revising of the manuscript: Nematollah Jonaidi-Jafari, Javad Tavallaei-Nosratabadi, Morteza Izadi, Reza Ranjbar, Seyed Moayed Alavian; final approval of the manuscript: all authors.

Conflict of Interest: Authors declare that there is no conflict of interest in this study.

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References

1. Kalantari H, Ferdowsi F, Yaran M. Prevalence of occult hepatitis B virus infection in hemodialysis patients in Isfahan, Iran. *Adv Biomed Res.* 2016;**5**:151. doi: 10.4103/2277-9175.188487. [PubMed: 27713872].
2. Vaezjalali M, Rashidpour S, Rezaee H, Hajibeigi B, Zeidi M, Gachkar L, et al. Hepatitis B viral DNA among HBs antigen negative healthy blood donors. *Hepat Mon.* 2013;**13**(3):ee6590. doi: 10.5812/hepatmon.6590. [PubMed: 23675384].
3. 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):ee34763. doi: 10.5812/hepatmon.34763. [PubMed: 27226802].
4. Sharifi-Mood B, Sanei-Moghaddam E, Ghasem-Zadeh I, Khosravi S. Occult hepatitis B virus infection among anti-HBc only positive individuals in the Southeast of Iran in high prevalence of HBV infection region. *Iran Red Crescent Med J.* 2009;**11**(1):90.
5. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol.* 2008;**49**(4):652–7. doi: 10.1016/j.jhep.2008.07.014. [PubMed: 18715666].
6. Ahmad Akhoundi MS, Momeni N, Norouzi M, Ghalichi L, Shamshiri AR, Alavian SM, et al. Prevalence of blood-borne viruses among Iranian dentists: Results of a national survey. *Int J Occup Med Environ Health.* 2015;**28**(3):593–602. doi: 10.13075/ijomeh.1896.00324. [PubMed: 26190734].
7. Adachi S, Shibuya A, Miura Y, Takeuchi A, Nakazawa T, Saigenji K. Impact of occult hepatitis B virus infection and prior hepatitis B virus infection on development of hepatocellular carcinoma in patients with liver cirrhosis due to hepatitis C virus. *Scand J Gastroenterol.* 2008;**43**(7):849–56. doi: 10.1080/00365520801935459. [PubMed: 18584524].
8. Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, Chen Z. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. *Liver Int.* 2012;**32**(2):231–40. doi: 10.1111/j.1478-3231.2011.02481.x. [PubMed: 21745272].
9. Hu KQ. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat.* 2002;**9**(4):243–57. [PubMed: 12081601].
10. Torbenson M, Thomas DL. Occult hepatitis B. *Lancet Infect Dis.* 2002;**2**(8):479–86. [PubMed: 12150847].
11. Allain JP. Occult hepatitis B virus infection: implications in transfusion. *Vox Sang.* 2004;**86**(2):83–91. doi: 10.1111/j.0042-9007.2004.00406.x. [PubMed: 15023176].
12. Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N Engl J Med.* 1999;**341**(1):22–6. doi: 10.1056/NEJM199907013410104. [PubMed: 10387938].
13. Fukuda R, Ishimura N, Niigaki M, Hamamoto S, Satoh S, Tanaka S, et al. Serologically silent hepatitis B virus coinfection in patients with hepatitis C virus-associated chronic liver disease: clinical and virological significance. *J Med Virol.* 1999;**58**(3):201–7. [PubMed: 10447413].
14. Mariscal LF, Rodriguez-Inigo E, Bartolome J, Castillo I, Ortiz-Movilla N, Navacerrada C, et al. Hepatitis B infection of the liver in chronic hepatitis C without detectable hepatitis B virus DNA in serum. *J Med Virol.* 2004;**73**(2):177–86. doi: 10.1002/jmv.20073. [PubMed: 15122790].
15. Sagnelli E, Coppola N, Scolastico C, Mogavero AR, Filippini P, Piccinino F. HCV genotype and "silent" HBV coinfection: two main risk factors for a more severe liver disease. *J Med Virol.* 2001;**64**(3):350–5. [PubMed: 11424125].
16. Abu El Makarem MA, Abdel Hamid M, Abdel Aleem A, Ali A, Shatat M, Sayed D, et al. Prevalence of occult hepatitis B virus infection in hemodialysis patients from egypt with or without hepatitis C virus infection. *Hepat Mon.* 2012;**12**(4):253–8. doi: 10.5812/hepatmon.665. [PubMed: 22690232].
17. Koike K, Nakamura Y, Kobayashi M, Takada S, Urashima T, Saigo K, et al. Hepatitis B virus DNA integration frequently observed in the hepatocellular carcinoma DNA of hepatitis C virus-infected patients. *Int J Oncol.* 1996;**8**(4):781–4. [PubMed: 21544426].
18. Uchida T, Kaneita Y, Gotoh K, Kanagawa H, Kouyama H, Kawanishi T, et al. Hepatitis C virus is frequently coinfected with serum marker-negative hepatitis B virus: probable replication promotion of the former by the latter as demonstrated by in vitro cotransfection. *J Med Virol.* 1997;**52**(4):399–405. [PubMed: 9260688].
19. Hollinger FB, Sood G. Occult hepatitis B virus infection: a covert operation. *J Viral Hepat.* 2010;**17**(1):1–15. doi: 10.1111/j.1365-2893.2009.01245.x. [PubMed: 20002296].
20. Shouval D. What is the clinical significance of the high prevalence of occult hepatitis B in US liver transplant patients with chronic hepatitis C?. *Liver Transpl.* 2008;**14**(4):418–9. doi: 10.1002/lt.21453. [PubMed: 18383088].
21. Arababadi MK, Hassanshahi G, Yousefi H, Zarandi ER, Moradi M, Mahmoodi M. No detected hepatitis B virus-DNA in thalassemic patients infected by hepatitis C virus in Kerman province of Iran. *Pak J Biol Sci.* 2008;**11**(13):1738–41. [PubMed: 18819629].
22. Plymoth A, Viviani S, Hainaut P. Control of hepatocellular carcinoma through hepatitis B vaccination in areas of high endemicity: perspectives for global liver cancer prevention. *Cancer Lett.* 2009;**286**(1):15–21. doi: 10.1016/j.canlet.2009.08.024. [PubMed: 19836128].
23. Windyga J, Brojer E, Gronowska A, Grabarczyk P, Mikulska K, Szczepanik AB, et al. Preliminary results of HBV DNA testing of Polish haemophilia patients–lack of occult HBV infection. *Haemophilia.* 2006;**12**(4):380–3. doi: 10.1111/j.1365-2516.2006.01293.x. [PubMed: 16834737].
24. Toyoda H, Hayashi K, Murakami Y, Honda T, Katano Y, Nakano I, et al. Prevalence and clinical implications of occult hepatitis B viral infection in hemophilia patients in Japan. *J Med Virol.* 2004;**73**(2):195–9. doi: 10.1002/jmv.20075. [PubMed: 15122792].
25. Singh H, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, et al. High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. *Vox Sang.* 2003;**84**(4):292–9. [PubMed: 12757503].
26. Hesamizadeh K, Sharafi H, Rezaee-Zavareh MS, Behnava B, Alavian SM. Next Steps Toward Eradication of Hepatitis C in the Era of Direct Acting Antivirals. *Hepat Mon.* 2016;**16**(4):ee37089. doi: 10.5812/hepatmon.37089. [PubMed: 27275164].

27. Alizadeh Z, Milani S, Sharifi Z. Occult hepatitis B virus infection among Iranian blood donors: a preliminary study. *Arch Iran Med.* 2014;**17**(2):106–7. [PubMed: 24527970].
28. Keechilot CS, Shenoy V, Kumar A, Biswas L, Vijayrajratnam S, Dinesh K, et al. Detection of occult hepatitis B and window period infection among blood donors by individual donation nucleic acid testing in a tertiary care center in South India. *Pathogens Global Health.* 2016;**110**(7-8):287–91. doi: 10.1080/20477724.2016.1248171.
29. Arababadi MK, Hassanshahi G, Pourfathollah AA, Zarandi ER, Kennedy D. Post-transfusion occult hepatitis B (OBI): a global challenge for blood recipients and health authorities. *Hepat Mon.* 2011;**11**(9):714–8. doi: 10.5812/kowsar.1735143X.694. [PubMed: 22235213].
30. Arababadi MK, Nasiri Ahmadabadi B, Yousefi Daredor H, Kennedy D. Epidemiology of occult hepatitis B infection among thalassemic, hemophilia, and hemodialysis patients. *Hepat Mon.* 2012;**12**(5):315–9. doi: 10.5812/hepatmon.5934. [PubMed: 22783342].
31. Squadrito G, Cacciola I, Alibrandi A, Pollicino T, Raimondo G. Impact of occult hepatitis B virus infection on the outcome of chronic hepatitis C. *J Hepatol.* 2013;**59**(4):696–700. doi: 10.1016/j.jhep.2013.05.043. [PubMed: 23751755].
32. Borhany M, Shamsi T, Boota S, Ali H, Tahir N, Naz A, et al. Transfusion transmitted infections in patients with hemophilia of Karachi, Pakistan. *Clin Appl Thromb Hemost.* 2011;**17**(6):651–5. doi: 10.1177/1076029611398122. [PubMed: 21406412].
33. Shaker O, Ahmed A, Abdel Satar I, El Ahl H, Shousha W, Doss W. Occult hepatitis B in Egyptian thalassemic children. *J Infect Dev Ctries.* 2012;**6**(4):340–6. [PubMed: 22505444].
34. Sabat J, Dwibedi B, Dash L, Kar SK. Occult HBV infection in multi transfused thalassemia patients. *Indian J Pediatr.* 2015;**82**(3):240–4. doi: 10.1007/s12098-014-1490-8. [PubMed: 24928107].
35. Chakrabarti S, Pradhan P, Roy A, Hira M, Bandyopadhyay G, Bhattacharya DK. Prevalence of anti HCV, HBsAg and HIV antibodies in high risk recipients of blood and blood products. *Indian J Public Health.* 2006;**50**(1):43–4. [PubMed: 17193761].
36. Alavian SM. Occult hepatitis B in thalassemia: a need for further study. *J Infect Dev Ctries.* 2012;**6**(8):650–1. doi: 10.3855/jidc.2864. [PubMed: 22910573].
37. Jang JY, Jeong SW, Cheon SR, Lee SH, Kim SG, Cheon YK, et al. Clinical significance of occult hepatitis B virus infection in chronic hepatitis C patients. *Korean J Hepatol.* 2011;**17**(3):206–12. doi: 10.3350/kjhep.2011.17.3.206. [PubMed: 22102387].