

Original Article

The Absence of Hepatitis C Virus Infection Among Patients with Hepatitis B virus in Mashhad, Iran

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Abstract

Background and Aims: Many studies have provided evidence for the role of hepatitis B and C viruses in the development of liver cancer. Although the routine treatment is available for both conditions, no definite guideline is available to treat patients dually infected with HBV and HCV. This study was performed to determine the frequency of HBV/HCV-coinfection in Mashhad, North-East of Iran.

Materials and Methods: In our previous study, 3198 participants were chosen for study of HBV infection from March 2010 to November 2011 in Mashhad, Iran. ELISA method was used to determine the existence of anti-HCV antibody among HBV infected cases.

Results: Of 34 HBsAg positive participants that included equal number of men and women (17 subjects of each gender) with the mean age of 49.9 years, none were positive for anti-HCV antibody.

Conclusion: According to the current study, it could be concluded that the prevalence of HBV/HCV coinfection is low in Mashhad. However, since occult HBV infection may go unnoticed by conventional HBsAg testing, high sensitive molecular techniques are required to investigate the presence of dual infection.

Keywords: Co-infection; hepatitis B virus; hepatitis C virus; Mashhad; Iran

Introduction

Worldwide, a substantial proportion of liver diseases are caused by Hepatitis B virus (HBV) and Hepatitis C virus (HCV). According to The World Health Organization (WHO) figures, an

estimated 170 million and 350 million individuals are chronic carriers of HCV and HBV, respectively (1). Patients coinfectd with hepatitis B virus (HBV) and hepatitis C virus (HCV), similar to HIV/HCV and HIV/HBV coinfectd individuals, are more likely to develop severe hepatic diseases (2-3). Mortality rate is higher among HBV/HCV-coinfectd individuals (7.1%) compared to HCV and HBV monoinfectd individuals with 3.2% and 5.3%, respectively (4). Compared to patients with HBV and HCV coinfection,

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patients monoinfected with HCV are more responsive to IFN therapy (5-6). Occult HBV infection, a condition in which HBV DNA and not HBsAg is detectable, may elevate the risk of hepatic diseases as well as the rate of non-response to conventional treatment of HCV (7). Due to shared routes of transmission, coinfection with HBV and HCV, particularly in endemic areas, is not uncommon (8). Dual infection is not limited to viral hepatitis C and B and clinical conditions may also vary. Senturk et al. concluded that HBV/HCV coinfection may have more serious implications compared to dual infection with HBV and HCV (9). Contradictory results have been reported regarding the extent to which HBV or HCV is dominant among patients with HBV and HCV coinfection; with more findings suggesting the suppression of HBV (10-14). Other *in vitro* findings have confirmed that HCV, through its core protein, brings about suppression of HBV replication (15-17). On the other hand, other studies showed that the dominance can change over the period of infection and some others reported the dominance of HBV (18-19). According to some limited studies, the treatment for HBV/HCV-coinfection is not straightforward and needs to be adjusted for individuals with different response rates (3, 5, 20). At the present time, no specific treatment guideline exists for this group of patients (21). A study by Liu in 2009 suggested that combination therapy with peginterferon alfa-2a and ribavirin is suitable for treating both HCV monoinfected and HBV/HCV coinfecting individuals to the same extent (21). In another study, PEG-IFN- α 2b and ribavirin was found to be effective in suppression of HCV among HBV/HCV-coinfecting individuals (22). The aim of this study was to determine the prevalence rate of HCV infection among patients with HBV infection in the general population of Mashhad.

Methods

According to multi-stage cluster sampling method, from March 2010 to November 2011, 3198 people from the general population of Mashhad were screened for HBV infection and HBsAg detection was determined by ELISA, as described previously (23). The current study was ethically approved by Mashhad University of Medical Sciences. Following obtaining of informed consent, 10 milliliters blood sample was collected from each participant. Thirty four patients positive for HBsAg were then subjected for anti-HCV antibody screening by ELISA method (Delaware Biotech, USA).

Results

In the studied group, patients who were positive for HBsAg included 17 (50%) men and 17(50%) women with the mean age of 47.1 ± 9.7 (range 19-63 years); none of them were found to be positive for HCV antibody by ELISA. Among 34 patients with HBV/HCV co-infection, one patient (2.94%), 26 patients (76.47%), and one patient (2.94%) were positive for HBeAg, HBeAb and HBc-IgM, respectively. HD-Ag was not detected in our infected patients. ELISA results for HBeAg, HBeAb and HBc-IgM in men and women were not statistically significant (P -value=0.999) (Table 1). Also one patient (2.94%) was positive for HBV using PCR method.

Discussion

Up to now, no large-scale studies have pointed out the global prevalence of HBV/HCV-coinfection. In addition to this fact, since occult HBV infection cannot be detected unless by sensitive molecular biology techniques, HBsAg screening, on its own, may not detect all cases of HBV (7, 24-25). Unlike this study which was performed in Iran and found no case of HBV/HCV coinfection, several studies in various regions of the world reported different prevalence rates. To mention some of them, In Taiwan, a prevalence rate of 3.4%, 12% and 18% was reported in 1991,

Table 1. ELISA results for HBeAg, HBeAb and HBc-IgM in HBsAg positive according to the gender.

		HBeAg				
ELISA result Gender	Positive		Negative		Total	
	Number	Percent	Number	Percent	Number	Percent
Female	1	5.9%	16	94.1%	17	100.00%
Male	0	0.0%	17	100.0%	17	100.00%
Total	1	2.9%	33	97.1%	34	100.00%
Statistical Test		Fisher's Exact Test p-Value=0.999				

		HBeAb				
ELISA result Gender	Positive		Negative		Total	
	Number	Percent	Number	Percent	Number	Percent
Female	13	76.5%	4	23.5%	17	100.00%
Male	14	82.4%	3	17.6%	17	100.00%
Total	27	79.4%	7	20.6%	34	100.00%
Statistical Test		Fisher's Exact Test p-Value=0.999				

		HBc-IgM				
ELISA result Gender	Positive		Negative		Total	
	Number	Percent	Number	Percent	Number	Percent
Female	1	5.9%	16	94.1%	17	100.00%
Male	0	0.0%	17	100.0%	17	100.00%
Total	1	2.9%	33	97.1%	34	100.00%
Statistical Test		Fisher's Exact Test p-Value=0.999				

1994 and 2001, respectively (26-28). Studies in Italy in 1991, 1999 and 2003 showed a prevalence rate of 15%, 14% and 7%, respectively (10, 29-30). Sato et al. and Ohkawa et al. reported a prevalence of 22% and 13%, respectively, in Japan in 1994 (11, 31). Two studies in China in 1994 and 1999 which included 193 and 103 individuals demonstrated the prevalence of 11% and 15%, respectively (32-33). Results from Spain, Thailand and India indicated the prevalence of

13%, 2.7% and 3%, respectively (34-36). Semnani et al study on 138 HBsAg-positive subjects in Iran, showed that 17 (12.3%) were positive for anti-HCV antibody (37). In another study in Iran, Cohan et al employed 207 chronic hepatitis C patients and evaluated the degree to which common HBsAg test is successful at detecting infection with HBV. Of the 207 patients, 23 (11.1%) were positive for HBV-DNA. However, only 6 of 23 were HBsAg positive (38). HBV/HCV coinfection

was observed in 21% of patients with injecting drug usage in Tehran, Iran (39). In another study in Tehran which was performed among patients with Human Immunodeficiency virus, the prevalence rate of 36.3% was observed for HBV/HCV coinfection (40). The authors' previous studies among the population of Mashhad showed the HBsAg positivity of 0.53% (23) and the prevalence rate of 0.13% for Hepatitis C virus infection (41). In the current study, none of the patients positive for HBsAg were positive for anti-HCV. Detection of dual infection with HBV and HCV can contribute to its clinical management. Further studies with more sensitive molecular biology techniques to detect all cases of HBV are required to better understand the prevalence of HBV/HCV-co infection in the population of Mashhad, Iran.

References

1. Raimondo G, Cacciato G, Saitta C. Hepatitis B virus and hepatitis C virus co-infection: additive players in chronic liver disease. *Ann Hepatol.* 2005;4:100-6.
2. Sagnelli E, Pasquale G, Coppola N, Scarano F, Marrocco C, Scolastico C, et al. Influence of chronic coinfection with hepatitis B and C virus on liver histology. *Infection.* 2004;32(3):144-8.
3. Weltman M, Brotodihardjo A, Crewe E, Farrell G, Bililus M, Grierson J, et al. Coinfection with hepatitis B and C or B, C and δ viruses results in severe chronic liver disease and responds poorly to interferon- α treatment. *Journal of viral hepatitis.* 1995;2(1):39-45.
4. Schiff ER. Prevention of mortality from hepatitis B and hepatitis C. *The Lancet.* 2006;368(9539):896-7.
5. Villa E, Grottola A, Buttafoco P, Colantoni A, Bagni A, Ferretti I, et al. High doses of α -interferon are required in chronic hepatitis due to coinfection with hepatitis B virus and hepatitis C virus: long term results of a prospective randomized trial. *The American journal of gastroenterology.* 2001;96(10):2973-7.
6. Zignego A, Fontana R, Puliti S, Barbagli S, Monti M, Careccia G, et al. Impaired response to alpha interferon in patients with an inapparent hepatitis B and hepatitis C virus coinfection. *Archives of virology.* 1997;142(3):535-44.
7. 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. *New England Journal of Medicine.* 1999;341(1):22-6.
8. DePaola LG, Carpenter WM. Bloodborne pathogens: current concepts. *Compend Contin Educ Dent.* 2002 Mar;23(3):207-10, 12, 14 passim; quiz 30.
9. Senturk H, Tahan V, Canbakan B, Dane F, Ulger Y, Ozaras R, et al. Clinicopathologic features of dual chronic hepatitis B and C infection: a comparison with single hepatitis B, C and delta infections. *Ann Hepatol.* 2008;7(1):52-8.
10. Fattovich G, Taggar A, Brollo L, Giustina G, Pontisso P, Realdi G, et al. Hepatitis C virus infection in chronic hepatitis B virus carriers. *Journal of Infectious Diseases.* 1991;163(2):400-2.
11. Sato S, Fujiyama S, Tanaka M, Yamasaki K, Kuramoto I, Kawano S, et al. Coinfection of hepatitis C virus in patients with chronic hepatitis B infection. *Journal of hepatology.* 1994;21(2):159-66.
12. Sheen IS, Liaw YF, Chu CM, Pao CC. Role of hepatitis C virus infection in spontaneous hepatitis B surface antigen clearance during chronic hepatitis B virus infection. *Journal of Infectious Diseases.* 1992;165(5):831-4.
13. Chu CM, Yeh CT, Liaw YF. Low-level viremia and intracellular expression of hepatitis B surface antigen (HBsAg) in HBsAg carriers with concurrent hepatitis C virus infection. *Journal of clinical microbiology.* 1998;36(7):2084-6.
14. Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, Galimany R, et al. Role of hepatitis B, C, and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. *Hepatology.* 2001;34(2):404-10.
15. Shih CM, Lo S, Miyamura T, Chen S, Lee Y. Suppression of hepatitis B virus expression and replication by hepatitis C virus core protein in HuH-7 cells. *Journal of virology.* 1993;67(10):5823-32.
16. Schüttler CG, Fiedler N, Schmidt K, Repp R, Gerlich WH, Schaefer S. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein. *Journal of hepatology.* 2002;37(6):855-62.
17. Chen SY, Kao CF, Chen CM, Shih CM, Hsu MJ, Chao CH, et al. Mechanisms for inhibition of hepatitis B virus gene expression and replication by hepatitis C virus core protein. *Journal of Biological Chemistry.* 2003;278(1):591-607.

18. Zarski JP, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, et al. Characteristics of patients with dual infection by hepatitis B and C viruses. *Journal of hepatology*. 1998;28(1):27-33.
19. Pontisso P, Ruvoletto M, Fattovich G, Chemello L, Gallorini A, Ruol A, et al. Clinical and virological profiles in patients with multiple hepatitis virus infections. *Gastroenterology*. 1993;105(5):1529-33.
20. Mazzella G, Saracco G, Festi D, Rosina F, Marchetto S, Jaboli F, et al. Long-term results with interferon therapy in chronic type B hepatitis: a prospective randomized trial. *The American journal of gastroenterology*. 1999;94(8):2246-50.
21. Liu CJ, Chuang WL, Lee CM, Yu ML, Lu SN, Wu SS, et al. Peginterferon alfa-2a plus ribavirin for the treatment of dual chronic infection with hepatitis B and C viruses. *Gastroenterology*. 2009;136(2):496-504. e3.
22. Potthoff A, Wedemeyer H, Boecher WO, Berg T, Zeuzem S, Arnold J, et al. The HEP-NET B/C co-infection trial: A prospective multicenter study to investigate the efficacy of pegylated interferon-[alpha] 2b and ribavirin in patients with HBV/HCV co-infection. *Journal of hepatology*. 2008;49(5):688-94.
23. Shakeri M, Foghanian B, Nomani H, Ghayour-Mobarhan M, Nabavinia M, Rostami S, et al. The Prevalence of Hepatitis B Virus Infection in Mashhad, Iran: A Population-Based Study. *Iran Red Crescent Med J*. 2013;15(3):245-8.
24. Zhang YY, Hansson BG, Kuo LS, Widell A, Nordenfelt E. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the presence of antibodies to HBsAg. *Hepatology*. 1993;17(4):538-44.
25. Squadrito G, Orlando ME, Pollicino T, Raffa G, Restuccia T, Cacciola I, et al. Virological profiles in patients with chronic hepatitis C and overt or occult HBV infection. *The American journal of gastroenterology*. 2002;97(6):1518-23.
26. Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. *Hepatology*. 1995;22(4):1101-8.
27. Chan CY, Lee SD, Wu JC, Hwang SJ, Wang YJ, Huang YS, et al. Superinfection with hepatitis C virus in patients with symptomatic chronic hepatitis B. *Scandinavian journal of infectious diseases*. 1991;23(4):421-4.
28. Dai CY, Yu ML, Chuang WL, Lin ZY, Chen SC, Hsieh MY, et al. Influence of hepatitis C virus on the profiles of patients with chronic hepatitis B virus infection. *Journal of Gastroenterology and Hepatology*. 2001;16(6):636-40.
29. Di Marco V, Iacono OL, Cammà C, Vaccaro A, Giunta M, Martorana G, et al. The long-term course of chronic hepatitis B. *Hepatology*. 1999;30(1):257-64.
30. Gaeta GB, Stornaiuolo G, Precone DF, Lobello S, Chiaramonte M, Stroffolini T, et al. Epidemiological and clinical burden of chronic hepatitis B virus/hepatitis C virus infection. A multicenter Italian study. *Journal of hepatology*. 2003;39(6):1036-41.
31. Ohkawa K, Hayashi N, Yuki N, Hagiwara H, Kato M, Yamamoto K, et al. Hepatitis C virus antibody and hepatitis C virus replication in chronic hepatitis B patients. *Journal of hepatology*. 1994;21(4):509-14.
32. Li W, Zhu Y, Hua Z. [Exploration on the association between the pattern of HBV markers and infection of HCV among population]. *Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi*. 1994;15(4):212-4.
33. Chen X, Xuan M, Wu D. [Study of superinfection of HBV and HCV]. *Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi*. 1999;20(3):141-3.
34. Crespo J, Lozano J, De La Cruz F, Rodrigo L, Rodriguez M, San Miguel G, et al. Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. *The American journal of gastroenterology*. 1994;89(8):1147-51.
35. Xess A, Kumar M, Minz S, Sharma H, Shahi S. Prevalence of hepatitis B and hepatitis C virus coinfection in chronic liver disease. *Indian Journal of Pathology and Microbiology*. 2001;44(3):253-5.
36. Pramoolsinsap C, Sirikulchayanonta V, Busakorn W, Poovorawan Y, Hirsch P, Theamboonlers A, et al. Coinfections with hepatitis g and/or c virus in hepatitis B-related chronic liver disease. *J Trop Med Public Health*. 1999;30:741-9.
37. Semnani S, Roshandel G, Abdolahi N, Besharat S, Keshtkar AA, Joshaghani H, et al. Hepatitis B/C virus co-infection in Iran: a seroepidemiological study. *Turk J Gastroenterol*. 2007;18(1):20-1.
38. Kohan N, Zandieh T, Samiei S, Ataie Z, Kavari M. The prevalence and clinical significance of hepatitis B and C coinfection. *Iranian Journal of Medical Sciences (IJMS)*. 2006;31(3):156-8.
39. 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. *International Journal of Infectious Diseases*. 2010;14(1):e28-e33.
40. SeyedAlinaghi SA, Jam S, Mehrkhani F, Fattahi F, Sabzvari D, Kourorian Z, et al. Hepatitis-C and hepatitis-B co-infections in patients with

human immunodeficiency virus in Tehran, Iran. *Acta Medica Iranica*. 2011;49(4):252-7.

41. Shakeri M, Nomani H, Mobarhan M, Sima H, Gerayli S, Shahbazi S. The Prevalence of Hepatitis

C Virus in Mashhad, Iran: A Population-Based Study. *Hepat Mon*. 2012;13(3):e7723.