

In the name of God



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Occult HBV Infection among Chronic Hepatitis C Patients.

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Abstract:

Transmission routs of both hepatitis B and hepatitis C viruses are similar and infection with both viruses is common. Occult hepatitis B is a new entity in which serum HBsAg is negative but HBVDNA is detectable in serum or liver tissues. In this study the frequency of occult hepatitis B among patients with chronic hepatitis C and also their biochemical and histological changes were investigated.

In this study, 27 patients with chronic hepatitis C with negative serum HBsAg were enrolled. These patients had been referred to Taleghani hospital and RCGLD (Research Center for Gastroenterology and Liver Diseases) or THC (Tehran Hepatitis Center) during years 2001 and 2002 and had been undertaken liver biopsy. Liver biopsies were reviewed and hepatitis B virus (HBV) DNA and HBsAg and HBcAg were assayed in liver tissue by polymerase chain reaction (PCR) and immunohistochemistry (IHC) respectively.

From 27 chronic hepatitis C patients studied, HBVDNA was detectable by PCR in 5(19%). Immunohistochemistry for both HBcAg and HBsAg were reported to be negative in all patients. Histological changes of cirrhosis and symptoms of decompensated cirrhosis were seen just in HBVDNA positive patients.

This study concludes that Occult HBV infection is common among chronic hepatitis C patients. Occult hepatitis B probably accelerates the evolution to cirrhosis in patients with chronic hepatitis C.

Key Words: Chronic hepatitis B, Chronic hepatitis C, Chronic liver diseases, HBVDNA.

Introduction:

Both HBV and HCV could transmit via transfusion or sexual contacts. Infection with both viruses is common especially in areas where viral hepatitis is endemic and among patients with high risk of parenteral infection. HCV infection is diagnosed by specific serum antibodies and serum HCVRNA. HBV infection is diagnosed by positive serum HBsAg. Frequent studies have been confirmed the presence of HBV infection among patients with negative serum HBsAg with or without serological evidences of previous HBV infection (HBcAb or HBsAb).¹⁻⁸

The reasons for lack of HBsAg in serum have not been yet known. But it may be due to changes or rearrangement of viral genome, which interfere with its expression, or due to production of a new S protein. Occult HBV infection has been reported commonly among chronic hepatitis C patients. Significant evidences have been shown that, occult HBV infection could aggravate liver pathology or induced development of hepatocellular carcinoma among this patients.¹⁻⁸

Despite clinical significance of occult hepatitis B, its frequency among chronic hepatitis C patients is unknown yet in Iran. In this study, the frequency of occult hepatitis B in addition to serological and pathological changes among chronic hepatitis C patients was determined.

Materials and Methods:

Patients: 27 patients with chronic hepatitis C (positive serum HCVAb and positive serum HCVRNA) were enrolled in the study. The samples of the study were obtained from laboratory archives of Research Center for Gastroenterology and Liver Diseases

(RCGLD (and Tehran Hepatitis Center) THC (during the years 2001-2002. All of them had negative HBsAg and had been undertaken liver biopsy. The paraffin-embedded liver tissue of these patients were investigated for presence of HLA-DR) humane genome(and HBV-DNA)viral genome(by PCR , and existence of HBs-Ag and HBc-Ag by immunohistochemistry (IHC) technique. All laboratory procedure (PCR and IHC) were performed in Iran Blood Transfusion Organization (IBTO) laboratory with high precision.

Epidemiological data (previous history of acute hepatitis, transfusion, intravenous drug addiction, acupuncture, tattooing, etc), clinical data (patient age, sex, body mass index (body weight in kg/height in meters)), and biochemical parameters, including glucose, triglycerides, cholesterol, and protein electrophoresis were obtained from patient's charts and recorded in questionnaire.

Liver Histopathology: Different histological findings, which were reported, included: fibrosis, portal inflammation, piecemeal necrosis, lobular inflammation, lobular necrosis, steatosis, sinusoidal dilation, and iron deposition. According to these data, liver biopsies were classified into two groups: 1. Chronic hepatitis and cirrhosis according to international criteria⁹; 2. Non-specific/minimal changes: a variety of mild abnormalities including, steatosis, sinusoidal dilation, and mild lobular inflammation or necrosis.

Immunohistochemistry:

Immunoperoxidase staining for HBV surface and core proteins were performed by kits from DAKO company (clone HBc Ag, lot No: 128, antibody concentration: 1/500 and code

No: BO586 and clone HBsAg: 3E7, lot No: 058, antibody concentration: 1/50 and code No: M3506) on all liver specimens. Then samples were interpreted by immunofluorescent microscope.

HBV DNA PCR: DNA was extracted from paraffin-embedded liver tissue using Greer .CE method¹⁰ with some modification .A fundamental safeguard to prevent cross contamination between block preparations have been done. Every extraction and PCR set included: 12 samples, seven patients paraffin-embedded block, three water as negative controls to detect cross contamination and two positive controls to establish sensitivity, corresponding 3000 and 300geq/ml (VQC Panel)

PCR was performed using the following primers:

Primer#1 (nt109-139)

ATACCACAGAGTCTAGACTCGTGGTGGACT.

Primer 2R (nt 555-586)

AAGCCCCTACGAACCACTGAACAAATGGCAC.

Briefly 6µl DNA was amplified for 35 cycle 95°C for 1min, 60°C for 1min and 72°C for 1min following by final extension at 72°C for 10 min in total reaction mixture of 20µl containing 10mM Tris-Hcl PH8.3, 1.5 mM Mgcl₂ (promega) 50mM Kcl 200µM dNTP (Roche) 1.5 U of Taq-polymerase and 0.5 µM each primer. 10µl of reaction mixture were loaded on a 2% agarose gel electrophoresis. All precautions for avoiding contamination were followed stringently. Each positive result was confirmed by a second independent determination. The sensitivity of assay was 300 gem/ml. Occult hepatitis B was defined as detectable HBV DNA in liver tissue.

Statistical Analysis: Fisher's exact test was used to compare proportions and the Student's t test to compare continuous variables. The Mann-Whitney U test was used to compare

non-parametric variables in independent samples. All statistical tests were two tailed. Correlations between the variables were calculated using Spearman rank order correlations. A P value of 0.05 (two-tailed) was considered to indicate significance.

Results:

Patients: 27 patients with chronic hepatitis C who had negative serum HBsAg including 19 men (70%) and 8 women (30%) were studied. The mean age of patients was 32.48 (±17.39). Most of our patients presented with non-specific symptoms (97%). Signs and symptoms of advanced liver diseases or cirrhosis were reported in 3% of patients.

Viral Markers: HBc Ab in 19 and HBs Ab in 21 patients were available. HBc Ab in 44% and HBs Ab in 11% of all patients with negative serum HBs Ag was positive. HBc Ab in 50% and HBs Ab in 25% of HBV DNA positive were reported to be positive. No significant correlation was found between HBV DNA positive in liver tissue and presence of HBc Ab or HBs Ab in serum.

Pathological Findings: According to pathological findings, chronic hepatitis in 25 patients (93%) and cirrhosis in 2 (7%) were reported.

Immunohistochemistry: HBs and HBc proteins were investigated in liver biopsies by immunohistochemistry. All cases were found to be negative.

Prevalence of HBV-DNA: HBVDNA was detectable in 5 of 27 patients with chronic hepatitis C. The patients were divided into two groups: HBVDNA positive and HBVDNA negative. As shown in table 1, no significant difference was found between two groups according to age, sex, clinical symptoms, serum aminoterasferases, and anti HBV

antibodies. Risk factors of viral infection were the same between two groups. Mild and non-specific symptoms were seen just in HBVDNA negative group and signs and symptoms of cirrhosis were reported just in HBVDNA positive group and no significant difference was found between two groups (Table1). Cirrhosis in 40% and chronic hepatitis in 60%

of 5 patients with HBVDNA positive group were found. Chronic hepatitis was more common in HBVDNA negative and cirrhosis was more common in HBVDNA positive group. But probably because of small number of patients studied, no significant difference was found between two groups according to statistical analysis.

Table 1: Clinical, biochemical and histological features of patients with chronic hepatitis C according to the presence of HBVDNA

	HBV DNA + (N=5)	HBV DNA - (N=22)	P Value
Age (Y)	41± 14.37	26.96 ±17.63	0.116
Sex (M/F)	1:4	7:15	1.000 ¹
Clinical presentation			
Decompensated Cirrhosis	1/5 ² (20)	0/22 (0)	0.780
Nonspecific symptoms	4/5(80)	22/22(100)	0.683
Biochemical			
AST	97.6(78.29)	118(87.42)	0.3.4
ALT	152.9(162.31)	157.3 (172.93)	0.356
Serology			
HBc Ab +	2/5(40)	10/20(50)	1.000 ¹
HBs Ab +	1/5(20)	2/20(5.9)	0.488
Pathology			
Cirrhosis	2/5(40)	0/22(0)	0.07
Chronic Hepatitis	3/5(60)	22/22(100)	0.28
Figures in parentheses are percentages. ¹ Fisher's exact test ² Number of positive patients/number of patients studied.			

Discussion

About 19% of our investigated chronic hepatitis C patients had detectable HBV genomes, despite the absence of circulating HBsAg. This frequency was significantly higher than that among HCV-negative patients with chronic liver disease.^{1,3,11-19} HBV co-infection has been reported to be 22% in Austria, 87% in Japan, 49% in Spain and only 5.5% in France. The discrepancy in the reported incidence of HBV DNA in HBsAg negative chronic

hepatitis C patients might be due to differences in the sensitivity of the methods used for detection of viral genome, different quantity of HBV viremia and geographical variation in prevalence of HBV infection.^{1,7,15} In our study no significant difference was found between HBVDNA positive and HBVDNA negative patients according to serum aminotransferases. But higher liver enzymes and more histological activities has

been shown in co-infection of HBV in chronic hepatitis C patients.^{1, 18}

At least 50 percent of our patients with chronic HCV infection had serological markers of previous exposure to HBV. HBcAb in 50% and HBsAb in 25% of patients with positive HBVDNA were reported to be positive. Other study, performed among Israeli patients, demonstrated that 30% of HbsAg negative patients with chronic liver disease had HBV DNA detectable by PCR in serum⁴. In that study, the positive rates of anti-HBc and anti-HBs were about 45%. Similar to the direct detection of HBV DNA, serological markers of past HBV infection are also frequently detectable in HBsAg negative patients with chronic hepatitis C (from 20% to 55%)⁷

Both cirrhotic patients in our study were found in HBVDNA positive group. It has been demonstrated that occult replication of HBV at low levels can accelerate the evolution to cirrhosis in patients with chronic HCV infection.^{1, 13, 18}. Patients with positive HBV DNA had a higher prevalence of cirrhosis and also signs of decompensated cirrhosis more frequently than those with undetectable HBVDNA.^{1, 13} In the other hand, incidence of co-infection of HBV and HCV, in non cirrhotic chronic hepatitis C patients is relatively low.^{1, 3, 13} In one study, the PCR methodology to detect an intact direct repeat region could amplify the CCC HBV DNA but not the incomplete HBV DNA and integrated HBV DNA and showed ongoing occult HBV infection and more advanced liver pathology.⁸ In other study, there was no significant difference regarding inflammatory disease activity or hepatic fibrosis in chronic hepatitis C patients with or without HBV co infections.⁷

Increased frequency of hepatocellular carcinoma and lack of response to interferon therapy have reported in patients with chronic hepatitis C and occult HBV infection.^{1, 3, 14} Genotype 1b has been

reported to be more common in co-infection of HBV and HCV.^{3, 14}

Immunoperoxidase staining for HBV surface and core proteins were negative in all the liver-biopsy specimens examined. These data suggest that occult HBV infection usually is associated with strong suppression of viral replication and gene expression.³

Viral or host factors allowing HBV persistence in the absence of HBsAg includes: viral interference in co-infection with HCV or a new viral agent, HBV mutations in the core promotor region leading to minimal HBV replication and rearrangements or mutations in the HBsAg-encoding region of the viral genome, particularly in the S gene¹⁶. It is also possible that in some cases host immune mechanisms can maintain HBV infection in a latent state until transmission to another individual who subsequently develops a more active infection especially when immunosuppressive therapy is employed.^{1-4, 8, 19, 20}

In conclusion, extensive studies have demonstrated that occult HBV infection represents a special form of HBV infection with clinical relevance. It seems to be common among patients with chronic hepatitis C in Iran and more investigation with larger number of patients is seriously needed in future.

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