The Effect Of Coinfection By Hepatotropic And Hepatomimetic Viruses In Physical Evolution Of Hcv Hepatitis

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Citation

Abstract

The liver is involved in infections by hepatotropic viruses that replicate in the liver and for which the liver is the main target. These include hepatitis A (HAV), B (HBV), C (HCV), D (HDV) and E (HEV) viruses. In all of these infections, hepatitis and liver damage arise as a consequence of the immune response to virus within the liver (1-6). We, retrospectively, studied 75 patients with HCV related chronic liver disease. We concluded that latent infections by hepatomimetic viruses may play a crucial role in the progression of liver disease in HCV-infected patients and may deteriorate the prognosis of those patients in relation with the response to combination therapy. However, further studies of a greater number of cirrhotic and HCC-related HCV patients are needed in order to clarify the role of "silent" CMV and/or EBV infection in HCV patients (75). As occult hepatitis B infection is an important clinical entity, which may progress to severe sequelae, some other yet-to-be determined factors, including hepatomimetic viruses, play a key role to the progression of chronic hepatitis C and its response to combination therapy. In agreement with most studies, environmental, genetic and immunologic determinants are involved in the development of occult hepatitis B, latent CMV and EBV infections and the consequent liver cirrhosis, HCC and the decreased response to combination therapy in HCV-infected patients. These certain risk factors should be included in a long-term follow-up study on HCV patients. Repeated measurements of HBV infection markers (antiHBs, antiHBc, HBV DNA) and PCR for CMV and EBV in combination with periodic health examinations of study subjects may provide useful information on their clinical outcome and identify development and progression of occult hepatitis B, CMV and EBV and its related liver diseases.

INTRODUCTION

The liver is involved in infections by hepatotropic viruses that replicate in the liver and for which the liver is the main target. These include hepatitis A (HAV), B (HBV), C (HCV), D (HDV) and E (HEV) viruses. In all of these infections, hepatitis and liver damage arise as a consequence of the immune response to virus within the liver (1-6).

HBV and HCV infections account for a substantial proportion of chronic liver disease worldwide, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Both are transmitted mainly through blood and by sexual contact (7-9).

HCV is an RNA virus of the Flaviviridae family. Global prevalence of chronic hepatitis C is estimated to average 3% (ranging from 0.1 to 5% in different countries). There are some 175 million chronic HCV carriers throughout the world, of which an estimated 2 million are in the USA and 5 million in Western Europe. The incidence of new symptomatic infections has been estimated to be 1-3 per 100.000 persons annually (6).

HBV is a DNA virus of the Hepadnaviridae family. It contains four opening reading frames: the S gene, coding for the envelope proteins; the core gene coding for the core and "e" proteins; the P gene, coding for a DNA polymerase; and the X gene, coding for a transcriptional transactivator. More than 350 million persons in the world are chronically infected with HBV (10).

HBV infection is usually diagnosed when circulating hepatitis B surface antigen (HBsAg) is identified. However, many studies have shown that HBV infection may occur in HBsAg-negative patients with or without serologic markers of previous infection, anti-HBc and/or anti-Hbs. The reasons

for the lack of circulating HBsAg in such patients are unknown. The so-called occult HBV infection can be defined as the presence of HBV DNA in blood or liver in the absence of detectable HBsAg (11-14). Two common findings and possible explanations for occult HBV are low levels of viral replication activity and/or mutations to the "a" epitope of the S gene that encodes amino acid residues 100-160 of HBsAg (S mutants or variants) (15-17). Although occult HBV infection is more common in individuals with serologic evidence of recovery from previous HBV exposure (anti-HBc and anti-HBs positive), it has also been described in those who are HBV seronegative (anti-HBc and anti-HBs negative). Occult HBV infection has frequently been identified in patients with HCV-related chronic hepatitis.

Considerable data suggest that HBV occult infection may contribute to chronic liver damage and the development of hepatocellular carcinoma (18-21). Despite its potential clinical importance, the prevalence of this occult infection in patients with hepatitis C is still undetermined (22).

The mechanism of HCV persistence and disease progression is multi-factorial with numerous host and viral factors contributing to the outcome of natural infection and antiviral therapy (23, 24). In addition, the liver can be affected as part of a generalized host infection with viruses that primarily target other tissues, particularly the upper respiratory tract. Examples of this phenomenon include the herpes viruses, such as Epstein Barr (EBV) and Cytomegalovirus (CMV). Liver involvement in hepatomimetic viral infections can range from mildly deranged liver biochemistry to fulminant liver failure. In most of these infections hepatitis is thought to be a consequence of an immune response to viral antigens with a close topographic association between the presence of viral antigens and the associated inflammatory infiltrates in the liver (27).

The purpose of our study was to evaluate the possible relation between occult HBV infection and the clinical outcome of liver disease in HCV patients (25-26) and their response to pegylated a-interferon and ribavirin therapy and also to reveal the clinical significance of a latent CMV and/or EBV infection in those HCV patients, the progression of liver disease and the response to the above combination therapy for hepatitis C.

MATERIALS AND METHODS

We, retrospectively, studied 75 patients with HCV related chronic liver disease. All of them had chronic HCV infection with negative HBsAg, positive antiHBc and either positive or negative antiHBs. The diagnosis of HCV infection was verified using the EASL International Consensus criteria (28). The anti-HCV antibodies were eveluated using a third generation immunoenzymatic method and HCV RNA with polymerase chain reaction (RT-PCR>600 IU/ml with HCV Monitoring Cobas Amplicor). All of the above patients had a histological verification of chronic HCV infection as well (29). Thirteen of them had normal levels of aminotransferases, 47 had chronic hepatitis C infection, 11 had HCV-related cirrhosis and 4 had hepatocellular carcinoma. All of our patients had received combination therapy for HCV infection with pegylated a-interferon once weekly plus oral ribavirin in the past. The presence of HCV RNA was determined before the initiation of combination therapy, at the end of therapy and 24 weeks after the therapy was discontinued. All 75 patients underwent liver biopsy before the initiation of treatment. Overall, the median time interval from acquisition of the tested sera to liver biopsy was 2.7 1.6 months. The response to combination therapy was classified into two patterns, according to the serum ALT levels and serum HCV RNA status. Patients who had normal serum ALT levels (< 40 U/L) and in whose serum HCV RNA was undetectable at the end of therapy and during follow-up period were considered to have a sustained viral response (SVR). A non-sustained viral response (non-SVR) was defined as serum ALT levels that could not be normalized either at the end of therapy or during the follow-up period, without clearance of HCV RNA from serum. None of our patients was receiving any special treatment for at least six months before entering the study, and all of them were investigated for the presence of HBV DNA in serum using a sensitive polymerase chain reaction kit (Cobas Amplicor HBV Monitor, Roche; cut off: 200 copies /ml).

The presence of HBV DNA was correlated with epidemiological, clinical, laboratory and histological data, HCV genotype, serological markers of HBV infection and response to therapy with pegylated a-interferon in combination with ribavirin.

EBV-specific antibody testing was used to determine past infection due to EBV. Titers of IgM and IgG antibodies to viral capsid antigen (VCA) were evaluated. None of our patients were positive for IgM anti VCA, which excludes any possibility of acute infectious mononucleosis. However, IgG antibodies to VCA were detected, which is a useful

marker to assess exposure to EBV in the past, because it persists for life.

Detection of CMV-specific IgM was used to exclude any active or recent CMV infection. None of our patients had positive titers of anti-CMV IgM. IgG antibodies to CMV were evaluated in all patients, which is a useful marker to assess exposure to CMV in the past.

None of the patients enrolled in the study were positive for HBsAg, anti-HIV

I-II, direct Coombs reaction and Venereal Disease Research Laboratory (VDRL) reaction. None of our patients had markers suggestive of autoimmune hepatitis including antinuclear antibodies, antimitochondrial antibodies and anti-smooth muscle antibodies. Metabolic liver disease including hemochromatosis,

Wilson's disease or a1-antitrypsin deficiency was excluded by clinical and laboratory data. None reported alcoholism, (alcohol intake of >50g/d), current injection drug abuse or hepatotoxic drug intake.

Serum samples taken from each subject were stored at –70 C until use. The values of AST, ALT, GT, albumin, PLT were detected by automated techniques. HCV genotypes were identified according to the Simmods et al criteria.

Statistical analysis was performed using SPSS version 17.0. Data were analyzed by the chi-square(x2) test in association with Kolmogorov-Smirnov criteria for non-parametric data. A P value of less than 0.05 was considered statistically significant.

RESULTS

From our 75 patients with chronic HCV infection, the sera of 33 (44.00 %) were positive for HBV DNA by the PCR assay, documenting an occult HBV infection. In addition, 2 (10.00%) HCV patients negative for all three serological markers of HBV infection were positive for HBV DNA by our PCR assays.

Twenty (26.66%) patients were negative for all markers, 31 (41.33%) patients were positive for antiHBc alone and 24 (32.00%) patients were positive for both antiHBc and antiHBs. However, the prevalence among the 75 HCV patients with different serological patterns for HBV infection ranged from 10 to 64 % (Table 1). The overall prevalence was significantly higher for those positive for antiHBc alone

than for those negative for all markers. None of the 31 HCV/antiHBc positive patients had a known history of acute or chronic HBV infection.

HBV DNA levels were low (mean 3152 viral copies/ml, range 260-8970 viral copies/ml).

Comparing the mean values of aminotransferases and -GT levels between HCV patients with positive HBV DNA levels and those negative for serum HBV DNA we observed that HCV patients with positive HBV DNA had slightly higher mean values than those with negative serum HBV DNA. In contrast, the difference between the mean values of ALP, -globulins, albumin and platelet level of HCV patients with positive serum HBV DNA and those with negative serum HBV DNA was not statistically significant (30).

The prevalence of occult HBV infection paralleled the severity of chronic liver disease. It was highest in patients with HCC and cirrhosis, but the difference was not significant. The mean age of HCC patients with HCV infection and occult HBV infections was similar to that of patients with HCV infection alone (55.66% Vs 44.21% years). So, there was some correlation between the clinicopathologic status of HCV patients and the negativity or positivity to HBV DNA in their serum, although it was not statistically significant (Table 1) (31).

Among 75 HCV patients genotypes 1, 2, 3 and 4 were found in 38 (50.66%), 11 (14.66%), 11 (14.66%) and 15 (20.00%) patients, respectively.

Overall, there was no significant difference in gender and age distribution, clinicopathologic features, serum markers of liver disease and distribution of HCV genotypes in all 75 HCV patients in relation with the presence or absence of occult HBV infection. However, the biochemical and virological responses to combination therapy with pegylated interferon and ribavirin were associated with better outcome in HCV patients who had negative serum HBV DNA (32-34). Overall, 30.70% of all 75 HCV patients had SVR and negative serum HBV DNA, in contrast to 25.30% of total that had a non-SVR or relapse. Twelve % of all 75 patients with positive serum HBV DNA had SVR, while 32.00% with positive serum HBVDNA had a non-SVR or relapse.

The prevalence of latent CMV or EBV infection paralleled the severity of liver disease of HCV patients. It was higher among patients with cirrhosis and HCC, and lower among

patients with normal aminotransferases. However, the difference was not statistically significant (Table 2).

Taking into account the presence or absence of hepatomimetic viruses in those 75 HCV patients and their response to combination therapy for HCV infection we can reveal the following results: 17 (22.70%) of our 75 HCV patients with positive serum anti-CMV IgG had SVR to HCV therapy, while 38 (50.70%) of total had a non-SVR or relapse. Simultaneously, 15 (20.00%) of all 75 HCV patients with positive serum anti-VCA IgG had SVR to combination therapy for HCV, while 36 (48.00%) of total had a non-SVR or relapse.

In conclusion, latent CMV and/or EBV infection may deteriorate the prognosis of HCV-infected patients. It is worthwhile to mention that all HCV patients with cirrhosis or HCC expressed a latent CMV and EBV infection.

Figure 1

Table 1. Prevalence of HBV DNA in sera of 75 HCV patients by serological patterns and liver status

Group and serological pattern or liver status	No. of individuals studied	No. of individuals positive for HBVDNA	% of individuals positive for HBVDNA
HCV - infected patients	75	33	44.00
Negative for antiHBC and antiHBS	20	2	10.00
Positive for antiHBC	31	20	64.51
Positive for antiHBC and antiHBS	24	11	45.83
HCV - infected patients	75	33	44.00
0 = "CHRONIC HEPATITIS"	47	19	40.43
1 = "CIRRHOSIS"	11	8	72.73
2 = "NORMAL TRANSAMINASES"	13	3	23.08
3 = "HEPATOCELLULAR CARCINOMA (HKK)"	4	3	75.00
HCV - infected patients	75	33	44.00
Positive for antiCMV IgG	55	28	50.91
Positive for antiVCA IgG	51	28	54.90
Positive for antiCMV IgG and antiVCA IgG	49	27	55.10

Figure 2

Table 2. Clinicopathologic data and response to pegylated a-interferon plus ribavirin therapy in 75 HCV patients with 'silent' HBV, CMV, EBV infections

CHARACTERISTIC	HBVDNA POSITIVE	HBVDNA NEGATIVE	antiCMVIgG POSITIVE	antiCMVIgG NEGATIVE	antiVCAIgG POSITIVE	antiVCAIgG NEGATIVE
No of patients	33	42	55	20	51	24
GENDER						
0 = "MALE"	18	21	34	5	31	8
1 = "FEMALE"	15	21	21	15	20	16
AGE	19 - 62	28 - 70	19 - 70	32 - 51	19 - 70	28 - 61
SURGERY No (%)						
0 = "NO"	6 (18.18%)	15 (35.71%)	14 (25.45%)	7 (35.00%)	12 (23.53%)	9 (37.50%)
1 = "YES"	27 (81.82%)	27 (64.29%)	41 (74.55%)	13 (65.00%)	39 (76.47%)	15 (62.50%)
Peak ALT level	21 - 211	12 - 178	12 - 178	21 - 211	12 - 178	21 - 211
CLINICAL_STAGE No (%)						
0 = "CHRONIC HEPATITIS"	19 (57.58%)	28 (66.67%)	37 (67.27%)	10 (50.00%)	33 (64.71%)	14 (58.33%)
1 = "CIRRHOSIS"	8 (24.24%)	3 (7.14%)	11 (20.00%)	0	10 (19.61%)	1 (4.17%)
2 = "NORMAL TRANSAMINASES"	3 (9.09%)	10 (23.81%)	3 (5.45%)	10 (50.00%)	4 (7.84%)	9 (37.50%)
3 = "HEPATOCELLULAR CARCINOMA (HKK)"	3 (9.09%)	1 (2.38%)	4 (7.27%)	0	4 (7.84%)	0
GONOTYPOS No (%)						
1	20 (60.61%)	18 (42.86%)	29 (52.73%)	9 (45.00%)	27 (52.94%)	11 (45.83%)
2	3 (9.09%)	8 (19.05%)	7 (12.73%)	4 (20.00%)	5 (9.80%)	6 (25.00%)
3	5 (15.15%)	6 (14.29%)	8 (14.55%)	3 (15.00%)	7 (13.73%)	4 (16.67%)
4	5 (15.15%)	10 (23.81%)	11 (20.00%)	4 (20.00%)	12 (23.53%)	3 (12.50%)
RESPONSE No (%)						
0 = "RELAPSE"	24 (72.73%)	19 (45.24%)	38 (69.09%)	5 (25.00%)	36 (70.59%)	7 (29.17%)
1 = "SUSTAINABLE VIROLOGICAL RESPONSE (SVR)"	9 (27.27%)	23 (54.76%)	17 (30.91%)	15 (75.00%)	15 (29.41%)	17 (70.83%)

DISCUSSION

HBV infection is diagnosed when circulating HBsAg is detected. However, we found that a unique persistent infection known as occult HBV infection, which is characterized by positivity for serum HBV DNA by using sensitive PCR assays, has been identified in HBsAg-negative patients with or without serological markers of HBV infection (anti-HBc or anti-HBs) (35-37). In our study we found that 33 (44.00%) of the patients with HCV-related chronic hepatitis had detectable HBV DNA, despite the absence of circulating HBsAg. The prevalence of occult HBV infection was particularly high among patients with anti-HBV antibodies. Occult HBV infection was also detected in only 2 (10.00 %) patients who were negative for all HBV serum markers.

Although occult hepatitis B has been well documented since 1978, it has not been well studied until PCR became available (38-40). The reasons for the disappearance of HBsAg and, in some cases, of all HBV markers despite the persistence of HBV infection are not known. The molecular and immunological mechanisms underlying occult hepatitis B and the inhibition of HBV activity remain undefined. Several hypotheses have been proposed for the occurrence of occult HBV infection. They included the mutation or rearrangements of the viral genome (surface, core and X

genes) the integration of HBV DNA into host genomes, which may be responsible for the failure to detect HBsAg (17), the HBV infection of peripheral blood mononuclear cells (41), the formation of circulating immune complex containing HBV, the altered host immune response to HBV (42-44), and the superinfection and interference of HBV by other viruses, e.g. HCV, CMV, EBV, HSV.

This occult HBV infection can be found in patients with chronic HCV infection at various frequencies (10.00 to 64.51%). Nevertheless, by using highly sensitive PCR assays, we found very low levels of viremia in patients with occult HBV infection. Serum HBV DNA was detected in 33 (44.00%) of the 75 HCV patients. The high prevalence of the occult HBV infection in such patients has been suggested to have clinical implications in the pathogenesis of HCV-induced chronic liver disease (45-48).

The relationship between occult HBV infection and serological markers of HBV infection showed that the prevalence of occult HBV infection in HCV patients was usually higher in subjects positive for either anti-HBc alone or for both anti-HBs and anti-HBc than in those negative for all serological markers (64.51 % vs 45.83 %) (49,50). These data were not statistically significant (Table 1). The possibility of persistent HBV infection in anti-HBc-positive patients has been supported by many studies showing that traces of HBV are often detectable in the blood for many years after clinical recovery from acute hepatitis, despite the presence of antibodies against HBV-specific cytotoxic T-lymphocytes in serum (51).

The clinical significance of occult HBV infection alone or in combination with HCV infection remains unsettled. Many epidemiological and molecular studies indicate that persistent HBV infection may have a critical role in the development of HCC in HBsAg-negative patients (52-57). This hypothesis is supported by studies showing that both woodchucks and ground squirrels that have once been infected by woodchuck hepatitis virus and ground squirrel hepatitis virus, respectively, are at high risk for HCC even after the apparent clearance of the virus (58, 59). Occult HBV and its potential oncogenicity are traditionally considered a consequence of the capacity of the virus to be integrated into the host genome, although many observations show that free episomal HBV genomes may persist in the liver cells during occult infection (60).

Our study demonstrates that occult HBV infection is

correlated with cirrhosis among HCV-infected patients. This suggests that a masked HBV infection may interfere with the clinical outcome of chronic hepatitis C and favor or accelerate the evolution to cirrhosis. Cirrhosis is generally considered the most important risk factor for the development of HCC. Thus, in addition to its possible direct oncogenic properties, occult HBV infection may favor neoplastic transformation in HCV-infected patients through its contribution to cirrhosis. However, the mean age of cirrhosis and /or HCC patients with HCV and occult HBV coinfection was comparable to that of patients with HCV infection alone. In addition, among patients with chronic hepatitis C, the demographic, clinical, histological and virological features were comparable between those with and those without occult HBV infection (Table 2). Suggestively, our observations showed that occult HBV infection has some influence on the clinicopathologic course of chronic HCV infection and in the promotion of HCV replication (61,62), although it was not statistically significant.

Occult HBV infection has been suggested to correlate with a lack of response to interferon therapy in patients with chronic hepatitis C (63-65). However, its impact on the response to combination therapy remains unknown (66-69). Our results showed that the sustained rates of response to combined pegylated alfa-interferon and ribavirin therapy were lower in chronic HCV patients with occult HBV infection and thus, low level HBV infection does interfere with the response to combination therapy against HCV (table 2).

It is common knowledge that HBV particles may persist for decades after self-limited acute hepatitis and clinical recovery. In addition, infections by either CMV or EBV are ranging from an asymptomatic subclinical infection to a mononucleosis syndrome in healthy individuals, and to disseminated disease in immunocompromised patients. Primary infection by hepatomimetic viruses is often associated with a vigorous T lymphocyte response. The hallmark of such infection is the appearance of atypical lymphocytes in the peripheral blood; these cells are predominantly activated CD8+ T lymphocytes. Once infected, an individual probably carries CMV or EBV for life. The infection usually remains latent. The sites of persistent or latent infection probably include multiple cell types and various organs. Data suggest that memory B-cells, not epithelial cells, are the reservoir for EBV in the body

(70). Autopsy studies suggest that salivary glands, bowel and liver may be areas of latent infection (71-77). However, CMV or EBV reactivation syndromes develop frequently when T lymphocyte-mediated immunity is compromised e.g. after organ transplantation, in association with lymphoid neoplasms and certain acquired immunodeficiencies, such as HIV infection and liver cirrhosis. CMV and EBV may contribute to further T lymphocyte hyporesponsiveness. Thus, according to some reports, occult infection may not have serious clinical consequences and may become injurious only when the virus is reactivated after immunosuppression.

However, the high prevalence of latent CMV and EBV infection in HCV patients probably suggest having clinical implications in the pathogenesis of HCV-induced chronic liver disease. Such evidence might lead to speculation about a possible pathogenic role of HBV, CMV and/or EBV in liver injury, despite the suppression of its activity.

Our study suggests that occult CMV and/or EBV infection has some influence on the clinicopathologic course of chronic HCV infection. In addition, occult CMV and/or EBV infection is suggested to correlate with a worse response to combination therapy for chronic hepatitis C (table 2).

CMV virus appears to replicate in a variety of cell types in vivo; in tissue culture it grows preferentially in fibroblasts. Although there is little evidence that CMV is oncogenic in vivo, the virus does transform fibroblasts in some instances, and genomic transforming fragments have been identified. In our study, there was a well-defined correlation between cirrhosis and hepatocellular carcinoma in HCV patients with latent CMV infection. Exactly the same observation was made when EBV latent infection was detected in HCV patients.

In conclusion, latent infections by hepatomimetic viruses may play a crucial role in the progression of liver disease in HCV-infected patients and may deteriorate the prognosis of those patients in relation with the response to combination therapy. However, further studies of a greater number of cirrhotic and HCC-related HCV patients are needed in order to clarify the role of "silent" CMV and/or EBV infection in HCV patients (75).

As occult hepatitis B infection is an imp009ortant clinical entity, which may progress to severe sequelae, some other yet-to-be determined factors, including hepatomimetic viruses, play a

key role to the progression of chronic hepatitis C and its response to combination therapy. In agreement with most studies, environmental, genetic and immunologic determinants are involved in the development of occult hepatitis B, latent CMV and EBV infections and the consequent liver cirrhosis, HCC and the decreased response to combination therapy in HCV-infected patients. These certain risk factors should be included in a long-term follow-up study on HCV patients. Repeated measurements of HBV infection markers (antiHBs, antiHBc, HBV DNA) and PCR for CMV and EBV in combination with periodic health examinations of study subjects may provide useful information on their clinical outcome and identify development and progression of occult hepatitis B, CMV and EBV and its related liver diseases.

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