OVERT AND HIDDEN COINFECTION WITH HEPATITIS B AND C VIRUSES IN CHRONIC LIVER DISEASE AND PORPHYRIA CUTANEA TARDA

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Summary. – The aim of this study was to assess the rate of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection („the coinfection") in chronic liver disease (CLD) and to reveal overt and hidden HBV infection in patients with antibodies to HCV (anti-HCV). A total of 209 untreated patients (64 with chronic hepatitis B, 79 with chronic hepatitis C and 66 with porphyria cutanea tarda (PCT)) were screened for serological markers of HBV and HCV infection in serum by third generation enzyme-linked immunosorbent assay (ELISA) methods and for HBV DNA and HCV RNA in serum by polymerase chain reaction (PCR). The rate of the overt coinfection in chronic hepatitis B was very low (2/64, 3%). However, in chronic hepatitis C, the rate of the hidden coinfection with HBV was relatively high (19/79, 24%); these patients had higher alanine transaminase (ALT) and asparagine transaminase (AST) levels in serum and a more advanced liver disease. In PCT patients, the rates of HBV and HCV infections were the same, 21% (14/66). In the PCT patients infected with HBV or HCV, the rate of the coinfection was 33% (7/21). The PCT patients with the coinfection had a high serum ALT level and the worst histological picture in the liver. The hidden HBV infection was more frequent than the overt one. The possibility of the overt or hidden coinfection in CLD renders a detailed analysis of all serum samples for both viruses mandatory. Vaccination against HBV infection should be offered to anti-HCV-positive individuals as well as to PCT patients not showing antibodies to HBV (anti-HBV).

Key words: hepatitis B virus; hepatitis C virus; antibodies; antigens; coinfection; porphyria cutanea tarda; chronic liver disease; vaccination

Introduction

HBV and HCV infections are the most common cause of CLD. In some patients with acute or chronic hepatitis B or C, the coinfection was observed. It seems that patients with concurrent chronic coinfection have a more severe liver disease, but this assumption has not always been confirmed in all patients and clinical consequences of the coinfection have not yet been fully clarified (Alberti *et al.*, 1995).

In the past ten years, HBV infection in Czech Republic was more frequently involved in the development of CLD than HCV infection (38% vs. 23%), and the anti-HCV reactivity was detected by ELISA-2 in liver cirrhosis in 27% and in chronic non-A, non-B hepatitis in 57%. Our previous results suggested that the coinfection was present in liver cirrhosis in 9% and in chronic HBsAg-negative hepatitis in 27% (Stránský *et al.*, 1997).

As concerns PCT, the rate of HBV infection in Italy is very high (about 70%), but HBsAg in serum is positive in 17% only (Rocchi *et al.*, 1986; Fargion *et al.*, 1992). The rate of HCV infection in Spain is also very high (79% in

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Abbreviations: ALT = alanine transaminase; AST = asparagine transaminase; anti-HBc = antibodies to HBc antigen; anti-HBe = antibodies to HBe antigen; anti-HBs = antibodies to HBs antigen; anti-HBV = antibodies to HBV; anti-HCV = antibodies to HCV; CLD = chronic liver disease; ELISA = enzyme-linked immunosorbent assay; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; PCR = polymerase chain reaction; PCT = porphyria cutanea tarda; URO-D = uroporphyrinogen decarboxylase

Table 1. Basic characteristic of patients with CLD

Group of patients	N	M	F	M/F Ratio	Mean age (years)	HBsAg-positive	HBeAg-positive	Liver biopsy[a]
Chronic hepatitis B	64	44	20	2.2:1	47 (16–82)	64	28	38
Chronic hepatitis C	79	41	38	1.1:1	49 (16–78)	0	0	30
PCT	66	51	15	3.4:1	59 (27–78)	0	0	56

[a]Number of patients with liver biopsy.

patients with sporadic PCT), and similar serological results were observed in Mediterranean Sea countries (Herrero *et al.*, 1993; De Castro *et al.*, 1993; Navas *et al.*, 1995). In Czech Republic, the incidence of HBV and HCV infections in chronic PCT patients is the same (21%), which is twice as much as in Germany or Great Britain (Malina *et al.*, 1998).

The aim of this study was to assess the rate of the coinfection in patients with CLD including PCT and to reveal hidden HBV infection in anti-HCV-positive patients with CLD.

Materials and Methods

Patients. In 1995–1997, a total of 209 untreated patients suffering from chronic hepatitis B or C and PCT were screened for serological markers of HBV and HCV infection: 64 patients with chronic hepatitis B (of whom 28 were HBeAg-positive), 79 patients with anti-HCV-positive chronic hepatitis, and 66 patients with PCT. The HBsAg and anti-HCV positivity in serum was present for more than 6 months in all the patients. Male to female ratio, mean age, HBV serology at presentation, and number of patients with liver biopsy is given in Table 1. All these patients were studied on the basis of a long-term follow-up.

Serum transaminases. ALT and AST, were assayed in all patients by standard procedures (normal values of ALT and AST were below 0.75 μkat/l). Ultrasonography of the liver was performed in all patients.

Liver biopsy specimens were examined by an experienced histopatologist. Chronic hepatitis was evaluated using the Knodell's histological activity index.

Serological analysis. Serological markers of HBV infection (HBsAg, HBeAg, anti-HBe, anti-HBc, and anti-HBs) were assayed by standard ELISA (Sanofi Diagnostics Pasteur, France). Anti-HCV were tested by a third generation ELISA (HCV+Sanofi Diagnostics Pasteur).

HCV RNA and HBV DNA in serum were assayed by Amplicor Set (Roche) and HBV Primer and Probe Capture Set (Boehringer Mannheim), respectively, according to the instructions of the manufacturers.

Statistical evaluation of differences between groups was done using the paired *t*-test.

Results

Chronic hepatitis B

HBeAg was present in 44% (28/64) of patients. Clinical and histologically proven diagnosis of chronic hepatitis B included chronic persistent hepatitis in 5, chronic active hepatitis in 34, cirrhosis in 16, and other diagnosis in 9 patients. Mean serum ALT and AST levels were 1.92 μkat/l (range 0.21–18.20) and 1.38 μkat/l (range 0.31–10.90), respectively.

HBV DNA in serum was present in 58% (37/64) of patients.

The coinfection (the HBV+HCV infection) was present in 3% (2/64) of patients. Both patients had liver cirrhosis. One had functional class Child A, the other had advanced liver cirrhosis with portal hypertension and transjugular intrahepatic portosystemic shunt since 1994. Both had positive HBsAg, HBeAg and HBV DNA in serum and mild elevation of the transaminases. Anti-HCV were repeatedly positive, but HCV RNA was negative.

Chronic hepatitis C

This group included 60 patients with repeatedly confirmed anti-HCV alone and 19 patients with anti-HCV and anti-HBV (19 anti-HBc-positive, 10 anti-HBs-positive, and 7 anti-HBe-positive). HCV RNA in serum was present in 75% (59/79) of patients.

In the 60 patients with anti-HCV, the mean serum levels of ALT and AST were 1.34 μkat/l (range 0.18–4.51) and 1.28 μkat/l (range 0.25–6.55), respectively.

The 19 patients with anti-HCV and anti-HBV represented the coinfection (19/79, 24%). In these 19 patients, the mean serum levels of ALT (2.10 μkat/l, range 0.25–4.25) and AST (1.54 μkat/l, range 0.31–4.86) were higher, but not significantly, than those in the patients with anti-HCV alone. Liver biopsy revealed chronic active hepatitis in 53% (10/19) of patients and liver cirrhosis in 32% (6/19) of patients (one case was complicated by hepatocellular carcinoma). HCV RNA in serum was present in 74% (14/19) of patients, but HBV DNA in 16% (3/19) of patients only.

Table 2. Subgroups of PCT patients with and without HBV infection or HCV infection, and with the coinfection

PC	N	%	Age (years)	Duration of PCT (years)	ALT (μkat/l)	Liver biopsy		
						ST	FI	CI
No HBV or HCV infection	45	68	58 (31–78)	13	0.66 (0.19–1.78)	23	14	0
HCV infection alone	7	11	59 (28–73)	7	0.66 (0.24–1.30)	1	6	0
HBV infection alone	7	11	54 (27–78)	6	0.55 (0.14–1.29)	3	3	0
Coinfection	7	11	67 (57–76)	20	1.25 (0.83–2.22)	0	2	4

N = number of patients. ST = steatosis. FI = fibrosis. CI = cirrhosis.

Porphyria cutanea tarda (PCT)

In 68% (45/66) of PCT patients, serological markers of HBV or HCV infection were not detected. These markers were present in serum of 32% (21/66) of PCT patients. Histological evaluation was performed in 37 of 45 patients without serological markers of either infection and in 19 of 21 patients with serological markers of HBV and/or HCV infection. Results of this analysis are shown in Table 2.

Anti-HBV were detected in serum of 21% (14/66) of the patients, none was HBsAg-positive, and 7 of 14 were concomitantly anti-HCV-positive. Data on the presence of anti-HBc (13/14, 93%), anti-HBe (10/14, 71%), and anti-HBs (7/14, 50%) were also obtained.

Anti-HCV were found in serum in 21% (14/66) of the patients, which is the same figure as in HBV infection, and also 7 of them were anti-HBV-positive. So we detected anti-HBV alone in 11% (7/66), anti-HCV alone in 11% (7/66), and anti-HBV together with anti-HCV (the coinfection) in 11% (7/66) of the patients. However, if only the 21 PCT patients with viral hepatitis were considered, then the coinfection was found in 33% (7/21) (Table 3).

A comparison between the subgroups of PCT patients with HBV or HCV infection alone and with the coinfection revealed that, in the case of the coinfection, the patients were older (67 vs. 54–59 years), the duration of PCT was longer (20 vs. 5–7 year), and the mean serum ALT level was higher (1.25 vs. 0.55–0.66 μkat/l, $p < 0.05$).

HCV RNA in serum was present in 86% (6/7) of patients with the coinfection but none of these sera was HBV DNA-positive.

In 56 patients with PCT which underwent liver biopsy, steatosis was present in 27 (48%) cases, fibrosis in 25 (45%) cases (11 of whom had non-stable fibrosis), hemosiderosis in 19 (34%) cases, and liver cirrhosis in 4 (7%) cases. Some patients had a combination of mild steatosis, fibrosis or hemosiderosis, and two had normal liver histology.

The subgroup of PCT patients with the coinfection showed the most severe histological picture, 4 patients had liver cirrhosis and 2 patients had severe and unstable liver fibrosis (in one case liver biopsy was not performed).

The rate of the coinfection in patients with CLD is summarized in Table 3. It shows a very low rate of the coinfection in patients with chronic hepatitis B (3%) and a relatively high rate of the coinfection in patients with chronic hepatitis C (24%) and in PCT patients with HBV or HCV infection (33%) together with 2-fold to 3-fold higher mean ALT and AST levels in serum, suggesting a liver damage in these patients.

About 75% of the CLD patients had HCV infection alone and these individuals should be potential candidates for vaccination against HBV infection, because the coinfection causes more severe liver disease than HBV or HCV infection alone. It seems that PCT patients with normal liver function tests and negative HBV serology are also at a great risk of chronic hepatitis B and C and therefore should be vaccinated against HBV infection.

Table 3. The rate of coinfection in patients with CLD

Group of patients	Rate of coinfection		Mean age (years)	ALT (μkat/l)	AST (μkat/l)
	N	%			
Chronic hepatitis B	2/64	3	58	1.94	1.64
Chronic hepatitis C	19/79	24	45	2.10	1.54
PCT patients with HBV or HCV infection	7/21	33	67	1.25	0.55
Total	28/164	17	57	1.76	1.24

N = numbers of patients.

Discussion

We observed in our anti-HCV-positive patients two types of HBV coinfection: the overt HBV coinfection with HBsAg in serum, and the hidden coinfection with positive anti-HBc, anti-HBe or anti-HBs but negative HBsAg in serum. In a retrospective analysis of patients with chronic hepatitis we cannot distinguish in most cases between coinfection and superinfection with both viruses.

Patients with the overt coinfection have the worst form of the disease (Alberti *et al.*, 1995). We also found chronic active hepatitis and liver cirrhosis in 84% (16/19) of patients, but the severity of the hidden coinfection was by no means milder. Chronic hepatitis patients with the coinfection were more likely to be cirrhotic and later to have decompensated liver disease (Fong *et al.*, 1991). An inapparent form of the coinfection may be implicated in cases resistant to interferon (Zignego *et al.*, 1997).

The rate of the coinfection was found very low in asymptomatic Chinese HBV carriers (0.7%, Lau *et al.*, 1998) similarly to US patients (2.8%, Kaur *et al.*, 1996), whereas in Spanish patients it was 13% (Crespo *et al.*, 1994). The coinfection in Israel is common (about 30%, Liang *et al.*, 1991). We found in our patients with chronic hepatitis C and PCT patients with HBV or HCV infection the rates of the coinfection 24% and 33%, respectively, figures very similar to that for Israel.

Liver disease seems to be more severe in patients with the coinfection and HCV is probably the main cause of the persistence of the disease. Both infections (which are often undetectable by conventional assays) may represent an important, so far unrecognized cause of idiopatic CLD.

In some patients, HBV may play a dominant etiological role in liver injury and a suppressive interaction between hepatitis B and C viruses may occur in the coinfection (Ohkawa *et al.*, 1994, 1995). On the other hand, HCV appears to suppress HBV replication and to cause more severe liver disease in patients with chronic HBV infection (Fong *et al.*, 1991). HCV seems to be dominant over HBV because it has been shown to influence the rate of HBsAg clearance (Koike *et al.*, 1995). Greek patients with the coinfection show a greater tendency to seroconversion of HBeAg and HBsAg than patients infected with HBV alone (Sougaridou *et al.*, 1996). Finally, replication of HBV is reduced in the patients with the coinfection compared to the patients with the HBV infection alone suggesting that HCV may exert a suppressive effect on HBV replication (Mathurin *et al.*, 1996).

In the group of anti-HCV-positive patients we found the coinfection in 24% and HCV RNA in serum in 74% but HBV DNA in 16% of patients only. We therefore believe that, in most patients, HCV is the dominant virus. HCV plays the major role in the immune response of patients with the coinfection (Tsai *et al.*, 1995). HCV may usurp the role of HBV in chronic hepatitis and act as the major cause of continuing hepatitis with ALT elevation after HBV/HBsAg clearance (Liaw *et al.*, 1994; Sato *et al.*, 1994). Although HBV may also suppress HCV, it appears to be less effective (Liaw, 1995).

Precise mechanism of viral interference is unknown. In superinfection, the second virus tends to suppress the first virus (Zairski *et al.*, 1998). Among patients who showed evidence of the coinfection, 78% had detectable levels of HBV or HCV genome alone in their sera and 15% had both HBV DNA and HCV RNA, but the concentrations of HCV RNA or HBV DNA were lower than those in patients with HCV RNA or HBV DNA alone.

HBV and HCV show alternating dominance in replication in most of the patients who have the coinfection, probably due to interference of these viruses (Koike *et al.*, 1995).

PCT is caused by a deficient hepatic activity of the enzyme uroporphyrinogen decarboxylase (URO-D), which leads to overproduction, hepatic accumulation, and increased urinary and faecal excretion of highly carboxylated porphyrins. The disease is clinically characterized by a cutaneous syndrome due to photosensitization of the skin, manifested by skin fragility, blister formation, hyperpigmentation, and hypertrichosis. Both the acquired (sporadic) and familial forms of PCT have been described. The URO-D activity is deficient in all tissues in the familial form, whereas, in the sporadic form, the enzyme activity is reduced only in the liver.

The iron ions accumulation in liver tissue seems to represent a connecting link between PCT and HCV infection (Bonkovsky *et al.*, 1998). Porphyrinogenic activity of HCV infection occurs either as direct result of viral liver changes or consequence of iron deposition in the liver. The majority of PCT patients suffer from hepatic siderosis; the presence of liver iron load may be due, in some patients, to the existence of the C282Y mutation of the hemochromatosis gene, responsible for hereditary hemochromatosis (Roberts *et al.*, 1997). The usual phlebotomy therapy leading to the depletion of these deposits and amelioration of clinical and laboratory signs of porphyria confirms the abovementioned assumption.

The rate of anti-HCV in PCT patients in the Mediterranean area is high (62–91%), but, in Germany and Ireland, it is relatively low (8–10%). In Czech Republic, we found anti-HCV positivity in 23% (21/92) of PCT patients by a second generation ELISA method, and 85% of these anti-HCV-positive subjects had viremia (positive HCV RNA in their serum by nested PCR). So the rate of anti-HCV-positive PCT patients in our country is about twice as high as in Germany (Malina *et al.*, 1997).

The rate of the coinfection in PCT patients with anti-HCV or anti-HBV was higher than that in patients with chronic hepatitis C (33% vs. 24%), and this small difference could be explained by age; the former patients were older than the latter (67 vs. 45 years).

As concerns the HCV genotype present in HCV-infected PCT patients, recent reports from Italy and France identified the 1b type in the vast majority of subjects hitherto studied (Rivanera *et al.*, 1998; Lamoril *et al.*, 1998).

About 75% of anti-HCV-positive CLD patients had HCV infection only. These individuals should be potential candi-

da[illegible] for vaccination against HBV infection, because the co[illegible]fection causes a more severe liver disease. PCT patients wi[illegible] normal liver function tests are also at a greater risk of ch[illegible]nic hepatitis B and C and therefore should be vaccinated ag[illegible]nst HBV infection.

Note. This work was presented at the 49th Annual Meeting of the American Association for the Study of Liver Diseases, Chicago, 1998 (Stránský *et al.*, 1998).

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