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Case report

Hepatitis D, B and C virus (HDV/HBV/HCV) coinfection as a diagnostic problem and therapeutic challenge

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Abstract

Coinfection with hepatitis D virus (HDV) in chronic hepatitis B is associated with more rapid progression to liver cirrhosis. We present two cases of infection with hepatitis D, B and C viruses. Both male patients were primarily diagnosed as infected with hepatitis B virus (HBV) and hepatitis C virus (HCV), HBsAg-positive and anti-HCV-positive. The first patient was treated with interferon, lamivudine and pegylated interferon. A full virological and biochemical response was achieved. The second patient was treated with interferon and ribavirin, lamivudine and twice with pegylated interferon. In the ultrasound elastography progression of liver fibrosis to F4 was described.

HDV infection should be considered in patients with HBV minireplication, high activity of aminotransferases and progression of liver disease despite a good virological response to anti-HBV treatment. Efficacy of interferon in HDV infection is severely limited.

Key words: hepatitis B virus, coinfection, hepatitis C virus, hepatitis D virus, HBV/HCV/HDV coinfection.

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Introduction

A new antigen "delta" was identified for the first time in the liver of patients infected with the hepatitis B virus (HBV) in 1977 by Mario Rizetto and colleagues [1]. Hepatitis D virus (HDV) is the smallest, human, defective RNA virus and it depends on HBV to propagate [2]. In the beginning of the 21st century hepatitis delta was considered "a vanishing disease" in Europe [3]. Acute hepatitis D has become infrequent in Western countries in the last two decades because of the introduction of anti-HBV vaccination programs. However, more reports from several European countries indicated rising prevalence areas due to migration from the ex-Soviet Union, Turkey, Far East and Africa. 15-20 million individuals are estimated to be anti-HDV positive worldwide [4]. The clinical course of hepatitis D is described as acute HBV/HDV co-infection with possible recovery in more than 90% of patients but also with a risk of a fulminant course or HDV superinfection. In the latter form HDV is cleared spontaneously in a minority of chronically HBV-infected patients, and in these cases it may lead to a more severe course of chronic hepatitis with higher incidence of cirrhosis compared to HBV monoinfection [5].

We present two cases of infection with hepatitis B, D and C (HCV) viruses. Both male patients were primarily diagnosed as infected with HBV and HCV, HBsAg-positive and anti-HCV-positive.

Case 1

A 33-year-old male patient was sent in 2001 to the Out-Patient Clinic of the Pomeranian Center of Infectious Dis-

eases because of an increase of aminotransferase activity (ALT 250 U/l, AST 150 U/l). At the age of 18 (15 years earlier) he had been treated for acute hepatitis B in the Department of Infectious Diseases. On admission in 2001, HBsAg and anti-HCV were detected but the patient was HCV RNA negative. No other comorbidities were found. The mode of acquiring HBV/HCV/HDV infection is unknown; the patient denied risky behaviors. The patient was observed to have a low HBV DNA load (200 IU/ml), hyperproteinemia, and hypergammaglobulinemia. In the liver biopsy specimens which were taken in the years 2001 and 2003 slow progression of inflammatory activity and fibrosis was confirmed (Table 1). Autoimmune hepatitis was ruled out. In 2003 he received 24 weeks of treatment with interferon α (Roferon) which was complicated by typical adverse events: weakness, fatigue, loss of weight, and abdominal pain. At the end of treatment partial improvement in activity of aminotransferases was observed. In the years 2003-2006 the patient fulfilled the criteria of qualification for treatment with lamivudine (Zeffix). This therapy was tolerated very well, but biochemical efficacy was not achieved despite low or not detected HBV DNA. The data are presented in Table 1. The treatment with lamivudine was discontinued in 2006 after detection of anti-HDV and HDV RNA.

Table 1. Case 1: laboratory and histopathological parameters during clinical observation and antiviral treatment in years 2001-2016

Patient 1	2001	2003	2006	2009	2010	2016
ALT (U/l)	252	290	236	314	80	35
GGTP (U/l)	84	107	93	78	145	57
Cholesterol (mg/dl)	283	253		274		
BMI (kg/m^2)	28.09	24.72		29.67		30.61
Total protein (g/l)	87	91		85	83	84
γ-globulin (%)	25.4	24.4		24	21	17.6
Liver histology						
Inflammation activity	1	1-2		2		
Fibrosis	1	1-2		3		
Steatosis	Minimal	2		1		
anti-HCV	Positive					
HCV RNA	Negative					
HBsAg	Positive				Positive	Positive
HBeAg	Negative				Negative	
HBV DNA (IU/ml)	265	182	< 26	130	Negative	13
anti-HDV, total			Positive			
HDV RNA			Positive		Negative	Not available

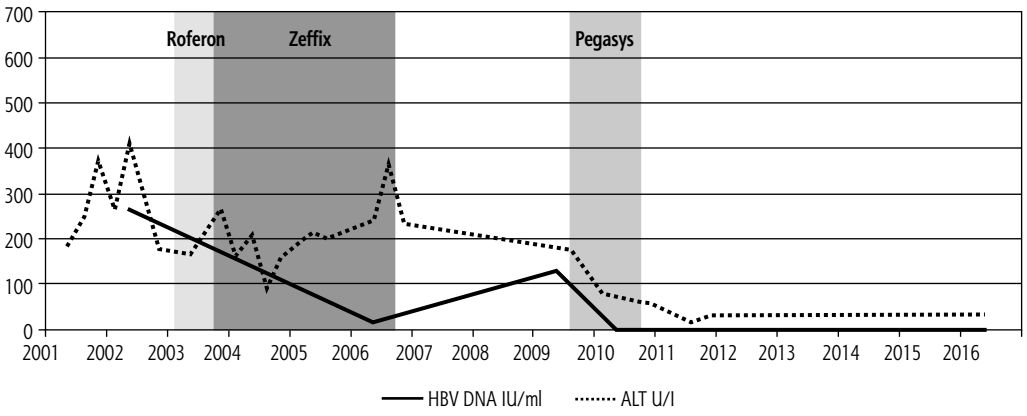

Fig. 1. Case 1: HBV DNA viral load and alanine aminotransferase activity during clinical observation and antiviral treatment

In 2009 significant progression of inflammation activity and liver fibrosis was confirmed in the control histopathological examination of the liver biopsy specimen. At this moment of observation the possible impact of metabolic syndrome on the rapid progression of liver disease was also considered in the patient with BMI ~30 kg/m^2, hypercholesterolemia (274 mg/dl) and moderate steatosis of hepatocytes. Still hyperproteinemia and hypergammaglobulinemia were found without presence of antibodies: ANA, SMA, LKM, SLA. There was no increase of HBV DNA load (130 IU/ml). In 2009 treatment with pegylated interferon α-2a (Pegasys) was administered for 48 weeks with good tolerance and a complete, sustained biochemical viral response. Neither HBV-DNA nor HDV-RNA was detected after the end of treatment. Liver function tests were normal. The patient remains under observation in the out-patient clinic because of hepatomegaly and moderate liver steatosis, which is observed in ultrasound examination. HBV DNA is detected in blood and slightly exceeds the detection threshold. The present level of HDV RNA is unknown.

Case 2

The 33-year-old man has been under the care of the Pomeranian Center of Infectious Diseases since 1998. HBV infection was diagnosed in early childhood; probably it was an iatrogenic infection associated with the course of treatment of serve pneumonia in a hospital. In the years 1998-2002 the patient was observed as a non-active carrier of HBV. In 2002 a significant increase of aminotransferase activity (ALT 300 U/l) and anti-HCV was detected. The route and time of HCV infection have not been determined. HCV-RNA testing was positive and biopsy of the liver showed minimal grading (0/1) and staging (1) according to Scheuer's classification. The patient, diagnosed with HCV/HBV coinfection, was qualified in 2003 for treatment with interferon and ribavirin (Intron and Rebetol). The therapy lasted 48 weeks, no significant side effects were observed, and a sustained viral response was achieved (HCV RNA was not detected 24 weeks after the end of treatment). In 2005 the patient presented symptoms of exacerbation of liver injury. He confirmed occupational exposure to hepatotoxic agents, but expected improvement of liver function tests was not observed. HCV reactivation was excluded but active HBV replication with a high rate of HBV DNA was detected (Table 2). In the second histopathological examination of a liver biopsy specimen (2006) significant progression of grading (G2) and staging (S3) was described. Treatment with lamivudine (Zeffix) was administered in 2006 and continued for the next two years. A quick viral response with negative HBV DNA in blood after six months of the therapy was not accompanied by improvement of liver function tests. As high activity of aminotransferases was constantly observed (ALT > 500 U/l), autoimmune hepatitis or autoimmune reaction was suspected due to persistent hypergammaglobulinemia, hyperglobulinemia and ag-

Table 2. Case 2: laboratory, histopathological parameters and liver stiffness during clinicaal observation and antiviral treatment in years 2002-2016

Patient 2	2002	2006	2009	2010	2012	2015	2016
ALT (U/l)	107	566	387	80	213	253	96
GGTP (U/l)	164	88	71	145	66	93	
Cholesterol (mg/dl)	170	180	139		131	136	
Total protein (g/l)	77	94	79	83	100	104	
γ-globulin (%)	19.2	13	25	27.1	41.1	42	
Liver histology							
Inflammation	0/1	2	2				
Fibrosis	1	3	2/3				
FibroScan (kPa)					20.6 (F4)	18.4 (F4)	
anti-HCV	Positive	Positive	Positive		Positive		
HCV RNA	Positive	Negative	Negative		Negative		
HBsAg	Positive	Positive	Positive	Positive	Positive	Positive	
HBeAg		Negative	Negative	Negative	Negative	Negative	
HBV DNA (IU/ml)		6.91×10^7	40	Negative	Negative	Negative	< 10
anti-HDV, total			Positive				
HDV RNA (co./ml)			6.2×10^3	Negative	6.7×10^6	1.2×10^8	

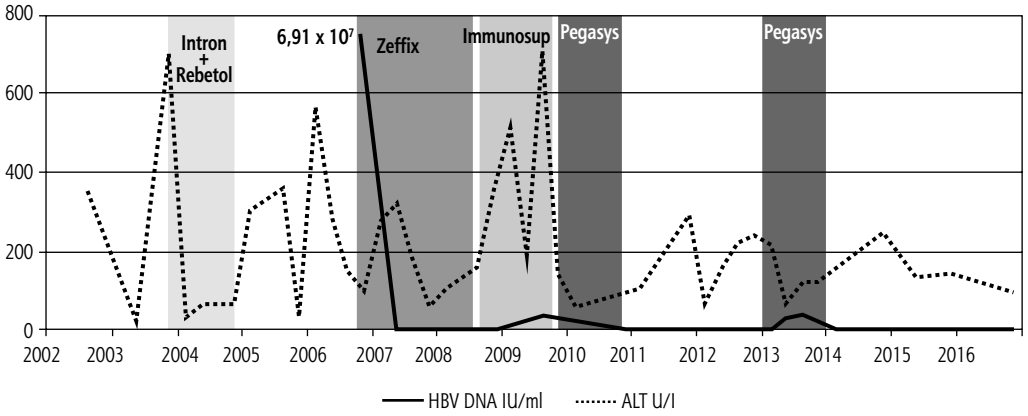

Fig. 2. Case 2: HBV DNA viral load and alanine aminotransferase activity during clinical observation and antiviral treatment

gressive hepatitis in liver biopsy. Despite lack of presence of autoantibodies – anti-nuclear (ANA), anti-smooth muscle (SMA), anti-liver-kidney microsomes (LKM) – immunosuppressive treatment with glucocorticoids and azathioprine was started. This form of therapy was ineffective and it was stopped at the moment of diagnosis of HDV with detection of anti-HDV and HDV RNA in blood (2009). No serious reactivation of HBV replication was found. In 2009/2010 and 2013 the patient received two courses of 48-week treatment with pegylated interferon (Pegasys). A significant decrease of aminotransferase activity was found during the first therapy. Resistant HDV infection is confirmed by persistent HDV viral load, at present with HBV DNA not detected. Stiffness of the liver (FibroScan 20 kPa/F4 in Metavir score) corresponds to advanced liver fibrosis. This patient is waiting for new therapeutic options.

Discussion

Both cases present different difficulties in diagnosis and treatment of hepatitis D. Epidemiological data that relate to HDV infection in Poland are not satisfactory. Recent research results were published in 2006 and they showed a 7.9% occurrence rate of HDV/HBV infection in the Pomeranian region of Poland [6]. Studies which were performed in a selected population of drug addicts in the 1990s suggested that the main transmission of HDV in Poland was associated with intravenous drug abuse [7]. Lack of evidence of HDV infection among chronic hepatitis C patients in the north-eastern part of Poland resulted in a conclusion that hepatitis delta was a rare disease [8].

In both presented cases, patients were first identified with HBV/HCV coinfection. Complete elimination of HCV was observed in two patients due to an effective antiviral treatment in one of them and as a result of spontaneous elimination in another. It is not possible to determine the sequence of all three infection events in time and to assess whether patients were infected with HBV, HDV and HCV simultaneously or rather superinfected with HCV. Also it cannot be excluded that HCV elimination acted as a trigger mechanism that led to loss of inhibition of HBV/HDV complex and further dynamic exacerbation of hepatitis and progression of liver disease. Active HCV replication may be involved in repression of propagation of other hepatotropic viruses. HCV may dominate in triple infection but it probably changes [9]. Other reports provide evidence for suppression of HCV replication by HDV and HBV [10, 11]. In both present cases the main clinical problem was associated with an observation of aggressive, quickly progressing hepatitis in patients who presented low or not detected HBV DNA viral load, including during antiviral treatment with lamivudine. The presence of incomplete exponents of autoimmune hepatitis (highly active inflammation in liver biopsy, hypergammaglobulinemia) caused misdiagnosis, use of immunosuppressive treatment and delay in diagnosis of active HDV infection in one of the patients.

HDV infection should always be considered in patients with a low HBV replication rate, active inflammation and progression of liver disease despite a good virological response to anti-HBV treatment. HDV infection is associated with suppression of HBV replication, which is explained by the inhibition of the host DNA-dependent RNA polymerase II by the large delta antigen [12].

An autoimmune reaction which requires differentiation from autoimmune hepatitis is not an unexpected condition in patients with chronic hepatitis B or C. According to the course of chronic HBV infection it can be assumed that triggering of an autoimmune reaction in HDV/HBV coinfection is possible and may play a significant role in progression of aggressive inflammation [13]. However, the role of HDV in autoimmunity is not clearly explained and needs further exploration [14].

At present, therapy with interferon remains the only available option, and it may lead to remission of the dis-

ease in 20-50% of cases depending on the dosage and the duration of treatment [15].

The HIDIT-1 study showed that PEG-INF-α2 displayed significant antiviral efficacy against HDV in more than 40% of patients (with 25% becoming HDV RNA negative after 48 weeks) [16].

The measurement of the level of HDV RNA at week 24 of treatment with pegylated interferon with or without adefovir for 48 weeks was identified as an additional prognostic factor. It appeared to be helpful when assessing the chance for negative HDV RNA 24 weeks after the end of treatment [17].

Unfortunately, late HDV RNA relapse after peginterferon alpha-based therapy of chronic hepatitis delta was described recently and a sustained virological response in HDV infection was denied [18].

Nucleoside and nucleotide analogues used in monotherapy for the treatment of HBV infection are ineffective against HDV. The efficacy of long-term combination of interferon with tenofovir is a subject of research but without a doubt new alternative treatment options are needed [19]. Among them, Myrcludex B as a first-in-class entry inhibitor inactivating HBV and HDV receptor sodium taurocholate co-transporting polypeptide seems to be a promising new drug which is under investigation [20].

As active chronic hepatitis B and D are associated with more rapid progression to liver cirrhosis, patients who do not respond to the antiviral therapy require further careful control and screening for hepatocellular carcinoma (HCC). HBV/HDV coinfection has been linked with a higher risk for the development of hepatocellular carcinoma [21].

Conclusions

HDV infection should be considered in patients with HBV minireplication, high activity of aminotransferases and progression of liver disease despite a good virological response to anti-HBV treatment. Chronic hepatitis in the course of HBV/HDV coinfection may be accompanied by an autoimmune reaction. The efficacy of interferon in HDV infection is significantly limited.

Disclosure

Authors report no conflict of interest.

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