HEPATITIS D

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Summary. – Hepatitis D virus (HDV) is a small, RNA-containing virus that requires the concomitant presence of Hepatitis B virus (HBV) in an obligate manner for its survival and pathogenicity. HDV infection is very uncommon in Czech Republic. The results of antiviral therapy of hepatitis D patients are not satisfactory. Alpha-interferon (alpha-IFN) in high doses (9–10 MU three times a week for 12 months) is usually recommended.

Key words: hepatitis D; alpha-interferon; neutropenia

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1. Introduction

HDV or Delta virus is a small, RNA-containing virus. It is an unique infectious agent that looks more like a plant virus than animal virus (Wang *et al*., 1986). It has been first described in 1977 in hepatitis B surface antigen (HBsAg)-positive patients (Rizzetto *et al*., 1977). HDV is classified as the species *Hepatitis delta virus,* the genus *Deltavirus* (van Regenmortel *et al*., 2000). The consensus is that HDV is a satellite virus, which requires a helper virus, Hepatitis B virus (HBV) for transmission and multiplication. There is no other satellite virus known among animal viruses. In the past, it has been believed that HDV is a viroid. However, this view had to be rejected after the discovery of hepatitis D antigen (HDAg), the antigen encoded by HDV.

2. Natural history

HDV infection has a variable influence on the course of hepatitis B. The clinical severity of dual infection probably depends on a number of factors, including the endemicity of HDV in a specific area, the degree of HBV viremia, and the genotypes of HBV and HDV. The co-infection is mostly seen in intravenous drug abusers and less frequently in the patients receiving multiple transfusions. As HBV infection resolves in most patients, also HDV infection disappears in most patients. The super-infection of chronic HBV infection

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Abbreviations: alpha-IFN = alpha-interferon; ALT = alanine transaminase; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; HBV = Hepatitis B virus; HDAg = hepatitis D antigen; HDV = Hepatitis D virus; ULN = upper limit of normal

with HDV often leads to a very high degree of HDV viremia, because HBV infection is already established. Due to intensive replication HDV super-infection leads to a more severe acute hepatitis; occasionally, this may lead to fulminate hepatitis. HDV replication exerts an inhibitory effect on HBV replication that results in a marked decline in HBV DNA levels in serum. The rate of HBeAg loss has been reported to be higher in HBV carriers who became super-infected with HDV. Very rarely, the HDV super-infection may lead to the disappearance of HBsAg (Wu *et al*., 1995b).

Chronic HDV infection occurs in 70% of super-infected patients. The most common pattern is persistent replication of HDV and HBV leading to progressive hepatitis that ends in liver cirrhosis within a few years. Rarely, particularly among intravenous drug abusers, a rapidly progressing liver disease may occur, leading to an end-stage liver disease within less than 2 years. In a minority of patients, the liver disease is slowly progressive. Based on a clinical study in 185 patients it has been proposed that the HDV super-infection can be divided into the following three stages: (i) acute phase, characterized by active HDV replication, suppression of HBV and high ALT activity, (ii) chronic phase with decreasing HDV replication, reactivating HBV replication and moderate ALT activity, and (iii) late phase, in which the patients either develop cirrhosis and hepatocellular carcinoma or enter into a remission resulting from a marked reduction of replication of both viruses (Wu *et al*., 1995b).

In liver transplant recipients, a latent type of HDV infection has been reported, in which HDV is expressed in hepatocytes in the absence of HBV. Such cases are due to a very low expression of HBV in the allograft rather than a true autonomous infection. The latent infection is usually silent but develops into clinical hepatitis after HBV infection becomes established in the allograft (Ottobrelli *et al*., 1991).

3. Virology

HDV virion is a 36–43-nm spherical particle consisting of a nucleocapsid surrounded by an envelope composed of hepatitis B surface antigen (HBsAg) and lipids. The nucleocapsid contains single-stranded, negative-sense, covalently closed, circular HDV RNA and HDAg. With only 1.7-kb size, HDV RNA is the smallest RNA genome among animal viruses. RNA genome forms an unbranched rod-like structure by folding on itself through extensive intramolecular base pairing (70%). HDV behaves like a ribozyme, similar to "hammerhead" ribozymes of plant viruses.

HDV RNA encodes only one protein, HDAg, which occurs in two isoforms, a small (195 aa) SHDAg and a large (214 aa) LHDAg. SHDAg transports RNA into the nucleus and is essential for HDV replication. LHDAg inhibits HDV RNA replication and participates in HDV assembly. Both isoforms are encoded by the same region in the antigenomic RNA, arise by RNA editing and differ in 19 amino acids at the C terminus (Kuo *et al*., 1989; Chao *et al.*, 1990). The protein envelope protects the HDV RNA-HDAg complex but is not required for HDV replication. Several specific structural domains were found in the delta antigens, including a nuclear localization signal (NLS) and leucine zipper RNA binding domain (Cunha *et al.*, 2003) (Fig. 1).

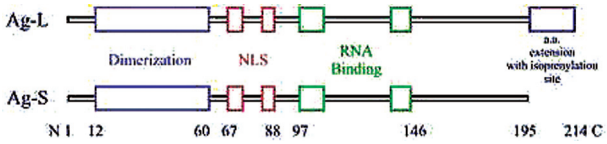

Fig. 1

Localization of structural domains in HDV antigen

According to Cunha *et al.* (2003).

HDV replication is limited to hepatocytes. HDV enters the host cell along with its HBsAg envelope. The HDV-RNA-HDAg complex migrates to the nucleoplasm aided by the nuclear localization signal. The mechanism of replication and transcription of HDV genome is unknown. It is believed that the genomic RNA of negative polarity is first transcribed into a linear anti-genomic transcript by a rolling circle model. Linear concatemeric products are cleaved by HDV ribozyme into monomers and circularized by a host ligase. This, in turn, replicates and generates negative genomic RNA, thus completing the cycle (Lai, 1995) (Fig. 2). The polymerase involved in HDV genome replication and transcription is most likely the host DNA-dependent RNA polymerase, because HDV itself does not encode such an enzyme. The antigenomic RNA can be found in infected hepatocytes and, to a lesser extent, in purified HDV particles.

So far three HDV genotypes have been identified: I, II and III. A 30–40% sequence variation is observed between different genotypes and a 10–15% variation within the genotypes. The genotype III produces the most severe disease. The genotype II is associated with a less severe disease compared with the genotype I (Wu *et al.*, 1995a).

4. Epidemiology

HDV is distributed worldwide with the highest endemicity in some South American countries, Mediterranean, Romania and some parts of South and Middle Africa (Fig. 3). It is believed that at least 5% of hepatitis B carriers worldwide

are infected with HDV. Based on blood donor screening, the seropositive rate among HBsAg-positive blood donors in the United States amounts to be 3.8%. The HDV infection is much more prevalent among intravenous drug users (20–53%) and hemophiliacs (48–80%).

HDV infection is transmissible only if the recipient is a carrier of HBV. In case of acute co-infection, HBV infection needs be established in the host prior to HDV infection. A super-infection in a host with previously established HBV infection is much more common than the co-infection. The co-infection and super-infection can occur, because the mode of transmission of HDV is similar to that of HBV. Parenteral transmission is the most efficient mode of spread in non-endemic areas of HDV infection. There is a definite risk of transmitting HDV through blood and blood products. Intravenous drug abusers are mainly in risk of HDV infection and epidemics among them have been documented in Scandinavia and elsewhere. Inadvertent parenteral transmission has been reported from households with overcrowding, as evidenced from the genomic homology among members of a household. This is probably a common mode of spread in underdeveloped countries. In the endemic countries of South America the most likely route to transmission to children is the contact with infected individuals who have skin breaks. The risk of vertical transmission is extremely low, and HDV infection is also very uncommon in populations with high rates of childhood and infant HBV infection. In contrast to HBV, sexual transmission is uncommon in developed countries, although it is a significant mode of transmission in endemic areas (Liaw *et al.*, 1990).

Different HDV genotypes have different geographic distribution. The most common is the genotype I that prevails in the Mediterranean countries, Africa, Europe, and North America. The infection caused by genotype II is most frequently reported from Japan and Taiwan. The genotype III has been isolated during the course of epidemics in the South America. Genotypic variations in HDV may also reflect variations in the prevalent HBV subtypes in a particular area (Nair and Perrillo, 2003).

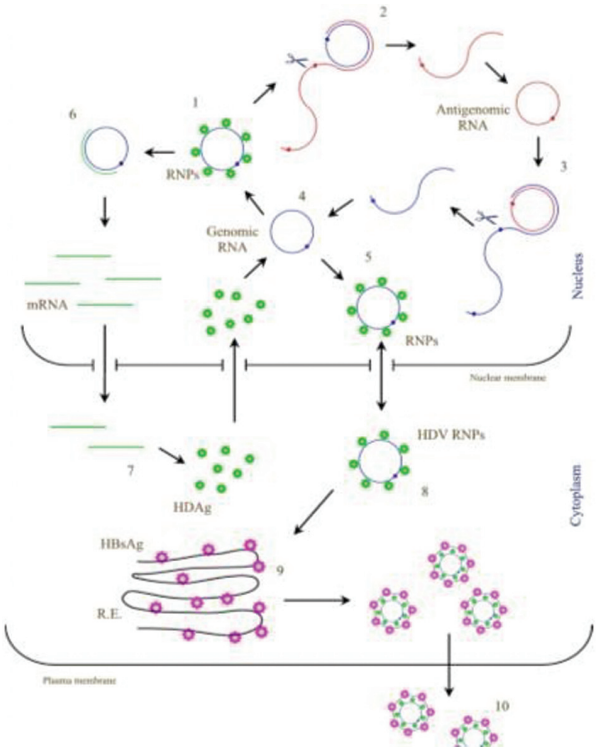

Fig. 2

HDV replication cycle

According to Cunha *et al.* (2003). 1: HDV ribonucleoprotein (RNP). 2: Synthesis of HDV antigenome by rolling circle. Linear concatemeric product is cleaved to monomers and these are circularized. 3: Synthesis of HDV genome by rolling circle. 4: HDV genome. 5: HDV RNP assembled from HDV genome and HDAg. 6: HDV genome is transcribed into mRNA for HDAg. 7: Synthesis of HDAg on its mRNA in cytoplasm and transport of HDAg into nucleus. 8: HDV RNP is assembled in nucleus and transported into cytoplasm. 9: HDV RNP binds HBsAg and is assembled into virions, which are released from cells.

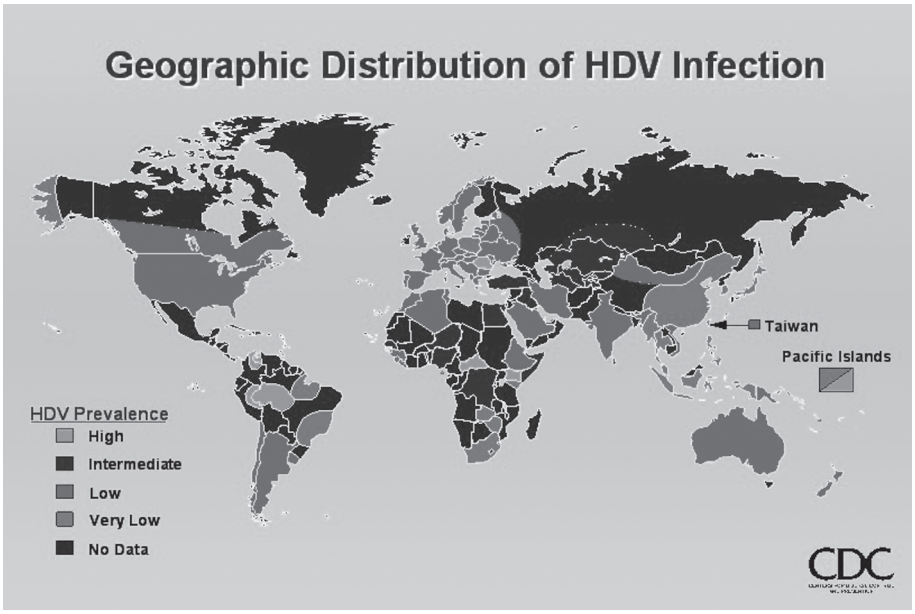

Fig. 3

Geographic distribution of Hepatitis D virus infection

According to http://www.cdc.gov/ncidod/diseases/hepatitis/slideset.

Several reports over the last decade have suggested that the incidence of hepatitis D is decreasing. Many epidemiologists have observed that the HDV epidemic that had began in the 1970s was declining. For example, in Italy, the prevalence of HDV infection among people suffering by chronic HBV infection was 25% 1987, 14% in 1992 and only 8% in 2000. Of course, it is connected with a global vaccination against HBV and with using of disposal injection syringes and needles (Sagnelli *et al*., 1997).

It has been found out repeatedly that this infection occurs in the Czech Republic only rarely (Stránský *et al.*, 1987, 1989; Summerová *et al*., 2000), but it is necessary to be aware of potential HDV infection of foreigners or our citizens who had resided for a long time in risk areas, especially if they had been given a blood transfusion or had undergone surgeries with a risk of HDV transfer. In the National Reference Laboratory for Viral Hepatitis, National Institute of Health, Prague HDV infection was proved only in 5 patients in last 10 years. Two cases were foreigners who had moved to the Czech Republic but had been probably infected while still in their native country, Ukraine or Romania. The remaining three cases were Czech citizens – one of them had a history of travelling and stays in Romania, Hungary, Greece and Croatia, while the two remaining cases had negative history in relation to a possible transmission of HDV infection.

5. Pathogenesis

The pathogenetic mechanism of HDV infection remains poorly understood. Because HBV is not known to be directly cytotoxic to hepatocytes, the severity of a co-infection of HBV and HDV may be attributed to either a direct cytotoxic effect of HDV or an enhanced immune response to the two viruses. Theoretically, because of similarities between HDV RNA and human RNA, the viral RNA could disrupt the translation process and hence have a direct cytotoxic effect. Reports of small-droplet steatosis in patients with HDV infection, cytoplasmic eosinophilia in chronic carriers, and an apoptotic promoting effect of HDAg *in vitro* in continuous HeLa and Hep G2 cell lines gave credence to a possible cytotoxic effect of HDV (Cole *et al*., 1991). On the other hand, studies on transgenic mice expressing either SHDAg or LHDAg showed no evidence of hepatocyte injury (Guilhot *et al*., 1994). The presence of lobular infiltration with lymphocytes in chronic hepatitis D and the finding of liver-kidney microsomal antibodies suggest a role for the host immune system in liver injury in HDV infection. The clinical relevance of these autoantibodies is not clear, and there is no correlation between the antibody titers and severity of infection (Philipp *et al*., 1994).

6. Diagnostics

The most useful markers of HDV infection include HDAg, antibodies to HDV (HDV antibodies), HDV RNA, and immunohistochemistry of the liver. Detection of HDV RNA by RT-PCR in most reliable, with nearly 100% sensitivity. It is also a reliable marker for monitoring the efficacy of treatment and documenting viral eradication (Jardi *et al*., 1995).

HDAg is another marker of HDV infection. HDAg can be demonstrated in the hepatocytes by immunohistochemistry, the predictive value of which decreases with chronicity of the disease. Many hepatocytes fail to produce adequate amounts of HDAg as the chronic HDV infection progresses; hence immunohistochemistry may yield negative results. Determination of serum HDAg is also problematic because of the presence of high titers of binding antibodies that interfere with its detection. Immunoblot analysis has been shown to be considerably more sensitive as compared to ELISA (Buti *et al*., 1989).

The most readily available markers of HDV infection are HDV antibodies. They can be detected either as IgM HDV antibodies or total HDV antibodies that comprise both IgM and IgG. IgM HDV antibodies appear at the time of acute infection, while IgG HDV antibodies develop later. IgM HDV antibodies persist as the infection becomes chronic, when they are often detectable in high titers. These antibodies have been regarded as a marker for serious liver damage. IgG HDV antibodies persist for years in immunocompetent patients and may represent a chronic or previous infection. HDV antibodies do not confer any protection against the virus. Both IgM and IgG HDV antibodies can persist even after the infection has resolved and in the absence of detectable HDV RNA. Hence the detection of HDV antibodies alone may lead to overestimation of the frequency of an active infection (Nair and Perrillo, 2003). Typical serological findings of co-infection of HBV and HDV and HDV super-infection on chronic HBV infection are shown in Figs. 4 and 5.

7. Treatment

Aim of the treatment in chronic hepatitis D patients is suppressing of HDV replication that is usually connected with normalization of ALT and histological improvement. In such patients, already before the treatment with alpha-IFN, the HBV DNA concentration is mostly below 10^3 copies/ml due to a suppressive influence of HDV on HBV replication. Thus the viremia is close also to the limit of sensitivity of PCR. In most of the countries in the world the only approved treatment of chronic hepatitis is a mono-

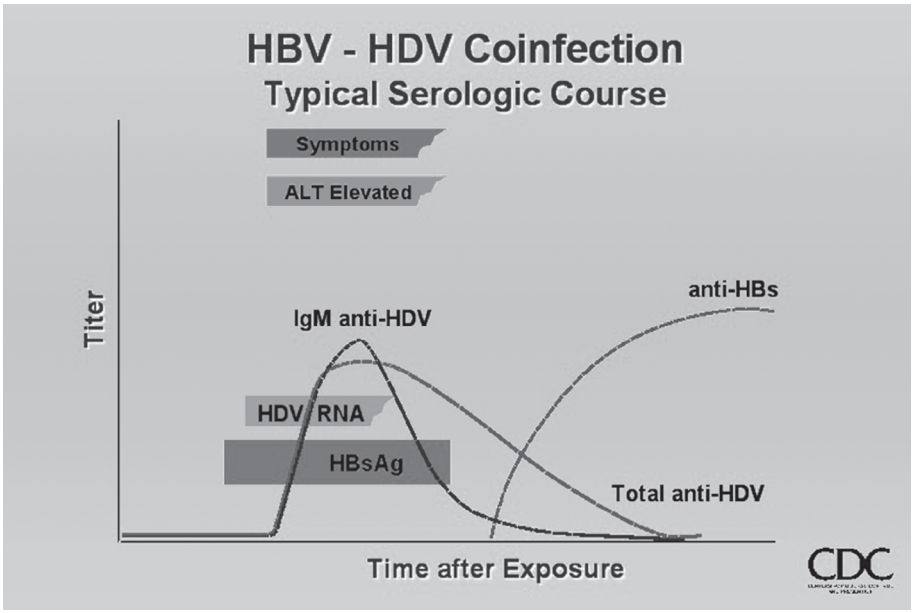

Fig. 4

Serological patterns of hepatitis B and D co-infection

According to http://www.cdc.gov/ncidod/diseases/hepatitis/slideset.

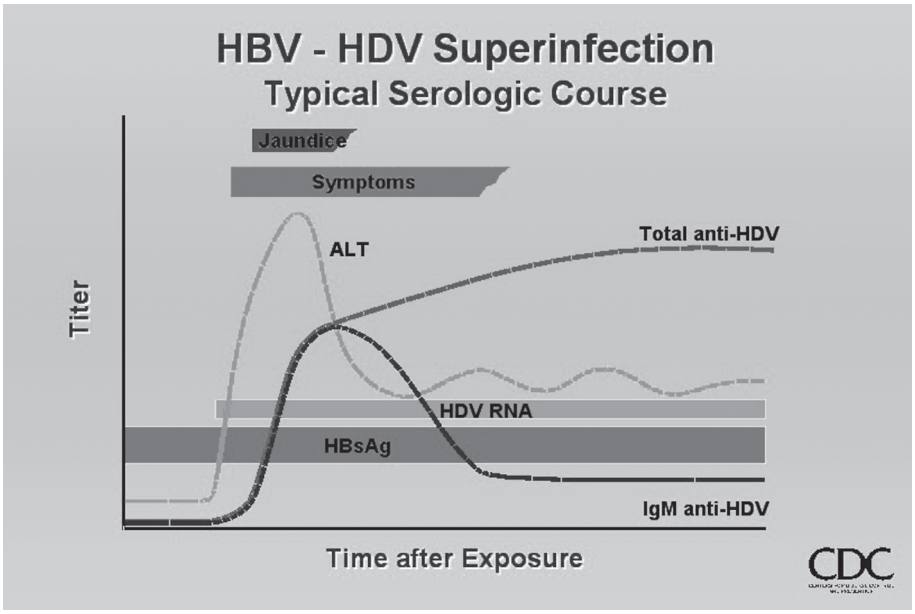

Fig. 5

Serological patterns of hepatitis B and D super-infection

According to http://www.cdc.gov/ncidod/diseases/hepatitis/slideset.

therapy with alpha-IFN. According to the results of antiviral treatments of only a small number of patients their successfulness was very low (Lok and McMahon, 2001; Nair and Perrillo, 2003; EASL International Consensus Conference on Hepatitis B, 2003). In Italy (Rosina *et al*., 2001), 61 patients were treated with alpha-IFN with 5 MU three times a week for 4 months and subsequently with 3 MU three times a week for 8 months. A control group of patients was without the treatment. No differences were found in influencing the HDV RNA level in serum and HDAg content in liver between the treated and control patients. Another study (Farci *et al*., 1994) proved that a higher dose of alpha-IFN (9 MU three times a week) was more effective regarding virology, biochemistry and histology than a lower dose of alpha-IFN (3 MU three times a week) or placebo. More specifically, the treatment resulted in reduction of virus replication (negative serum HDV RNA and disappearance of IgM HDV antibodies) in 10–30% of the patients. A normal activity of ALT was proved in 70% of patients at the time of treatment termination. In most of the patients, a virological relapse occurred after the termination of the treatment; however, a normal ALT activity still remained in roughly 50% of the patients and a histological improvement was still obvious after 10 years since the treatment termination.

There is no sufficient experience in treatment of chronic hepatitis D with lamivudine, but, according to preliminary results, lamivudine has in this indication no beneficial effect even in combination with alpha-IFN (Lau *et al*., 1999; Wolters *et al*., 2000). Adefovir dipivoxil has not been tested in these patients so far (EASL International Consensus Conference on Hepatitis B, 2003).

According to available results it is necessary to administer alpha-IFN in higher doses (9–10 MU three times a week) at least for a period of 12 months. In vast majority of patients, the virological relapse appears after this treatment, but a histological progression of hepatic inflammation is probably slower. It is not clear so far, whether it would be appropriate to administer alpha-IFN for a period even longer than 1 year, either to all patients or only to those with a normal ALT activity after one year of the treatment, or to those, in whom the virological symptoms appeared again after the treatment termination (Lok and McMahon, 2001; Nair and Perrillo, 2003; EASL International Consensus Conference on Hepatitis B, 2003).

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