Hepatitis D : virology, clinical and epidemiological aspects

M. Rizzetto

Department of Gastroenterology, University of Torino, Italy.

The Hepatitis Delta Virus (HDV) is a defective circular RNA viroid -like agent requiring functions from the Hepatitis B Virus (HBV) including provision of the HB surface coat for virion assembly. Like viroids of plants, HDV is transcribed by a host RNA polymerase and has an autocatalitic (self-cleavage and self-ligation) site.

Infection is present worldwide ; however, with the increased control of HBV, the circulation of HDV has decreased in recent years in the Western World. HDV infection can be acquired by coinfection with HBV or through superinfection on a pre-existing HBV infection, the latter acting as a magnet for the rescue and activation of HDV.

The clinical expression of coinfection and superinfection is variable but patients with hepatitis D, either acute or chronic, usually have serious and progressive liver disorders.

Usually, chronic Hepatitis D progresses to cirrhosis in 5 to 10 years ; once established, cirrhosis may remain clinically stable for decades.

Interferon (IFN) is the only available therapy but is of limited effect with less than 15% of chronic hepatitis D cases responding to the cytokine in clinical trials performed in the 1980s.

With the recent decline of HDV, the ratio of long-standing advanced disease non responsive to therapy over fresh forms of the disease responding to IFN has increased. Liver transplantation offers a valid therapeutic option for end-stage disease.

Taxonomy

The hepatitis D virus (HDV) is a negative-stranded RNA virus that depends on HBV for propagation but not for genome replication. It is the only member of the genus Deltavirus from the Deltaviridae family. The HDV shares structural similarities with viroids and virusoids, which belong to the world of plant viruses (1).

The virion particle of 35 to 37 nm is made of the circular ribonucleic acid (HDV-RNA) and of the delta antigen (HDAg) coated by the surface antigen (HBsAg) of HBV (2). There are three major genotypes of HDV. Infection with mixed genotypes has been reported (3).

The HDV genome is a 1.7-kb single-stranded circular RNA that can fold itself into an unbranched rodlike structure caused by base pairing of over 70% of its nucleotides. It codes for an antigen (HDAg) which consists of two closely related proteins with molecular weights of 24 kd and 27 kd. Replication of the virus, is based on the rolling-circle replication strategy.

HDV has autocatalytic capacity (self-cleavage and self-ligation) in both the genomic and antigenomic strands ; this is required for the processing of linear transcripts to circular replication molecules.

The host range of HDV infection includes man, chimpanzee, and woodchucks carrying the HBV-related hepadnavirus. HDV infection can be initiated by direct injection of cDNA clones in the liver or transfection into cells. No evidence of lymphotropism has been demonstrated so far for HDV.

Epidemiology

HDV is transmitted by the parenteral route, whether overt or covert (4). The efficiency of transmission is primarily due to whether the person exposed to the infection is or is not an HBV carrier. In the first case, as HDV cannot be transmitted unless HBV has been previously established, HDV depends on the infectious titre of coinfecting HBV. In the second, the preexisting HBsAg state acts as a magnet, rapidly activating and amplifying even a tiny amount of HDV. The HDV transcapsidates from the HBsAg coat worn in the original inoculum to the HBsAg coat made available by the superinfected host's HBV. The most efficient mode of transmission is the direct parenteral route, thus infection prevails in parenteral drug addicts exposed to unchecked blood (5).

In developed countries HDV infection has significantly declined in groups such as medical staff, institutionalised patients, hemodialysis patients and prisoners, due to the more effective control of HBV.

A major form of HDV transmission is sexual contact, which is attested to by the prevalence of anti-HDV in prostitutes and in the sexual partners of HDV infected carriers. Cohabitation with an HDV carrier was identified as a critical risk factor (7). Molecular studies of the HDV genome among infected partners of family members have confirmed the mechanism of sexual and household transmission of the HDV (8).

Correspondence : M. Rizzetto, University of Torino, Department of Gastroenterology, Corso Bramante 88, Molinette Torino, Italy.
Presented at the International Symposium on Viral Hepatitis beyond the Millennium Session of December 11, 1999.

Although HDV infection occurs worldwide, its geographical distribution varies considerably. The variations in prevalence reflect local prevalence rates of HBV but do not depend on them. In North America and Northern Europe the prevalence of HDV infection is low, as are overall prevalence rates in Alaska, Japan and South Africa despite consistent local rates of HBV infection. In Okinawa, North India, and Albania new foci of the infection have been identified in the last years, and in several tropical and subtropical countries where HBV is still hyperendemic hepatitis D predominates.

Of the 3 well-characterised genotypes of the virus genotype I is predominant and is found in many parts of the world (3) ; it is the predominant genotype in Italy and Spain. Genotype II, which was first identified in Japan, predominates in Taiwan, while genotype III has so far only been found in the Amazon Basin.

Recent surveys show that HDV infection rate has considerably diminished in Southern Europe, which represented an area of HDV endemicity in the 1970s-1980s. Prevalence rates of chronic hepatitis D in HBsAg carriers in Italy have declined from 25% at the beginning of the 1980s to 10% now, and a similar drop in prevalence rates has been reported in Spain. Better public health standards, HBV vaccination and the effect of measures introduced to control the spread of HIV (which is transmitted in the same way as HBV/HDV) have brought about a decline in the numbers of HBsAg carriers and therefore a fall in hepatitis D (9,10).

Diagnosis

Finding the HDV-RNA and/or its genome product HDAg in the liver or in serum is the most accurate way of diagnosing HDV infection (11,12,13). Immunohistology may turn up false negative results in the advanced stages of the disease, however, as the percentage of liver cells expressing HDAg decreases with the progression of chronic hepatitis D. The diagnostic method of choice is now reverse transcription PCR which can detect 10 to 100 copies of the viral genome in serum.

The highest degree of efficacy is obtained through the amplification of the C-terminal segment of the HDAg coding region. Though the genomes for both small and large delta proteins of HDV have been characterised in the serum of chronic hepatitis D patients, no specific correlation has been found between the ratio of the molecular forms and the clinical outcome. Direct in situ-hybridisation assays to detect HDV-RNA in fixed liver tissue with non-radioactive procedures have been developed but these are not available outside research laboratories.

Detection of indirect antibody markers is the first step in diagnosing HDV (11,15). These markers are the IgM antibody to HDAg (IgM anti-HD), measured with μ capture immunoassays, and total antibody to the HDAg (anti-HD) which predominantly detects the IgG antibody and is measured with competitive radioimmunoassays ; the standard antigen used in the various assays is recombinant HDAg. The first reaction to arise in primary infection is the IgM anti-HD. This persists as the disease progresses to chronicity and is usually detectable in high titres in patients with chronic hepatitis D ; it is principally composed of monomeric 7S IgM molecules in chronic infections, in contrast to the predominance of 19S pentameric molecules in primary infection (16).

In immunocompetent patients with chronic hepatitis D the IgG antibodies develop a few weeks after primary infection and rise to high titres as the infection progresses. These antibodies do not protect against the disease. They may also be a serological scar to double HDV/HBV infections that resolved ; the IgG antibody titres are low in past infections.

Natural history

Infection with HDV comes about through coinfection with the HBV or through superinfection of an HBV carrier. People with the HBsAg antibody (anti-HBs) in serum are also protected from HDV (4,11).

The activation of HDV is dependent on the prior activation of HBV in ordinary people coinfected simultaneously by the two viruses. In this dynamic situation HDV expression can vary from a very virulent to an abortive one. The underlying HBV infection is usually selflimited, however, and the HDV coinfection resolves, with only 2% of coinfections becoming chronic. In carriers superinfected with HBsAg, the HBV colonises the infected host thus providing HDV with the complementary biological support it requires to develop. Such carriers run a strong risk of becoming chronic carriers of HDV as in over 90% of cases the disease becomes chronic.

HDV infection can produce a broad spectrum of clinical conditions which range from the asymptomatic carrier of the virus to the most severe forms of hepatitis and there is considerable variation in the natural history of chronic HDV infections (4). In early studies from clinical centres HDV was linked to progressive and severe disease, while surveys in open populations have shown that HDV infection is also possible without liver damage. Anti-HD-positive people without liver disease largely outnumbered those with hepatitis in two studies reporting in American Samoa and Archangelos on Rhodes Island in Greece.

By examining all of the data collected in previous studies, together with the findings of studies on the evolution of chronic hepatitis D, the following may be inferred (17).

1) In a minority of patients, mostly drug addicts, with double active HBV and HDV infections, the disease can run a rapidly progressive course which results in liver failure from a few months to 2 years. However

retrospective analysis of patients with a rapidly progressive course of hepatitis D published in the 1980s has revealed that several of them also had HCV coinfection ; thus rather than representing a separate clinical entity of HDV infections, this rapidly progressive form may be the result of HCV/HDV/HBV coinfections.

2) In about 15% of patients the course of the disease is benign and not progressive.
3) In most HDV carriers the disease quickly causes alterations of the liver architecture which within a few years leads to anatomical cirrhosis (43), whereupon the cirrhosis often remains clinically stable for decades. Nevertheless in endemic areas the majority of HBsAg carriers with chronic hepatitis D have been exposed to HDV in adolescence or as young adults and present clinically with signs of decompensated cirrhosis in their forties or fifties, which is ten to twenty years earlier than patients presenting with HBV or HCV cirrhosis. The risk of hepatocellular carcinoma in patients with HDV cirrhosis is comparable to that of cirrhotic HBV patients.

Hepatitis D is indistinguishable from ordinary hepatitis B on both clinical and histological grounds. The key to accurate diagnosis is specific serologic testing which reveals markers of HDV infection coexisting with markers of HBV infection and their combination varies according to the degree of virus and disease expression. The HBsAg and IgM anti-HBc are the markers of HBV infection, and the presence of IgM anti-HBc in high titres is a requisite for diagnosing HBV/HDV coinfection as opposed to superinfection. An early antigenemic phase is usually not detectable in ordinary coinfection hepatitis, and diagnosis relies on the increase in IgM anti-HD.

The IgG antibody also rises, but this takes place several weeks after the rise in the IgM antibody and sometimes appears during convalescence. The IgM anti-HD may not appear at the onset of the disease, emerging from one to two weeks later, and it may be necessary to repeat testing for this marker over several weeks to diagnose a coinfection (15).

In acute HBV/HDV coinfections the clinical presentation and course vary according to the degree of HBV and HDV expression and the interplay between the two viruses. As a rule, clinically overt coinfection hepatitis is severe and icteric, with the full, though transient, expression of the two viruses.

The more severe forms of hepatitis D are accompanied by the full battery of HDV markers including early HD-antigenemia. It is possible to detect HDV-RNA very early on in the course of infection, which quickly disappears with resolution of the disease. Persistence of the HBsAg and increased titers of antibodies to HDV mark evolution to chronicity (4).

HDV superinfection usually presents as overt hepatitis in previously healthy carriers, or it may result in hepatitis flares in carriers with preexisting chronic hepatitis B. The acute disease is usually severe and sometimes fulminant. Both the clinical and the histological features of the disease are the same as those of acute hepatitis B. Diagnosis is based on the early presence of HD-antigenemia and viremia and on the prompt and vigorous rise in both the IgG and IgM antibodies to HDV. The IgM antibody to HBcAg is usually absent or present in low litres.

Histology shows a mild portal and periportal hepatitis with little or no fibrosis in about 15% of cases of chronic hepatitis D. In the other cases histology reveals extensive inflammation and necrosis with widespread lobular involvement, and the hepatocytes can show an eosinophilic type of degeneration which is not accompanied by the peripolesis of lymphocytes (18). Roughly 40% of these patients will develop cirrhosis in 3 to 5 years (18). HDV carriers with normal liver enzymes can also have the disease.

Though it is true that no clinical feature distinguishes chronic hepatitis D from other types of chronic viral hepatitides, chronic hepatitis D patients with long standing disease frequently present a marked enlargement of the spleen and often report an episode of acute hepatitis in the past, which probably corresponds to primary superinfection.

Prevention and therapy

A safe and efficacious prophylaxis against hepatitis D is vaccination against HBV, as it prevents the helper infection on which HDV thrives. The dramatic decline in endemic HDV infection after the introduction of HBV vaccination in Southern Europe supports this conclusion.

As yet Interferon alfa (IFN) is the only drug licensed for the treatment of chronic hepatitis D (19,20).

However, only a minority of patients appear to benefit fully from IFN, as even though from 40% to 70% of treated patients may normalise liver enzymes during therapy, a very high percentage - from 60% to 97% - of the responders relapse when treatment is suspended. Permanent response is indicated by progressive decline of IgM anti-HD, the disappearance of HDV-RNA during treatment and by the subsequent seroconversion from HBsAg to anti-HBs, a serological event which marks the ultimate endpoint of successful therapy.

At present the best approach is treatment with doses of from 5 to 9 MU three times a week, prolonging therapy for 12 months after normalisation of aminotransferases has been obtained. This regimen may, however, cause significant side effects.

Generally speaking, there is more likely to be a good response in patients with the early disease, i.e. without fibrosis at histology and with an infection of short duration, than in patients with scarring and long-standing disease. Thus the already mentioned recent epidemiologic decline of HBV and HDV infection in Southern

Europe and the marked fall in the numbers of new cases of both infections has meant that chronic hepatitis D has become more resistant to IFN and the results of therapy are even less promising than those reported in the clinical trials of the 1980s. This is probably because there are proportionately fewer fresh forms of HDV infection than patients with long-standing advanced disease.

Transplantation

A valid therapeutic measure for end-stage HDV disease is liver transplantation (21,22). Paradoxically enough, compared to ordinary HBV infection alone the dual HBV/HDV infection provides protection from subsequent graft reinfection. The concomitant HDV infection represses HBV synthesis in most cases to the extent that HDV transplants are not able to transmit the HBV required to rescue HDV in the liver graft. In the two largest reported studies of HDV transplants, the reinfection rate has dropped to 9% and 12% thanks to the introduction of prophylaxis with standard hepatitis B immunoglobin. Survival has been excellent. No patients have died in 45 liver transplants in Turin over 8 to 10 years of follow up and in Paris in 76 transplants the survival rate has been 88% after five years. HDV recurrences are apparently accompanied by less severe disease than ordinary HBV infection. There is no established therapy for recurrent hepatitis D.

References

1. GERIN J.L. The taxonomy of Hepatitis Delta Virus. *In* : Nishioka K., Suzuki H., Mishiro S., Oda T. (eds). Viral Hepatitis and Liver Disease. Springer-Verlag Tokio, 1994 : 63-64.
2. TAYLOR J.M. Structure and replication of hepatitis delta virus. *Seminars in Virol.*, 1990, **1** : 135-141.
3. CASEY J.L., POLSON A.G., BASS B.L., GERIN J.L. Molecular biology of HDV : analysis of RNA editing and genotype variations. *In* : RIZZETTO M., PURCELL R.H., GERIN J.L., VERME G. (eds). Viral Hepatitis and liver disease, Minerva Medica, Torino, 1997 : 290-294.
4. SMEDILE A., RIZZETTO M., GERIN J.L. Advances in Hepatitis D Virus biology and disease. *In* : BOYER J.L., OCKNER R.K. (eds). Progress in Liver Disease, WB Saunders Company, Vol XII, 1994 : 157-175.
5. RIZZETTO M., PONZETTO A., BONINO F., SMEDILE A. Hepatitis Delta virus infection : clinical and epidemiological aspects. *In* : ZUCKERMAN A.J. (eds). Viral Hepatitis and Liver Disease Alan Liss, New York, 1988 : 389-394.
6. LIAW V.F., CHIN K.W., CHEN C.M., *et al.* Heterosexual transmission of hepatitis delta virus in the general population of an area endemic for hepatitis B virus infection : a prospective study. *J. Infect. Dis.*, 1990, **162** : 1170-1172.
7. SAGNELLI E., STROFFOLINI T., ASCIONE A. *et al.* The epidemiology of hepatitis delta infection in Italy. *J. Hepatol.*, 1992, **15** : 211-15.
8. NIRO G.A., CASEY J.L., GRAVINESE E. *et al.* Intrafamilial transmission of hepatitis delta virus : molecular evidence. *J. Hepatol.*, 1999, **30** : 564-569.
9. HADZIYANNIS S.J. Hepatitis Delta : an overview. *In* : RIZZETTO M., PURCELL R.H., GERIN J.L., VERME G. (eds). Viral Hepatitis and liver disease, Minerva Medica, Torino, 1997 : 283-289.
10. GAETA G. Hepatitis D in Europe ; a vanishing disease ? Submitted.
11. NEGRO F., RIZZETTO M. Diagnosis of hepatitis delta virus infection. *J. Hepatol.*, 1995, **22** (suppl.) : S 136-139.
12. ZIGNEGO A.L., DENY P., FERAY C. *et al.* Amplification of hepatitis delta virus RNA sequences by polymerase chain reaction : a tool for viral detection and cloning. *Mol. Cell. Probes*, 1990, **1** : 43-51.
13. TANG J.R., COVA L., LAMELIN J.P. *et al.* Clinical relevance of the detection of hepatitis delta virus RNA in serum by RNA hybridization and polymerase chain reaction. *J. Hepatol.*, 1994, **21** : 953-960.
14. JARDI R., BUTI M., COTRINA M., RODRIGUEZ F. Determination of hepatitis delta virus RNA by polymerase chain reaction in acute and chronic delta infection. *Hepatology*, 1995, 21 : 25-29.
15. FARCI P., ARAGONA M., CRIVELLI O. *et al.* Diagnostic and prognostic sign)ficance of the IgM antibody to the hepatitis delta virus. *JAMA*, 1986, **225** : 1443-1446.
16. MACAGNO S., SMEDILE A., CAREDDA F., OTTOBRELLI A., RIZZETTO M. Monomeric (7S) immunoglobulin M antibodies to hepatitis delta virus in hepatitis type D. *Gastroenterology, 1990,* **98** : *1582-1586.*
17. ROSINA F., CONOSCITORE P., CUPPONE R. *et al.* Changing pattern of chronic hepatitis D in southern Europe. *Gastroenterology*, 1999, **117** : 161-166.
18. RIZZETTO M., VERME G., RECCHIA S. *et al.* Chronic HBsAg hepatitis with intrahepatic expression of delta antigen. An active and progressive disease unresponsive to immunosoppressive treatment. *Ann Inter. Med.*, 1983, **98** : 437-41.
19. ROSINA F., RIZZETTO M. Treatment of chronic type D (delta) hepatitis with alpha interferon. *Semin. Liver Dis.*, 1989, **9** : 264-266.
20. FARCI P., MANDAS A., COIANA A. *et al.* Treatment of chronic hepatitis D with interferon alfa2a. *N. Engl. J. Med.*, 1994, **330** : 88-94.
21. FAGIUOLI S., LEANDRO G., BELLATI A. *et al.* Liver transplantation in Italy : preliminary 10-year report. *Ital. J. Gastroenterol.*, 1996, **28** : 343-350.
22. WRIGHT T.L., PEREIRA B. Liver transplantation for chronic viral hepatitis. *Liver Transplant Surgery*, 1995, **1** : 30-42.