

Hepatitis D in Children

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ABSTRACT

Hepatitis D virus (HDV) is an uncommon, defective, single-stranded circular RNA virus that is dependent on the hepatitis B virus' surface antigen envelope proteins for transmission. It is highly pathogenic and associated with high rates of progression to cirrhosis and associated complications. HDV continues to ravage endemic parts of Asia and Europe, and its prevalence in the United States, although low, has not decreased in frequency, despite universal hepatitis B virus vaccination, because of lack of testing and underrecognition. There are few reports on the prevalence and characteristics of HDV infection in the pediatric population. We present 2 patients with HDV infection at our institution; both were from eastern Europe and were treated with pegylated interferon- α . The present standard of care treatment for HDV yields suboptimal results, but insights into the virology of hepatitis D are stimulating the search for novel therapeutic approaches, particularly the development of prenylation inhibitors and viral entry inhibitors.

Key Words: hepatitis D virus, hepatitis delta, pediatrics

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Hepatitis D virus (HDV) is an uncommon, defective, single-stranded circular RNA virus that is dependent on the hepatitis B virus' surface antigen (HBsAg) envelope proteins for transmission (1). HDV is the smallest virus known to infect humans, yet it is highly pathogenic with a rapid evolution to cirrhosis (2). There are ≥ 8 known genotypes of HDV, with genotype 1 being the most common in the world and the United States (15,16). Mutations of the virus, however, can be associated with variable clinical outcomes (17). HDV can be transmitted perinatally and through sexual body fluids and blood. The transmission of HDV exists in 2 patterns: co-infection known as simultaneous hepatitis B virus

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What Is Known

- Hepatitis D is a highly pathogenic RNA virus dependent on the hepatitis B virus surface antigen for transmission.
- Existing literature on the presentation and treatment of hepatitis D virus in hepatitis B virus–infected children is limited.
- Hepatitis D virus screening via hepatitis D virus antigen, anti-hepatitis D virus antibodies, and RNA testing remains important in patients with chronic hepatitis B, with goals to quantitatively measure hepatitis D virus RNA levels in the near future.

What Is New

- Recently, increasing hepatitis D virus infection rates have been noted in the United States and abroad.
- Although treatment with pegylated interferon is limited in efficacy, newer alternative antiviral agents are being studied, including prenylation inhibitors, bile salt transporters, and nucleic acid-based amphiphathic polymers.

(HBV) and HDV infection, and superinfection, which is typically HDV infection of a patient with chronic hepatitis B (CHB) (2). Co-infection has been associated with more serious acute liver disease such as liver failure. In most patients, co-infection of HBV and HDV results in the clearance of both infections. Superinfection of HBsAg-positive individuals, however, may lead to acute hepatitis but frequently progresses to chronic infection with higher risk of cirrhosis.

In the 1980s, the worldwide prevalence of chronic HDV infection was ~ 15 to 20 million (3). HDV is generally more prevalent in populations of low socioeconomic status. Its prevalence is the highest in the Mediterranean basin, the Middle East, the Amazonian basin, and selected regions of central Asia and sub-Saharan Africa (Fig. 1) (2). In the 1990s, there was a reported decline in the prevalence of chronic HDV infection in several countries, including Italy (4), Spain (5), Turkey (6), and Taiwan (7), largely attributed to better control of HBV infection (8). Initial declines in prevalence rates in certain regions of Europe, however, have been offset by increases because of immigration from endemic areas. Vaccination against HBV is an effective means to protect against HDV infection because HBsAg is required for HDV to infect hepatocytes. More recently however, there seems to be a growing prevalence of HDV infection in the United States and abroad. Gish et al (9) found that an alarming 8% of patients with chronic HBV in northern California were co-infected with HDV; 63% of these patients were born in the United States. Higher than expected prevalence rates have also been reported in Europe, the Brazilian Amazon region, the Mediterranean basin, and Vietnam (10–13), with rates ranging from 3.7% of patients with CHB in

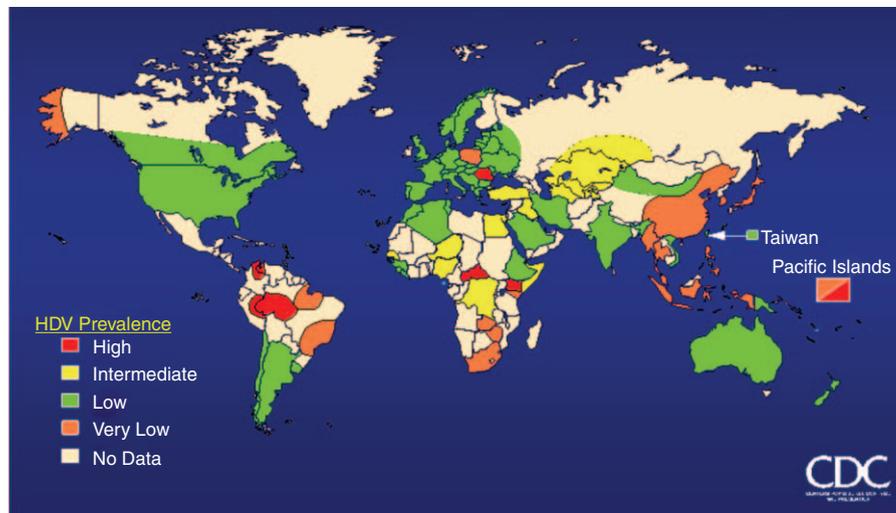


FIGURE 1. Worldwide distribution of hepatitis delta infection. HDV = hepatitis D virus. Reprinted from the Centers for Disease Control and Prevention, Atlanta, GA.

Belgium to 10.7% in Vietnam, 13.5% in the Brazilian Amazon region, and 27.8% in the Mediterranean basin.

There is limited literature on the prevalence of perinatal HDV transmission. A study of 185 HBsAg carrier pregnant Saudi women revealed that 9.7% were anti-HDV positive. A follow-up of 17 infants of anti-HDV positive mothers revealed that none of the infants at 7 months showed evidence of HDV infection. Thus, it has been suggested that vertical transmission is relatively uncommon and that acquisition of HDV infection occurs primarily through horizontal transmission (14).

There are few reports on the prevalence and characteristics of HDV infection in the pediatric population (Table 1) (18,19,21,23,64). A case series of 4 HDV-infected Taiwanese children treated from 1977 to 1986 reported 3 patients who acquired HDV through co-infection and 1 through superinfection. Two of the patients were infected through horizontal transmission from caregivers. All of the 4 children had relatively benign courses, leading to the authors' conclusion that the natural course of HBV infection in Taiwanese children was minimally affected by HDV infection. Their data suggests that high levels of HBV replication may have resulted in the suppression of HDV in their patients (18). A Turkish study reported that the prevalence of HDV in chronically HBV-infected children of western Turkey was 1.76% (3/170). The prevalence of histological cirrhosis demonstrated a positive linear relationship with the number of years following acute HDV infection, and all of the 3 children infected with HDV had cirrhosis on biopsy (19). In another Turkish study, 6 of the 206 children with CHB (mean age 7.76 years [SD 3.70]) were infected with HDV: 3 with cirrhosis, 2 with chronic active hepatitis, and 1 with chronic persistent hepatitis. In contrast to chronic persistent hepatitis, which is considered benign and defined by uncomplicated portal inflammation, chronic active hepatitis takes a more aggressive course and is associated with hepatocellular necrosis and fibrosis leading to cirrhosis (20). Four of the patients tested positive for hepatitis B e-antigen (HBeAg) and 2 tested positive for hepatitis B e-antibody (anti-HBe). During a 4- to 7-year follow-up period, none of these patients decompensated (21). Craxi et al (22) reported that children infected with HDV typically have minimal symptoms and high levels of HBV replication in the liver with the presence of hepatitis B core antigen (HBcAg) in the liver and HBeAg and HBV DNA in the serum. In a case series by Kay et al (23), both of their patients infected with HDV were adopted from eastern Europe

(Romania and Albania) and treated with interferon (IFN)- α for 1 year. Although both had biopsies consistent with chronic active hepatitis and initially had elevated HBV viral loads and alanine transaminase (ALT) before treatment, only 1 of the 2 patients responded favorably to IFN- α with HBV seroconversion and undetectable HBV DNA after 33 weeks of treatment. This resulted in decreased inflammation of the liver parenchyma on repeat biopsy, and immunohistochemical stains showed no detectable HBsAg or HBeAg.

In adults, co-infection of HBV with HDV is usually self-limited but can also be associated with a severe form of acute hepatitis and may lead to fulminant hepatitis. Approximately 20% of patients with co-infection progress to cirrhosis (36). More common, however, is the superinfection route of acquiring HDV. Superinfection of HDV leads to chronic HDV infection 90% of the time, resulting in cirrhosis in 5 to 10 years in 70% of patients (37). The progression to cirrhosis is more rapid, and the incidence of cirrhosis is 3 times higher with HDV infection when compared with HBV infection alone (37).

Typically, the adult patient with HDV has HBsAg in the serum but no markers of HBV replication, in addition to an elevated ALT and a liver biopsy showing significant hepatitis (44). HDV has been shown to suppress HBV replication. It is postulated that liver damage is primarily from HDV (45). Viral replication of HBV, however, varies during the disease course of HDV. During the initial phase, HDV replicates, and HBV is suppressed. This is followed by a second phase in which HDV starts to decrease with reactivation of HBV. Finally, the late phase occurs when replication of either virus causes cirrhosis or hepatocellular carcinoma (HCC), or reduction of both viruses leads to remission (46). Some patients with HDV and HBV seroconvert spontaneously or following IFN- α treatment, as previously reported (47). Even with seroconversion and years of disease remission, the persistence of occult HBV infection can lead to HCC development in patients with cirrhosis (48). The association between HDV infection and HCC risk is controversial, with some studies showing no correlation and others citing a 3-fold increase in risk (49). Decreased liver size and signs of more severe portal hypertension have been associated with HDV-associated HCC compared with HBV mono-infection (50).

A recent retrospective cross-sectional study of 426 Chinese patients co-infected with HDV and HBV (total 6604 HBV positive patients) revealed that HBV/HDV co-infected patients tend to

TABLE 1. Pediatric case reports of hepatitis delta virus

Author	No. patients	Country	Age, y	Follow-up period	HBV status	Cirrhosis	Treatment	Outcome
Hsu et al (18)	4	Taiwan	0.3–8	1–4 y	3 acute; 1 chronic	—	—	All stable; 3 seroconverted
Kay et al (23)	2	Romania; Albania	2 and 4	0.5 y	2 chronic	0	IFN- α , 33 wk	1 seroconverted; 1 with persistent chronic inflammation
Yuce et al (21)	6	Turkey	8–13	4–7 y	6 chronic	3	—	All stable
Di Marco et al (64)	26	Italy	—	12 mo	26 chronic	—	IFN- α , 12 or 24 mo	Normalization of ALT in 12; 8/10 remained HDV RNA+; 2 cleared HDVAg from liver
Ozgenç et al (19)	3	Turkey	—	—	3 chronic	3	—	—

— Indicates unavailable or undocumented information. ALT = alanine aminotransferase; HBV = hepatitis B virus; HDV = hepatitis D virus; HDVAg = hepatitis D virus antigen; IFN = interferon.

progress to more severe liver disease. HBV/HDV co-infected patients had less HBV DNA levels and were less likely to have positive HBeAg compared with HBV-infected patients. HBV/HDV co-infected patients with end-stage liver disease (ESLD), however, had similar HBeAg and HBV DNA profiles compared with HBV-infected patients. Therefore, the rapid progression of ESLD in co-infected patients may be mediated by increasing HBV DNA levels in the late phase. Furthermore, compared with patients with CHB, patients with chronic HDV had higher transaminase levels, lower platelet counts and prothrombin activity, and higher frequencies of necroinflammation and significant fibrosis on liver biopsy (82).

Between 40% and 60% of cases of fulminant hepatitis in adults have positive delta markers, indicating that HDV infection plays a role in massive liver necrosis (38). Fulminant liver failure has been reported to occur in ~1% of patients with co-infection, compared with 5% of superinfected patients (39). Initial signs of HDV infection are generally nonspecific. Most patients who become superinfected with HDV have a chronic persistent course (40). Chronic HDV infection is a risk factor for both cirrhosis and HCC, and persistent HDV replication is a predictor of liver failure–related mortality (41). Levels of HDV viremia are higher in patients with early chronic hepatitis compared with patients with cirrhosis (42). Data suggests that liver transplant (LT) in patients co-infected with HDV has a better outcome compared with patients with HBV alone (43).

PATIENT PRESENTATIONS

Because literature on the presentation and treatment of HDV in children is scarce, we present 2 patients with HDV infection at our institution who were treated with pegylated IFN- α . Patient 1 was an adopted Ukrainian 11-year-old girl who presented to our institution in 2010 with CHB after being diagnosed at an outside institution at the age of 6 and having reportedly failed treatment with pegylated IFN 1 year before her HBV. Her first liver biopsy performed at age 8 had revealed moderately active chronic hepatitis without fibrosis (Fig. 2A), but a repeat biopsy done 1 year later because of persistently elevated transaminases showed early bridging fibrosis, prompting her treatment. There was no significant medical or surgical history and no reported family history from her biological parents. She continued to demonstrate elevated liver biochemistries, and upon presentation, her ALT was 93 U/L and aspartate transaminase (AST) was 86 U/L despite evidence of seroconversion (HBeAb+ and HBeAg– with an undetectable viral load). Given her negative HBV DNA quantitative polymerase chain reaction (PCR), nucleos(t)ide analogues were not considered. Subsequently, a repeat liver biopsy revealed more severe bridging fibrosis, prompting us to rule out HDV and other concomitant liver diseases such as autoimmune hepatitis, Wilson disease, and α -1 antitrypsin deficiency. A qualitative serum HDV RNA PCR yielded a positive result, and HDV Ag staining of the liver biopsy performed at Stanford returned positive, prompting repeat treatment with pegylated IFN- α -2a (180 μ g \cdot 1.73 m² \cdot week) because ~25% of patients achieve HDV viral suppression following 48 weeks of repeat therapy (24). The patient reported some fatigue and anorexia but had no jaundice, icterus, pruritus, easy bruising, or bleeding. She reported compliance with her medications. The patient achieved undetectable HDV RNA levels 8 months into the treatment course with normalization of liver biochemistries, but at 48 weeks, the patient demonstrated the presence of HDV RNA, at which point, the patient’s father decided to discontinue treatment. At that time, HBV DNA also became detectable at 39 IU/mL for the first time with elevated liver biochemistries (ALT 46 and AST 55). Two months later, the patient continued to have detectable HDV RNA with HBV

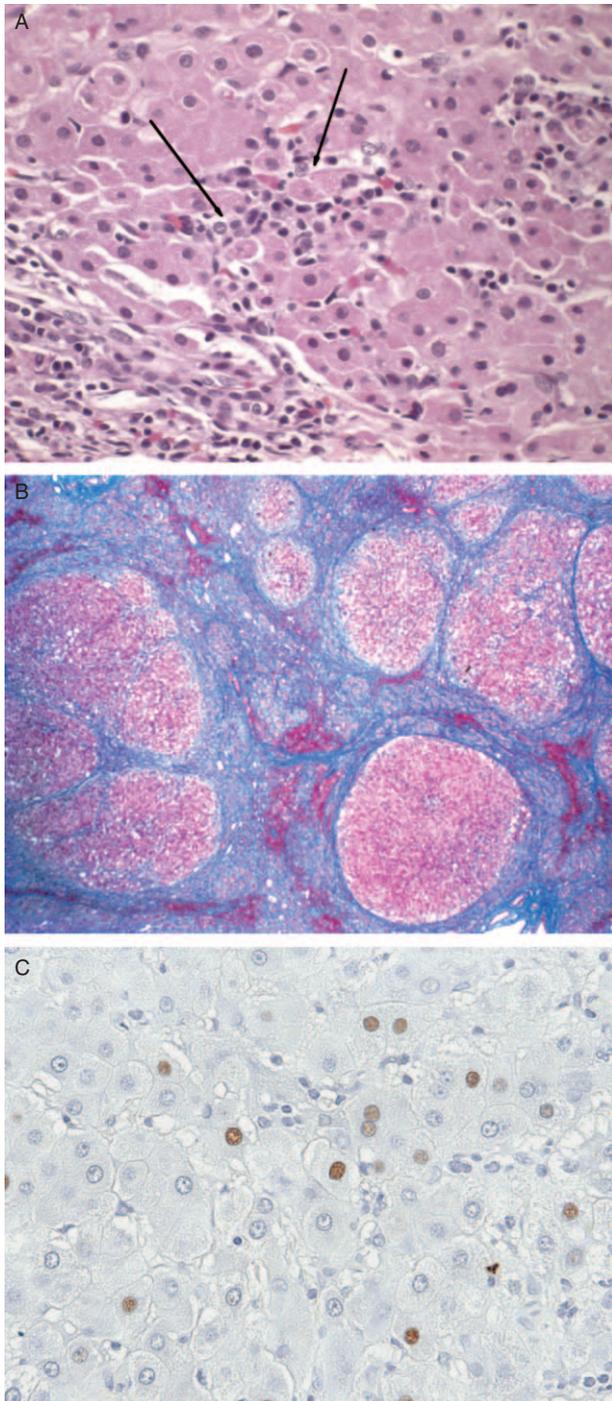


FIGURE 2. Histopathological features of hepatitis delta. A, Hematoxylin & eosin stain; original magnification $\times 400$. Infected hepatocytes undergoing cytototoxic changes (arrows) including shrunken eosinophilic cytoplasm, pyknotic nuclei, and mild inflammation in the surrounding parenchyma. B, Trichrome stain; original magnification $\times 25$. Multifocal biliary cirrhosis with collagen in blue. C, Original magnification $\times 40$. Immunohistochemical stain of infected hepatocytes positive for hepatitis D surface antigen. Images courtesy of Dr Milton Finegold.

DNA elevated at 98 IU/mL, ALT 54, and AST 54. She has been lost to follow-up since mid 2012.

Patient 2 first presented to our institution in 2008 from Uzbekistan with a diagnosis of HBV at age 15. Despite laboratory reports consistent with inactive chronic HBV (HBsAg+, HbeAg–, and HBV DNA <100 IU/mL), he had developed significant liver disease, however, with hepatosplenomegaly, jaundice, and elevated liver biochemistries (AST 474 and ALT 205). Ultrasound demonstrated recanalization of the periumbilical vein, consistent with portal hypertension. As done with patient 1, we excluded other concomitant liver diseases, and serum HDV Ag was negative. Ten days later, liver biopsy revealed micronodular cirrhosis (Fig. 2B) with active chronic hepatitis and cholestasis, and weak focal hepatitis B surface antigen positivity. Delta virus antigen, however, was found in the nuclei of scattered hepatocytes (Fig. 2C), consistent with HDV superinfection. His liver function was well preserved however. The patient was started on empiric entecavir treatment for HBV given the detectability of viral load on repeat testing (230 IU/mL) but continued to have detectable HDV RNA, so the patient was started on pegylated IFN- α -2a (180 $\mu\text{g} \cdot 1.73 \text{ m}^2 \cdot \text{week}$). Subsequently, he developed worsening jaundice, ascites, thrombocytopenia, leukopenia, and coagulopathy with intermittent gingival bleeding and epistaxis. The patient was urgently listed for LT in 2009 because of acute-on-chronic liver failure. The patient has remained HBsAg and HbeAg negative with undetectable HBV DNA for >5 years while on tenofovir despite being on sirolimus and mycophenolate mofetil.

In summary, 1 patient developed liver failure and required LT at 16 years of age, whereas the other was an 11-year-old precirrhotic patient who failed treatment with pegylated IFN on 2 occasions. Of note, both were from eastern Europe. Patients with HDV in addition to HBV demonstrate a much faster progression of liver fibrosis, as seen in our 2 patients. Unlike reports of HDV in pediatric patients, our 2 patients had undetectable HBV levels at the time of HDV detection with signs of advanced liver disease, indicating the rapid progression of fibrosis, cirrhosis, and its complications associated with HDV infection.

Although the incidence of HDV infection has decreased in western Europe because of improvements in HBV vaccinations and socioeconomic conditions, it remains a significant cause of morbidity in eastern Europe (36). Because the prevalence of HDV infection is relatively low in the United States, immigrants from eastern Europe to the United States (eg, our 2 young patients from Ukraine and Uzbekistan) require a higher index of suspicion. Greater suspicion of HDV infection is also warranted in HBV-infected pediatric patients with aggressive liver disease who may be out of proportion to what is expected.

HDV LIFE CYCLE AND PATHOLOGY

In natural infections, HDV replicates solely in hepatocytes, with both direct cytotoxic and immune-mediated liver damage implicated in disease. Anti-HDV antibodies do not always persist after acute infection is cleared. The serological evidence of past HDV infection is therefore not always easy to demonstrate (Fig. 3). The life cycle of HDV begins following the uncoating of the delta virus particle within the hepatocyte (Fig. 4). This is followed by RNA-dependent RNA replication of the genome within the cell's nucleus that complexes with delta antigen while acquiring their HBV surface antigen proteins within the endoplasmic reticulum. Finally, HDV exits the hepatocyte via a secretory pathway. The pathogenesis of HDV has been reported to include IFN- α signaling inhibition, HDV-specific T cell activation and cytokines responses, tumor necrosis factor- α and nuclear factor κB signaling, and cell proteome modification mechanisms (25–27). It is known from in

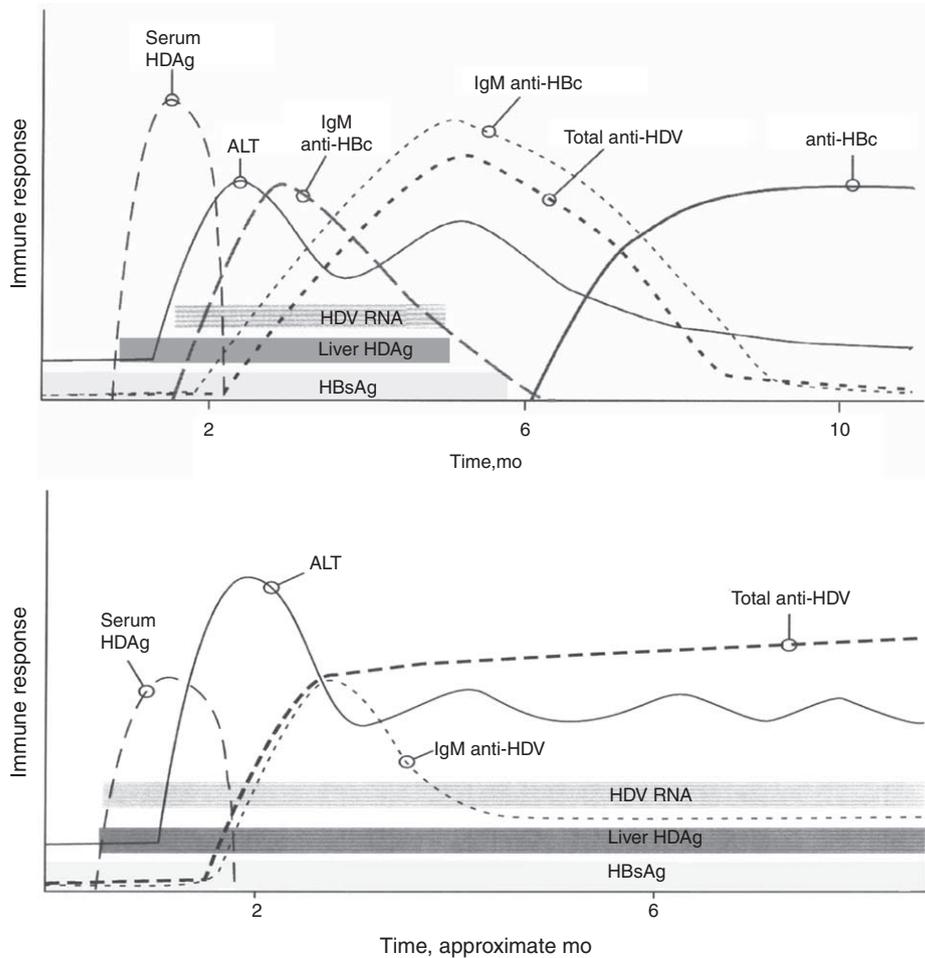


FIGURE 3. Typical serological course of HBV-HDV co-infection (top) and HBV-HDV superinfection (bottom). ALT = alanine transaminase; anti-HDV = HDV antibody; HBc = hepatitis B core antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HDV = hepatitis D virus; HDAG = hepatitis delta antigen; IgM = immunoglobulin M.

vitro and human studies that during acute HDV infection, infected hepatocytes undergo cytotoxic changes including shrunken eosinophilic cytoplasm, pyknotic nuclei, and mild inflammation in the surrounding parenchyma (34,35). Immune-mediated responses are thought to have both acute and chronic components involving both cytotoxic T cells and delayed or insufficient immune responses.

The first animal model for HDV was the chimpanzee, in which key experiments were performed on mechanisms of transmission (28,29). Interestingly, these experiments demonstrated that HDV derived from a human inoculum could be pseudotyped with the woodchuck hepatitis virus envelope proteins to generate infectious HDV-RNA-containing particles. The woodchuck system has also been used to assess candidate antiviral agents (30). A variety of mouse-based models have been used to study HDV and candidate antivirals (31,32). The most recent of these are so-called humanized mice, in which human liver cells are engrafted into the livers of immunodeficient mice. Such mice can support HDV infections and be used to assess novel antiviral strategies (33).

SCREENING AND MONITORING OF HDV

With higher than expected prevalence rates of HDV infection in many countries including the United States, it is recommended that all adult patients with chronic HBV infection be screened for

HDV and that special attention be given to patients with higher risk. We believe these adult risk factors (2) include coming from endemic regions (Mediterranean basin, eastern Europe, Middle East, Amazonian basin, Asia, Africa), intravenous drug use or exposure, men who have sex with men, hemodialysis patients, health care workers, or patients with chronic HBV and clinical deterioration have relevance in children and adolescents. The diagnosis of HDV is made serologically through the detection of HDV antigen and anti-HDV antibodies (Table 2) often in combination with HBV serologies (41). Within 2 months of HDV infection, >90% of patients will demonstrate HDV antibody. Total anti-HDV can be detected by commercially available radioimmunoassay or enzyme immunoassay kits. HDV immunoglobulin (Ig) G is produced in all patients with HDV and can persist long after the infection is cleared. Quantitation of HDV RNA via real-time PCR techniques is available commercially in Europe but only on a research basis in the United States at present. Specimens from patients suspected to be infected with HDV can be sent to the Centers for Disease Control and Prevention or other specialized HDV research laboratories for HDV RNA testing. The development of an international standardization will enable reporting in international units per milliliter and provide for uniform assessments for diagnostic and treatment monitoring purposes (51). A 1-step real-time PCR assay for the detection and quantitation of

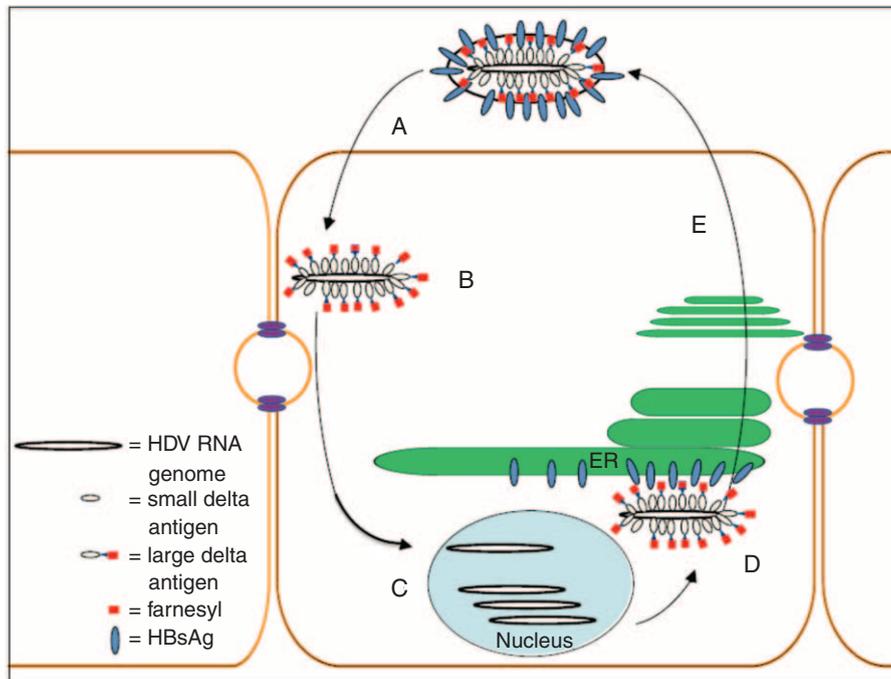


FIGURE 4. A, Hepatitis delta virus particle enters its target, the hepatocyte, and (B) after an uncoating event, (C) transports its genome to the cell's nucleus where RNA-dependent RNA replication of the HDV genome occurs. D, Nascent particles of replicated genomes complexed with delta antigen acquire their envelope containing HBV surface antigen proteins at the level of the endoplasmic reticulum in a prenylation-dependent manner, and (E) exit the cell via the secretory pathway. ER = endoplasmic reticulum; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HDV = hepatitis D virus.

HDV RNA of all of the 8 genotypes was recently developed (52). Owing to the variability of the HDV genome sequence, HDV RNA assays may yield false-negative results, if inadequate primers are used. Therefore, HDV IgM should be tested in patients with negative HDV RNA levels if there is a clinical suspicion for

HDV infection or high-risk exposures. Furthermore, quantitative assays of HDV in serum do not correlate with disease activity, although serial measurement of HDV may be useful in monitoring response in treatment (53). HDV genotyping is not widely available, although it may be clinically useful because genotype 1 is

TABLE 2. Interpretation of serological and nucleic acid testing for viral hepatitis B and D

HBV	
HBsAg	A reactive result indicates that an individual has HBV infection and is infectious
HBcAb, total	Presence indicates past or present HBV infection
HBc IgM	Presence usually indicates HBV infection within the preceding 4 to 6 months (acute infection)
HBeAb	Presence indicates resolving infection (seroconversion) or response to therapy
HBsAb	Presence indicates recovery and immunity against HBV infection or history of immunization
HBV DNA	Presence indicates active infection with viral replication
HDV	
HDV Ab, total	Reactive results, coincident with the presence of HBsAg, indicate past or present HBV/HDV co-infection or superinfection
HDV IgM	Reactive results, coincident with the presence of HBsAg, indicate past or present HBV/HDV co-infection or superinfection. Negative results in the presence of positive HDV total Ab indicates resolved infection
HDV RNA (Qual)	Presence indicates active infection

Hepatitis virus	HAV IgM	HBsAg	HBV DNA	HBc IgM	HBc total	HCV Ab	HCV RNA	HDV Ab	HDV RNA
Acute HBV	-	+	+	+	-	-	-	-	-
Acute HBV with D co-infection	-	+	+	+	-	-	-	+	+
Chronic HBV with D "superinfection"	-	+	+	-	-	-	-	+	+

HAV = hepatitis A virus; HBc = hepatitis B core; HBcAb = hepatitis B core antibody; HBeAb = hepatitis B e antibody; HBsAb = hepatitis B surface antibody; HBsAg = hepatitis B virus surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HDV = hepatitis D virus; IgM = immunoglobulin M; Qual = qualitative.

associated with a higher risk of ESLD and may have implications on therapeutic outcomes (54). The remaining genotypes are more region-specific. Genotype 2 HDV infection, found mainly in Asia, is less commonly associated with fulminant hepatitis in the acute phase or cirrhosis or HCC in the chronic phase. Genotype 3 infections, present in northern South America, are associated with particularly severe disease. Patients with genotype 4, found in Taiwan and Okinawa islands, have similar clinical courses as genotype 2 patients (54,55). Other genotypes have been most commonly of African origin.

Screening of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) is important in patients with HDV because these viruses share common transmission routes. A study in Central Europe showed that approximately one-third of the HDV-infected patients tested positively for HCV (56). In Spain, 5% of HIV infected patients with hepatitis had multiple hepatitis virus infections, of which the most common multiple hepatitis was triple B, C, and D (57).

There are no substantial pediatric series to justify liver biopsy timing. In adults, following HDV confirmation, liver biopsy to grade inflammation and stage liver fibrosis is recommended because the clinical literature has demonstrated that these patients progress to more severe liver disease relatively rapidly (60). Particularly in the setting of an HBV/HDV-co-infected child with persistently elevated ALT or clinical deterioration out of proportion to known disease, we advocate liver biopsy to guide clinical decision making (more aggressive HBV therapy, primary HDV treatment, or LT consideration).

A Baseline-Event Anticipation (BEA) score was developed to assess the risk of developing liver-related complications (decompensation, HCC, liver transplantation, and/or death) in adults with chronic HDV infection (Table 3) (59). The BEA score factors in age, sex, region of origin, bilirubin, platelets, and international normalized ratio, and stratifies patients with high accuracy into 3 risk groups: BEA-A (mild), BEA-B (moderate), and BEA-C (high). Points were allocated according to hazard ratios for the above liver-related morbidity and mortality. Although not yet validated as a tool in the pediatric population, given the aggressive nature of HDV and younger onset of cirrhosis, particularly in HDV superinfection, its utility as a risk-stratifying measure is intriguing. Applying this tool to the 2 pediatric case reports presented from our center, patient 1 would have been correctly classified as moderate risk, and patient 2 met the criteria for high risk. A modified pediatric scale merits further investigation.

PRESENT STANDARD OF CARE AND EFFICACY OF HDV TREATMENT

The goal of treating HDV infection is a sustained HDV virological response (negative HDV RNA following 6 months of

stopping treatment). Ultimately, this may be achieved via the eradication of HBV infection and clearance of HBsAg (60). Oral HBV antivirals alone, however, have not been shown to be effective in clearing HDV infection, which is to be expected given their inability to efficiently achieve clearance of HBsAg (24). First-line treatment for HDV is pegylated IFN- α , although its effects are modest, with only 25% to 30% of patients showing a sustained virological response after 1 to 2 years of therapy as measured by HDV RNA (40). Clearance rates of HDV RNA are even lower with posttreatment relapse (38). IFN- α is naturally produced by monocytes and B lymphocytes, and can be synthetically produced by genetic engineering. Patients with the best response to IFN- α are those with low HBV viral replication, high initial ALT, short duration of the chronic carrier state, and no antibody to HIV (61,62). At our site, HBV/HDV-infected children with clinical decompensation or fibrosis on liver biopsy out of proportion to their HBV disease are considered for treatment.

A meta-analysis of 5 studies using recombinant IFN in the treatment of HDV showed that although serum aminotransferase levels decreased with treatment, the response was not sustained following the discontinuation of treatment, and HDV RNA clearance was not achieved (63). Furthermore, higher doses of IFN yielded better responses: ≥ 5 MU/day or 9 MU 3 times a week for a longer period of time (12 vs 6 months). Treatment beyond 12 months, however, has not been shown to be superior (64). IFN-induced clearance of HDV RNA is also associated with a reduction in HBsAg levels, although HDV RNA levels tend to decrease first (65). It has been proposed that treatment failure with IFN- α may be secondary to interference of HDV with IFN- α intracellular signaling mechanisms, leading to impaired activation and translocation of STAT 1 and STAT2 (66). A recent study published by Lunemann et al (58) examined the effects of pegylated IFN- α treatment for chronic HDV on the phenotype and function of natural killer (NK) cells. It found that IFN- α treatment leads to a selective loss of terminally differentiated NK cells in addition to a functional impairment of NK cells and impaired STAT4 signaling. It appears, however, that a high frequency of NK cells before treatment and retained high numbers of NK cells during treatment are positively associated with treatment outcomes. In adults, NK cell count and trend may serve as an immunological biomarker for outcomes following IFN- α therapy. This has not been demonstrated in children.

Although there are few published studies on the effectiveness of IFN- α against HDV in children, a retrospective study on IFN- α treatment in Greek children with chronic HDV showed that treatment with IFN- α was safe with few adverse effects but ineffective (67). The patients were treated with IFN- α , 6 MU/m² body surface area 3 times weekly by intramuscular or subcutaneous injection. Adverse effects included mild fever, arthralgias, malaise, and

TABLE 3. BEA score algorithm for patients with HDV (59)

1 Point per condition met	Total points	BEA score	Risk group
Sex: male	0	BEA-A	Mild risk
Age ≥ 40 y	1		
Region of origin: eastern Mediterranean	2	BEA-B	Moderate risk
INR ≥ 1.2	3		
Platelets $\leq 100 \times 10^3$ /mL	4	BEA-C	Severe risk
Platelets $\leq 50 \times 10^3$ /mL	5		
Bilirubin \geq ULN	6		
	7		

BEA = Baseline-Event Anticipation; HDV = hepatitis D virus; INR = international normalized ratio; ULN = upper limit of normal.

reversible neutropenia and thrombocytopenia, with no detrimental effects on weight and height development. An observational study in Pakistan showed that in 11 children (mean age 15 ± 2.92 years) and 14 young adults treated with IFN- α for a period of 1 year, 80% were HDV-RNA negative after 1 year with minimal adverse effects from treatment and a decrease in ALT levels (68). A dosing of $6 \text{ MU} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ was applied to the children, and neither growth parameters nor hematological parameters were remarkable during and following treatment.

Pegylated IFN has been increasingly used because of its longer half-life, allowing for once weekly dosing. The HIDIT-1 trial, which studied the treatment of HDV with pegylated IFN- α in Germany, Turkey, and Greece, showed that treatment led to a 28% sustained virological response. The addition of adefovir led to a decrease in HBsAg levels but no change in virological response. Furthermore, treatment with IFN- α and additional HBV-targeted drugs (famciclovir, lamivudine, and adefovir) has not proven to be more efficacious than IFN- α alone (69). Yurdaydin et al (70) classified the response to IFN therapy into 3 groups: complete response (or sustained virological response), with negative RNA levels at 6 months; partial response, with incomplete RNA suppression at 6 months and replication following the discontinuation of treatment; and no response, with a lack of RNA reduction during treatment and follow-up.

The present standard of care for the treatment of chronic HDV is subcutaneous pegylated IFN weekly (1.5 MU/kg for α -2b), although it has not been FDA approved for use in children. Data are lacking for pegylated IFN dosing and efficacy studies in children, but in our experience with HBV and HCV, a dose of $180 \mu\text{g} \cdot 1.73 \text{ m}^2 \alpha$ -2a per week for ≥ 48 weeks in patients with HDV RNA replication and evidence of liver disease on biopsy has been found to be safe and can be effective. There are presently no pediatric recommendations to treat HDV in an effort to avoid future liver fibrosis progression. Because the published pediatric case series are unable to justify optimal timing and dosing of HDV therapy, we share our site-specific indications for HDV treatment in children based on our review of the literature: significant fibrosis on liver biopsy with or without ALT elevation despite successful suppression of HBV viral load. Particularly in the setting of unexplained elevated transaminases with evidence of hepatitis B seroconversion (HBeAg-, HBeAb+), HDV treatment should be considered. These recommendations are derived from our institution's treatment experiences in addition to extrapolation from adult literature. We advocate practical and individualized therapy, based on the clinical and virological responses during treatment.

Following a PCR-based assay at 48 weeks, patients with persistent RNA replication, anti-HDV IgM, and elevated ALT are unlikely to benefit from further treatment up to ≥ 72 weeks compared with patients with decreasing RNA, IgM, and transaminase levels. Anti-HBV nucleotide analogues are indicated in patients with reactivation of HBV following suppression of HDV replication or in patients with detectable HBsAg who cannot be treated with pegylated IFN therapy.

FUTURE OF HDV THERAPY

Present HDV treatment options are limited because HDV has no enzymatic proteins such as polymerases or proteases that can be targeted by conventional antiviral strategies. Nucleos(t)ide analogues, including famciclovir, ribavirin, tenofovir, and entecavir, which block the HBV polymerase, have also been studied with mixed results (71–74). As alluded to above, this is not surprising given the inability of these agents to efficiently eradicate HBsAg—which is necessary and sufficient for enabling the continuous spread of HDV infection. Because medical treatment for HDV is limited in

efficacy, some patients, including our male patient from Uzbekistan, decompensate from advanced liver disease, requiring LT. The only treatment option in patients with ESLD is LT, which is necessary in patients with acute liver failure with poor prognosis. To reduce the risk of reinfection following LT, long-term administration of hepatitis B immunoglobulin and antivirals can help suppress HBV, in particular HBsAg, and thus HDV infection.

Alternative antiviral agents are being studied for the treatment of HDV infection (75). Prenylation is a site-specific modification of proteins whereby a prenyl lipid—farnesyl or geranylgeranyl—is covalently attached to a cysteine within a characteristic signature motif encoded within the carboxyl terminus of prenylated proteins. Large hepatitis delta antigen (L-HDAg) contains such a motif directing the addition of farnesyl, and farnesylation is necessary for the interaction of L-HDAg with HBsAg and subsequent HDV viral assembly and secretion (76). Farnesylation inhibitors, which inhibit protein farnesyltransferase, are presently being developed to inhibit viral assembly and secretion, with promising in vitro and in vivo study responses thus far (Fig. 5) (2,83). Presently, a phase IIa double-blinded randomized placebo-controlled study is being conducted at the National Institutes of Health to investigate the effectiveness of 28 days of treatment with lonafarnib, an oral farnesyltransferase inhibitor followed by 6 months follow-up of therapy to measure sustained virological response (77).

Viral attachment targets including myristoylated synthetic peptides for the N-terminal region of the pre-S1 domain of HBsAg are also being studied for their impact on HDV infectivity (2). Sodium taurocholate cotransporting polypeptides, a group of bile salt transporters, are important in the binding of myristoylated

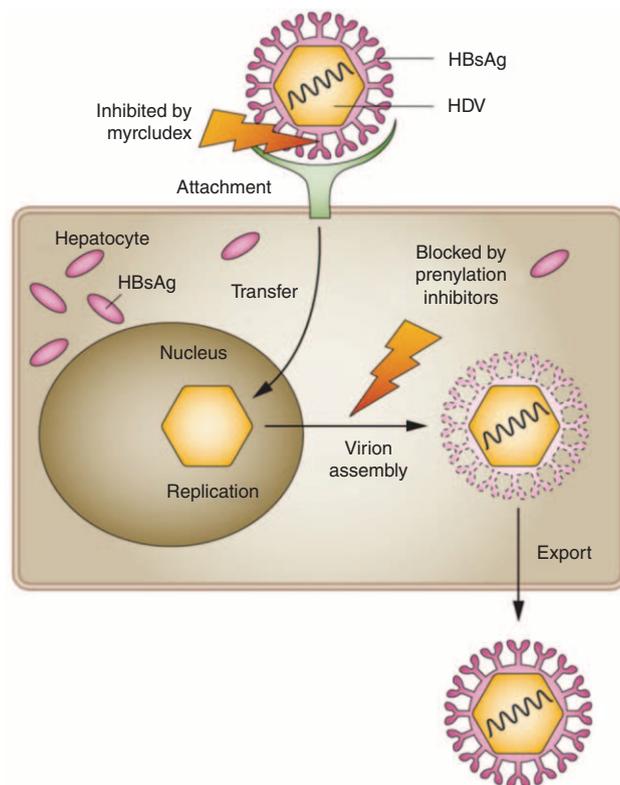


FIGURE 5. Potential targets to inhibit HDV replication. HBsAg = hepatitis B virus surface antigen; HDV = hepatitis D virus. Reprinted with permission from Nature Publishing (83).

TABLE 4. Present HDV therapeutics in development and potential future agents

	Mechanism of action	Potential agents	Phase 1, 2, or 3
Prenylation inhibitors	Inhibit protein farnesyltransferase. Prevent interaction of HDV antigen with HBsAg	EBP921 EBP994 (lonafarnib)	Phase 1 Phase 2
NAPs	Inhibit release of HBsAg from infected hepatocytes	REP 9AC	
NTCP blockers	Prevent binding of the myristoylated pre-S1 domain of the large envelope protein to hepatocytes	Cyclosporin A, Myrcludex B, ezetimibe, irbesartan, ritonavir	

HBsAg = hepatitis B virus surface antigen; HDV = hepatitis D virus; NAP = nucleic acid-based amphipathic polymer; NTCP = sodium taurocholate cotransporting polypeptide.

pre-S1 domain of the HBV large envelope protein to hepatocytes and are blocked by antibodies, cyclosporin A, ezetimibe, and Myrcludex B. Therefore, these agents and others (Table 4) may be useful in preventing HBV and HDV infection and reinfection (78). Inhibiting the release of HBsAg from infected hepatocytes is another target because nucleic acid-based amphipathic polymers are being developed for HBsAg clearance (79). Furthermore, RNA interference-based therapies may be useful in treating HBV/HDV co-infection by targeting conserved HBV sequences leading to repression of viral RNA, proteins, and viral DNA, and maybe HDV viral release (80). Cytokine levels may also be mapped in the future to monitor patients' responses to new immunotherapy agents. Additional pathway targets such as posttranslational modification of hepatitis delta antigen and virion assembly may also yield effective therapies in the future (81). Pediatric trials studying novel HDV therapies are being considered, even though none presently.

CONCLUSIONS

Although HDV is the smallest virus known to infect humans and is dependent on HBV for transmission, it is highly pathogenic, with a rapid onset of disease. The outcome of disease largely depends on whether the HBV and HDV infect simultaneously (co-infection) or whether the newly HDV-infected patient is a chronically infected HBV carrier (superinfection). Up to 70% of patients with chronic hepatitis D develop cirrhosis with mortality ranging from 2% to 20%, values that are 10 times higher than for hepatitis B. Progression to cirrhosis only takes 5 to 10 years, but can occur as early as 2 years after infection, whereas superinfection may lead to fulminant HDV, which carries a mortality rate of 80%. HDV continues to ravage endemic parts of Asia and Europe, and prevalence in the United States, although low, has not decreased in frequency despite universal HBV vaccination as a result of the lack of testing and underrecognition. All children with HBV, particularly those from high-prevalence countries or with risk factors for HDV, should be screened with total HDV Ab. In the setting of clinical deterioration, unexplained or significant ALT elevation, HDV IgM should also be performed. Children with confirmed HDV infection (total HDV Ab+ or HDV IgM+) merit an individualized evaluation, which may include a liver biopsy to guide clinical decision making. Severe or rapidly progressing fibrosis (if serial biopsies are available) warrants consideration for treatment. The present standard-of-care treatment for HDV yields suboptimal results, but insights into the virology of hepatitis D are stimulating the search for novel therapeutic approaches, particularly the development of prenylation inhibitors and viral entry inhibitors.

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