Hepatitis D Virus: A Call to Screening

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Abstract: Hepatitis D virus causes an aggressive viral hepatitis with a virulent course of progression to cirrhosis and hepatic decompensation. It relies on hepatitis B coinfection for its pathogenesis and propagation. Hepatitis D virus had become the forgotten virus, with reduced public awareness, medical interest, and research support. Recently, there has been a resurgence of awareness and interest in hepatitis D, with improvements in diagnostic testing and establishment of international collaborative efforts to improve therapy. This article provides a framework to understand the impetus for increased screening as well as to identify key issues toward which collaborative efforts can be directed.

Hepatitis D virus (HDV) causes the most aggressive form of viral hepatitis, with rapid progression to cirrhosis and hepatic decompensation in comparison with its better-known counterparts, hepatitis B virus (HBV) and hepatitis C virus (HCV). The D in HDV stands for delta, but it can be thought of as defective or destructive, given its dependence on HBV coinfection for its pathogenesis and potent clinical impact. Yet, in contradistinction to its devastating clinical effect, this unassuming virus, the smallest of all animal viruses, has been forgotten and neglected over the past 10 years by many clinicians. The discovery of the delta antigen by Dr Mario Rizzetto and colleagues in 1977 led to a period of great clinical and research interest and study with subsequent sequencing of the HDV RNA genome in 1986.1,2 The ensuing development of diagnostic antibody and molecular tests combined with treatment reports based on interferon alfa (IFN−) led to the perception that with progressive implementation of universal HBV vaccination, HDV would cease to be a significant clinical challenge. As clinical interest waned with time, there was diminished effort toward the development and standardization of diagnostic testing for both antibody and polymerase chain reaction testing for HDV. This contributed to reduced testing and decreased detection of HDV in the various populations, reinforcing the perception that HDV was not a significant clinical problem. As a result, there was a further reduction in public awareness, medical community interest, and government and industry support for HDV research and, in turn, decreased efforts to improve the accuracy and
availability of diagnostic testing, expand treatment options, and improve success rates. However, there has been a recent resurgence of awareness and interest in HDV with the work of the Hepatitis Delta International Network (www.hepatitis-delta.org) and publication of the HIDIT-2 (Hepatitis Delta International Intervention Trial 2) study. HDV has also been granted orphan disease status by the US Food and Drug Administration and European Medicines Agency, signaling the hopeful return of HDV into the consciousness of the medical community and the public at large. Much work, however, still needs to be done.

**Epidemiology**

Given the unique nature of HDV, it is important to remember that HBV patients serve as the host reservoirs of HDV superinfection. Thus, the estimate of HDV prevalence is based on the calculation that 5% of HBV patients, approximately 15 to 20 million people, are infected with HDV worldwide. However, an accurate estimate of HDV prevalence cannot be ascertained because data are not available from many parts of the world and updated data are even more scarce. HDV has been reported to be endemic in the Mediterranean Basin, Middle East, Turkey, Central Africa, and parts of South America. Early prevalence studies reported a high 20% to 30% HDV coinfection rate in HBV patients. Subsequent reports suggested a reduction in prevalence rates, generally attributed to the wider implementation of HBV vaccination. More recently, rising prevalence rates have been reported from countries that have not traditionally been known to have a significant HDV prevalence, underscoring the need for further prevalence testing. A surprising 8% to 12% prevalence was reported in Germany, attributed in part to immigration of patients from areas of high endemicity. Reports from London of 8.5% prevalence and from Greece of 4.7% prevalence bolstered the evidence that HDV has not gone away, and may, in fact, be heading toward a resurgence. In the United States, the National Health and Nutrition Examination Survey’s report of a low 3.6% (1/28) prevalence in 2003 contributed to the surprise stemming from a 2013 report from northern California showing an 8.4% (42/499) HDV coinfection rate in HBV patients. The finding that cirrhosis was present in 73% of the coinfected patients compared with only 22% of the monoinfected patients highlights the concept that HDV has an aggressive course. What was more surprising was that half (21 of 42 HBV/HDV subjects) were tri-infected with HCV and that these patients had an even higher rate of cirrhosis (80%). Interestingly, HDV prevalence does not appear to merely mirror HBV prevalence, as even within the same geographic region, there can be striking differences in reported HDV infection rates. For example, a recent report from Korea showed a low prevalence of only 3.2% (3/93), whereas a surprisingly high prevalence of 10.3% was reported from Vietnam, a striking finding compared with previous reports from this country.

These disparate rates are attributed to differences in genotypes and strains, socioeconomic differences, and various unknown differences in the risk factors and susceptibility of the local population. Higher-risk populations include injection drug users and those with high-risk sexual behavior or exposure to unregulated medical procedures or tattoos. Some of the at-risk HBV populations such as injection drug users have been reported to have an HDV rate of up to 50%, whereas the prevalence in low-risk HBV patients is 1% to 2%. In addition, it is important to recognize that reports of HDV prevalence are mutable, based not only on the region being tested and the risk factors in the screened subjects but also on the time or era in which the subjects are tested.

Due to the increased utilization of HBV vaccination, which confers protection against HBV and HDV, as well as improving health awareness, health practices, and socioeconomic conditions, the general impression has been that HDV prevalence has decreased. In fact, this is a dangerous misperception. It has actually been proposed that HDV prevalence has not decreased but that, due to diminished awareness, testing has decreased, leading to reports of reduced prevalence rates and further decreased awareness. In addition, the reported prevalence data must be interpreted with the caveat that recent data are largely lacking, with no systematic or comprehensive surveys in most countries, especially in developing areas with traditionally high HBV endemicity. The axiom that unknown does not mean unaffected holds true in this regard, particularly in this era of globalization and global migration.

This is important because knowing the true scale of the clinical problem at hand by having accurate prevalence data helps guide mobilization of public health measures to mount successful diagnosis, treatment, and prevention programs. This can help dispel the misperception that HDV is not a clinically significant problem and break the vicious cycle of diminished awareness and testing.

**Virology and Pathogenesis**

HDV is the only member of the Deltavirus genus. Derived from a plant virus, it is the smallest animal virus that affects humans, with 1679 bases encompassing a covalently closed, circular RNA genome. The HDV virion is a 36-nm spherical particle composed of an inner nucleocapsid surrounded by an outer envelope. The outer envelope is comprised of host lipids and 3 types of borrowed HBV surface antigen (HBsAg): large, medium,
and small. This dependence on HBV’s HBsAg for forming the protein outer coat of HDV virions is the reason for the dependence of HDV on HBV for propagation. The sharing of the HBsAg envelope proteins by both HBV and HDV virions underlies the assumption that HDV virion attachment and entry into cells rely on the same cell receptors. Upon entry into the hepatocyte, the virion is uncoated and the inner nucleocapsid is exposed. This inner nucleocapsid is made up of ribonucleoproteins surrounding the HDV RNA genome complexed with approximately 200 molecules of the hepatitis D antigen (HDAg) protein.20,21 The nucleocapsid is translocated to the nucleus reliant on signaling associated with HDAg.22 In the nucleus, HDV hijacks the host cell machinery for replication, translation, and posttranslational modifications to make a “complete” virion that cannot exist without HBsAg.23

The HDV RNA replication process has not been completely elucidated, but it is described as a double rolling circle mechanism and has recently been reviewed in detail elsewhere.23 The replication process involves 3 species of HDV RNA: genomic RNA (original), antigenic RNA (complement of genomic RNA), and messenger RNA that is formed without HDV replicase or RNA-dependent RNA polymerase. However, genomic and antigenic RNA do contain a ribozyme domain of approximately 80 to 100 nucleotides that is capable of self-cleavage and ligation.24,25 HDV is able to hijack the host’s replication machinery by the HDV genomic RNA’s conformation into an unbranched, rodlike formation that is able to mimic the host’s double-stranded DNA.26,27 This conformation is able to redirect the host DNA-dependent RNA polymerase into accepting HDV genomic RNA and drive replication to create multimeric linear structures that contain multiple copies of antigenic RNA.27 The multimeric structure is self-cleaved by HDV’s ribozyme domain to produce linear HDV antigenic RNA that is then re-ligated by the ribozyme to form circular antigenic RNA. Antigenic RNA then acts as a template for replication of genomic RNA for packaging into virions. HDV’s lack of an endogenous enzyme for replication means that there is no obvious HDV enzyme target for the development of direct-acting antiviral therapy.

HDV messenger RNA encodes for 2 isoforms of the only encoded protein from HDV RNA, called long HDAg (L-HDAg) or short HDAg (S-HDAg). They differ only in an extra 19 amino acids on L-HDAg and share many functional domains. L-HDAg is postulated to be critical for assembly of HDV RNA into new virions and to serve as an inhibitor of HDV RNA replication.28 Posttranslational modification of L-HDAg through processes such as prenylation has been found to be key to L-HDAg’s ability to bind to HBsAg and assist in virion assembly.29 S-HDAg is thought to support accumulation of HDV RNA in the nucleus and to facilitate HDV RNA replication.27,30 The ratio between L-HDAg and S-HDAg appears to provide the balance between viral synthesis and virion assembly.

The pathogenesis of HDV is thought to be host immune-mediated by the presentation of HDAg on infected cell surfaces, activating CD4 and CD8 pathways.31,32 Some reports have suggested a direct cytotoxic mechanism, especially in severe, acute hepatitis presentations, called replication-associated cytopathogenicity, attributed to the impact of intracellular accumulation of HDAg.33 However, follow-up reports have discounted this cytotoxic mechanism.34 Regardless, HDV appears to interfere with the host’s immune response by blocking IFN-α signaling to mute or silence the antiviral action of IFN-α.35 Thus, treatment with exogenous IFN-based therapy appears to be a logical beginning point to combat HDV and reduce HDV viremia.

Eight genotypes have been identified for HDV, with genotype 1 being the most common worldwide and in the United States. There is a 15% to 40% divergence in nucleotide sequencing between the genotypes as well as more than 15% variation within the same genotype.36 Genotypes 2 and 4 have been reported in Asia, genotype 3 in the Amazon Basin, and genotypes 5 through 8 in Africa.37 These genotypes appear to have geographic localization and a variable association with clinical presentation. One study reported a higher association with adverse outcomes and decreased survival with genotype 1. The researchers postulated that genotype 1 had higher efficiencies in assembly and replication than other genotypes, such as genotype 2, leading to more rapid and aggressive HDV virion formation and dissemination.38 However, given the limited data on the natural history of HDV, especially data correlated with genotypes, as well as the difficulty in obtaining HDV genotype testing, it appears that the full impact of HDV genotype on the clinical course and outcomes remains to be confirmed.

Interestingly, both isoforms of HDAg suppress HBV DNA replication, suggesting a mechanism for HDV dominance over HBV while maintaining HBsAg production for HDV virion envelope assembly.39 HDV also appears to be dominant over HCV, which is found in up to 30% of HDV cases, by suppressing HCV replication.40,41 Although HDV is thought to be the dominant virus over HBV and HCV, it is important to remember that viral dominance and hierarchies are fluid over time and by stage of fibrosis.42 If HDV RNA is cleared either endogenously by the host or through treatment, HBV may reactivate. HDV replication may also decrease after the host becomes cirrhotic, and HBV DNA levels may increase. In this setting, HBV reactivation and
HBV DNA replication can lead to progressive development of hepatic decompensation and/or hepatocellular carcinoma (HCC). This highlights the importance of appropriately managing the HBV component of HBV/HDV coinfection, using HBV treatment guidelines.

Clinical Presentation and Natural History

Hepatitis D is transmitted parenterally, sharing the same routes of transmission as HBV, HCV, and HIV, especially injection drug use. Only a small inoculum appears to be sufficient for infection, but perinatal transmission appears to be uncommon. Intrafamilial spread, as well as sexual transmission, is thought to be an underappreciated mode of transmission, especially in endemic regions. As HDV can be transmitted only in the presence of HBV, the percentage of individuals in a population who are infected with HBV, and the physical proximity of this HBV-infected network, are thought to have a direct influence on the risk and rapidity of HDV transmission.

There are 2 main forms of HDV presentation: coinfection and superinfection. Acute infection with HBV and HDV can occur concomitantly, leading to a hepatitis presentation after a 3- to 7-week incubation period, which is dependent on the titer of the HBV/HDV inoculum. Patients may subsequently develop nonspecific symptoms, including malaise, fatigue, lethargy, and nausea. Some also develop jaundice. Testing is positive for HBV antibodies (ie, anti-HBV core immunoglobulin [Ig] M) and also anti-HDV IgM, which is consistent with acute exposure. The majority of patients, especially adults, are able to clear HBsAg and, thus, subsequently clear the HDV infection as well. Only 5% are believed to progress to chronic HDV infection. However, acute coinfection has a higher risk of progression to acute liver failure compared with superinfection. Superinfection with HDV occurs in patients with antecedent chronic HBV. These patients present with exacerbations of previously stable chronic HBV (eg, having acute hepatitis and/or hepatic decompensation). Superinfection can also lead to acute liver failure, but the majority of these patients—up to 90%—progress to chronic HDV infection, in contradistinction to the 5% rate in patients with acute coinfection.

In either scenario, once HDV infection becomes chronic, it exacerbates disease activity and contributes to a brisk progression to cirrhosis. Three phases of progression have been proposed, beginning with an early active phase of HDV replication and HBV suppression, followed by a moderately active phase with a decline in HDV replication and reactivation of HBV, and ending with a late phase of cirrhosis or HCC due to the impact of both HBV and HDV. In general, HDV coinfection has been found to confer nearly 3 times the risk of more severe presentation and increased progression to cirrhosis than HBV monoinfection. The subsequent risk of hepatic decompensation and liver-related death is also increased compared with HBV monoinfection. However, the impact of HDV infection on the risk for HCC appears less straightforward, with conflicting reports of up to a 3-fold greater risk as well as no difference. These discordant results may be due to differences in the studied HDV populations and the years in which the studies were conducted, which may be an indirect reflection of the duration of HDV/HBV coinfection. What is clear is that further, well-planned studies are needed to clarify the issue of HDV impact on HCC risk.

Diagnosis

Currently, HDV antibody testing is not routinely performed in patients with chronic HBV infection in the United States despite updated prevalence data that suggest a higher-than-expected prevalence in these patients. As mentioned previously, this only serves to perpetuate the cycle of inaccurate prevalence data about, indifference toward, and neglect of HDV. Based on the available prevalence data and the clearly recognized negative impact of HDV on clinical outcomes, we believe that HDV testing should be obtained in all patients with chronic HBV infection, especially those with a history of injection drug use or high-risk sexual behavior, as well as all hemodialysis patients, immigrants from high-prevalence countries, and, most importantly, all patients with advanced liver disease regardless of their ethnicity or place of birth.

Screening can be performed with the commercially available test for HDV antibodies (anti-HDV IgG and IgM), which appear starting approximately 4 weeks after exposure. A positive test result suggests previous or ongoing exposure to HDAg but does not imply protective immunity against HDV. A positive result should be followed with an HDV RNA test to confirm viremia. However, HDV RNA qualitative and quantitative tests have been limited by a lack of standardization, calibration, and broad commercial availability. Many tests have relied on specialized laboratory in-house protocols that lacked internal controls or had limited genotype coverage, leading to concerns of false-negative results. These tests have also been used for treatment monitoring despite their limitations, further contributing to the uncertainty regarding diagnostic parameters for treatment initiation, monitoring, and cessation. However, multiple recent studies have reported the development of improved HDV RNA testing, raising hope that these tests will offer improved pan-genotypic accuracy and will have widespread commercial availability. The availability of an accurate, standardized, reproducible HDV RNA test will allow improved clinical treatment by facilitating determi-
nation of treatment response, formulation of parameters for treatment initiation, and prediction of treatment response. In the interim, HDV testing is available through the Centers for Disease Control and Prevention at http://www.cdc.gov/hepatitis/HDV/.

Routine testing of HDAg and HDV genotype is not recommended, as the tests are not widely available and have limited usefulness in the clinical setting due to problems related to sensitivity and reproducibility; however, HDAg and HDV genotype, as with anti-HDV IgM, are indicators of ongoing infection/replication. Of note, anti-HDV IgM test results can be positive in both chronic and acute HDV. Anti-HDV IgM can also be obtained if HDV RNA testing is negative but HDV is still suspected, a scenario that can occur because some HDV RNA tests do not detect all HDV genotypes accurately.68 Testing for anti-HBV core IgM can sometimes help distinguish between acute HDV coinfection (positive) vs superinfection (negative). HDV patients should be screened for HCV and HIV, given the shared transmission routes and significant triple infection rates.12 A liver biopsy may be useful, especially for staging and if the diagnostic laboratory test results are conflicting or not available. An HDV baseline event-anticipation clinical score to predict mild, moderate, or high risk of outcomes is available at http://hepatitis-delta.org/physicians-and-scientists/calculators/ and may be used in discussions with patients regarding their prognosis. This score was evaluated in 75 patients and validated in 2 separate cohorts of 77 and 62 patients; the following factors were found to be significant predictors of worse outcome: male gender, age over 40 years, Eastern Mediterranean origin, international normalized ratio for prothrombin time greater than 1.2, platelet count less than 100, and total bilirubin greater than the upper limit of normal.59 The application and usefulness of this score has yet to be determined in clinical practice.

Treatment

The goal of HDV treatment is HDV RNA clearance and HBsAg clearance with the ultimate goal of reducing progression to cirrhosis, hepatic decompensation, liver cancer, and death. However, there are many unanswered questions and limited data regarding treatment endpoints and treatment impact.66 The lack of standardization and limited availability of HDV RNA tests have restricted the ability to compare different clinical trial results and have contributed to the heterogeneity of reported outcomes. HBsAg clearance is a logical but impractical treatment endpoint, given its rarity, especially in a short time frame. HDV RNA quantification tests remain limited and have not been proven to be reliable for predicting and monitoring treatment response to guide treatment duration. It remains to be demonstrated that the surrogate markers of HDV RNA and HBsAg clearance—along with markers of biochemical response, such as normalization of liver enzyme levels or histologic improvement—correspond to a reduction in cirrhosis, hepatic decompensation, and HCC and an improvement in survival.60,61,62

Acute hepatitis D patients can be monitored for spontaneous recovery and for referral for transplantation in case of progression to acute liver failure. A patient’s hepatitis B can be managed independently based on HBV management guidelines. Patients requiring liver transplantation for acute liver failure or end-stage liver disease due to HDV have done well with HBV Ig and HBV nucleoside and nucleotide treatment.63

The currently accepted treatment for chronic HDV is pegylated IFN-α (PEG-α), based on common practice patterns that began with empiric IFN-α use rather than with robust randomized clinical trial data. However, PEG-α and IFN-α are the only currently available treatments with data demonstrating any appreciable effectiveness against HDV. IFN-α has been replaced by PEG-α, mainly due to the ease of dosing and tolerability rather than based on studies showing superiority.64,65 The expected rate of sustained HDV RNA suppression after 1 year of PEG-α therapy is only 15% to 20%.66-68 Therefore, inclusion in clinical trials should be offered to patients whenever possible, given these relatively low virologic response rates. Otherwise, in patients with active hepatitis D with elevated liver enzymes, histologic evidence of hepatitis, or persistently elevated HDV RNA or anti-HDV IgM who do not have contraindications to PEG-α, PEG-α treatment can be initiated. An individualized, pragmatic approach has often been used to determine the possibility of extending the duration of treatment beyond the 1-year mark. Patients reaching 1 year of treatment who have undetectable HDV RNA with normal liver enzyme levels can stop receiving treatment, with close monitoring for relapse. Those who have persistently high HDV RNA levels or anti-HDV IgM with abnormal liver enzyme levels can have their treatment discontinued, as it is unlikely to lead to a clinical response. The decision is difficult in those with declining HDV RNA or anti-HDV IgM levels with improving liver enzyme levels, who may be treated for another 24 to 48 weeks based on tolerability. It is important to remember that tolerability of PEG-α is an important limiting factor and that in patients with cirrhosis, hepatic decompensation is possible and has been reported to have led to death or the need for liver transplantation.61,68

Thus, prolonged PEG-α treatment must be undertaken only with thoughtful stratification of the risks and benefits.

Reports on the use of oral HBV antiviral therapies, such as lamivudine, adefovir, entecavir, and tenofovir, have been uniformly disappointing.69,70 This is unsurprising because nucleoside and nucleotide analogues affect
HBV DNA synthesis but do not have an impact on HDV RNA replication. In addition, they do not directly diminish the production of HBsAg, which is critical for HDV virion envelope formation. Similarly, ribavirin has not been shown to have any significant effect when given with or without IFN-α or PEG-α. Excellent summaries of IFN-α and PEG-α–based study results have been published recently.60,67 These studies are notable for the small numbers of patients included, variable treatment regimens, short durations of follow-up, and heterogeneity of results.

Results of the combination of PEG-α–plus oral antivirals such as adefovir and tenofovir have recently been reported. The HIDIT-1 study randomly assigned 90 patients to receive PEG-α with or without adefovir vs adefovir alone and found that the addition of adefovir provided no benefit.61 Although the subsequent HIDIT-2 study of PEG-α with or without tenofovir reported a slight improvement in treatment response with the tenofovir combination, it was not statistically significant.62 Moreover, extended treatment beyond 1 year was difficult to maintain due to intolerability and was associated with significant dropouts and adverse events. Furthermore, in the HIDIT-1 trial, extended follow-up showed a significant relapse rate of more than 50% among those who were noted to have an HDV RNA virologic response, suggesting that relapse is highly likely after PEG-α discontinuation.74 All of these trials support the fact that new treatments and clinical trials assessing them are greatly needed. Nevertheless, the best way to reduce hepatitis D may still remain the prevention of HBV and HDV infection through universal HBV vaccination efforts.

Future Directions

New treatments based on the growing understanding of the unique virology and pathophysiology of HDV are being developed. Because HDV lacks a specific viral enzymatic target, studies are being directed toward targeting the HDAg and interaction of the HBsAg virion coat with cell entry and exit. Prenylation inhibitors that target the prenylation step of L-HDAg by farnesyltransferase are being developed. In vitro and in vivo studies have demonstrated that inhibition of prenylation can lead to HDV clearance.75–77 A National Institutes of Health–sponsored study investigating the effect of the prenylation inhibitor lonafarinib, an oral farnesyltransferase inhibitor, is ongoing.78 HBV entry inhibitors such as Myrcludex B and HBV release inhibitors such as REP 9AC are also currently being studied.79–82 Other possible pathways include RNA interference–based therapies, antisense-based agents, lambda interferon, and toll-like receptor 7 agonists. These are all in the preliminary stages of study and require further development.83–85

Conclusion

Further work must be done to improve the standardization and availability of HDV diagnostic tests in order to have reliable tools for the screening of HDV. Screening all HBV patients will allow a better understanding of HDV’s prevalence and its impact on outcomes and will identify patients who can be offered treatment within or outside clinical trials. Furthermore, the resultant increased awareness of HDV can lead to redoubled efforts aimed at prevention of and early intervention against progressive fibrosis and cirrhosis. It is heartening that the European Association for the Study of the Liver has recently awarded a registry study grant to the Hepatitis D International Network. However, further support must be sought and garnered. When we consider the revolutionary change that has occurred in the management of HCV, with the astonishing development and success of direct-acting antiviral therapy, there is hope that our concerted efforts can also push HDV toward effective patient identification and successful treatment.

The authors have no relevant conflicts of interest to disclose.

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