Hepatitis D Virus: An Important Viral Agent in Hepatitis B Virus Endemic Regions a Review

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Abstract: More than 370 million individuals worldwide are Hepatitis B virus (HB V) carriers and about 5% of these individuals are co-infected with Hepatitis D virus (HDV). Hepatitis D virus is a defective virus that requires the obligatory help of Hepatitis B virus for its replication and expression. Hepatitis D virus is known to induce acute or chronic liver diseases. Individuals having HBV-HDV co-infection are prone to present with more severe acute disease and higher risk of fulminant hepatitis, cirrhosis and hepatocellular carcinoma (HCC) than those having Hepatitis B virus infection alone. Based on phylogenetic analysis, Hepatitis D virus is classified into 8 genotypes. Except genotype 1 which is found worldwide, genotypes 2-8 have specific geographical distribution. The transmission of Hepatitis D virus is similar to that of Hepatitis B virus (i.e. by blood and blood products, perinatal and also sexual intercourse). Hepatitis D virus can be diagnosed by serological analysis, molecular techniques and histo-immunochemistry. Several antiviral agents are under trial with varying degrees of efficacy. However, vaccination against Hepatitis B virus has helped in the control of Hepatitis D virus.

Keywords: Hepatitis D virus (HDV), Hepatitis B virus (HBV), Hepatocellular carcinoma (HCC), Fulminant hepatitis, Cirrhosis

Introduction

Hepatitis D virus (HDV) is a ribonucleic acid (RNA) virus that requires the help of hepatitis B virus (HBV) which is a DNA virus for its replication (WHO, 2016). Hepatitis D virus was first identified in the 1970s by an Italian gastroenterologist and colleagues (Rizzetto *et al.*, 1977). It is the only member of the deltavirus genus and the *Deltaviridae* family (Botelho-Souza *et al.*, 2017). HDV is a hepatotropic virus that replicate exclusively in the liver just as HBV (Yurdaydin 2017). Hepatitis D virus can only be transmitted in the presence of Hepatitis B virus infection because of its association with a Hepatitis B virus infected individual. It is transmitted either as a simultaneous infection of a susceptible individual with Hepatitis B virus and Hepatitis D virus (Co-infection pattern) or with an individual already chronically infected with Hepatitis B virus (Super-infection) depending on prior hepatitis B surface antigen (HBsAg) status of the infected individual (Rizzetto and Smedile 2002;Opaleye*et al.*,2016).Thus, every individual not immunized for the hepatitis B vaccine is at risk of both HBV and HDV (WHO, 2016).More than 15 million people worldwide are estimated to be chronically infected with HDV (Hughes *et al.*, 2011). Geographical distribution ranges, with very high prevalence rates of (>20%) in the Amazon Basin in South America, Central Africa, Iran, Pakistan, Eastern Turkey and Mongolia (Lempp and Urban 2017).

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Hepatitis D virus is transmitted via blood and blood products (such as blood transfusion, incisions), perinatal and also sexual intercourse (via semen or vaginal secretion) (Noureddin & Gish, 2014). Hepatitis D virus can be diagnosed by, Radioimmunoassay (Noureddin & Gish, 2014), Enzyme Linked Immunosorbent Assay (Opaleye *et al.*, 2016),reverse transcriptase polymerase chain reaction (RT-PCR) (Opaleye *et al.*, 2016) and liver biopsy (Wede meyer and Manns, 2010).Several antiviral agents such as lamivudine, ribavirin, adefovir, and tenofovir have been tried with varying degrees of disappointments when compared to pegylated interferon PEG-IFN-α which is the only drug of proven benefit for the treatment of chronic hepatitis D with an efficacy of about 20% (Lempp and Urban 2017; Wedemeyer *et al.*, 2011).

Hepatitis B virus (HBV) is an enveloped double stranded DNA virus that belongs to the *Hepadnaviridae* family (WHO, 2016).Globally, HBV accounts for over 370 million chronic infection out of which 65 million individuals reside in Sub-Saharan Africa. Approximately more than1 million deaths globally are attributed to chronic HBV infections annually (Lin and Zhang, 2017). It causes the disease hepatitis B (WHO, 2016) which is a liver disease and also with a progression of a more severe development and complication of hepatocellular carcinoma (Zampino *et al.*, 2015). In highly endemic areas, hepatitis B is most commonly spread from mother to child at birth (perinatal transmission) (WHO, 2016), or through infected blood and other body fluid, especially from an infected child to an uninfected child (Zampino *et al.*, 2015). Hepatitis B virus is usually detected by the presence of HBsAg which is a protein on the surface of HBV and it can be detected in high levels in serum

during acute or chronic HBV infection (Watts *et al.*, 2017). Vaccination is essential to control HBV infection. Significant progress made regarding the Expanded Programme on Immunization started in some African countries, with the monovalent anti-HBV vaccine being administered to children and various adult vaccination and treatment strategies had influenced the reduction of infections particularly among children. This had also reduced the burden, morbidity and mortality in adults (Burnett *et al.*, 2012).

History of Hepatitis D Virus

Hepatitis D virus was first identified in 1977 by an Italian gastroenterologist named Mario Rizzetto and his colleagues who detected an unrecognized antigen in hepatocytes of patients with chronic hepatitis B infection. The antigen resembled hepatitis B core antigen (HBcAg) in its association with hepatitis B virus (HBV) infection (Rizetto *et al.*, 1977). The antigen was initially interpreted as a novel hepatitis B–specific antigen; it was called delta antigen (HDAg) and its antibody, anti-delta (Rizzetto et al., 1977). A radioimmunoassay for anti-delta was developed and a high prevalence of anti-delta was shown among drug users and hemophiliacs (Rizzetto et al., 1980).The virus-like delta agent was shown to be associated with the most severe forms of acute and chronic hepatitis in many HBsAg-positive patients (Smedile *et al.*, 1982). Thus, in some cases, severe disease thought to be caused by HBV was actually the result of concurrent infection with the delta agent. By 1983, evidence for infection with the delta agent had been found on every populated continent (Rizzetto, 1983). Also in 1983, the delta agent was considered unique enough to obtain the status of a distinct viral hepatitis virus with the official name of hepatitis delta virus (HDV) (Pascarella and Negro, 2010). Cloned in 1986 and reported same year, the sequencing of the HDV genome confirmed its uniqueness among animal viruses (Wang *et al.*, 1986).The HDV outbreak, which started in Italy (25% in 1983) to, has been brought under control in industrialized countries during the past 31 years (9% in 2014) (Lempp and Urban 2017).Nevertheless, still more than 15 million people worldwide are estimated to be chronically infected with HDV (WHO 2016), especially in developing continents like Asia and Africa where HDV infections remain a major health problem (Ciancio and Rizzetto, 2014).

Virology of Hepatis D Virus

Hepatitis D virus is the only species in the genus deltavirus and the only member of the Deltaviridae family (Ahn and Gish, 2014; Botelho-Souza et al., 2017). The HDV genome is a small circular single stranded negative sense RNA agent (ssRNA-) (Flores *et al.*, 2012). Like the satellite RNA viruses of plants, HDV is coated by the envelope containing proteins derived from another virus that acts as a helper virus, and in this case, it is the hepatitis B surface antigen (HBsAg) of the hepatitis B virus(Alvarado-Mora *et al.*, 2013). Since HDV and HBV share the same envelope proteins, it is often assumed that attachment and cell entry occur via similar mechanisms (Alves *et al.*, 2013).

The hepatitis delta virion is the smallest infectious RNA virus known to humans (Rizzetto, 1983; Lempp and Urban 2017). The inner nucleocapsid of the virus contains approximately 1,700 nucleotide (Wedemeyer and Manns, 2010) and around 200 molecules of hepatitis delta antigen (HDAg), which is the only known protein encoded by the HDV RNA (Lempp and Urban 2017). Within the hepatocyte, replication leads to the accumulation of three HDV RNAs: the circular genomic RNA, the antigenomic RNA, and a smaller linear mRNA, which is the template for the translation of HDAg (Botelho-Souza *et al.*, 2017). A unique open reading frame on the antigenomic HDV RNA leads to the synthesis of the HDAg, which occurs in two different forms: small HDAg and large HDAg. The small HDAg (24 kDa) is important for virus replication, whereas the large form (27 kDa) inhibits replication and leads to virion assembly (Abbas and Afzal 2013). HDV is a defective virus, whose genome is surrounded by three HBV envelope proteins and host lipids. HBV plays an essential role as a helper virus for HDV, since its envelope proteins are stringently necessary for HDV propagation (Rizetto *et al.*, 1980) therefore, the release of hepatitis delta virions from the infected hepatocytes can only occur if the cells are coinfected with HBV or when HDV super-infection occurs in individuals already infected with HBV (Rizzetto and Smedile 2002; Opaleye *et al.*, 2016).

HDV Genotypes

Till date, eight genotypes (1 to 8) of Hepatitis D virus have been identified and described so far which are distributed over different parts of the world (Alvaro-Mora *et al.*, 2013; Yurdaydin, 2017), except with HDV genotype 1 which is represented worldwide, HDV genotypes 2 to 8 are mostly found in specific geographical areasas illustrated in figure 1 below (Alvaro-Mora *et al.*, 2013). HDV genotype 2 prevails in Japan (Imazeki *et al.*, 1990), Taiwan and Russia (Yurdaydin 2017), genotype 3 in the Amazonian region (Alvaro-Mora *et al.*, 2011), genotype 4 in Japan (Ahn and Gish, 2014) and genotypes 5-8 in Africa (Deny, 2006; Yurdaydin, 2017).

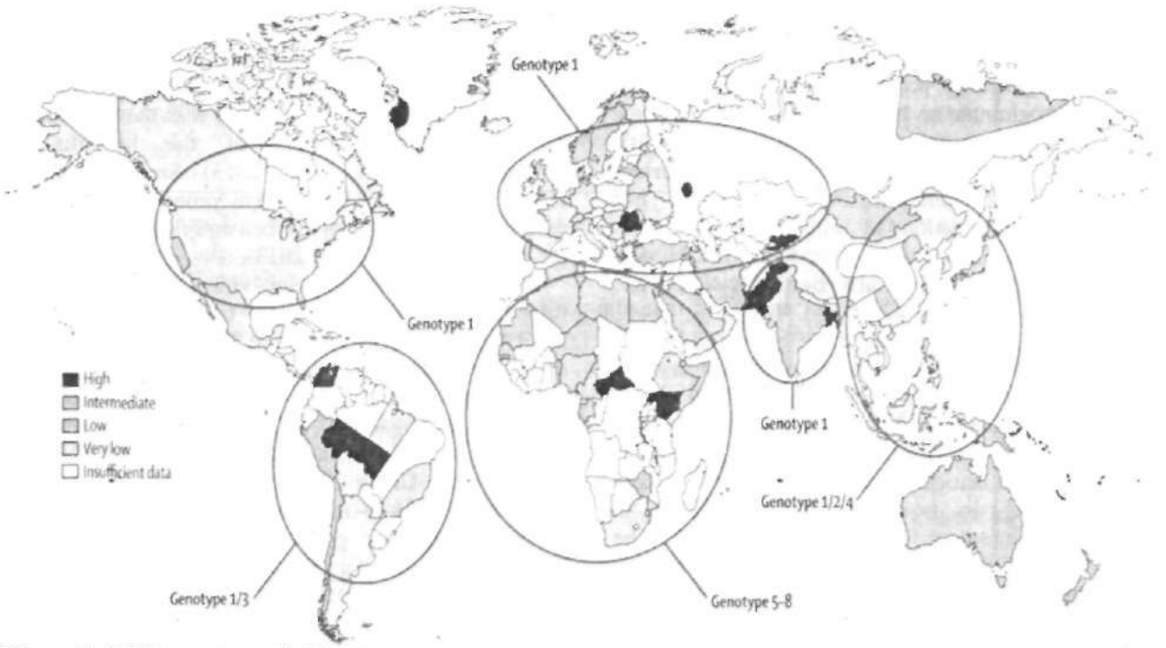

Figure 1: HDV genotype distribution

Source: Wedemeyer and Manns 2010

Epidemeology of Hepatitis D Virus

More than 370 million individuals worldwide are Hepatitis B Virus (HBV) carriers and at least 5% of these are co-infected with Hepatitis D virus (WHO, 2016). Current estimates suggest that 15–20 million people are infected with HDV (Lempp and Urban. 2017). However, these estimates are inaccurate and difficult to perform as systematic screening is not performed in all HBV-infected individuals (Cross *et al.*, 2008). Main areas of prevalence are the Mediterranean basin, the Middle East, Central and Northern Asia, West and Central Africa, the Amazonian basin, Venezuela, Colombia and certain islands of the Pacific as shown in figure 2 (Rizzetto and Alavian, 2013).

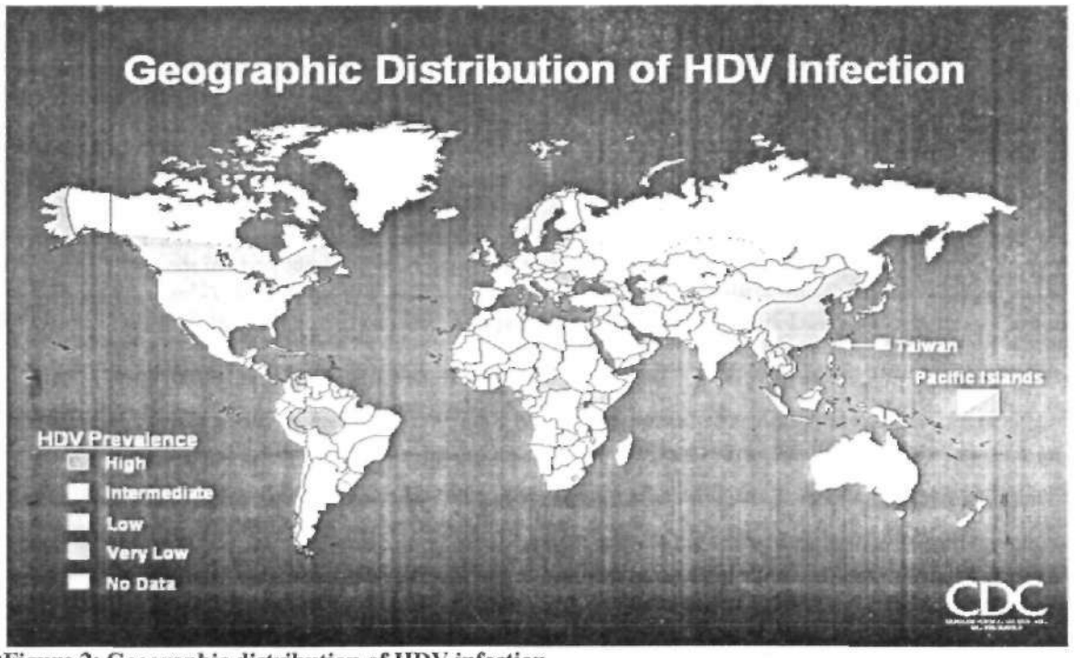

***Figure 2: Geographic distribution of HDV infection**

Source: CDC 2015

Regardless of the fact that HDV needs HBV for its replication, the distribution pattern of each virus is different. Approximately 90% of HBV carriers are infected with both viruses in the Pacific Islands, 8% in Italy and 5% in Japan (Rizzetto, 2015). Despite the prevalence of hepatitis D falling in southern Europe, the disease still represents a major health burden in Central Europe where its prevalence is mostly attributable to the immigration of individuals from highly endemic areas (Wedemeyer et al., 2007). In Greece, Samoa, and the Far East, HDV was also associated with benign clinical conditions, suggesting that disease expression may vary, possibly related to different HDV genotypes (Ciancio and Rizzetto 2012). The prevalence remained stable for example in London (Cross et al., 2008), Hanover (Wedemeyer et al., 2007) and Italy (Gaeta et al., 2007) and seemed to be increasing in France (Le Gal et al., 2007). This recrudescence in industrialized countries is mainly observed because of an increased immigration from Eastern Europe, Africa, the Middle East, Turkey and the former Soviet Union (Wedemeyer and Manns 2010). Immigration from endemic regions is not the only cause: intravenous drug use, sexual practices and body modification procedures may also be involved (Pascarella et al., 2011). A study by Brancaccio et al in Italy reported the prevalence of anti-HD was 8.4%; the antibody was found in 7.4% of Italians, but in 11.5% of immigrants (Cianco and Rizzetto et al. 2014). . In Germany, 8%–10% of the HBsAg-positive patients had anti-HD; 75% were coming from Turkey Eastern Europe, and the former Soviet Union (Wedemeyer and Manns 2010). The prevalence of HDV has increased over the last 15 years in blood donors in France (Servant-Delmas *et al.*, 2014). Immigrants also account for the larger proportion of chronic hepatitis D in Greece (Ciancio andRizzetto2012).

In Africa, the anti-HDV antibody prevalence in HBsAg carriers was reported in Cameroon (17.6 %) and Gabon (15.6 % to 70.6 %) (Makuwa *et al.*, 2009). In a paper by (Andernach *et al.*, 2014) HDV prevalence in sub-Saharan Africa was estimated from 1.3 % to 50 %.A study in 2017 estimated the prevalence of anti-hepatitis D virus among general HBsAg-positive populations in sub-Saharan Africa was 8.39%, which exceeded the estimated global prevalence of 5% (Lempp and Urban 2017). Relating these findings to the prevalence of hepatitis B virus infection would provide an estimated population prevalence of HBV and HDV co-infection in sub-Saharan Africa of 0.7%, corresponding to around 7 million people (Stockdale *et al.*, 2017).

Although HBV is endemic in Nigeria, data on HDV seroprevalence are limited. A recent study showed that HDV antigen was detectable in 9 % of patients with chronic hepatitis B in Southwest Nigeria (Opaleye *et al.*, 2016). In addition, another study in Nigeria reported an anti-HDV prevalence of 12.5 % in 96 HBsAg positive patients (Nwokediuko and Ijeoma2009). Moreover, a recent study showed that HDV1 prevails with 53.3 % in Southwestern Nigeria followed by the HDV5 (33.3 %) and HDV6 (13.3 %), which were more restricted to the northern part of Nigeria (Andernach *et al.*, 2014). Early childhood transmission is considered to be the most important route of HBV infection in high endemic areas therefore HDV super-infections contribute considerably to the high burden of chronic liver disease (Makuwa *et al.*, 2009). Although the HBV vaccination program in the routine children immunization schedule has been introduced since 2004 (Abdulraheem *et al.*, 2011), HBV infection still remains a major public health problem in Nigeria with 10–15 % of the population positive for HBsAg (Okoror *et al.*, 2017)

Transmission of Hepatitis D Virus

The route of hepatitis D virus transmission are the same as for hepatitis B, the most important being parenteral exposure and the trans-mucosal (sexual or intra-familial) spread vertical transmission is rare. A study in Romania (which is a hyper endemic HDV HBV country) by Gheorghe et al 2013in a multicenter survey performed in HBsAg-positive subjects showed that the high endemicity of HDV infection in Romania was generated by unsafe medical procedures in the past associated with blood transfusion, illegal abortion procedure, contaminated needles and reuse of medical or surgical instruments and even endoscopy (Lempp and Urban 2017).

Early childhood transmission is considered to be the most important route of hepatitis B virus infection in high endemic areas, therefore hepatitis D virus super infections contribute considerably to the high burden of chronic liver disease (Opaleye et al., 2016).

Pathogenesis of Hepatitis D Virus

Hepatitis D virus infection is dependent on the obligatory presence of Hepatitis B virus within the same hepatocytes which results in a frequent aggressive form of chronic liver damage (Yurdaydin, 2017). In endemic areas, such as northern South America, (Venezuela) outbreaks of severe hepatitis D have been linked to unusual histological features of liver disease that could represent a cytopathic viral nature (Romeo *et al.*, 2009 and Botelho-Souza *et al.*, 2017). Pathogenesis of HDV is also thought to be immune-mediated by the presentation of HDAg on infected cell surfaces, activating CD4 and CD8 pathway (Ahn and Gish 2014). Hepatitis D virus infection is known to occur either as a co-infection or a super-infection. A simultaneous co-infection with both HBV and HDV leads to acute hepatitis (Lin *et al.*, 2017), while a super-infection frequently progresses into chronic hepatitis D infection (de Sousa and Cunha 2010). A third pattern called helper-independent latent infection has been reported after liver transplantation (Negro, 2014). Clinical studies from all continents have shown that HDV

infection aggravates the natural history of the underlying HBV infection. Hepatitis D is considered the most severe form of viral hepatitis in humans, accelerating progression to cirrhosis and leading to early decompensation of liver function compared with HBV mono-infection (Rizzetto and Alavian, 2013).

Hepatitis Dvirus and cirrhosis

Progression of liver disease in Hepatitis D virus infection has been demonstrated to be influenced by many factors, including modes of infection, specific HDAg variants and HDV and HBV genotypes (Kiesslich *et al.*, 2009). Super infection with HDV in chronic HBV is associated with a more severe form of liver disease because of pre-existing liver damage due to HBV (Choi *et al.*, 2007).

Hepatitis Dvirus and hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the second most common cause of cancer related death in men worldwide (Jemal *et al.*, 2011). Persistent HDV replication and hepatic inflammation end up with cirrhosis and HCC formation (Romeo, 2010).

Hepatitis D virus co-infection pattern

Simultaneous infection of a susceptible individual with Hepatitis B virus and Hepatitis D virus results in both acute hepatitis B and acute hepatitis D (Farci 2003). As a defective virus depending on HBsAg synthesis, HDV infection starts only after HBV has infected hepatocytes; its expression relies on and is limited by the virulence of the concomitant HBV infection (Farci and Niro, 2012). As a result of the complex interplay between the two viruses, the clinical expression of HBV and HDV co-infection varies from mild to severe or even fulminant hepatitis (Negro, 2014). The incubation period of hepatitis D is dependent on the titer of the co-infecting HBV inoculum (Taylor 2012).

Hepatitis D virus super-infection pattern

In this setting, the preexisting HBsAg status provides a perfect biologic background for the rapid expression of the defective delta virus, which immediately establishes infection using the HBsAg from the preexisting HBV infection (Rizzetto and Smedile 2002). The clinical outcome of HDV super-infection is a rapid progression to cirrhosis (Farci and Niro, 2012), increased liver decompensation, and eventually death (Fattovich *et al.*, 2000), when compared with HBV monoinfection (Alves *et al.*, 2013). Chronic HBsAg carriers super infected by HDV develop progressive chronic hepatitis D in over 90% of the cases (Rizzetto, 2015).

Helper-independent latent infection

The liver transplantation setting has provided evidence for the existence of a third form of HDV infection defined latent infection (Ottobrelli *et al.*, 1991). It is characterized by the presence of markers of HDV infection associated with very low levels of HBV replication, (Smedile *et al.*, 1998) which initially went undetected due to the low sensitivity of the tests used (Ottobrelli *et al.*, 1991). Thus, this pattern cannot be considered a form of bona fide viral latency and reiterates the strict dependence of HDV upon HBV for its replication (Farci and Niro, 2012).Helper-independent HDV infection is now believed clinically irrelevant and probably of limited virological significance (Negro, 2014).

Laboratory Diagnosis of Hepatitis D Virus

The first step in the diagnosis of Hepatitis D virus is the screening of all patients with chronic HBV infection (Botelho-Souza *et al.*, 2017), especially those with a history of injection druguse 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 (Ahn and Gish 2014). In patients that are positive with HDAg the next step is to screen for HDV RNA which efficiently evaluates HDV replication by means of reverse transcription PCR assay (RT-PCR) (Botelho-Souza *et l.*, 2017). A liver biopsy may be useful, especially for staging and if the diagnostic laboratory test results are conflicting or not available (Wedemeyer and Manns 2010).

Enzyme Linked Immunosorbent Assay (ELISA)

One or more serological markers are used to ascertain Hepatitis D virus infection: HDAg, which marks an ongoing and acute infection; anti-HDV antibody (anti-HD) is the screening test for past or chronic infection. IgM anti-HD, is detectable during the 'window' phase of the infection, that is the period between the appearance of HDAg and the development of IgG anti-HD; when detected at high titres, it is indicative of chronic infection (Hughes et al., 2011).

Indirect Immune Fluorescent Assay

Indirect immune fluorescent assay can be used on slides of mouse tissue (kidney, liver, stomach) to evaluate the main antibodies: ANA (anti-nuclear antibodies), ASMA (anti-smooth muscle antibodies), LKM (liver-kidney microsomes antibodies), ARA (anti-reticulin antibodies), AMA (anti-mitochondrial antibodies), anti-ribosomal antibodies, APCA (anti-parietal cell antibodies), and ABBA (anti-brush border antibodies). Polyclonal rabbit anti-human IgA, IgG, IgM, Kappa, Lambda/FITC is used as fluorescent conjugate (Ghamari et al., 2013).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Hepatitis D virus replication is most efficiently evaluated by testing with molecular based assays for HDV RNA in serum by means of reverse transcription-polymerase chain reaction assays (RT-PCR) (Opaleye et al., 2016). In the mid-1980s, cloning of HDV RNA provided genetic probes and reagents for the measurement of delta viraemia in serum and for its detection in the liver by means of nucleic acid hybridization methods (Denniston et al., 1986). Polymerase chain reaction is useful for monitoring the efficacy of antiviral therapy as well as for investigating the molecular events during acute and chronic hepatitis D (Yamashiro et al., 2004). Because of genetic heterogeneity of HDV it is important to select primers from highly conserved region among the eight major genotypes (e.g. primers from ribozyme) (Le Gal et al., 2007).

Treatment, Prevention and Control

The goal of Hepatitis D virus treatment is HDV RNA and HBsAg clearance with the ultimate goal of reducing progression to cirrhosis, hepatic decompensation, liver cancer, and death (Ahn and Gish, 2013). Reports on the use of oral HBV antiviral therapies, such as lamivudine, adefovir, entecavir, and tenofovir, have been uniformly disappointing (Kabacam et al., 2012). This is unsurprising because nucleoside and nucleotide analogues affect HBV DNA synthesis but do not have an impact on HDV RNA replication (Ahn and Gish, 2014). Therefore, as long as HBsAg protein is expressed by HBV, HDV can thrive regardless of the presence of HBV and the titre of HBV DNA (Cianco and Rizzetto, 2014). Similarly, ribavirin has not been shown to have any significant effect when given with or without Interferon alpha (IFN-α) or pegylated interferon alpha (PEG-IRN-α) (Niro et al., 2006). Evidence suggests that Pegylated interferon alpha (Peg-INF) is effective in reducing the viral load and the effect of the disease during the time the drug is given, but the benefit generally stops if the drug is discontinued (Abbas et al., 2011). The efficiency of the pegylated interferon treatment does not usually exceed ~20% (Pascarella and Negro 2011).

Prenylation inhibitors like Lonafarnib targets the prenylation step of L-HDAg by farnesyl transferase which is a key enzyme in the prenylation pathway. The inhibition of prenylation can lead to HDV clearance (Lempp and Urban 2017). The drug myrclurdex, which is an HBV entry inhibitor, may also hinder the establishment of HDV infection by inhibiting virus entry into hepatocytes and possibly re-infection (Lutgehetmann et al., 2012). Myrcludex B received "prime eligibility status" by the European medical association (EMA) in May 2017 (Lempp and Urban 2017). REP 9AC which is a nucleic acid based amphipathic polymer which inhibits the release of HBsAg from infected hepatocyte is being evaluated in patients with chronic HBV (Mahtab et al., 2010). Liver transplantation is the only therapy for patients with end-stage liver disease, hepatocellular carcinoma, or fulminant hepatitis due to hepatitis D virus (HDV) and hepatitis B virus (HBV) coinfection or super infection (Roche and Samuel 2012).

There has been decrease in hepatitis D virus (HDV) prevalence in some endemic areas such as Italy, Spain, Taiwan, Japan and Turkey. The decline in HDV in these regions was substantial in young individuals as a result of the introduction of anti-HBV vaccination programs and factors such as general improvement in hygiene, socio-economic conditions, awareness of the virus and its mode of transmission and better implementation of preventive measures in healthcare system (change to disposable needle, syringe and other medical equipment) have possibly contributed (Gheorghe et al., 2013).

Conclusion

Hepatitis D represents a major health problem in Hepatitis B virus endemic regions around the world. Hepatitis D virus is a highly pathogenic virus that causes severe acute hepatitis, which may run a fulminant course, and the most progressive form of chronic viral hepatitis leading to cirrhosis in up to 70% of the cases. HDV is a unique agent because of its defective nature that allows it to survive and replicate only in association with HBV. Over the past two decades, the implementation of universal HBV vaccination programs in many countries has led to a dramatic decline in the prevalence of HDV infection, particularly in the industrialized world. But it still remains a major public health problem in underdeveloped areas of the world where HBV is not under control. Considering the high pathogenicity of HDV, the fact that chronic hepatitis D is a difficult target for antiviral therapy, and the lack of an effective vaccine specific for HDV, which would be the only means to eliminate the risk of HDV super-infection for the 370 million HBsAg carriers infected worldwide, HDV should still be regarded as a major public health concern.

A global outreach program should be organized in conjunction with governments of endemic countries to educate people about HDV on the increase in HBV vaccination (which confers protection against HBV and HDV), improving health awareness, health practices, and socioeconomic conditions.

References

Abbas, Z. and Afzal, R. (2013). Life cycle and pathogenesis of hepatitis D virus: A review. *World J Hepatol* 5:666–675.

Abbas, Z., Khan, M., Salih, M. and Jafri, W. (2011). ed. "Interferon alpha for chronic hepatitis D". *Cochrane Database Syst Rev* (12): CD006002.

Abdulraheem, I., Onajole, A., Jimoh, A. and Oladipo, A. (2011). Reasons for incomplete vaccination and factors for missed opportunities among rural Nigerian children. *J Public Health Epidemiol*; 3:194–203.

Alvarado-Mora, M., Locarnini, S., Rizzetto, M. and Pinho, J. (2013). An update on HDV: virology, pathogenesis and treatment. *AntivirTher.* ;18(3 Pt B):541-8.

Alvarado-Mora, M., Romano, C., Gomes-Gouvea, M., Gutierrez, M., Carrilho, F., Pinho J. (2011). Dynamics of hepatitis D (delta) virus genotype 3 in the Amazon region of South America. *Infect Genet Evol*; 11: 1462-1468.

Alves, C., Branco, C. and Cunha, C. (2013). Review Article Hepatitis Delta Virus: A Peculiar Virus.*Advances in Virol*;

Ahn, J. and Gish, R. (2014). Hepatitis D Virus: A Call to Screening. *Gastroenterol Hepatol* (N Y) 10(10).

Andernach, I., Leiss, L., Tarnagda, Z., Tahita, M., Otegbayo, J. and Forbi, J. (2014) Characterization of hepatitis delta virus in sub-Saharan Africa. *J Clin Microbiol*; 52:1629–36.

Botelho-Souza, L., Vasconcelos, M., dos Santos, A., Salcedo, J., and Vieira, D. (2017). Hepatitis delta: virological and clinical Aspects *Virol Journ* 14:177

Burnett, R., Kramvis, A., Dochez, C., and Meheus, A. (2012). An update after 16 years of hepatitis B vaccination in South Africa. Vaccine; 30 Suppl 3: C45-C51.

Casey, J., Brown, T., Colan, E., Wignall, F. and Gerin J. (1993). A genotype of hepatitis D virus that occurs in northern South America. *Proc. Natl. Acad. Sci. U S A*; 90: 9016-9020.

Cao, D., Haussecker, D. and Huang, Y. (2009). Combined proteomic-RNAi screen for host factors involved in human hepatitis delta virus replication. *RNA*;15:1971–1979.

Chang, J., Gudima, S. and Tam, C. (2005). Development of a novel system to study hepatitis delta virus genome replication. *J Virol*;79:8182–8188.

Choi, S., Jeong, S. and Hwang, S. (2007). Large hepatitis delta antigen modulates transforming growth factor-beta signaling cascades: implication of hepatitis delta virus-induced liver fibrosis. *Gastroenterology*; 132:343–357.

Ciancio, A. and Rizzetto, M. (2014). Chronic hepatitis D at a standstill: where do we go from here?.*Nat Rev Gastroenterol Hepatol*;11:68–71.

Cross, T., Rizzi, P. and Horner, M. (2008). The increasing prevalence of hepatitis delta virus (HDV) infection in South London. *J Med Virol*; 80: 277–82.

Denniston, K., Hoyer, B., Smedile, A., Wells, F., Nelson, J. and Gerin, J. (1986). Cloned fragment of the hepatitis delta virus RNA genome: sequence and diagnostic application. *Science*; 232: 873-875.

Dény, P. (2006). Hepatitis delta virus genetic variability: from genotypes I, II, III to eight major clades? *Curr Top Microbiol. Immunol*; 307: 151-171

de Sousa, B. and Cunha, C. (2010). Development of mathematical models for the analysis of hepatitis delta virus viral dynamics. *PLoS One*;5

Farci, P. (2006). Treatment of chronic hepatitis D: new advances, old challenges. *Hepatology*;44:536–539.

Farci, P and Niro, G. (2012). Clinical Features of Hepatitis D. *Semin Liver Dis*; 32:228–236.

Fattovich, G., Giustina, G and Christensen, E. (2000). Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B, *Gut*, vol. 46, no. 3, pp. 420–426.

Flores, R., Ruiz-Ruiz, S. and Serra, P. (2012). Viroids and hepatitis delta virus. *Semin Liver Dis* ;32:201–10.

Gaeta, G., Stroffolini, T., Chiaramonte, M., Ascione, T., Stornaiuolo, G. and Lobello S. (2000). Chronic hepatitis D: a vanishing Disease? An Italian multicenter study. *Hepatology*;32:824–827.

Gaeta, G., Stroffolini, T., Smedile, A., Niro, G. and Mele, A. (2007). Hepatitis delta in Europe: vanishing or refreshing? *Hepatology*; 46: 1312–3.

Gheorghe, L., Csiki, I., Iacob, S., Gheorghe, C., Trifan, A., Grigorescu, M., Motoc, A., Suceveanu, A., Curescu, M., Caruntu, F., Sporea, I., Brisc, C.,

Rogoveanu, I., Cerban, R., Tugui, L., Alexandrescu, A. (2013). Hepatitis Delta virus infection in Romania: Prevalence and risk. *J Hepatol*; 58: S169.

Glenn, J. (2006). Prenylation of HDAg and antiviral drug development. *Curr Top Microbiol Immunol*; 307:133-149

Hughes, S., Wedemeyer, H. and Harrison, P. (2011). Hepatitis delta virus. *Lancet*; 378:73–85.

Hwang, E. and Cheung, R. (2011). Global epidemiology of hepatitis B virus (HBV) infection. *N Am J Med Sci*; 4(1):7-13.

Imazeki, F., Omata, M. and Ohto, M. (1990). Heterogeneity and evolution rates of delta virus RNA sequences. *J. Virol* 1990; 64:5594–9.

Ivaniushina, V., Radjef, N. and Alexeeva M, (2001). Hepatitis delta virus genotypes I and II cocirculate in an endemic area of Yakutia, Russia. *J Gen Virol* 2001; 82(Part 11): 2709 18

Jemal, A., Bray, F., Center, M., Ferlay, J., Ward, E. and Forman, D. (2011). Global cancer statistics. CA Cancer *J Clin;* 61: 69-90

Kabaçam, G., Onder, F. and Yakut, M. (2012). Entecavir treatment of chronic hepatitis D. *Clin Infect Dis*; 55(5):645–650.

Le Gal, F., Castelneau, C., and Gault, E. (2007). Hepatitis D virus infection – not a vanishing disease in Europe!. *Hepatology*; 45: 1332–3.

Le Gal, F., Gault, E. and Ripault, M. (2006). Eighth major clade for hepatitis delta virus. *Emerg Infect Dis*; 12: 1447–50.

Le Gal, F., Gordien, E. and Affolabi, D. (2005). Quantiication of hepatitis delta virus RNA in serum by consensus real-time PCR indicates different patterns of virological response to interferon therapy in chronically infected patients. *J Clin Microbiol*;43:2363–2369.

Lee, C., Changchien, C., Chung, J. and Liaw, Y. (1996). Characterization of a new genotype II hepatitis delta virus from Taiwan. *J Med Virol*: 49: 145-154.

Lempp, F., Ni, Y and Urban, S. (2016). Hepatitis delta virus: insights into a peculiar pathogen and novel treatment options. *Nat Rev Gastroenterol Hepatol.*;13:580–589.

Lempp, F. and Urban, S. (2017). Hepatitis Delta Virus: Replication Strategy and Upcoming Therapeutic Options for a Neglected Human Pathogen. *Viruses*, 9, 172

Lin, C., Lee, C., Lin, S., Huang, P., Li, H., Chang, Y, Tang, P and Chao, M. (2017). RNA recombination in Hepatitis delta virus: Identification of a novel naturally occurring recombinant. *Journ of Microbiol, Immunol and Infection* ; 50, 771e780

Lin, S and Zhang, Y. (2017). Interference of Apoptosis by Hepatitis B Virus. *Viruses*; 9, 230

Lutgehetmann, M., Mancke, L. and Volz, T. (2012). Humanized chimeric uPA mouse model for the study of hepatitis B and D virus interactions and preclinical drug evaluation. *Hepatol*;55:685–94.

Mahtab, M., Bazinet, M. and Vaillant, A. (2010). REP 9 AC: a potent HBsAg release inhibitor that can rapidly restore immune-competence in patients with chronic hepatitis B. *Hepatol*;52:559A–60.

Makuwa, M., Mintsa-Ndong, A., Souquiere, S., Nkoghe, D., Leroy, E. and Kazanji, M. (2009). Prevalence and molecular diversity of hepatitis B virus and hepatitis delta virus in urban and rural populations in northern Gabon in central Africa. *J Clin. Microbiol*; 47:2265–8

Mansour, W., Malick, F., Sidiya, A., Ishagh, E., Chekaraou, M. and Veillon P. (2012). Prevalence, risk factors, and molecular epidemiology of hepatitis B and hepatitis delta virus in pregnant women and in patients in Mauritania. *J Med Virol*; 84:1186–98

Noureddin, M. and Gish, R. (2014). Hepatitis Delta: Epidemiology, Diagnosis and Management 36 Years After Discovery.*CurrGastroenterol Rep*; 16:365

Niro, G., Rosina, F. and Rizzetto, M. (2005). Treatment of hepatitis D. *J Viral Hepat*; 12:29. Niro, G. and Smedile, A. (2012). Current concept in the pathophysiology of hepatitis delta infection. *Curr Infect Dis Rep*; 14:9–14.

Nwokediuko, S. and Ijeoma, U. (2009). Seroprevalence of antibody to HDV in Nigerians with hepatitis B virus-related liver diseases. *Niger J Clin Pract*;12:439–42

Okoror , L., Ajayi, A., and Ijalana, O. (2017). Elevated serum β2 microglobulin in Individuals coinfected with hepatitis B and hepatitis D virus in a rural settings in Southwest Nigeria. *BMC Res Notes* 10:719

Ottobrelli, A., Marzano, A. and Smedile, A. (1991). Patterns of hepatitis delta virus reinfection and disease in liver transplantation. *Gastroenterol*; 101(6):1649–1655

Opaleye, O., Japhet, O., Adewumi, O., Omoruyi, E., Akanbi, O., Oluremi, A., Wang, B., Hoang, T., Thirumalaisamy, P., Velavan, C. and Bock, T. (2016). Molecular epidemiology of hepatitis D virus circulating in Southwestern Nigeria. *Virol. Journ* 13:61.

Opaleye, O., Fagbami, A., Lalremruata, A. and Kun J. (2011). Prevalence and association of human parvovirus B19V with hepatitis B and C viruses in Nigeria. *J Med Virol;*83:710–6

Pascarella, S. and Negro, F. (2011). Hepatitis D virus: an update. *Liver Int*. 31 (1): 7–21.

Rizzetto, M. (1983). The delta agent. *J Hepatol*; 3:729–737.

Rizzetto, M. (2010). Delta hepatitis: Forgotten, but not gone. *Curr. Hepatitis Rep*; 9:239-242

Rizzetto, M. (2015). Hepatitis D Virus: Introduction and Epidemiology. *Cold Spring Harb Perspect Med*;5:a021576

Rizzetto, M. and Alavian, S. (2013). Hepatitis delta: the rediscovery. *Clin Liver Dis;* 17(3):475–487

Rizzetto, M. and Smedile, A. (2002). Hepatitis D. In: Diseases of the Liver. Philadelphia, PA: Lippincott, Williams and Wilkins; 863–875

Rizzetto, M., Shih, J. and Gerin J. (1980). The hepatitis B virus associated delta antigen: isolation from liver, development of solidphaseradioimmunoassays for delta antigen and antidelta and partial characterization of delta antigen. *J Immunol* ;125:318–324.

Rizzetto, M., Canese, M. and Arico J. (1977). Immunofluorescence detection of a new antigen-antibody system associated to the hepatitis B virus in the liver and in the serum of HBsAg carriers. *Gut* 1977:18:997–1003.

Rizzetto, M., Purcell, R. and Gerin J. (1980). Epidemiology of HBV associated delta agent: geographical distribution of antid and polytransfused HBsAg carriers. *Lancet* ;1:1215–1218.

Rizzetto, M., Canese, M., Gerin, J., London, W., Sly, D. and Purcell R. (1980). Transmission of the hepatitis B virus-associated delta antigen to chimpanzees. *J Infect Dis*;141:590–602.

Roche B and Samuel D. (2012). Liver transplantation in delta virus infection. *Semin Liver Dis*; 32(3):245-55.

Romeo R. (2010). Role of the hepatitis Delta virus on the pathogenesis of hepatic cirrhosis and hepatocellular carcinoma. Recent advances. *Recenti Prog Med*; 101: 52-56.

Romeo, R., Foglieni, B., Casazza, G., Spreafico, M., Colombo, M. and Prati D. (2014). High Serum Levels of HDV RNA Are Predictors of Cirrhosis and Liver Cancer in Patients with Chronic Hepatitis Delta. *PLoS ONE* 9(3): e92062.

Romeo, R., Del Ninno, E., Rumi, M., Russo, A., Sangiovanni, A. and de Franchis, R. (2009). A 28-year study of the course of hepatitis delta infection: a risk factor for cirrhosis and Hepatocellular carcinoma. *Gastroenterology*; 136:1629–38.

Sakugawa, H., Nakasone, H. and Nakayoshi T. (1999). Hepatitis delta virus genotype IIb predominates in an endemic area, Okinawa, Japan. *J Med Virol*; 58: 366–72.

Scholtes, C., Icard, V. and Amiri M. (2012). Standardized one-step real-time reverse transcription-PCR assay for universal detection and quantification of hepatitis delta virus (HDV) from clinical samples in the presence of a heterologous internal control RNA. *J Clin Microbiol;* 50: 2126-2128.

Servant-Delmas, A., Le Gal, F., Gallian, P., Gordien, E. and Laperche S. (2014). Increasing prevalence of HDV/HBV infection over 15 years in France. *J Clin Virol;* 59: 126–128.

Shiva, G., Alavian, S., Rizzetto, M., Olivero, A., Smedile, A., Khedive, A., Alavian, S., Zolfaghari, M. and Jazayeri S. (2013). Prevalence of Hepatitis Delta Virus (HDV) Infection in Chronic Hepatitis B Patients with Unusual Clinical Pictures.*Hepat Mon* e6731.

Smedile, A., Casey, J. and Cote P. (1998). Hepatitis D viremia following orthotopic liver transplantation involves a typical HDV virionwith a hepatitis B surface antigen envelope. *Hepatol*;27(6): 1723–1729

Smedile, A., Ciancio, A. and Rizzetto, M. (2002). Hepatitis D. In: Richmann DD, Whitley RJ,

Hayden FG, eds. Clinical Virology: Hepatitis Delta Virus. Washington, DC: *ASM Press*;1227–1240

Smedile, A., Farci, P. and Verme G. (1982). Inluence of delta infection on severity of hepatitis B. *Lancet*; 2:945–947.

Sollievodella Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Foggia, Italy *Semin Liver Dis* 2012;32:228–236.

Stockdale, A., Chaponda, M., Beloukas, A., Philips, R., Matthews, P., Papadimitropoulos, A., King, S., Bonnett, L and Geretti, A. (2017). Prevalence of hepatitis D virus infection in sub-Saharan Africa: a systematic review and meta-analysis. *The Lancet. Global Health*5.10 : e992–e1003.

Sureau C. (2010). The use of hepatocytes to investigate HDV infection: the HDV/HepaRG model Methods. *MolBiol*; 640:463–473.

Sureau, C., Guerra, B. and Lee H. (1994). The middle hepatitis B virus envelope protein is not necessary for infectivity of hepatitis delta virus. *J Virol*; 68:4063–4066.

Sureau, C., Guerra, B. and Lanford R. (1993). Role of the Large Hepatitis B Virus Envelope Protein in Infectivity of the Hepatitis Delta Virion. *Jour. Virol.*, Vol. 67; 366-372

Taylor J. (2012). Virology of hepatitis D virus. *Semin Liver Dis*;32:195–200

Taylor, J., Purcell, R. and Farci, P. (2013). Hepatitis D (Delta) Virus. Sixth edition. Fields virology. pp 2222-2241

Wang, K., Choo, Q. and Weiner A. (1986). Structure, sequence and expression of the hepatitis delta viral genome. *Nature*;323:508–513.

Watanabe, H., Nagayama, K. and Enomoto N. (2003). Chronic hepatitis delta virus infection with genotype IIb variant is correlated with progressive liver disease. *J Gen Virol*: 84(Part 12): 3275–89.

Watts, A., Bennett, W., Molleston, J., Gupta, S., Croffie, J., Waseem, S., McFerron, B., Steiner, S., Kumar, S., Vanderpool, C., Hon, E., Bozic, M., Subbarao, G. and Pfefferkorn M. (2017). The Incidence of Low Seroimmunity to Hepatitis B Virus in Children with Inflammatory Bowel Disease. *J Pediatr Gastroenterol Nutr.*

Webb, C. Riccitelli, N., Ruminski, D. and Luptak A. (2009). Widespread occurrence of self-cleaving ribozymes. *Science*, vol. 326, no. 5955, p. 953.

Wedemeyer, H., Heidrich, B. and Manns, M. (2007). Hepatitis D virus infection not a vanishing disease in Europe. *Hepatology* 45, 1331–1332.

Wedemeyer, H. and Manns M. (2010). Epidemiology, pathogenesis and management of hepatitis D: Update and challenges ahead. *Nat Rev Gastroenterol Hepatol*; 7:31–40.

Wedemeyer H, Yurdaydin C, Ernst S, et al.: 96 weeks of pegylated-Interferonalpha- 2a plustenofovir or placebo for the treatment of hepatitis delta: the HIDIT-2 study (abstr). *Hepatology*. 2013; 58: 222–3.

World Health Organization: Hepatitis B, Fact Sheet July 2016

World Health Organization: Hepatitis D, Fact Sheet July 2016

Yamashiro, T., Nagayama, K. and Enomoto N. (2004). Quantitation of the level of hepatitis delta virus RNA in serum, by real-time polymerase chain reaction – and its possible correlation with the clinical stage of liver disease. *J Infect Dis*; 189: 1151-1157.

Yan, H., Zhong, G. and Xu G. (2012). Sodium taurocholateco transporting polypeptide is a functional receptor for human hepatitis B and D virus. *eLife*;1:e00049.

Yurdaydin, C. (2017). Recent advances in managing hepatitis D [version 1; referees: 2 approved] *F1000Research* 2017, 6 (*F1000* Faculty Rev):1596

Zaidi, G., Muhammad, Fayyaz, A., Irum, A., Muhammad, S., Saima, Y., Rashid, H., Zunaira, A., Aaliyah, T. and Khalida P. (2010). Prevalence of hepatitis delta virus infection among hepatitis b virus surface antigen positive patients circulating in the largest province of Pakistan. *VirolJourn*: 7:283

Zampino, R., Boemio, A., Sagnelli, C., Alessio, L., Adinolfi, L., Sagnelli, E. and Coppola N. (2015). Hepatitis B virus burden in developing countries. *World J Gastroenterol;* 21(42): 11941-11953.