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# Current Management and Novel Therapeutic Strategies to Combat Chronic Delta Hepatitis

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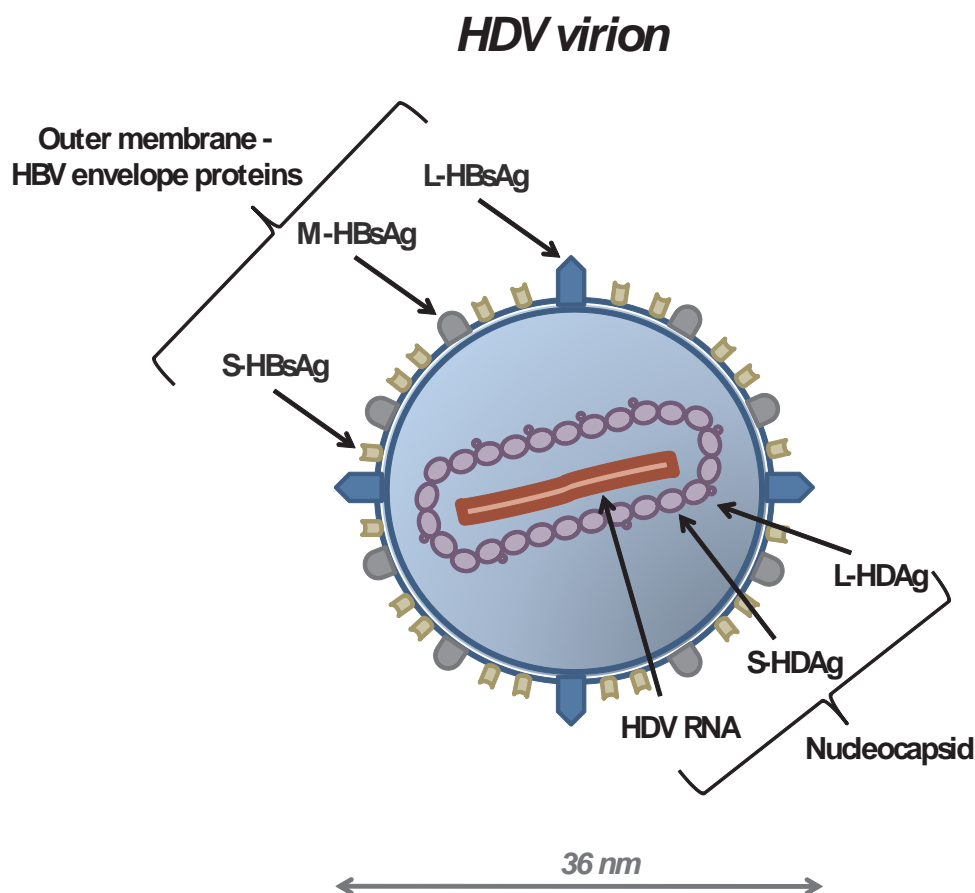
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## 1. Introduction

Forty years ago Mario Rizzeto's group identified a new antigen-antibody system (delta antibody and delta antigen (HDAg)) in HBsAg carriers with severe hepatitis [1, 2]. Further experiments in chimpanzees showed that this HDAg marked transmissible pathogen requires coexistence of HBV infection for its life cycle, proving its defective nature which requires HBsAg for transmission and replication [3]. As the cause of hepatitis D was identified, a virion particle (Figure 1.) composed of outer coat containing HBV envelope proteins (HBsAg) and inner nucleocapsid was described [4]. Internal nuclear like structure is comprised of single stranded, circular RNA molecule of 1700 nucleotides associated with two distinctive forms of hepatitis D antigen (HDAg), small and large subunit [5, 6]. Although HDV in its structure possesses HBsAg, HDV is classified as separate pathogen with own genus called *Deltavirus* [7]. This unusual virus is the smallest infectious pathogen in human virology, with unique replication cycle unknown to animal cells. During replication process a viral RNA is transcribed by hosts RNA polymerases [8], which usually transcribe DNA molecules, and after transcription HDV RNA is cleaved by its own ribozyme [9].

Dependence on the HBsAg presence results in two patterns of HDV infection. HDV can be transmitted simultaneously with HBV (co-infection pattern) or infection may occur at preceding HBV infected individual (super-infection pattern). Due to differences of HDV acquisition clinical course and outcome of HDV infection varies. However, many studies have shown that HDV infection causes more severe liver disease [10] and more rapid progression to cirrhosis [11] than HBV mono-infection alone.



**Figure 1.** Structure of HDV virion. HDV is composed of outer membrane, HBV envelope proteins within a phospholipid bilayer, and inner nucleocapsid consisting of viral RNA and hepatitis delta antigens. L-HBsAg=large hepatitis B surface antigen. M-HBsAg=medium hepatitis B surface antigen. S-HBsAg=small hepatitis B surface antigen. HDV RNA=hepatitis D virus RNA. L-HDAg=large hepatitis D antigen. S-HDAg=small hepatitis D antigen.

Although many details regarding HDV viral cycle are revealed, therapy of HDV has not progressed. To date, treatment options of HDV infection are limited to interferon regimes with open issues about effectiveness of current approaches. Due to the unsatisfactory results further studies of novel drugs and therapy protocols are required. In this chapter current treatment procedures as well as novel therapeutic strategies to combat chronic HDV infection will be discussed.

## 2. Epidemiology

It is estimated that 15-20 million of HBsAg positive individuals are infected with HDV, but these numbers are not accurate because of absence of systematic screening in HBV infected individuals [12]. Interestingly, HDV infection is worldwide distributed; however the distribution is not uniform. Areas of HDV high prevalence are: Central Africa, Mediterranean countries, Middle East and South America, while in Western world HDV infection is limited

to intravenous drug abusers [13]. Decrease of HDV incidence in industrialized world is caused by improvement of socioeconomic conditions such as implementation of HBV vaccination and systematic screening of blood and blood products. However, reduction in the number of infected individuals in developed countries has stopped, mainly due to the increased immigration from endemic areas [14]. Prevalence of anti-HD among HBsAg positive individuals in Western Europe has been stable during last decade ranging from 8.5 to 11.0 % [15, 16].

So far, eight HDV genotypes have been described. Most frequent is genotype 1 [17], which is prevalent worldwide, while genotypes 2 and 4 are mainly found in the Far East and genotype 3 is limited to Amazonian basin [18]. HDV genotypes 5-8 are found in patients originating from Africa [19]. Different HDV genotypes have impact on variations in clinical course of disease, thus genotypes 1 and 3 cause more severe disease, while genotype 2 is associated with milder form. Also, multiple genotypes of HDV can be found in high risk patients [20].

### 3. Transmission of HDV

HDV, as its helper HBV, is transmitted parenterally through the exposure to the blood and body fluids. In the developed countries the main route of transmission is by infected syringes among intravenous drug users. Although, there is an evidence for sexual transmission [21], it seems that HDV does not have the same rate of sexual transmission as HBV [22]. Due to the screening of the blood products, there is no more risk of HDV infections for the blood receiving patients. Despite the fact that HDV is parenterally transmitted, inapparent parenteral transmission within household [23] represents major route of transmission in the areas of the high prevalence. Therefore vaccination against HBV of all household members of infected individual is the crucial step in prevention of HDV infections. Vertical transmission from mother to the newborn is rare.

### 4. Clinical features

As defective virus, HDV replication depends on HbsAg synthesis, therefore HDV can be transmitted only in the presence of accompanied HBV infection. Based on the previous HBsAg status of the infected individual two major patterns of infection are distinguished: simultaneous HBV/HDV co-infection or superinfection by HDV of chronic HBsAg carriers [18].

In the simultaneous infection the fate of HDV depends on the host response to the HBV. When the expression of HBsAg is restrained, the HDV infection may result with abortive response, while abundance of HBsAg enables the full expression of HDV pathogenicity. As a result of this interaction between the two viruses, disease expression may vary. Clinically, HDV/HBV co-infection is usually similar to HBV mono-infection, although acute co-infection can be more severe and can be manifested as acute liver failure [24]. The rate of progression of HDV infection to chronic form is equal to that for acute hepatitis B, less than 5% [19].

Superinfection is defined as HDV infection of a chronically HBV infected individual. Preexisting chronic HBV infection represents perfect environment for replication of defective hepatitis D virus, resulting with abundant HDV expression and suppression of HBV replication. HBV suppression will become permanent, if HDV infection progress to chronic form [20]. Furthermore, superinfection is generally presented as severe acute hepatitis with shorter incubation time and will exceed to chronic HDV infection in high percentage of patients, up to 80% [25]. Clinically, HDV superinfection is manifested as a worsening of present HBV disease or as a new hepatitis in previously undiagnosed HBV infected individual. Along with detection of HDV serum markers, correct diagnosis of superinfection is clarified by negative IgM anti-HBc [21].

## 5. Symptoms and course

Symptoms of acute hepatitis D infection are similar to the other forms of viral hepatitis. Initial phase of acute infection is characterized by nonspecific symptoms such as: fatigue, anorexia, nausea and lethargy accompanied with high increase of serum alanine aminotransferase and aspartate aminotransferase. Sometimes this phase is followed with icteric phase, characterized by jaundice, dark urine, clay-colored stools and elevated levels of serum bilirubin. Acute hepatitis is more severe in superinfection pattern, due to the fact that preceding HBV infection facilitates multiplication of HDV. Furthermore, acute disease can occur as fulminant hepatitis, named as acute liver failure (ALF). ALF is a rare form of acute hepatitis; which is more often seen in acute hepatitis caused by HDV than other hepatotropic viruses. Clinically, ALF starts with nonspecific symptoms, such as fatigue and malaise followed by jaundice and hepatic encephalopathy and ultimately leading to coma. ALF is characterized by massive necrosis of hepatocytes, rapid clinical course; ultimately result with death of individual in 2-10 days. Without liver transplantation, ALF has lethal outcome in 80% of cases [26].

Chronic hepatitis D is the rarest form among chronic viral hepatitis, although it is the most severe and most progressive one. Chronic hepatitis D has three times higher risk for cirrhosis development than HBV chronic infection [27]. Clinically it is initially expressed as acute hepatitis in the half of the cases, probably due to initial acute superinfection [28]. However, symptoms of chronic hepatitis D are variable. Chronic hepatitis D can be manifested as fatigue, malaise and anorexia or its clinical course can be without any symptoms [9]. When cirrhosis is developed disease may be stable and asymptomatic for a long period or it can be manifested with complications related to the cirrhotic process. Once developed, a high number of patients with HDV cirrhosis will die of liver failure or hepatocellular carcinoma unless liver transplantation is performed [29].

## 6. Diagnosis

First step in diagnostic procedures is detection of HDV markers in the blood of HBsAg positive individual, due to the fact that HDV replication is possible only in the presence of HBV.

Actually, guidelines suggest that all HBsAg positive individuals should be screened for HDV infection, as well as members of high-risk group's like intravenous drug users, hemodialysis patients, health care and public safety workers. Also in the case of clinical deterioration of chronic HBV infection, superinfection with HDV should be considered.

Specific markers of HDV infection in serum are: Hepatitis D virus RNA, HDAg and anti-HDV. HDV RNA is detected in serum by molecular hybridization [30] or more sensitive RT-PCR [31], thus serum HDAg and anti-HDV are detected by enzyme-linked immunosorbent assay (ELISA) [32] and radioimmunoassay (RIA). During the diagnostic procedure, along with confirmation of HDV presence, it is necessary to clarify the stage of the infection due to the differences in clinical course and prognosis. Based on the interactions between two viruses, three patterns of HDV infections are distinguished: acute HDV/HBV co-infection, acute HDV infection of HBsAg positive carrier and chronic HDV infection.

Acute HBV/HDV co-infection is characterized with a high titre of IgM anti-HBc, a marker of acute HBV infection which enables to distinguishing infection from acute HDV superinfection. HDAg appears in the early phase of the acute infection and it is only transiently detectable in serum, unless patient is immunodeficient. Then HDAg can be detected for a longer period due to the weak immune system [33]. HDV RNA is also detectable in the early phase of acute infection [34] and represents a sensitive marker for virus replication present in 90% of patients. Nowadays, an active HDV infection is confirmed by detection of HDV RNA in the serum by sensitive real time PCR assays [35]. Although, HDV RNA test can be false negative due to the variability of the genome sequence, so that sero-conversion and detection of IgM anti-HDV is still helpful to establish diagnosis of acute infection.

In chronic HDV infection the high titre of IgG anti-HD antibodies persist even after viral clearance. Also, a large proportion of patients has positive IgM antiHD, a characteristic marker of acute infection. Persistence of anti-HD IgM antibodies indicates progression of disease to the chronic form. HDV RNA is present in the serum of chronically infected patients as HDAg.

Individual's positive for HDV serum markers should be subjected to the liver biopsy to determine histological stage of the liver disease, due to the fact that HDV serum markers or values of liver test do not reflect severity of liver damage [36]. Also, all the HDV positive patients should be tested for HCV and HIV because of the high frequency of co-infection with these parenterally transmitted viruses [37].

## 7. Treatment

Presence of HBsAg is necessary for the replication of the HDV, hence the therapeutic aim is to eradicate both pathogens. HDV is considered eradicated if HDV-RNA in serum and HDAg in the liver are negative 6 months after therapy [24]. Despite the sustained viral response, there is still a possibility of reactivation of HDV in HBsAg positive individual, due to the limitations of diagnostic procedures to detect a low level of HDV copies (1000 copies/ml). Experimentally in animal model, the possibility to transfer HDV infection with serum diluted up to  $10^{-11}$  was



demonstrated [38]. Although HDV is considered eradicated when serum HDV RNA and HDAg in the liver are persistently undetectable, only eradication of HBsAg represents a complete cure and it is ultimate goal of HDV treatment. Eradication of virus results in normalization of ALT levels and stopping of liver fibrosis process, while developed anti HD antibodies will prevent re-infections [24].

So far only approved therapy for Hepatitis D is standard interferon- $\alpha$  (IFN- $\alpha$ ). Long-term administration of high-dose standard IFN- $\alpha$ , 5 million units daily or 9 million units three times per week for 12 months, results in normalization of alanine aminotransferase serum values, clearance of serum HDV RNA, and histological improvement in 50 percent of patients with chronic hepatitis D [39]. High-dose IFN therapy improves the long-term clinical outcome and survival rate of the patients, even if they have advanced disease and active cirrhosis before therapy induction [40]. There are still arguments going on with regard to duration of interferon treatment. Interferon therapy administered through 12 months has better results comparing to 6 months therapy [39], although prolongation of IFN therapy to 24 months does not result with increasing response to the treatment [41]. Unfortunately, large number of patients will appear relapse usually 2-6 months after termination of treatment [39]. Thus interferon therapy is insufficient for the majority of patients with chronic HDV, characterized with incomplete sustained viral response and common biochemical and virological relapses after cessation of treatment [42, 43]. Interferon treatment is often accompanied with numerous side effects, which requires a continuous supervision of the patients during treatment. Most common side effects are influenza like symptoms such as fatigue and weight loss [44]. Severe psychiatric disorders can appear as a result of prolonged high dose interferon therapy [42, 45], which disables interferon application to the certain number of patients. Another compulsory reason for cessation of interferon therapy is decompensation of liver disease, due to the fact that high number of patients has advanced disease and cirrhosis [46, 47].

Lately, pegylated form of interferon- $\alpha$  (Peg-IFN- $\alpha$ ) is introduced in therapy of HDV. It is characterized by longer half-life, which allows longer intervals between drug administrations. Treatment with Peg-IFN- $\alpha$  showed better response in naive patients and in previous nonresponders compared to standard IFN- $\alpha$  treatment [48, 49]. Patients not achieving SVR with standard interferon therapy, may eradicate serum HDV RNA after a 6-month treatment with Peg-IFN- $\alpha$  [50]. For the lowering of HDV RNA beyond detectable level, it is demonstrated that even standard doses of Peg-IFN- $\alpha$  are more successful than high doses of standard IFN- $\alpha$ , although in that case seroconversion of HBsAg is not taken into consideration [51]. However, the rate of clearance of HBsAg is greater with Peg-IFN- $\alpha$ , but overall clearance and seroconversion of HBsAg is low, only in 3-5% of cases [52]. Generally, it is difficult to assess the effectiveness of IFN- $\alpha$  therapy due to differences in study strategies that examined the effect of the treatment. Studies usually differ in forms of drugs, doses, duration of the treatment and patient follow-up period, making comparison of results a difficult task (Table 1.). Considering these differences in the previous studies, overall sustained viral response varies from 17 to 43 % [53]. Currently the largest hepatitis delta multicenter study is HIDIT I trial, which is carried out by the German Network of Competence for Viral Hepatitis (Hep-Net) in collaboration with centers from Turkey and Greece. In total of 90 patients with hepatitis D, the effect of Peg-IFN-

$\alpha$ -2a in combination with adefovir versus either drug alone was examined. Overall, 28% of the patients had sustained viral response after treatment with Peg-IFN- $\alpha$ -2a for 48 weeks, with no difference in efficacy between combined therapy compared with Peg-IFN- $\alpha$ -2a monotherapy [54]. From the results of current studies it is evident that treatment with Peg-IFN- $\alpha$ -2a has limited efficacy as a therapy of hepatitis delta, thus further investigations of potential treatment options are needed.

Study and year	Patients (n)	Therapy	Duration (weeks)	Results
Yurdaydin, 2008. [74]	39	1st group (n=14), Lamivudine (100mg/day) plus IFN- $\alpha$ -2a (9 MU/3x week) vs. 2nd group (n=8), IFN- $\alpha$ -2a vs. 3rd group (n=17), Lamivudine	48	BR: 1st(64%), 2nd(63%), 3rd(18%) EOTR: 1st(50%), 2nd(50%), 3rd(12%) SVR: 1st(36%), 2nd(50%), 3rd(12%) Combination treatment was not superior to IFN therapy.
Gheorghe, 2011. [75]	49	Peg-IFN- $\alpha$ -2b (1.5 $\mu$ g/kg/week)	48	BR: 50% EOTR: 33.3% SVR: 25%
Ormeçi, 2011. [76]	18	Peg-IFN- $\alpha$ -2b (1.5 $\mu$ g/kg/week)	96 weeks (n=11) and 48 weeks (n=7)	No significant difference between two groups in terms of HDV-RNA suppression and ALT normalisation.
Wedemeyer, 2011. [77]	90	1st group (n=31) Peg-IFN- $\alpha$ -2a (180 $\mu$ g/week) plus Adefovir (10 mg/daily) vs. 2nd group (n=29) Peg-IFN- $\alpha$ -2a vs. 3rd group(n=30) Adefovir	48	BR: 1st(32%), 2nd(28%), 3rd(7%) EOTR: 1st(23%), 2nd(24%), 3rd(0%) SVR: 1st(26%), 2nd(31%), 3rd(0%)
Karaca, 2012. [78]	32	Peg-IFN- $\alpha$ -2a (180 $\mu$ g) or Peg-IFN- $\alpha$ -2b (1.5 $\mu$ g/kg) per week	96	EOTR: 50% SVR: 47%
Kabaçam, 2012. [79]	13	Entecavir (1 mg/day)	48	Ineffective in chronic hepatitis delta.
Samiullah, 2012. [80]	238	Peg-IFN- $\alpha$ -2b (1.5 $\mu$ g/kg/week)	48	BR: 51.3% EOTR: 29.8% SVR: 29.4%

**BR:** Biochemical response is determined by a normalization of ALT at the end of the treatment.

**EOTR:** The end of treatment response is defined by a HDV-RNA negative status.

**SVR:** A sustained virological response is defined by undetectable serum HDV-RNA at six months after the end of treatment.

**Table 1.** Recent studies for treatment of chronic delta hepatitis



Last 30 years many antiviral drugs are tested in the therapy of hepatitis D, but with limited success. Particularly it was tested efficiency of nucleoside and nucleotide analogues (NUCs) such as: lamivudine, adefovir, famciclovir and entecavir; due to the fact that NUCs have some therapeutic efficacy against HBV. The effect of tenofovir was observed in a group of patients with concomitant presence of HCV, HBV and HDV infection. It seems that the prior long term treatment with lamivudine and tenofovir before introduction of IFN therapy might help faster decline of HDV RNA copies in such patients. However, seroconversion of HBsAg was not observed. Patients who suffer from multiple infections with HCV, HBV and HDV present another group difficult to treat. Unfortunately, the consequence is a progressive liver fibrosis. It is shown that neither IFN monotherapy or combination therapy with NUCs and IFN are effective. Those patients are less sensitive to IFN therapy [55]. Babiker et al. report the case of successful depletion of serum HDV RNA in a patient with acute HDV superinfection due to 65 weeks treatment with tenofovir and lamivudine. But, after the cessation of the treatment HDV RNA levels began to increase [56]. Another combination therapy is the therapy with entecavir and PEG IFN $\alpha$ -2a. In this case also, quantitative HBsAg was used as the treatment response guidance for dual infection with HBV and HDV. The seroconversion of HBsAg and undetectable HDV RNA levels were achieved after 35 months of such therapy. In this patient, the stage of liver fibrosis has also improved significantly. The consolidation therapy during next 12 months after the seroconversion was continued and the patient remained seronegative during 12 months after cessation of the therapy [57].

Possible new drug candidates for the therapy are the ones affecting the interaction between HD virion and HBsAg, as well as posttranslational modifications of HDV proteins, such as prenylation. Also, it seems IFN- $\lambda$  could be possible alternative for IFN- $\alpha$  because in the treatment of chronic hepatitis C, IFN- $\lambda$  proved to cause less side effects [53].

## 8. Liver transplantation in HDV patients

HDV infection is characterized with more severe disease than HBV mono-infection. Studies showed two times higher relative risk of cirrhosis and threefold risk increase of hepatocellular carcinoma in patients coinfecting with HBV and HDV compared with HBV mono-infection [27, 58]. Consequently, liver transplantation (LT) represents only therapeutic option for the patients with end-stage liver disease, as well as for hepatocellular carcinoma and fulminant hepatitis due to the coinfection or superinfection with HDV and HBV.

Prevention of allograft reinfection is the main requirement for the long term survival. Major risk factor predicting HBV-HDV recurrence after LT is a high level of HBV DNA ( $>10^5$  copies/ml) at transplant, fortunately that is unusual feature of HDV disease course [59]. Therefore, patients coinfecting with HBV and HDV generally do not require pretransplant antiviral B therapy. In case when pretransplant antiviral treatment is needed, entecavir or teneofovir are preferred rather than lamivudine, while IFN therapy is not recommended during the pretransplant period. Other predictors of a low risk of HBV-HDV recurrence are low levels of HBV replication markers, HDV coinfection and fulminant hepatitis [60]. Coinfection with

human immunodeficiency virus and recurrence of HCC represent a risk factors for HBV recurrence [61, 62].

The patient's prognosis and overall outcome after LT is on satisfactory level with current posttransplant therapy. Golden standard for prevention of recurrent disease is combination of hyperimmune serum against HBV(HBIg) and potent nucleoside analogue. Therapeutic strategy of low dose intramuscular HBIg in combination with lamivudine is the most cost-effective profilaxis [63], with the rates of the recurrence level as low as 4% at 4 years [64]. Therefore, the outcome of LT due to HDV related liver disease is similar to or better than in other indications of LT [65, 66].

Due to LT, HDV RNA becomes negative within the first days after transplantation, followed by a decline of HbsAg with almost identical pattern. However, HDVAg can be detected in the hepatocytes of the graft for several months after treatment [54]. This HDV latency in the graft represents a potential source of HDV recurrence, because of possible HBV superinfection and reexpression of HBsAg. Thus, transplanted patients should be monitored for HbsAG and HBV DNA every 3 months and for HDV RNA every 6 months.

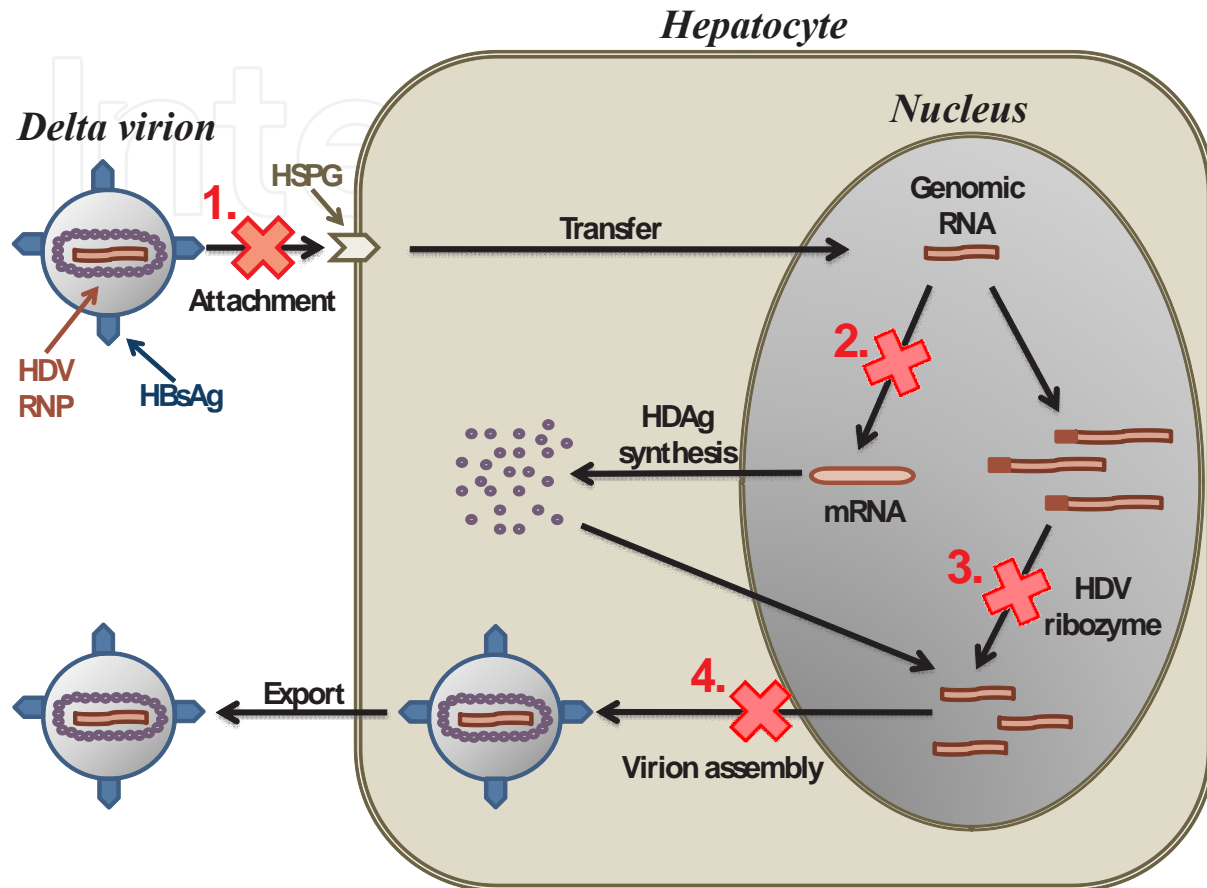
## 9. Perspectives for a vaccine development against HDV

Since the details in pathophysiologic response to HDV infection still aren't enlightened, there are difficulties in finding the effective vaccine against it. In the case of HDV infection, the antigen is nucleoprotein and the immunization means the activation of T cells (CD4+ and CD8+) which would destroy infected cells and prevent replication of the virus. Preclinical studies have been done on woodchucks. In this model of chronically infected woodchucks with woodchuck hepatitis virus (WHV), it is possible to achieve the superinfection with HDV. So far, T cell vaccine prevented the coinfection with WHV- HDV, but it failed to prevent the superinfection of chronic carriers of WHV. Further studies have to be done to resolve the problem of preventing the superinfection by stimulating T cells. This would mean that chronic carriers would have to be vaccinated frequently to activate a large number of T cells before the patient is exposed to HDV. [67]

## 10. Novel therapeutic strategies for future treatment

So far, the treatment outcome of hepatitis delta is not satisfactory. Thus, new therapy approaches are necessary (Figure 2.). Interferon- $\alpha$  targets the HbsAg, whose depletion is crucial for successful treatment of hepatitis delta. The major difficulty with such therapy are numerous adverse effects, since IFN- $\alpha$  receptors are also present in other tissues than hepatic. Additional problem is the necessity of long term application of IFN- $\alpha$  to achieve therapeutic response. Better candidate could be IFN- $\lambda$ , since its receptors are present only on epithelial cells [68]. It has been shown that pegylated IFN- $\lambda$  used as monotherapy, or in combination with ribavirin, has significant antiviral activity against hepatitis C virus. Also, as expected, it causes less

undesirable side effects. [69] Further studies must be done to evaluate the effectiveness of IFN- $\lambda$  in treatment of chronic hepatitis delta.



HDV RNP= hepatitis D virus ribonucleoprotein. HBsAg= hepatitis B surface antigen. HSPG=highly sulphated proteoglycans. HDVAg=hepatitis D antigen.

**Figure 2.** Potential drug targets in HDV treatment. 1. Neutralizing of negatively charged HSPG may prevent HDV and HBV attachment to hepatocyte membrane. 2. Gene therapy targets genes which encode HDVAg 3. Inhibition of HDV ribozymes would prevent virus replication, 4. Interfering with posttranslational modifications of HDVAg enables virion assembly.

Other strategy for therapy development is to interfere with posttranslational modifications of HDVAg. Such modifications are prenylation, acetylation, methylation and phosphorylation of HDVAg. Prenylation inhibitors proved effective in cell culture model and are currently in clinical studies. [54] Drugs interfering with other types of HDV posttranslational modifications haven't been developed yet. [70]

Since HDV genome is too small to code all the necessary particles for its own replication, it almost entirely depends on the host's replication mechanisms. For example, it deceives host's RNA polymerases so they copy HDV genome. HDV replication is known as rolling replication

mechanism, meaning that HDV circular genome is elongated into multimeric linear transcripts and then cleaved into multiple genome size monomers by its own RNA. This type of RNA is known as ribozyme, which is actually HDV RNA with enzymatic activity.

Small interfering RNAs (siRNAs) are up to 25 nucleotides long double stranded RNAs which silence particular gene by binding to mRNA. After binding to target mRNA, siRNA causes its degradation. In therapy of HDV infection, silencing gene which codes LHD-Ag would disable HD virion replication [16].

Ribozymes can also serve as target for therapy. It has been shown that amoxicillin, apramycin and ristomycin in complex with copper (II) bind to HDV ribozymes and inhibit them. [71] Further studies are necessary to investigate the therapeutic potential of such treatment.

Not only large HDAg (LHDAg) is important for the lifecycle of hepatitis delta virus, but also the small delta antigen protein (SHDAg). Therefore, it can also be the target for new drugs in development for HDV treatment.

It has been shown that the negatively charged highly sulphated proteoglycans (HSPG) play a role in both HBV and HDV attachment to hepatocyte membrane. Those weak forces between opposite charged subjects enable virus attachment to the cell. [72] This kind of target could be good for developing the drug which would prevent binding of both HBV and HDV to hepatocyte surface by neutralizing the negative charge of HSPG.

Gene therapy would target HDVAg, which is proved to have crucial role in lifecycle of HDV. Design of specific molecule which binds to gene which encodes delta antigen is in progress. Some computational analyses have been done to simulate the silencing of target gene. [73]

## 11. Conclusions

HDV is an unusual, defective hepatotropic virus which causes severe acute hepatitis and most progressive chronic viral hepatitis. Despite the efforts in eradication of HDV, as its obligatory helper HBV, prevalence of HDV in developed countries remains stable and represents a relevant public health concern. Current conventional therapy of hepatitis delta is characterized with poor overall response, thus further investigations of novel treatment options are needed. Continuous research of virology and pathogenesis is necessary to provide fundamentals for development of novel approaches in treatment of HDV.

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Authors declare no conflict of interest

## References

- [1] Rizzetto M, Canese MG, Aricò S, Crivelli O, Trepo C, Bonino F, et al. Immunofluorescence detection of new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. *Gut*. 1977;18(12):997-1003.
- [2] Rizzetto M, Shih JW, Gocke DJ, Purcell RH, Verme G, Gerin JL. Incidence and significance of antibodies to delta antigen in hepatitis B virus infection. *Lancet*. 1979;2(8150):986-90.
- [3] Rizzetto M, Canese MG, Gerin JL, London WT, Sly DL, Purcell RH. Transmission of the hepatitis B virus-associated delta antigen to chimpanzees. *J Infect Dis*. 1980;141(5):590-602.
- [4] Rizzetto M, Hoyer B, Canese MG, Shih JW, Purcell RH, Gerin JL. delta Agent: association of delta antigen with hepatitis B surface antigen and RNA in serum of delta-infected chimpanzees. *Proc Natl Acad Sci U S A*. 1980;77(10):6124-8.
- [5] Gudima S, Chang J, Moraleda G, Azvolinsky A, Taylor J. Parameters of human hepatitis delta virus genome replication: the quantity, quality, and intracellular distribution of viral proteins and RNA. *J Virol*. 2002;76(8):3709-19.
- [6] Ryu WS, Netter HJ, Bayer M, Taylor J. Ribonucleoprotein complexes of hepatitis delta virus. *J Virol*. 1993;67(6):3281-7.
- [7] Alvarado-Mora MV, Locarnini S, Rizzetto M, Pinho JR. An update on HDV: virology, pathogenesis and treatment. *Antivir Ther*. 2013;18(3 Pt B):541-8.



- [8] Lai MM. RNA replication without RNA-dependent RNA polymerase: surprises from hepatitis delta virus. *J Virol.* 2005;79(13):7951-8.
- [9] Taylor JM. Structure and replication of hepatitis delta virus RNA. *Curr Top Microbiol Immunol.* 2006;307:1-23.
- [10] Govindarajan S, Chin KP, Redeker AG, Peters RL. Fulminant B viral hepatitis: role of delta agent. *Gastroenterology.* 1984;86(6):1417-20.
- [11] Fattovich G, Boscaro S, Noventa F, Pornaro E, Stenico D, Alberti A, et al. Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. *J Infect Dis.* 1987;155(5):931-5.
- [12] Hadziyannis SJ. Review: hepatitis delta. *J Gastroenterol Hepatol.* 1997;12(4):289-98.
- [13] Rizzetto M, Ponzetto A, Forzani I. Hepatitis delta virus as a global health problem. *Vaccine.* 1990;8 Suppl:S10-4; discussion S21-3.
- [14] Wedemeyer H, Heidrich B, Manns MP. Hepatitis D virus infection--not a vanishing disease in Europe! *Hepatology.* 2007;45(5):1331-2; author reply 2-3.
- [15] Heidrich B, Deterding K, Tillmann HL, Raupach R, Manns MP, Wedemeyer H. Virological and clinical characteristics of delta hepatitis in Central Europe. *J Viral Hepat.* 2009;16(12):883-94.
- [16] Ciancio A, Rizzetto M. Chronic hepatitis D at a standstill: where do we go from here? *Nat Rev Gastroenterol Hepatol.* 2014;11(1):68-71.
- [17] Shakil AO, Hadziyannis S, Hoofnagle JH, Di Bisceglie AM, Gerin JL, Casey JL. Geographic distribution and genetic variability of hepatitis delta virus genotype I. *Virology.* 1997;234(1):160-7.
- [18] Ito T, Tahara SM, Lai MMC. The 3'-untranslated region of hepatitis C virus RNA enhances translation from an internal ribosomal entry site. *Journal of Virology.* 1998;72(11):8789-96.
- [19] Radjef N, Gordien E, Ivaniushina V, Gault E, Anaïs P, Drugan T, et al. Molecular phylogenetic analyses indicate a wide and ancient radiation of African hepatitis delta virus, suggesting a deltavirus genus of at least seven major clades. *J Virol.* 2004;78(5):2537-44.
- [20] Su CW, Huang YH, Huo TI, Shih HH, Sheen IJ, Chen SW, et al. Genotypes and viremia of hepatitis B and D viruses are associated with outcomes of chronic hepatitis D patients. *Gastroenterology.* 2006;130(6):1625-35.
- [21] Wu JC, Chen CM, Sheen IJ, Lee SD, Tzeng HM, Choo KB. Evidence of transmission of hepatitis D virus to spouses from sequence analysis of the viral genome. *Hepatology.* 1995;22(6):1656-60.



- [22] Weisfuse IB, Hadler SC, Fields HA, Alter MJ, O'Malley PM, Judson FN, et al. Delta hepatitis in homosexual men in the United States. *Hepatology*. 1989;9(6):872-4.
- [23] Niro GA, Casey JL, Gravinese E, Garrubba M, Conoscitore P, Sagnelli E, et al. Intrafamilial transmission of hepatitis delta virus: molecular evidence. *J Hepatol*. 1999;30(4):564-9.
- [24] Pascarella S, Negro F. Hepatitis D virus: an update. *Liver Int*. 2011;31(1):7-21.
- [25] Smedile A, Farci P, Verme G, Caredda F, Cargnel A, Caporaso N, et al. Influence of delta infection on severity of hepatitis B. *Lancet*. 1982;2(8305):945-7.
- [26] Lee WM. Acute liver failure. *N Engl J Med*. 1993;329(25):1862-72.
- [27] Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). *Gut*. 2000;46(3):420-6.
- [28] Farci P, Niro GA. Clinical features of hepatitis D. *Semin Liver Dis*. 2012;32(3):228-36.
- [29] Rosina F, Conoscitore P, Cuppone R, Rocca G, Giuliani A, Cozzolongo R, et al. Changing pattern of chronic hepatitis D in Southern Europe. *Gastroenterology*. 1999;117(1):161-6.
- [30] Zignego AL, Dubois F, Samuel D, Georgopoulou U, Reynes M, Gentilini P, et al. Serum hepatitis delta virus RNA in patients with delta hepatitis and in liver graft recipients. *J Hepatol*. 1990;11(1):102-10.
- [31] Wu JC, Chen TZ, Huang YS, Yen FS, Ting LT, Sheng WY, et al. Natural history of hepatitis D viral superinfection: significance of viremia detected by polymerase chain reaction. *Gastroenterology*. 1995;108(3):796-802.
- [32] Shattock AG, Morgan BM. Sensitive enzyme immunoassay for the detection of delta antigen and anti-delta, using serum as the delta antigen source. *J Med Virol*. 1984;13(1):73-82.
- [33] Gripon P, Ribiere O, Cadranel JF, Pelletier S, Pillot B, Emerit J, et al. Long-term delta antigenaemia without appearance of delta antibody in two immunodeficient patients. *Lancet*. 1987;1(8540):1031.
- [34] Buti M, Esteban R, Roggendorf M, Fernandez J, Jardi R, Rashafer R, et al. Hepatitis D virus RNA in acute delta infection: serological profile and correlation with other markers of hepatitis D virus infection. *Hepatology*. 1988;8(5):1125-9.
- [35] Mederacke I, Bremer B, Heidrich B, Kirschner J, Deterding K, Bock T, et al. Establishment of a novel quantitative hepatitis D virus (HDV) RNA assay using the Cobas TaqMan platform to study HDV RNA kinetics. *J Clin Microbiol*. 2010;48(6):2022-9.

- [36] Zachou K, Yurdaydin C, Drebber U, Dalekos GN, Erhardt A, Cakaloglu Y, et al. Quantitative HBsAg and HDV-RNA levels in chronic delta hepatitis. *Liver Int.* 2010;30(3):430-7.
- [37] Boyd A, Lacombe K, Miaillhes P, Gozlan J, Bonnard P, Molina JM, et al. Longitudinal evaluation of viral interactions in treated HIV-hepatitis B co-infected patients with additional hepatitis C and D virus. *J Viral Hepat.* 2010;17(1):65-76.
- [38] Ponzetto A, Hoyer BH, Popper H, Engle R, Purcell RH, Gerin JL. Titration of the infectivity of hepatitis D virus in chimpanzees. *J Infect Dis.* 1987;155(1):72-8.
- [39] Farci P, Mandas A, Coiana A, Lai ME, Desmet V, Van Eyken P, et al. Treatment of chronic hepatitis D with interferon alfa-2a. *N Engl J Med.* 1994;330(2):88-94.
- [40] Farci P, Roskams T, Chessa L, Peddis G, Mazzoleni AP, Scioscia R, et al. Long-term benefit of interferon alpha therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. *Gastroenterology.* 2004;126(7):1740-9.
- [41] Yurdaydin C, Bozkaya H, Karaaslan H, Onder FO, Erkan OE, Yalçın K, et al. A pilot study of 2 years of interferon treatment in patients with chronic delta hepatitis. *J Viral Hepat.* 2007;14(11):812-6.
- [42] Gaudin JL, Faure P, Godinot H, Gerard F, Trepo C. The French experience of treatment of chronic type D hepatitis with a 12-month course of interferon alpha-2B. Results of a randomized controlled trial. *Liver.* 1995;15(1):45-52.
- [43] Madejón A, Cotonat T, Bartolomé J, Castillo I, Carreño V. Treatment of chronic hepatitis D virus infection with low and high doses of interferon-alpha 2a: utility of polymerase chain reaction in monitoring antiviral response. *Hepatology.* 1994;19(6):1331-6.
- [44] Romeo R, Del Ninno E, Rumi M, Russo A, Sangiovanni A, de Franchis R, et al. A 28-year study of the course of hepatitis Delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. *Gastroenterology.* 2009;136(5):1629-38.
- [45] Lau JY, King R, Tibbs CJ, Catterall AP, Smith HM, Portmann BC, et al. Loss of HBsAg with interferon-alpha therapy in chronic hepatitis D virus infection. *J Med Virol.* 1993;39(4):292-6.
- [46] Rizzetto M. Hepatitis D: thirty years after. *J Hepatol.* 2009;50(5):1043-50.
- [47] Niro GA, Rosina F, Rizzetto M. Treatment of hepatitis D. *J Viral Hepat.* 2005;12(1):2-9.
- [48] Castelnau C, Le Gal F, Ripault MP, Gordien E, Martinot-Peignoux M, Boyer N, et al. Efficacy of peginterferon alpha-2b in chronic hepatitis delta: relevance of quantitative RT-PCR for follow-up. *Hepatology.* 2006;44(3):728-35.

- [49] Erhardt A, Gerlich W, Starke C, Wend U, Donner A, Sagir A, et al. Treatment of chronic hepatitis delta with pegylated interferon-alpha2b. *Liver Int.* 2006;26(7):805-10.
- [50] Ferenci P, Formann E, Romeo R. Successful treatment of chronic hepatitis D with a short course of peginterferon alfa-2a. *Am J Gastroenterol.* 2005;100(7):1626-7.
- [51] Alavian SM, Tabatabaei SV, Behnava B, Rizzetto M. Standard and pegylated interferon therapy of HDV infection: A systematic review and meta-analysis. *J Res Med Sci.* 2012;17(10):967-74.
- [52] Li WC, Wang MR, Kong LB, Ren WG, Zhang YG, Nan YM. Peginterferon alpha-based therapy for chronic hepatitis B focusing on HBsAg clearance or seroconversion: a meta-analysis of controlled clinical trials. *BMC Infect Dis.* 2011;11:165.
- [53] Rizzetto M. Current management of delta hepatitis. *Liver Int.* 2013;33 Suppl 1:195-7.
- [54] Mederacke I, Filmann N, Yurdaydin C, Bremer B, Puls F, Zacher BJ, et al. Rapid early HDV RNA decline in the peripheral blood but prolonged intrahepatic hepatitis delta antigen persistence after liver transplantation. *J Hepatol.* 2012;56(1):115-22.
- [55] Riaz M, Idrees M, Kanwal H, Kabir F. An overview of triple infection with hepatitis B, C and D viruses. *Virology.* 2011;8:368.
- [56] Babiker ZO, Hogan C, Ustianowski A, Wilkins E. Does interferon-sparing tenofovir disoproxil fumarate-based therapy have a role in the management of severe acute hepatitis delta superinfection? *J Med Microbiol.* 2012;61(Pt 12):1780-3.
- [57] Chen GY, Su TH, Kao JH. Successful treatment of chronic hepatitis B and D with pegylated-interferon plus entecavir. *J Formos Med Assoc.* 2013.
- [58] Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol.* 2008;48(2):335-52.
- [59] Samuel D, Muller R, Alexander G, Fassati L, Ducot B, Benhamou JP, et al. Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med.* 1993;329(25):1842-7.
- [60] Degertekin B, Han SH, Keeffe EB, Schiff ER, Luketic VA, Brown RS, et al. Impact of virologic breakthrough and HBIG regimen on hepatitis B recurrence after liver transplantation. *Am J Transplant.* 2010;10(8):1823-33.
- [61] Faria LC, Gigou M, Roque-Afonso AM, Sebah M, Roche B, Fallot G, et al. Hepatocellular carcinoma is associated with an increased risk of hepatitis B virus recurrence after liver transplantation. *Gastroenterology.* 2008;134(7):1890-9; quiz 2155.
- [62] Coffin CS, Stock PG, Dove LM, Berg CL, Nissen NN, Curry MP, et al. Virologic and clinical outcomes of hepatitis B virus infection in HIV-HBV coinfecting transplant recipients. *Am J Transplant.* 2010;10(5):1268-75.

- [63] Roche B, Samuel D. Liver transplantation in delta virus infection. *Semin Liver Dis.* 2012;32(3):245-55.
- [64] Gane EJ, Angus PW, Strasser S, Crawford DH, Ring J, Jeffrey GP, et al. Lamivudine plus low-dose hepatitis B immunoglobulin to prevent recurrent hepatitis B following liver transplantation. *Gastroenterology.* 2007;132(3):931-7.
- [65] Steinmüller T, Seehofer D, Rayes N, Müller AR, Settmacher U, Jonas S, et al. Increasing applicability of liver transplantation for patients with hepatitis B-related liver disease. *Hepatology.* 2002;35(6):1528-35.
- [66] Kim WR, Poterucha JJ, Kremers WK, Ishitani MB, Dickson ER. Outcome of liver transplantation for hepatitis B in the United States. *Liver Transpl.* 2004;10(8):968-74.
- [67] Roggendorf M. Perspectives for a vaccine against hepatitis delta virus. *Semin Liver Dis.* 2012;32(3):256-61.
- [68] Donnelly RP, Dickensheets H, O'Brien TR. Interferon-lambda and therapy for chronic hepatitis C virus infection. *Trends Immunol.* 2011;32(9):443-50.
- [69] Muir AJ, Shiffman ML, Zaman A, Yoffe B, de la Torre A, Flamm S, et al. Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. *Hepatology.* 2010;52(3):822-32.
- [70] Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet.* 2011;378(9785):73-85.
- [71] Stokowa-Sołtys K, Gaggelli N, Nagaj J, Szczepanik W, Ciesiołka J, Wrzesiński J, et al. High affinity of copper(II) towards amoxicillin, apramycin and ristomycin. Effect of these complexes on the catalytic activity of HDV ribozyme. *J Inorg Biochem.* 2013;124:26-34.
- [72] Lamas Longarela O, Schmidt TT, Schöneweis K, Romeo R, Wedemeyer H, Urban S, et al. Proteoglycans act as cellular hepatitis delta virus attachment receptors. *PLoS One.* 2013;8(3):e58340.
- [73] Singh S, Gupta SK, Nischal A, Khattri S, Nath R, Pant KK, et al. Identification and characterization of novel small-molecule inhibitors against hepatitis delta virus replication by using docking strategies. *Hepat Mon.* 2011;11(10):803-9.
- [74] Yurdaydin C, Bozkaya H, Onder FO, Sentürk H, Karaaslan H, Akdoğan M, et al. Treatment of chronic delta hepatitis with lamivudine vs lamivudine + interferon vs interferon. *J Viral Hepat.* 2008;15(4):314-21.
- [75] Gheorghe L, Iacob S, Simionov I, Vadan R, Constantinescu I, Caruntu F, et al. Weight-based dosing regimen of peg-interferon  $\alpha$ -2b for chronic hepatitis delta: a multicenter Romanian trial. *J Gastrointestin Liver Dis.* 2011;20(4):377-82.

- [76] Ormeci N, Bölükbaş F, Erden E, Coban S, Ekiz F, Erdem H, et al. Pegylated interferon alfa-2B for chronic delta hepatitis: 12 versus 24 months. *Hepatogastroenterology*. 2011;58(110-111):1648-53.
- [77] Wedemeyer H, Yurdaydın C, Dalekos GN, Erhardt A, Çakaloğlu Y, Değertekin H, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med*. 2011;364(4):322-31.
- [78] Karaca C, Soyer OM, Baran B, Ormeci AC, Gokturk S, Aydin E, et al. Efficacy of pegylated interferon- $\alpha$  treatment for 24 months in chronic delta hepatitis and predictors of response. *Antivir Ther*. 2013;18(4):561-6.
- [79] Kabaçam G, Onder FO, Yakut M, Seven G, Karatayli SC, Karatayli E, et al. Entecavir treatment of chronic hepatitis D. *Clin Infect Dis*. 2012;55(5):645-50.
- [80] Samiullah S, Bikharam D, Nasreen. Treatment of chronic hepatitis delta virus with peg-interferon and factors that predict sustained viral response. *World J Gastroenterol*. 2012;18(40):5793-8.