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Virological Responses Evaluated During Antiviral Therapy in Chronically Infected HBV and HDV Patients

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Abstract

The aim of thisinvestigation was to evaluate the various virological responses at several time points of therapy in chronically infected patients with dual HBV and HDV infection.A total of 23 patients, 8 (34.8%) women and 15 (65.2%) men with serologically proved chronic HBV and HDV infection, at the Gastroenterology Department of the University Hospital St. Marina, Varna, Bulgaria, were investigated in the hospital's laboratories of Clinical Virology and Clinical Immunology. Quantitative determination of HBV DNA and HDV RNA was performed by Real-time PCR, on several occasions during and after therapy from 2012 to 2017.HBV viremia ranged from $1.0x10^{2}$to $1.6x10^{6}$cps/ml. HDV viremia ranged from $2.5x10^{2}$ to $9.6x10^{6}$cps/ml. HDV replication dominated in 16 patients (69.6%), accompanied by low HBV viremia. HDV-RNA and HBV-DNA levels showed no direct inverse correlation in the less part of the investigated patients, although higher HDV levels were accompanied by lower HBV viremia. HBV DNA correlates positively with HBeAg positivity. IFN is efficient in reducing transaminanses (ALT), decreasing HDV RNA levels at some point, but it is not operative in HDV RNA clearance. Lamivudine alone is a potent inhibitor of HBV DNA replication but does not improve disease activity or lower HDV RNA levels in patients with chronic delta hepatitis. It did not increase sustained virological response when combined with IFN. When virological breakthrough during Lamivudine therapy occurs, Tenofovir is a means of choice for treatment. Biochemical parameters did not accurately indicate the stage and grade of liver disease in chronic HDV patients as they often fluctuate over time.

Key words: *HBV DNA,HDV RNA,virological responses, INF, Lamivudine, Tenofovir*

Резюме

Да се оценят различните вирусологични отговори, в няколко етапа от терапията, при пациенти с хронична смесена HBV и HDV инфекция. Изследвани са 23 пациенти на гастроентерологичната клиника на УМБАЛ "Св. Марина" Варна, от които 8 жени (34.8%) и 15 мъже (65.2%), със серологично-

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гични данни за хронична смесена HBV и HDV инфекция в Лабораторията по клинична вирусология и Лабораторията по клинична имунология на УМБАЛ ”Св. Марина”. Количествено определяне на вирусния товар за HBVДНК, както и HDVРНК беше извършено посредством Real-time PCR, на различни етапи от провежданата терапия, в периода 2012-2017 год. HBV вирусният товар се движи от$1.0 x 10^2$до $1.6 x 10^6$cps/ml. HDV виремия доказахме в обсег от $2.5 x 10^2$до $9.6 x 10^6$cps/ml. HDV репликацията доминира при 16 пациента (69.6%), съпроводена от ниска HBV виремия. Регистрираните нива на HDV РНК и HBV ДНК не показаха директна обратна корелация, при по-малка част от пациентите, въпреки че по-високите HDV нива се свързват обичайно с по-ниска HBV виремия. HBVДНК корелира положително с HbeAg-позитивността. IFN ефективно води до снижаване на нивата на трансаминазите (ALAT), намалява нивата на HDV РНК, но не води до трайно негативиране на HDV РНК. Монотерапията с Lamivudine не подобрява хода на болестта при пациенти с хроничен делта хепатит и не намалява трайно нивата на HDV РНК, въпреки че е потенциален инхибитор на HBV ДНК репликацията. Lamivudine в комбинация с IFN не задържа нивата на HBV ДНК под 2000 IU/ml за поне12 месеца след края на терапията. При наличие на вирусологичен пробив на фона на терапия с Lamivudine, се включва Tenofovir, като средство на избор. Биохимичните показатели не отразяват коректно стадия на чернодробното заболяване, при пациентите с хронична HBV и HDV инфекция, т.к се варират през цели период на лечението и след това.

Introduction

Chronic hepatitis B is a major global problem, affecting more than 350 million worldwide and leading to 1 million deaths each year (Lee, 1997; Wright *et al.,* 2006; Ganji *et al.*, 2011). There are 15 million of chronic HBV carriers in Europe (Ott *et al.*, 2012). The prevalence of chronic carriage generally increases from North to South and from West to East, varying between 0.1% in the Netherlands and > 7 % in the Balkan countries (Todorova *et al.*, 2016). According to the data about the overall Bulgarian population, the Northeastern region belongs to the high intermediate endemic area (Fitzsimons *et al.*, 2011). Hepatitis delta virus (HDV) infection is the least frequent but the most severe type of viral hepatitis (Bielawski *et al.*, 2006; Manesis *et al.*, 2007). Discovered and isolated in Italy in the mid-1970s, the virus was demonstrated to be endemic worldwide, with prevalence rates varying greatly in different geographical areas, regardless the prevalence of HBV, whose presence is mandatory for HDV propagation (Romeo *et al.*, 2015). The chronic dual infection HBV/HDV has lately regained clinical importance because of the recent evidence of increasing prevalence in several European countries, due to immigration from highly endemic areas, cheap air travel and globalization (le Gal *et al.*, 2007; Gaeta *et al.*, 2007; Degertekin *et al.*, 2008; Tahaei *et al.*, 2014). There are two models of HDV infection: coinfection, which results from acute infection with both hepatitis B virus and HDV, whereas super infection results from HDV infection of patients with underlying chronic hepatitis B infection (Hepactive, 2009). The clinical expression of HBV/HDV infection is wide and although it sometimes follows a benign course, more often HDV superinfection suppresses HBV and above 70% of the patients are at greater risk of developing chronic active hepatitis, cirrhosis, liver decompensation and death, as compared to HBV monoinfection (Fattovich *et al.,* 2000; Ivanova, *et al.*, 2012; Ghamari *et al.*, 2013). The treatment of chronic HDV (CHD) is non-specific and inefficient (Niro *et al.,* 2005).

Responses to antiviral therapy of chronic HBV and HDV can be divided into biochemical, serological, virological and histological. All responses can be estimated at several time points during and after therapy. The definitions of virological responses vary according to the timing (during or after therapy) and type of therapy (EASL, Practice guidelines).

The aim of our study is to assess the significance of the quantitative determination of HBV DNA and HDV RNA levels and to evaluate the various virological responses at several time points on-therapy, in chronically infected patients with dual HBV and HDV infection. The virological responses of the patients included in the study were evaluated every 3-6 months during the course of treatment, at the end of therapy and 3 months after the post-treatment follow-up period.

Materials and Methods

Study population

A group of 23 patients- 8 (34.8%, 95% CI: 16.4; 57.3) women and 15 (65.2%, 95% CI: 42.7; 83.6) men with serologically proved chronic HBV and HDV infection, patients at the Gastroenterolo-

gy Department of the University Hospital St. Marina, Varna, Bulgaria, were investigated in the hospital's laboratories of Clinical Virology and Clinical Immunology.

Twenty-one of our patients were HBeAg negative (91.3 %), one of them HBeAg (+) and one patient with HBeAg seroconversion and reversion to HBeAg positive status.

Past and resolved HCV infection was evident in one of them with positive anti-HCV antibodies, but negative for HCV RNA. One patient was with HBV/HDV/HCV triple infection, anti HCV positive and HCV RNA with level 233IU/ml.

Methods

Virological assays were performed at baseline, during the course of therapy and during the post-treatment follow-up period. The serum samples were investigated for HBsAg, anti HBctotal, HBeAg/anti-Hbe Ab, HCV, and anti HDV by ELISA, using commercially available test kits. Quantitative determination of HBV DNA and HDV RNA was performed by Real-time PCR, on several occasions during and after therapy from 2012 to 2017.

ELISA for detection of HBsAg (DIAPRO Milano Italy; ETI-MAK-4 DiaSorin Italy; SURASE B-96 Taiwan), anti HBc-total (HBcAb Dia.Pro Milano Italy; Anti-HBc total Elisa BIOSOURCE Belgium), HBeAg (DIA.PRO Milano Italy; ETI-EBK-2 DiaSorin Italy)A, anti HBe (Hepatitis B e Ab Enzyme Immunoassay ClinPro. International USA), anti-HCV (DIA.PRO Milano Italy; ETI-AB-HCVK-3 DiaSorin Italy, anti HDV (HDV Ab Enzyme Immunoassay Test Kit ClinPro. International USA; ETI-AB-DELTAK-2-Diasorin Italy), according to the manufactures' recommendations were performed.

Real-Time PCR for quantitative detection of HBV-DNA were carried out after extraction of the nucleic acid from 500 µl serum samples using a commercially available kit (Bosphore Magrev 24 Manual Magnetic Bead Nucleic Acid Extraction Stand) and were tested for HBV-DNA by Bosphore® Ultra HBV Quantitation/ Detection Kit(-sensitivity threshold 10 IU/ml). Both tests are IVD CE marked according to 98/79/EC Directive.

Real-Time PCR for quantitative detection of HDV-RNA were performed after extraction of the nucleic acid from 200 µl serum samples using a commercially available kit (Viral DNA/RNA Purification Kit 50, Fermentas, Life Sciences. The extracted RNAs were processed in Reverse transcription PCR with commercially available kit (First Strand cDNA Synthesis kit, Thermo Scientific). Quantification of HDV-RNA was performed by Real time PCR for HDV-RNA, Primer Design TM Ltd, Genesig).

Table 1. Demographic, clinical and laboratory characteristics of patients at baseline (n=23)

Mean age ± SD range, years	43,9± 11,9 (28-68)
Male/Female sex, n(%)	15(65,2)/8(34,8)
Hb(g/l) ± SD range	133,3(±24,5)
AST(U/L, UNL:41U/L) ± SD range	75,8(±35,4)
ALT(U/L, UNL:41U/L) ± SD range	104,5(±75,3)
γ-GT(U/L, UNL: 49U/L) ± SD range	92,9(±90,4)
ALP(U/L, UNL: 129 U/L) ± SD range	114,8(±57,1)
HBV DNA, log_{10}, copies/ml Median range	$7{,}0.10^{2}$
HDV RNA, log_{10}, copies/ml Median range	$8{,}7.10^{4}$

*UNL-upper normal limit

Virological responses

Virological responses were evaluated every 3- 6 months during the course of treatment, at the end of therapy and 3 months after the post-treatment follow-up period.

Liver biopsy

Histological necroinflammation and fibrosis in liver biopsies were graded according to METAVIR scoring system (Hepactiv, 2009).

Statistical analyses

Statistical analysis was performed by using the "R commander" software (R foundation for statistical computing, https://www.R-project.org). Results are expressed as mean (±) SD or median (range) as appropriate. Data were analyzed by t-test, Pearson's χ^2test, Spearman's rank correlation (r). Two-sided p-values< 0,05 were considered statistically significant.Confidence intervals (95% CI) were determined using the formula $P=p\pm1{,}96 (pq/n)^{1/2}$, where p is the frequency, q is 1-p, and n is the number of individuals tested in each group.

Results

Twenty-three patients of whom 34.8% (95% CI: 16.4 - 57.3, n=8) women and 65.2% (95% CI: 42.7 – 83.6, n=15), chronically infected with HBV and HDV were enrolled in this study. The demographic, clinical and laboratory characteristics of the patients at baseline can be seen in Table 1.

All of the participants in this survey are Bulgarians, from 28 to 68 years (mean age 43.9). Hb (133.3±SD) is given as mean value in g/dl. Biochemical testing included serum liver enzymes: AST, ALT, γ-GGT and AF. They were performed at baseline, during the course of therapy and during the post-treatment follow-up period.

The ALT levels, for every one of the patients, were abnormal, comprised between 1.2 and 7.6 times (median 4.4) the upper limit of normal-GT, and ALP were 92.9 (±90.4) and 114.(±57.1), respectively.No significant correlation was noted between any of the enzyme activities and patient age or sex,

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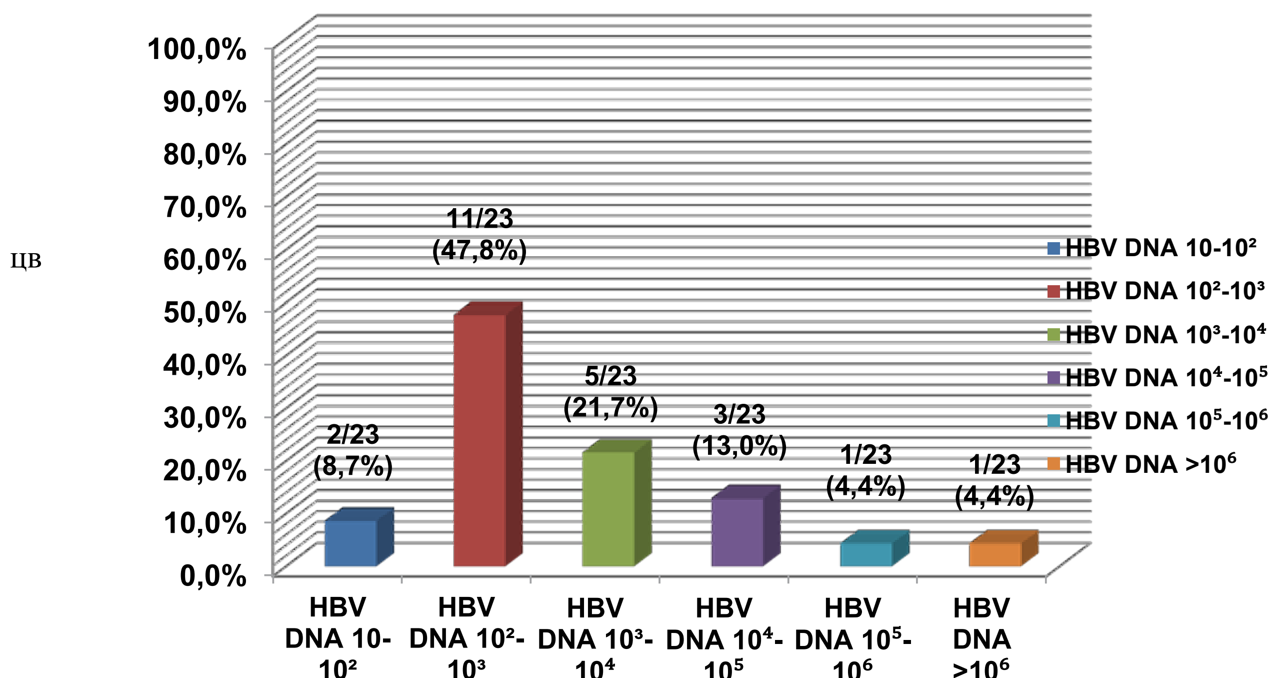

Fig.1. HBV DNA levels (copies/ml)

- According to the HDV RNA levels (Fig. 2) five groups of patients were indentified:
- Patients with HDV RNA from $2{,}5.10^2$ to $8{,}9.10^2$copies/ml (n=3; 13%, 95% CI: 2,8; 33,6)
- Patients with HDV RNA from $1{,}4.10^3$ to $5{,}2.10^3$copies/ml (n=5; 21,7%, 95% CI: 7,5; 43,7)
- Patients with HDV RNA from $1{,}5.10^4$ to $8{,}7.10\ ^4$copies/ml (n=4; 17, 4%, 95% CI: 5,0; 38,8)
- Patients with HDV RNA from $2{,}3.10^5$ to $8{,}9.10^5$ copies/ml (n=5; 21,7%, 95% CI: 7,5; 43,7)
- Patients with HDV RNA from $1{,}2.10^6$ to $9{,}6.10^6$copies/ml (n=6; 26,1%, 95 % CI: 10,2; 48,4)

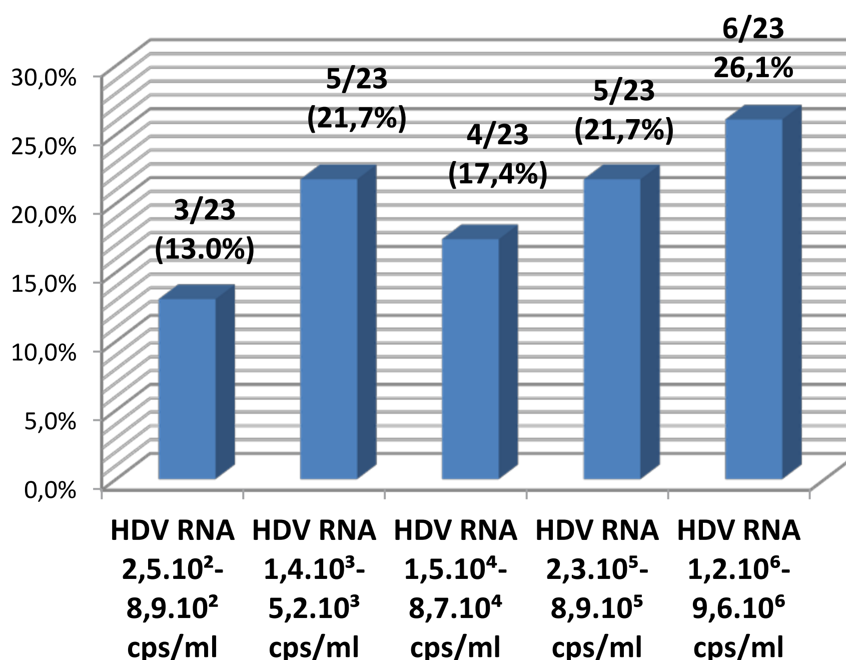

Fig. 2. HDV RNA levels (copies/ml)

- According to the HBV DNA and HDV RNA viral loads four group of patients were defined:
- Patients with high HDV RNA levels (>10^5 copies/ml) and low HBV DNA levels (<10^5 copies /ml) (n=11). 47.8%)
- Patients with both low HDV RNA and HBV DNA levels (< 10^5 copies/ml) (n=8, 34.8%)
- Patients with both high HDV RNA and HBV DNA levels (>10^5 copies/ml) (n=1, 4.4 %)
- Patients with low HDV RNA levels (<10^5) and high HBV DNA levels (>10^5) (n=3, 13%).

the histological stage of fibrosis and the presence of cirrhosis. Both patients with HBeAg positivity were with HBV DNA levels > 10^5. ForHBeAgpositive patients, the opportunity of a sustained virological response to antiviral therapy is < 20%-30%, but the long-term response depends upon HBeAg seroconversion. All of the patients were HBsAg, anti-HBc total and anti HDV positive.

According to the HBV DNA levels (Fig. 1) six groups of patients were indentified:

- Patients with HBV DNA from 0-100 copies/ml (n= 2; 8,7%, 95% CI: 1,1; 28,0)
- Patients with HBV DNA from 10^2 to 10^3 copies/ml (n= 11; 47,8%, 95% CI: 27,4; 68,2)
- Patients with HBV DNA from 10^3 to 10^4 copies/ml (n= 5; 21,7%, 95% CI: 7,5; 43,7)
- Patients with HBV DNA from 10^4 to 10^5 copies/ml (n=3; 13,0%, 95 % CI: 2,8; 33,6)
- Patients with HBV DNA from 10^5 to 10^6 copies/ml (n=1; 4,4, 95% CI: 1,0; 21,9)
- Patients with HBV DNA >10^6 copies/ml (n=1; 4,4, 95% CI: 1,0; 21,9)

HBV viremia ranged from 1.0x10 ^{2} to 1.6x10^6 cps/ml. HDV viremia ranged from 2.5x10^2 to 9.6x10^6 cps/ml. HDV replication dominated in 16 69.6% (95% CI: 50.8 – 88.4) patients accompanied by low HBV viremia. Quantitative HBV DNA levels (median range 7.0x10^2copies/ml) were not correlated with any demographic, clinical, serological and histological characteristics of the patients. Similarly, HDV RNA levels (median range 8.7x10^4) did not differ significantly according to any demographic and histological characteristics, but correlated with the enzyme activities: Spearman's rank correlation coefficient between γ-GT and HDV RNA = 0.42, p-value = 0.05; AST and HDV RNA = 0.33, p-value = 0.12; ALT and HDV RNA = 0.25, p-value = 0.25; ALP and HDV RNA = 0.36, p-value = 0.09. HBV DNA and HDV RNA levels showed no direct inverse correlation (rho = -0.01, p-value = 0.94).

Twenty-one of our patients were HBeAg negative (91.3 %), one of them was HBeAg positive and one patient was with HBeAg seroconversion

and reversion to HBeAg positive status. Both patients with HBeAg positive status were with HBV DNA levels > 10^5cops/ml.

Fibrosis was observed in 52.2% (95% CI: 30.6% - 73.2%, n=12).Liver biopsies were achieved to 43.5% (95% CI: 23.2% - 65.5%, n=10) patients from the investigated group and results were assessed with METAVIR scoring system. All patients had moderate necroinflammatory activity (median METAVIR score 2, ranging from 1 to 3), associated with severe fibrosis (median score: 3, ranging from 2 to 4). Cirrhosis was evident in 47.8% (95% CI: 26.8% - 69.4%, n=11).Patients with high HBV DNA or high HDV RNA levels (>10^5 copies/ml) were not predominantly affected by cirrhosis (Chi-square of 0.1, p = 0.75 and 0.38, p = 0.54, respectively) when compared to patients with low HBV DNA or HDV RNA levels.Cirrhosis progression and mortality rate was evaluated via Child-Pugh score (Ruseva, 2015). Five patients with cirrhosis were at class A – 45.5% (95 % CI: 16.7% - 76.6%), four- at class B (33.3%, 95% CI: 6.7% - 60.0%) and two at class C (16.7%, 95% CI: 21.0% - 48.4%). Six patients (26.1%, 95% CI: 10.2% - 48.4%) died during the survey period and for all of them HDV RNA replication dominated HBV DNA. No significant difference was found in enzyme activities, HBV DNA levels and HDV RNA levels between patients with cirrhosis of different score.

Treatment efficacy

In this study the antiviral treatment efficacy was monitored mainly with virological markers, as well as biochemical and histological markers. During the period of investigation (2012-2017) an average decline of HBV DNA and HDV RNA was observed. Virological responses were evaluated every 3-6 months during the course of treatment (M3,M6,M9,M12) at the end of therapy and at 6th month of post-treatment follow-up period.

Eight patients (34,8 %, 95 % CI: 16,4; 57,3) had previously been treated with standard IFN(6 MU, 3 times a week) for 6 to 12 months (median treatment duration 9 months). Summarized information about the viral load changes at baseline, last-on treatment and post-treatment follow- up (median range) can be seen in Table.2.

- Patient treated with IFN 2α (Rofferon) (n=9; 39,1%, 95% CI: 19,7; 61,5)
- Patients treated with Peg IFN (Pegasys) (n=4; 17,4%, 95% CI: 5,0; 38,8)
- Patients treated with Lamivudine (Zeffix) (n=8; 34,8%, 95% CI: 16,4; 57,3)
- Patients treated with Tenofovir (Viread) (n=2; 8,7%, 95% CI: 1,1; 28,0).

Table 2. HBV DNA and HDV RNA copies/ml are given as median range (interquartile range)

Treatment	Baseline		Last on treatment		Post treatment follow up	
	HBV log_{10} copies/ml.	HDVlog_{10} copies/ml.	HBVlog_{10} copies/ml	HDVlog_{10} copies/ml.	HBVlog_{10} copies/ml	HDVlog_{10} copies/ml.
IFN2α (Rofferon) (n=9)	$4,6.10^2$	$5,3.10^5$	$1,3.10^2$	$2,5.10^4$	$2,3.10^2$	$4,3.10^5$
Peg.IFN (Pegasys) (n=4)	$1,1.10^3$	$2,2.10^4$	8.10^2	$6,1.10^3$	$1,7.10^5$	$2,2.10^4$
Lamivudine (Zeffix) (n=8)	$3,5.10^3$	$3,6.10^5$	$7,3.10^2$	$3,1.10^5$	$1,3.10^2$	$3,7.10^4$
Tenofovir (Viread) (n=2)	$3,4.10^6$	$5,6.10^2$	$4,2.10^5$	Neg.	$2,4.10^5$	$1,8.10^2$

*n-№ of patients

Discussion

In this research we retrospectively analyzed a well-characterized group of 23 patients with dual chronic HBV/HDV infection. Serum samples were available at baseline, during treatment and as well as at least one sample collected over follow-up, with a median range of 6 samples/patient.

The purpose was to display whether sensitive, quantitative determinations of serum HBV DNA and HDV RNA are a sufficient marker for initiation of therapy and if so, whether they provide an early insight of anticipated response to treatment.

The interpretation of serum HBV DNA levels is not easy because we do not know the cut-off value for defining indication for and response to treatment. An arbitrary value of 20000 IU/ml (10^5 copies/ml) had been chosen. As for the majority of viral infections, HDV replication should be evaluated by the detection and quantification of the viral genome in serum, because the serological approach alone lacks sensitivity (Zachou *et al.*, 2010). Levels of HDV RNA in the serum were distributed in a relatively wide range as was HBV. It is known that host- derived double-stranded-RNA adenosine deaminase modifies HDV's mRNA and changes the balance of large HDAg and small HDAg, causing a change in the level of HDV RNA, even though this mechanism needs further study (Yamashiro *et al.,* 2004).As HBV DNA and HDV RNA have a fluctuating nature, monitoring changes in serum levels of HDV RNA and HBsAg is an essential tool (Lok *et al.,* 2001). In our study more than half of the patients had high HDV-RNA and low HBV-DNA levels (56.5%).HDV replication dominated in 16 patients (69.6%, 95% CI:50.8% - 88.4%), accompanied by low HBV viremia. This datum can be explained with the fact that HDV proteins p24 and p27 repress the HBV enhancer by inhibiting HBV replication and HDV p27 transactivates the interferon alpha-inducible Mx1 gene, also known as MxA gene, which also inhibits replication (Williams *et al.*, 2009; Romeo *et al.*, 2014).

HDV is encapsidated by the HBsAg, which is the only known function provided by the helper virus. This function, however, is essential for HDV assembly and propagation. Therapeutic trials showed that the decline of HBsAg titre corresponded to HDV-RNA decrease in patients with response to interferon therapy, while stable HBsAg and HDV-RNA levels characterized thenon-responders to nucleoside analogue treatment (Zachou *et al.,* 2010). This implies that, in any long term treatment trials of chronic hepatitis B and D, following HDV RNA and HBsAg serum levels is of paramount importance (Taylor, 2006).

Up to one-third of European patients with hepatitis D are co-infected with HCV (Heidrich *et al.,* 2009). In this context it is important to note that HDV cannot only suppress HBV replication but also suppresses HCV replication in patients with triple infection (Wedemeyer *et al.,* 2001). Chronic HCV infection may even be cleared in patients who are superinfected with HBV and HDV. However, it is not clear how many of the individuals who are anti-HCV antibody positive and HCV RNA negative have truly recovered from HCV infection or whether HCV replication is just suppressed in the context of viral co-infection (Wedemeyer and Manns, 2010).

Antiviral therapy in patients with chronic hepatitis B mono infectioncan delay disease progression and contribute to resolution of fibrosis (WHO 2015; Papachrysos *et al.,* 2015). As a general concept, cirrhosis occurs within a few years from HDV infection in about two thirds of the cases, and there is a three-fold higher risk of progression to cirrhosis as compared to patients with chronic HBV monoinfection only (Romeo *et al.,* 2015). A baseline high HBV-DNA level >10 000 copies/ml was associated with a significantly increased risk of HCC and with progression towards cirrhosis (le Gal *et al*., 2007; Marugan and Garzon, 2009). Specifically, in this research HBV DNA and HDV RNA levels did not differ significantly between patients with mild necroinflammatory activity (METAVIR score from A1 to A3) and severe fibrosis (METAVIR score from F2 to F4). We did not find connection between viral load and histological markers and biochemical markers as well.

The treatment of HBV/HDV infection is difficult. It is indicated if there is a high risk of liver-related mortality and morbidity and a high likelihood of maintained viral suppression after a defined course of therapy. This risk is variable during dual HBV/HDV infection. Hepatitis D is the only form of viral hepatitis for which there is no established treatment. The ideal drug would be able to induce HDV clearance but also HBV clearance. There are different therapeutic strategies (IFN, PEG IFN, nucleotide analogs - NAs) but none of these achieves complete HBV eradication and they have limited long-term efficacy. In the majority of HBeAg negative patients, HBV is suppressed but not eradicated by treatment, and relapses occur when treatment is interrupted (Marugan and Garzon, 2009).

Although virological response (negative se-

rum HDV RNA or ≥ 2log $_{10}$ decrease) with IFN therapy might not be sustained, it indicates that treatment is effective in decreasing HDV replication and encourages the patient to continue (Manesis *et al*., 2007). IFNs are administered for predefined durations and do not select antiviral-mutants.

In all cases, the onetime treatment had been interrupted at least one year before signing up in this study. PEG IFN is well tolerated and leads to virological response, even in the case of previous failure of standard IFN therapy. The best responder in our cohort was treated with 2 courses of IFN and 2 courses of Peg IFN afterwards. We marked an excellent effect with negative HBV DNA and HDV RNA subsequently, as well as normal aminotransferases. Virological and biochemical breakthrough was noted one year after the end of therapy. One patient was defined with sustained virological response (undetectable HDV RNA levels six months after treatment). This confirms results from other research that treatment of HDV infection with pegylated interferon results in SVR six months post-treatment in one fourth of the patients (Abbas *et al.,* 2014). In the follow-up period serum levels of HBV DNA and most of HDV RNA viral loads returned to baseline values within 3 to 6 months of stopping therapy and there were no increases in serum aminotransferases that accompanied this rise in viral levels.

Lamivudine led to a marked decrease in serum levels of HBV DNA and was with weaker effect on HDV RNA levels. NA treatment is costly and its long term efficacy has yet to be proven. The hardest challenge is the selection of antiviral-resistant mutants, with long-term treatment. Lamivudine has the highest rate of resistance, 70% after 4 years of treatment in both HBeAg positive and HBeAg negative patients (Marugan and Garzon, 2009). All patients in our study tolerated the therapy well. One patient was defined with mutations (I204, I80) and virological breakthrough while treated for one year with Lamivudine. In this case Tenofovir was recommended. Lamivudine led to a rapid and marked decrease in serum HBV DNA levels, but had little or no effect on HDV-RNA levels. The activity of the liver disease and cell injury did not change. Three patients (37.5%, 95% CI: 8.5% - 75.5%) were treated with a combination of Lamivudine and IFN. This combination did not emerge as effective and did not increase sustained virological response.

Tenofovir is highly efficient in suppressing HDV replication. In our trial all of the patients on Tenofovir were virological responders with undetectable HDV RNA at the end of therapy and both of them relapsed during post-treatment follow-up. The authors observed an average decline in serum HDV RNA levels from $1.8x10^5$ to $1.5x10^5$ copies/ml and four of 23 patients became HDV RNA negative (17.4%, 95% CI: 1.9% - 32.9%). Thus, prolonged treatment with potent HBV polymerase inhibitors may lead to beneficial effects in patients with HDV (Wedemeyer and Manns, 2010).

In any treatment trial, the best way to follow response is to monitor small changes of parameters on which the response depends (Manesis *et al*., 2007). According to EASL, there are three criteria for beginning HBV/HDV therapy: serum HBV-DNA and HDV RNA levels, serum aminotransferase levels, and histological grade and stage. Diagnosis of chronic dual HBV/HDV infection is challenging and requires deeper knowledge of viral interactions between these two viruses. Larger scale studies are needed to understand the pathophysiology of HBV and HDV and to develop a novel treatment options for this major health problem.

Conclusions

Viral load information is essential. Quantitative detection of HBV DNA and HDV RNA in serum improves monitoring and their analysis might help to adjust the duration of therapy. HDV-RNA and HBV-DNA levels showed no direct inverse correlation in the lesser part of the patients, although higher HDV levels were accompanied by lower HBV viremia. HBV DNA correlates positively with HBeAg. Defining of HBsAg serum levels together with HDV RNA viral load is of primary importance for any long-term treatment trial.

No correlation between HDV RNA levels, age and histological necroinflammation and fibrosis was established. Quantitative HBV DNA level were not correlated with any demographic, clinical, serological and histological characteristics of the patients. Similarly, HDV RNA levels did not differ significantly according to any demographic and histological characteristics, but correlated with the enzyme activities.

Biochemical parameters did not accurately indicate the stage and grade of liver disease in chronic HDV patients as they often fluctuate over time.

Among the various biochemical markers only γ-GT was independently associated with the presence of cirrhosis. There is statistically significant correlation between HDV RNA levels and γ-GT.

IFN is efficient in reducing transaminanses

(ALT), decreasing HDV RNA levels at some point, but inoperative in HDV RNA clearance.

Lamivudine alone is a potent inhibitor of HBV DNA replication but does not improve disease activity or lower HDV RNA levels in patients with chronic delta hepatitis.Lamivudine did not increase sustained virological response when combined with IFN.

Tenofovir is a means of choice for treatment when virological breakthrough during Lamivudine therapy occurs.

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