Hepatitis Delta coinfection in persons with HIV: misdiagnosis and disease burden in Italy

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Word count: 3024

Abstract

Objectives Hepatitis Delta virus (HDV) causes severe liver disease. Due to similarities in transmission routes, persons living with HIV (PLWH) are at risk for HDV infection. This analysis investigates prevalence and long-term clinical outcome of people with HDV in a large cohort of PLWH .

Design and Methods We retrieved HBsAg ± anti-HDV positive PLWH enrolled from 1997 to 2015 in the multicentre, prospective ICONA study The primary endpoint was a composite clinical outcome (CCO=having experienced ≥1 of the following: Fib4 score >3.25; diagnosis of cirrhosis; decompensation; hepatocellular carcinoma or liver-related death). Kaplan Meier curves and unweighted and weighted Cox regression models were used for data analysis.

Results Less than half of HBsAg positive patients had been tested for anti-HDV in clinical practice. After testing stored sera, among 617 HBV/HIV cases, 115 (19%) were anti-HDV positive; 405 (65%) HBV monoinfected; 99(16%) undeterminate. The prevalence declined over the observation period. HDV patients were more often males, intravenous drug users, HCV coinfected. After a median of 26 months, 55/115 (48%) developed CCO among HDV+; 98/403 (24%) among HBV monoinfected; 18/99 (18%) in HDV unknown ($p<0.001$). After controlling for geographical region, alcohol consumption, CD4 count, anti-HCV status and IFN-based therapies, the association with HDV retained statistical significance [HR=1.67 (1.15, 2.95; p=0.025)].

Conclusions HDV infection among PLWH is underdiagnosed, although HDV entails an high risk of liver disease progression. Because effective drugs to treat HDV are now available, it is even more crucial to identify PLWH at an early stage of liver disease.

Key words: HIV infection; hepatitis B; hepatitis delta; clinical outcome; anti-HBV therapy

Introduction

Hepatitis Delta virus (HDV) is a small defective RNA virus which needs human polymerase to replicate and Hepatitis B virus (HBV) surface protein to generate the complete transmissible virion [1]. It shares the main routes of transmission with HIV, HBV and HCV, so dual or multiple coinfections are not rare in subjects exposed to parenteral or sexual risks [2]. The prevalence of HBV/HDV coinfection varies among different areas in the world, due to the presence of clusters in East Europe, Sub-Saharan Africa, South America and Asia, resulting in a heterogeneous epidemiological and clinical picture [3,4]. Recent meta-analyses estimate a global prevalence of HDV infection between 40 and 60 million chronically infected individuals [5-7]; however, population estimates remain uncertain due to under-diagnose of HDV infection. In Europe, the prevalence of HDV infection has decreased in clinical settings over the last two decades, mainly due the extensive vaccination campaigns against HBV that deprived the virus of its biological background; however, a re-emergence has been noticed in various countries in recent years due to the immigration from endemic areas [4,8-11].

HDV is commonly acquired through superinfection of a chronic HBV carrier and rarely by simultaneous infection by both viruses; in the former case, it causes a chronic infection characterized by a rapid progression towards end-stage liver disease and liver cancer [12-14]. Overall, coinfected patients appear to have a higher mortality rate as compared with HBV mono-infected [15]; in a cohort of 17 patients with HDV the rate of liver decompensation was higher as compared to that seen in HBV mono-infected [16]. A recent meta-analysis showed that the presence of HDV infection increased the risk of hepatocellular carcinoma in comparison to HBV monoinfection, both in HIV-negative or HIV-positive subjects [17].

Due to its unique replication pattern, HDV is a difficult target for current antiviral drugs [18]. In the recent years, the only available treatment was Peg-IFN which was administered with different designs and protocols and caused a transient suppression of HDV viremia in up to half of the patients; however, late viral relapse was common after therapy, leading to a sustained control of HDV replication in less than one fourth of the patients [19-22]; in addition, the eligibility to treatment is limited by IFN-related toxicities. Nucleos(t)ide analogues active against HBV did not reduce plasma levels of HDV-RNA; even in combination with Peg-IFN long-term clinical outcome remained severe [23-25]. However, one study reported virological and clinical benefit in patients with HIV/HDV coinfection receiving long-term tenofovir treatment [26].

New antiviral strategies against HDV are on development [18], which adds interest to quantifying the impact of HDV infection on liver disease progression among individuals at risk. The present analysis aims at estimating the yearly prevalence of HDV infection in the HIV positive patients enrolled from the year 1997 in the Italian Cohort of Individuals Naïve for Antiretrovirals (ICONA) and to compare the clinical outcome of HBV/HDV coinfected patients with that of HBV mono-coinfected persons; in addition, the study explores the attitude to anti-delta antibody testing in a clinical practice setting.

Patients and methods

The ICONA Foundation Study is a multicentre prospective open observational study of patients living with HIV seen for care in 52 infectious disease clinics across Italy, enrolling in a continuous manner since 1997. Of note, because Icona Foundation Study is an open cohort, deaths and loss to follow-up are over-compensated with new enrolments over time. For the purpose of this analysis, data of patients who were diagnosed with chronic HBV infection from 1997 to 2015 were retrieved. Presence of anti-HDV antibodies was determined from routine serologic testing performed at the participating sites or retrospective testing of stored samples. Indeed, for each participants in the cohort a serum sample is stored at -40° at entry in the study and then at least yearly according to the Icona Foundation Study protocol which has remained substantially unchanged since 1997. Serum storage is centralized at the biobank of the National Institute for Infectious Diseases L. Spallanzani in Rome. For HBsAg positive patients who had not been tested for anti-HDV in routine care, 279 sera stored at enrolment were retrieved and tested for anti-HDV using a commercial EIA kit (Diasorin, Saluggia, Italy) [27]; anti-HDV positive sera were tested for HDV-RNA as described recently [25] and for anti-HDV-IgM (Diasorin, Saluggia, Italy). HDV-RNA concentrations were expressed as IU/mL; the reference standard was WHO N.7657/12, Paul Ehrlich Institut. As internal control, a sequence of mRNA coding for 18s subunit of human ribosomal RNA was used.

Patients were classified as having an active HDV disease if HDV RNA and/or anti-HDV-IgM were positive [28,29]. Clinical data of the patients were recorded at baseline and during a long-term follow-up every 3-6 months following the Icona Study protocol as described elsewhere [30]; alcohol consumption was assessed by physician interview and categorized according to the Italian National Institute for Food and Nutrition into: abstainer; moderate; hazardous; unknown as

described elsewhere [31]; hazardous drinking was the consumption of >3 alcohol units/day for men and >2 for women. A unit corresponded to 14 g of pure alcohol.

The clinical endpoint was a composite clinical outcome (CCO) defined as the time of first occurrence of any of the following events: Fib4 score >3.25; clinical diagnosis of cirrhosis; decompensation (i.e. ascites, encephalopathy; haemorrhage from esophageal varices); hepatocarcinoma (HCC) or liver-related death, as ascertained from clinical records. There was no linkage with regional or national mortality registry to validate the accuracy of the medical reports. In order to avoid misclassification of the exposure, participants with HBV serology testing conducted after December 2015 are not included here because the work of retrospectively filling the gap in people with missing HDV test results was interrupted at the end of 2015.

Ethical considerations

The Icona Foundation study was approved by the Ethics Committee (institutional review board) of each participating institution. All of the individuals enrolled provided a written informed consent at the time of the enrolment. All procedures of the study were performed in accordance with the 1964 Helsinki declaration and its later amendments.

Statistical analysis

Baseline for this analysis was the date of the first HBV serology test. Only participants with a HBsAg+ results were included and grouped according to the exposure of interest (HBV+/HDV+ vs. HBV+/HDV-). We performed a cross-sectional analysis to compare characteristics of HBV+/HDV+ vs. HBV+/HDV-patients measured at baseline. Statistical testing was conducted to compare population ranking distributions (non-parametric Mann-Whitney U test) and chi-square test for proportions by serology group. The yearly proportion of patients who were HDV positive at baseline was also calculated.

We also performed a prospective analysis comparing liver disease progression according to HDV status. The primary endpoint of this analysis was time to severe liver disease which was defined as the development of a Fib4 score >3.25; clinical diagnosis of cirrhosis; decompensation (any among ascites, GI bleeding, encephalopathy); hepatocellular carcinoma (HCC) or liver-related death. A composite clinical outcome (CCO) was defined as the occurrence of any of the events mentioned above. Participants' follow-up accrued from baseline up to the time of developing the CCO or last clinical visit.

Kaplan Meier method was used to plot the time to develop the CCO, stratified by anti-HDV status, log-rank test and cumulative probability of developing the outcome given with 95% CI.

Univariable and multivariable standard Cox regression models were used. Potential confounding at baseline were carefully examined by making assumption on the underlying causal structure of the data. These assumptions are described by means of a direct acyclic graph (DAG, Figure 1).

According to these assumptions all backdoor confounding pathways could be blocked by controlling in the standard Cox regression model for the following set of time-fixed potential confounders measured at baseline: geographical region, alcohol consumption, baseline CD4 count, anti-HCV status and use of IFN-based therapy. By IFN-based therapy we included the following therapies: pegylated interferon, ribavirin, interferon-alpha and any other non specified interferon-based treatment. Geographical region is the region of the participating site where the participant was receiving care divided in North, Centre and South regions of Italy (see Supplementary Table 1).

To handle missing data for the variable alcohol consumption, we have used the so-called 'Missing Indicator Method' which is often applied to categorical exposures. This amounts to including an extra category of the exposure variable for those individuals with missing HCV test results. Then indicator variables are created for inclusion in the regression models, including an indicator for the missing data category.

In order to control time-varying factors that could have changed post baseline we also fitted a marginal Cox regression model controlling for use use of IFN-based post baseline and censoring by means of inverse probability of weighting (IPW).

Results

Among 13,558 HIV positive patients enrolled at the time of their first HBsAg test after entering the cohort, 10,988 were HBsAg-negative, 1,953 had not been tested for HBsAg and 617 patients were HBsAg-positive; of the latter, 378 (61%) had not been tested for anti-HDV (Figure 2). The proportion of patients untested for HDV was 41% over the period 1997-1999 and 85% over the period 2012-2015. Among stored sera 38/279 (14%) were anti-HDV positive of whom 32 (85%) had HDV-RNA and/or anti-HDV IgM detectable [21 (55.3%) were HDV-RNA positive; range $3.9x10^4$-$15.6x10^6$ IU/mL]. After filling the gaps from testing available stored samples, overall, 115 patients (19%) were anti-HDV positive, 403 (65%) anti-HDV negative while 99 (16%) for whom a sample was not available or serology failed remained undetermined. None of the patients had

decompensated cirrhosis. Proportions of anti-HDV positive cases decreased from the year 1997 to 2011 (from 28% to 4%) then rebounded to 8% in the period 2012-15 (Figure 3).

The characteristics of the patients at baseline by detection of anti-HDV are summarized in Table 1; HDV patients were more often males and of Italian nationality; drug injection was by far the predominant cause of HIV acquisition in HDV positive group (71.3 vs 21.1%, p<0.001); HCV coinfection was more frequent among anti-HDV positive patients (67.8 vs 21%, p<0.001).

Overall, 171 patients (28%) developed the CCO over a median of 26 months from baseline. The breakdown of the CCO was as follows: 161 Fib4 elevations (94.2%), 2 HCC (1.2%), 4 decompensated cirrhosis and 4 deaths (2.3%).

Of the total number of events, 55/115 (48%) occurred among anti-HDV positive cases, 98/403 (24%) among anti-HDV negative and 18/99 (18%) in unknown HDV test result group (log-rank p<.001). Cumulative risk estimates by KM curves are shown in Figure 4; by 3 years the proportion of people who experienced CCO were 43%, 20% and 22% respectively in the HDV-positive, HDV-negative and HDV-unknown groups. The corresponding KM cumulative probability of CCO at 1,2,3 and 4 year is shown in Supplementary Table 2.

Table 2 shows the relative hazards of developing CCO; unadjusted HR (95% C.I.) was 2.34 (1.68, 3.26). After controlling for baseline factors at time-fixed covariates geographical region, alcohol consumption, baseline CD4 count, anti-HCV status and use of IFN-based therapy the association was attenuated but effect size was still large and retained statistical significance HR=1.67 (95% CI:1.15-2.95, p=0.025). Overall 6 patients (1%) received an IFN-based therapy prior to baseline and additional 23 (3.7%) at any time during the study period. To control for the use of IFN-based therapy post baseline and potential informative censoring, we fitted a Cox regression marginal model, by means of inverse probability of weighting (Table 2). If anything, in this analysis the difference in hazards between HDV-positive and HDV-negative groups was even larger and the association stronger (HR=2.02, 95% CI:1.39-2.95).

In detail, the other factors independently associated with higher risk of CCO were: age (HR:1.26 per 10 years older, 95% CI(1.13-1,63, p<0.001, baseline CD4 count (HR:0.58, 95% CI:0.39-0.87, p=0.009 comparing people with >500 vs. 0-300 cells/mm^3), and alcohol use (hazardous vs. abstain HR:3.27, 95% CI:1.52-7.03).

The exposure to anti-HBV nucleos(t)ide analogues was recorded in 320/403 (79.4%) HDV negative and in 79/115 (68.7%) HDV positive patients; CCO qualifying events were 84 (26%) and 43 (54%), respectively (HR=2.07 (95% C.I. 1.34-3.20).

Discussion

HDV infection remains a cumbersome event due to the progressive course of the HDV related liver disease and the lack of an effective antiviral therapy until now. The overall proportion of anti-HDV positive patients in our cohort was 19%; of note, while decreasing from 1997 to 2011, then the prevalence tended to rebound. A similar prevalence was recorded in the Swiss cohort of HIV positive patients [15], while it was slightly greater than the 15% reported in the EuroSIDA cohort (32). An heterogeneous geographical prevalence might explain the differences; indeed, a recent European survey among HIV/HBV coinfected patients found a 13% prevalence in North-Western Europe and about double prevalence in Southern Europe, where HDV had been endemic in the past (33); in the latter study only half of HBsAg positive patients had been tested for anti-HDV in routine practice. Looking at the impact of Delta infection from another point of view, overall hospital admissions for HDV were stable over the recent years in Spain, while rates of decompensation and liver cancer were on the rise (34).

In the present cohort, the proportion of HBsAg positive patients tested for anti-HDV in clinical practice decreased from 59% in 1997 to only 15% in 2015; this trend continued in the period 2016-2019 (data not shown). Of note, when sera of untested patients were retrieved and tested for Delta serology, the proportion of anti-delta positive subjects was in the same order of magnitude than in originally tested patients, which seems to exclude a selection for testing in clinical practice based on the severity of the liver disease. The false perception that HDV infection has disappeared in Europe and the consequent reduced availability of dedicated laboratory facilities might have contributed to the scarce attitude to testing; there is urgent need to tackle the phenomenon in order to identify HDV-positive individuals in view of the introduction of new drugs active against HDV.

The progression of the liver disease was evaluated by a composite clinical outcome which comprised the progression of the Fib4 score to ≥3.25, decompensation, hepatocellular carcinoma, liver-related death, liver transplant. We acknowledge that data on Fib-4 for detecting fibrosis in patients with HBV infection are still scanty, and even more for patients with HDV, since the test was validated initially in HCV infection. However, in a series of 937 HBV patients the AUROC for

Fib-4 in detecting cirrhosis was 0.818 (35); the performance of the test was lower in detecting any significant fibrosis stage (36).

Since the only treatment that could influence the disease course is interferon, we carefully analysed the potential impact of this treatment and found that it only slightly attenuated the effect size of HDV infection. Indeed, the use of IFN-based therapies remained marginal in this setting, mainly due to its poor tolerability. As expected, the enhanced risk of progression of the liver disease in HDV patients was not affected by nucleoside analogues active against HBV, mainly received as part of anti-HIV therapy. In most studies, nucles(t)ide analogues had no effect on HDV viremia in HIV positive patients [37,38]; a beneficial effect was reported sporadically [26]. In a recent study in HIV-negative patients the clinical outcome after 50 months of therapy with either entecavir or tenofovir was by far worse in patients with Delta infection as compared to pair matched HBV monoinfected patients receiving the same therapy [25].

In the present cohort 68% of HDV infected patients were also coinfected with HCV vs only 21% in HBV mono-infected; the high prevalence of subjects with history of intravenous drug use among HDV-positive patients (71% vs 21%) explains the risk of acquiring multiple hepatitis virus infections [3,4]. Although the presence of HBV plus HCV coinfection was previously associated with the risk of a worse prognosis [2,39], in the present cohort HDV remains the main driver for the worse outcome. Similarly, in the Swiss cohort the presence of HCV together with HBV and HDV coinfection had no or marginal effect on mortality rate [15], HDV remaining the main driver for mortality; this finding was confirmed by the European study mentioned above (33). The complex reciprocal interactions of the three viruses and the timing and duration of the infections might explain the finding [40]. Very recently, it was shown in experimental models, including the humanized mice, that HCV as other HBV-unrelated viruses, can act as helper viruses for HDV dissemination [41]; interestingly, an analysis of a database of Italian patients with a positive HBsAg is serum, without HIV, showed that the coinfection with HDV and HCV was strongly associated with the presence of cirrhosis, with a multiplicative effect as compared with single HCV or HDV coinfections [42].

A limitation of this analysis is the observational, multicentre nature of the study, so the results are valid under the usual assumptions of having controlled for all measured potential confounders, that these are measured without errors and that no additional sources of unmeasured confounding exist. Importantly, although we have carefully examined the confounding at baseline, with respect of time-varying covariates we have restricted to control only for the key confounding

of use of IFN-based therapy post baseline. This is a simplification as there could be other time-varying factors affected by serology who could have biased the estimate (e.g. alcohol consumption). Also, there could be residual confounding due to HCV infection, although only 29 HDV positive participants were non co-infected with HCV and HCV-RNA was only available in 16 of the HCVAb-positive patients. A further limit of the study could be the lack of systematic testing for HDV-RNA , which was available only for a subgroup of patients. It must be noted that HDV-RNA in plasma may fluctuate over time, thus a single spot testing might not reflect the real presence of an active HDV infection [43]. Thus, there is still uncertainty in the evaluation of virological endpoints in Delta infection and repeated determinations of HDV-RNA are recommended for patients with a positive anti-HDV antibody test result [44]. In the present study, HDV RNA was detected in 55.3% of the patients at a single point measure. To overcome these shortcomings, we considered the presence of anti-HDV IgM as a surrogate marker of disease activity [28,29], which led to an overall 85% prevalence of active HDV; the same proportion of patients were found with an active HDV infection in the Swiss Cohort during a five year follow-up [15] and in a French cohort [38], on the basis of repeated HDV-RNA testing.

In conclusion, while confirming in a large cohort that HDV infection identifies a subgroup at high risk of liver disease progression among HIV/HBV positive persons, the study also underlines the poor attitude to anti-HDV testing in clinical practice. In the view of the availability of new drugs active against HDV (45,46), screening of HBsAg positive persons should be encouraged. Recently, the first of these drugs, bulevirtide (Hepcludex®; information at: ema.europa.eu/medicines/human/EPAR/hepcludex), has been approved by the European Medicine Agency for the therapy of HDV infection, with the exclusion of patients with decompensated liver disease; thus, efforts should be made urgently to foster the identification of the patients at an early stage of the liver disease.

Acknowledgments

The study was supported by the ICONA Foundation

There are no relevant financial or non-financial competing interests to report.

The authors thanks Dr. Alessandro Tavelli for his valuable help in retrieving patient sera from the biobank of the ICONA study

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Figure legends

Figure 1. Direct acyclic graph (DAG) which describes the assumptions regarding the underlying causal structure of the data

Figure 2. Flow of enrolment

Figure 3 . Proportions of anti-HDV positive cases by calendar years over the study period

Figure 4 . Kaplan Meier estimates of reaching the composite clinical outcome defined as any of the following events: Fib4 score >3.25; clinical diagnosis of cirrhosis; decompensation (i.e. ascites ver transplant. , ence phalopathy; hemorrhage from esophageal varices); hepatocellular carcinoma; liver related death , li