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Prevalence of Hepatitis D in a cohort of chronic Hepatitis B patients

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Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

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Porto, Maio de 2024

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Dedication

Ao meu avô Zé, ao meu avô Manel e à minha avó Lucinda, partiram cedo demais, mas nem por isso me deixaram de acompanhar ao longo deste percurso. Estejam onde estiverem, esta dissertação é para vocês, espero ter-vos orgulhado.

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Resumo

Introdução: O vírus da Hepatite D (VHD) é o responsável pela forma mais grave de hepatite viral em humanos. Os indivíduos infetados com VHD experienciam uma evolução mais rápida para doença hepática avançada, comparativamente com os doentes unicamente infetados com vírus da Hepatite B (VHB). As recomendações de 2023 da *European Association for the Study of the Liver* recomendam o doseamento dos anticorpos contra o VHD (anti-VHD) em todos os doentes com antigénio de superfície do vírus da hepatite B (AgHBs). Estudos recentes estimam que a hepatite D afeta globalmente cerca de 5% dos portadores de VHB.

Objetivos: Identificar numa coorte de doentes com infeção crónica por VHB seguidos na consulta de Hepatologia de um centro terciário a prevalência de doentes com VHD e estudar as características demográficas, clínicas, bioquímicas e virológicas nesta população, bem como *outcomes* relativos ao desenvolvimento de cirrose e carcinoma hepatocelular (CHC).

Métodos: Os dados dos doentes AgHBs positivos seguidos na consulta de Hepatologia entre janeiro de 1983 e fevereiro de 2024 foram recolhidos retrospetivamente. A análise estatística foi efetuada com recurso ao IBM *Statistical Package for the Social Sciences* versão 29.0 com um nível de significância de 0,05.

Resultados: A presença de anti-VHD não foi testada em 300 dos 541 (55,5%) doentes positivos para o AgHBs, o que reduziu a nossa amostra para 241 casos. Dentro dos que foram rastreados, o resultado foi positivo em apenas 4,15% (n=10) dos doentes. A coinfecção VHB/VHD estava presente em 3 doentes (1,24%) e os restantes 7 (2,90%) apresentavam superinfeção VHB/VHD. Cofatores para a doença hepática notáveis, como a infeção por vírus da Hepatite C e consumo de álcool, foram mais frequentes nos casos positivos para o anti-VHD (p<0,001 em ambos). A análise estatística revelou valores significativamente elevados de alanina aminotransferase, aspartato aminotransferase e elastância hepática nos doentes anti-VHD positivos, particularmente nos casos de coinfecção (p-valores de 0,021, 0,007 e 0,041, respetivamente). O consumo de drogas injetáveis também foi mais frequente nos doentes com coinfecção e superinfeção VHB/VHD, comparativamente aos doentes com monoinfecção VHB (33,3%, n=1 e 42,9%, n=3, respetivamente, vs 0,9%, n=2, p<0,001). Todos os doentes com coinfecção desenvolveram cirrose, enquanto 71,4% daqueles com superinfeção progrediram para cirrose após o diagnóstico de VHD. Um número substancialmente mais elevado de doentes coinfectados com VHB/VHD desenvolveu CHC (66,7%, n=2), quando comparado com os doentes superinfectados com VHB/VHD (14,3%, n=1) e monoinfectados com VHB (3,5%, n=8) (p=0,010). As taxas de mortalidade foram mais elevadas e a

idade aquando da morte foi notavelmente inferior na infeção pelo VHD (*p*=0,020 e *p*=0,031, respetivamente).

Conclusões: Os nossos resultados enfatizam uma lacuna na adesão às atuais orientações sobre a infeção por VHD. Demonstrou-se que a infeção por VHD está associada a pior prognóstico, incluindo morte e doença hepática terminal. A implementação da testagem de anti-HDV de forma metódica ou reflexa é fundamental, de modo a efetuar o diagnóstico o mais precocemente possível para evitar a progressão para resultados desfavoráveis.

Palavras-chave: VHD, Hepatite D, VHB, Hepatite B, Cirrose Hepática, Carcinoma Hepatocelular, Prevalência, Epidemiologia.

Abstract

Introduction: Hepatitis D virus (HDV) is considered the most aggressive hepatitis virus. Individuals infected with HDV progress to advanced liver disease faster than hepatitis B virus (HBV) monoinfected patients. Latest guidelines advocate for HDV antibody (anti-HDV) screening at least once in all individuals positive for Hepatitis B virus surface antigen (HBsAg). Global prevalence of anti-HDV has been estimated to be 5% in HBsAg-positive people.

Objectives: Evaluate the prevalence of HDV in a cohort of patients with chronic HBV infection followed in Hepatology consultation of a tertiary center, as well as patient's demographic, clinical and laboratory characteristics, and to assess outcomes regarding cirrhosis and hepatocellular carcinoma (HCC) development.

Methodology: Data were retrospectively collected from HBsAg-positive patients undergoing Hepatology consultation at our center from January 1983 to February 2024. Statistical analyses were conducted using IBM Statistical Package for the Social Sciences version 29.0 with a significance level of 0.05.

Results: Anti-HDV testing was not performed in 300 of the 541 (55.5%) HBsAg positive patients, leaving a sample size of 241 cases. Among those screened, only 4.15% (n=10) were anti-HDV positive. HBV/HDV coinfection was present in 3 patients (1.24%) and the remaining 7 (2.90%) had HBV/HDV superinfection. Noteworthy, co-factors for liver disease, like Hepatitis C virus (HCV) infection and alcohol consumption, were more frequent in anti-HDV positive cases (p<0.001 in both). Statistical analyses revealed significantly elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST) and liver elastography values in anti-HDV positive patients, particularly in cases of coinfection (p-values of 0.021, 0.007 and 0.041, respectively). Intravenous drug use (IVDU) was also more frequent in patients with HBV/HDV coinfection and superinfection, comparing with those with HBV monoinfection (33.3%, n=1 and 42.9%, n=3, respectively, vs 0.9%, n=2, p<0.001). All patients with coinfection developed cirrhosis, while 71.4% with superinfection progressed to cirrhosis post-HDV diagnosis. A substantially higher number of HBV/HDV coinfected patients developed HCC (66.7%, n=2), when compared with HBV/HDV superinfected (14.3%, n=1) and HBV monoinfected (3.5%, n=8) patients (p=0.010). The mortality rates were higher and age at death was remarkably lower in the HDV infection (p=0.020 and p=0.031, respectively).

Conclusions: Our findings underscore a gap in adherence to current HDV infection guidelines. It demonstrated HDV infection is associated with a worse prognosis, including death and end-stage liver disease. Implementation of anti-HDV testing in a methodical way or reflex testing is critical, as

this diagnosis must be carried out as early as possible to avoid progression to unfavorable outcomes.

Keywords: HDV, Hepatitis D, HBV, Hepatitis B, Hepatic Cirrhosis, Hepatocellular Carcinoma, Prevalence, Epidemiology.

Abbreviations

AASLD - American Association for the Study of Liver Diseases

ADEFO - Adefovir

ALT - Alanine aminotransferase

Anti-HBc - Total hepatitis B core antibody

Anti-HBe - Hepatitis B e antigen antibody

Anti-HDV - Hepatitis D virus antibody

APASL - Asian Pacific Association for the Study of the Liver

AST - Aspartate aminotransferase

CAP - Controlled attenuation parameter

CKD - Chronic kidney disease

COMB - Combination of antiviral treatments

DM2 - Type 2 Diabetes Mellitus

DNA - Deoxyribonucleic acid

EASL - European Association for the Study of Liver Diseases

ENT - Entecavir

HAV - Hepatitis A virus

HBV - Hepatitis B virus

HBsAg - Hepatitis B virus surface antigen

HCC - Hepatocellular carcinoma

HCV - Hepatitis C virus

HDAg - Hepatitis delta antigen

HDV - Hepatitis delta virus

HIV - Human immunodeficiency virus

IFN/PEG - Pegylated interferon

INR - International normalized ratio

IQR - Interquartile range

IVDU - Intravenous drug use

LAM - Lamivudine

PLT - Platelets

TENO - Tenofovir

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Tables

Introduction

Hepatitis delta virus (HDV) is responsible for the most severe viral hepatitis in humans. However, it is also the smallest known virus capable of infecting humans. HDV is an RNA virus that depends on Hepatitis B virus (HBV) surface antigen (HBsAg) to enter hepatocytes and continue its replication cycle. The single-stranded RNA codifies one single protein, the hepatitis delta antigen (HDAg).[1]

HDV is transmitted parenterally, sexually, or perinatally, with some known risk factors being intravenous drug use (IVDU), high-risk sexual behaviors, multiple past transfusions, and emigration to hyperendemic areas (sub-Saharan Africa, Central Asia and Eastern Europe, for example).[2,3]

A person must have a prior HBV infection in order to develop a HDV infection. We are in the presence of an HDV coinfection if both infections occur in an individual not yet exposed to either virus.[4] A coinfection typically leads to an acute hepatitis similar to HBV, with spontaneous healing in 95% of cases. On the other hand, an HDV infection in a person already infected with HBV is called a superinfection, associated with chronicity rates above 80% and higher risk of early development of cirrhosis and hepatocellular carcinoma (HCC). Chronic hepatitis D is the most rapidly progressive and severe form of viral hepatitis, resulting in cirrhosis within 5 to 10 years in approximately 70% of the patients (usually young).[5]

The data regarding HDV prevalence is highly variable. According to the World Health Organization, hepatitis D affects 5% of HBV carriers.[6] The same study estimates that around 0,16% of the general population (12 million people) is anti-HDV positive. That number rises to 16,4% among those with follow-up in hepatology centers, with estimates that vary between 3,3% in America and 19,5% in Europe.[7] The first Portuguese study about this matter dates back to 1987, with HDV infecting about 17% of HBsAg-positive individuals[8] A recent study from 2022, reported that, in a cohort of HBsAg-positive patients followed in Centro Hospitalar Universitário de São João, a tertiary center, 3.4% were anti-HDV positive. [9]

HDV prevalence numbers are affected by various factors: lack of awareness among health professionals regarding this issue, hepatitis D label as a rare disease that only affects those with high-risk behavior and difficulty in diagnosis. Other players play part in the great geographical variation in HDV prevalence like coverage of HBV vaccination, migration flows, hygienic and socio-economic conditions, and heterogeneity of the virus itself.[3]

The different approaches regarding screening also impact HDV detection. The 2023 European Association for the Study of the Liver (EASL) Guidelines and Asian Pacific Association for the Study

of the Liver (APASL) recommend anti-HDV testing in all HBsAg-positive individuals while the American Association for the Study of the Liver Diseases (AASLD) only mentions screening in high-risk individuals.[3] There is still no cure for hepatitis D. However, there are therapeutic alternatives that can alter the natural history of disease. Therefore, early screening, diagnosis, and treatment of HDV are essential.

Objectives

The aim of this study was to evaluate the prevalence of HDV in a cohort of patients with chronic HBV infection followed in Hepatology consultation of a tertiary center, as well as patient's demographic, clinical and laboratory characteristics, and to assess outcomes regarding cirrhosis and HCC carcinoma development.

Methodology

This retrospective cohort study was conducted in the Gastroenterology Department of Unidade Local de Saúde de Santo Antonio. Adult patients (age superior to 18 years-old) with chronic hepatitis B infection, defined as HBsAg positivity for more than 6 months, followed in Hepatology consultation at this center from January 1983 to February 2024 were included. The study was approved by the Ethics Committee of Unidade Local de Saúde de Santo António – Hospital de Santo António [2023.235(198-DEFI/187-CE)].

Data was obtained from each patient´s digital medical records, via SClínico® and PCE®. Information regarding sex, age, nationality, date and cause of death, date of first and last Hepatology consultation, personal history of chronic arterial hypertension, type 2 Diabetes Mellitus (DM2), dyslipidemia, tobacco and alcohol consumption, IVDU, unprotected sexual intercourse, psychiatric disorders, human immunodeficiency virus (HIV) infection, Hepatitis A virus (HAV) and Hepatitis C virus (HCV), chronic kidney disease (CKD), dialysis and kidney transplantation were obtained. Data about HBsAg, total hepatitis B core antibody (anti-HBc), Hepatitis B e antigen antibody (anti-HBe), HBV viral load, HBV genotype, anti-HDV, HDAg, HDV viral load, duration and type of treatment used for both infections was collected to better characterize the HBV and, if present, HDV infection. Clinical outcomes (cirrhosis (assessed by transient elastography [Fibroscan®] or other imaging modalities), endoscopic portal hypertension, HCC, liver transplantation and acute decompensations) were evaluated. Laboratory parameters (serum creatinine, total bilirubin, liver enzymes - aspartate aminotransferase (AST]) and alanine aminotransferase (ALT), albumin and sodium levels, international normalized ratio (INR) and platelets (PLT) count were also obtained. Child-Pugh and MELD-Na scores were calculated using the published formulas.[10]

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences, version 29.0. Continuous variables were described with median and interquartile range (IQR) while categorical variables were described with frequency and percentage. The sample was deemed non-normal distributed, given the small number of HDV positive patients. The Pearson chi-square test and the Montecarlo exact test were used to compare categorical variables between HBV monoinfected patients, HDV-HDV coinfected and super infected patients. Independent Samples T-test and Kruskal-Wallis test were used to compare continuous variables between the previously mentioned groups. A *p-value* of <0.05 was considered statistically significant.

Results

Anti-HDV testing was not performed in 300 of the 541 (55.45%) HBsAg positive patients, leaving a sample size of 241 cases.

Demographic and Clinical Characteristics

Out of 241 patients, 61.41% (n=148) were male and 89.63% (n=216) were Portuguese. The median of age at last follow-up was 57.00 (46.00-64.50) years old and follow-up lasted a median of 14.50 (7.50-23.50) years. The most common risk factor was immunosuppression (4.56%, n=11), followed by IVDU (2.49%, n=6). The vast majority of the cases (81.74%, n=197) reported alcohol consumption. HCV coinfection was present in 14 individuals (5.81%), while HAV coinfection was diagnosed in 4 patients (1.66%). There was one case of HIV infection (0.41%).

Among those screened, only 4.15% (n=10) were anti-HDV positive. HBV/HDV coinfection was present in 3 patients (1.24%) and the remaining 7 (2.90%) had HBV/HDV superinfection. The sample was divided into 3 subgroups: HBV monoinfected (95.5%, n=231), HBV/HDV coinfected and HBV/HDV superinfected.

The median of age at last follow-up was 57.00 (46.00-65.00) years old in the HBV monoinfected group, 52.00 years old in those with HBV/HDV coinfection and 53.00 (50.00-58.00) years old in the HBV/HDV superinfected group. The majority of the included patients was male: 60.17% (n=139) in the HBV monoinfected group, 85.71% (n=6) in the HBV/HDV superinfection group, and all (n=3) in HBV/HDV coinfection group. The predominant nationality was Portuguese, corresponding to 89.61% (n=207), 100.00% (n=3) and 85.71% (n=6) in the HBV monoinfection, HBV/HDV coinfection and HBV/HDV superinfection groups, respectively. There were no differences concerning age, sex or nationality between the 3 subgroups.

Comorbidities and Risk Factors

Patients had follow-up in Hepatology consultation for a median of 14.50 (7.50-23.50), 4.50 and 14.50 (4.50-23.50) years in the HBV monoinfected, HBV/HDV coinfected and HBV/HDV superinfected groups, respectively.

Table I summarizes patients' comorbidities and risk factors. The prevalence of chronic arterial hypertension was higher in patients with HDV coinfection and superinfection (66.67%, n=2 and 57.14, n=4, vs 19.48%, n=45, p=0.020). Twenty-eight HBV monoinfected patients (12.12%) had personal history of DM2, while only 1 (14.30%) of those HBV/HDV superinfected had DM2; none of

the HBV/HDV coinfected patients had DM2. Dyslipidemia and mental health disorders were also more prevalent in patients with HDV infection, but with no statistically significant differences among the three groups of patients.

HCV coinfection was also more frequent in patients with HBV/HDV coinfection (100.00%, n=3) and HBV/HDV superinfection (71.42%, n=5), with a *p value* <0.001. Regarding HAV coinfection, HIV infection and CKD, there were no statistically significant differences between the 3 groups of patients, as described in Table I.

Regarding risk factors, alcohol consumption was higher in HBV/HDV coinfected and superinfected patients (66.67%, n=2 and 71.43%, n=5 vs 16.02%, n=37, *p*<0.001). The median of alcohol grams consumed per day was 40.00 (37.50-50.00) in the HBV monoinfected group. On the other hand, there was no information related to this metric in the HDV infected groups. IVDU was also more frequent in patients with HBV/HDV coinfection and superinfection, comparing with those with HBV monoinfection (33.33%, n=1 and 42.86%, n=3, respectively, vs 0.87%, n=2, *p*<0.001). Past or current smoking habits were present in 7.79% (n=18) of HBV moninfected subjects and 14.30% (n=1) of those with HBV/HDV superinfection. No tobacco consumption was registered in the HBV/HDV coinfection group. Information concerning other risk factors is described in Table I.

Data concerning HBV infection is reported in Table II. The route of transmission remained unknown in most cases. Among those with determined routes, IVDU transmission was significantly higher among HDV infected individuals (33.33%, n=1 in those HBV/HDV coinfected and 28.57%, n=2 in those HBV/HDV superinfected, *p*=0.004). Sexual transmission was also significantly higher (28.57%, n=2) in HBV/HDV superinfection cases than in the remaining groups (p=0.008). Vertical transmission was the most common route in those HBV monoinfected (17.75%, n=41). HBV genotype was determined in 118 cases, with genotype D (66.10%, n=78) and A (19.49%, n=23) being the most frequent. Anti-HBe was present in the majority of the patients: 85.71% (n=198) in HBV monoinfection group, 66.67% (n=2) in HBV/HDV coinfection group and 100.00% (n=7) in HBV/HDV superinfection group. HBV-DNA was detectable exclusively in the HBV monoinfected cases, with a median of 704.00 (155.00-13900.00) IU/ml, and in HBV/HDV superinfection, with a median of 91.50 IU/ml. All patients with HBV/HDV coinfection (n=3) were treated for HBV, while only 42.86% (n=3) of those with HBV/HDV superinfection were. The most recent treatments more commonly used consisted of Entecavir (ENT) and Tenofovir (TENO).

Laboratory parameters

Laboratory parameters are summarized in Table III. HDV infected individuals presented with significantly higher levels of AST (p=0.007), ALT (p=0.021), INR (p=0.042) and Liver Stiffness (p=0.041) alongside with lower levels of albumin (p=0.002) and PLT count (p=0.021), when compared with the HBV monoinfection group. When comparing HBV/HDV superinfection with those with HBV/HDV coinfection, the latter also presented significantly higher levels of AST, ALT, INR and liver stiffness with lower levels of albumin and PLT count. There were no differences concerning total bilirubin and controlled attenuation parameter (CAP) (determined through liver elastography) between the 3 groups.

Clinical Outcomes

Various outcomes were analyzed and are reported in Table IV. Endoscopic evaluation of portal hypertension was performed in 66 patients (27.39%). From these, 12 patients with HBV monoinfection, 1 with HBV/HDV coinfection and 1 with HBV/HDV superinfection had endoscopic portal hypertension. Both HDV infected cases presented with esophageal varices and hypertensive gastropathy. The most common finding among the HBV monoinfected patients was esophageal varices (75.00%, n=9).

The evolution to cirrhosis occurred in all HBV/HDV coinfection cases (n=3) and in 71.43% (n=5) of those with HBV/HDV superinfection. This number was remarkably lower in the HBV monoinfection group (13.85%, n=32) (p<0.001). Regarding Child-Pugh and MELD-Na scores, there were no statistically significant differences found between the three groups. Most of the patients were classified as Child-Pugh A: 73.08% (n=19) in HBV monoinfection group, 66.67% (n=2) in HBV/HDV coinfection group and 66.67% (n=2) in HBV/HDV superinfection group. None of the HDV infected individuals were reported as Child-Pugh C. MELD-Na scores medians were 10.00 (7.00-21.00), 9.00 and 18.00 in the HBV monoinfection, HBV/HDV coinfection and HBV/HDV superinfection groups, correspondingly. A substantially higher number of HBV/HDV coinfected patients developed HCC (66.67%, n=2), when compared with HBV/HDV superinfected (14.29%, n=1) and HBV monoinfected (3.46%, n=8) patients (p=0.010). Cirrhosis was present in all the HDV positive individuals who developed HCC and in 62.5% (n=5) of those HBV monoinfected. All the HDV positive patients (n=3) who developed HCC also presented with a HCV infection, a number vastly larger than the 12.5% (n=1) of those HBV monoinfected who also had both diseases (p=0.023). There was only 1 case of liver transplantation in the whole sample, a HBV monoinfected patient.

The mortality rates in the HDV infection groups were higher: 66.67% (n=2) in HBV/HDV coinfection group, followed by 28.57% (n=2) in the HBV/HDV superinfection group and 5.63% (n=13) in HBV monoinfection group (p=0.020). HCC was the main cause of death in 50.00% (n=2) of the HDV infected cases and in 46.15% (n=6) of the HBV monoinfected cases. The age at death was remarkably inferior in the HDV infection groups (median of 44.00 and 52.00 years old in HBV/HDV coinfected and superinfected group, respectively), comparing with HBV monoinfection group (median of 63.00 (54.50-72.50) years old) (p=0.031).

Discussion

Despite current guidelines from EASL and APASL recommending testing all individuals with HBV for HDV at least once in their lifetime, anti-HDV testing was overlooked in 55.45% of the patients. [11,12] Although this study did not collect information about patient compliance to such tests, it has been established that this practice is relatively uncommon among physicians, with an Italian study reporting anti-HDV testing in only 16.6% of the HBsAg positive individuals.[13] Such a small number may be explained by lack of awareness regarding the disease, recently published guidelines or accessibility of the test.[14] The lack of tests conducted led to a quest for ways to automate anti-HDV testing. The main focus has been placed on anti-HDV reflex testing, in other words, anti-HDV automatic testing of all HBsAg-positive individuals by the laboratory.[15] This strategy increased the number of tests carried out and individuals diagnosed with HDV infection, with an almost total coverage of HBsAg-positive patients.[13] While it might seem like a costly measure, a recent paper of Polaris Observatory Group estimated that only 34,500 HBV patients within the European Union would require testing. Thus, nationally, the number requiring anti-HDV testing will be even lower, with prevention of end-stage HBV/HDV liver disease providing further cost savings.[15]

Current meta-analysis concerning this matter estimated a HDV seroprevalence in HBsAg positive patients between 4.5% and 14.57%, while a 2022 Portuguese study reported 3.4% of Anti-HDV positivity.[9,14,16,17] Our calculations of 4.15% HDV seroprevalence seems to be in line with the recent literature.

The majority of the individuals were male in their fifties, as expected in European countries, unlike other regions where HDV infection takes place earlier in life.[18,19] It is suggested that vaccination coverage above 80% is an effective measure in the fight against HBV and therefore HDV.[14] The high anti-HBV vaccination rates in Portugal (97.5%) also plays a part in protecting the youngest from HDV.[20] As a matter of fact, vaccination was a key factor in the decline of HBV and HDV cases in Europe. This decline is now threatened and even ceased to exist and most studies name migration as a key factor, with immigrants from high endemicity (and low vaccination coverage) areas contributing to the HDV infection burden in Mediterranean countries at an increasing rate.[14,21] The same cannot be said about our study, where more than 80% of the individuals were Portuguese.

In what concerns comorbidities, significant differences between groups were only found regarding arterial hypertension and HCV infection. Both HDV, HCV and HIV share routes of transmission and risk populations, which most likely explain the statistical difference in HCV infection. On the other hand, HIV prevalence was not higher in HDV infected cases in our analysis.[16,17] As expected, alcohol

consumption, a well-known hepatotoxic, and IVDU, the main HDV transmission route in developed countries, were also more likely present among HDV infected individuals.[17,21,22]

HBV transmission routes remained unknown in most cases. In intermediate prevalence areas, like Western Europe, sexual intercourse is responsible for most cases of HBV infection, followed by IVDU.[23,24] Unexpectedly, vertical transmission was the most common route in the HBV monoinfection group, while IVDU and unprotected sexual intercourse played a big role in HBV transmission among those with HDV infection. The low number of known transmission routes reduces the strength of the conclusions drawn. Nevertheless, they highlight the need to target these high-risk behaviors with public health measures, such as providing disposable medical equipment and contraception.

HDV infected individuals presented with higher levels of transaminases, INR and Liver Stiffness alongside with lower levels of albumin and PLT count, when compared with those HBV monoinfected. These findings where similar to those enumerated in Cossiga et al.,[13] (but our sample size is much smaller) and are mostly related with HDV's aggressive form of viral hepatitis.[17]

HDV infection leads to a faster and more severe course of disease, in comparison to HBV monoinfection, through mechanisms that have not yet been fully studied.[11] This study demonstrates that the evolution to cirrhosis is more frequent in those with HDV infection. The same findings were reported in other papers, including a meta-analysis encompassing 634 studies.[17,19,21]

Unlike other cancers, HCC's incidence and mortality rates are still rising and viral hepatitis, including hepatitis D, are responsible for more than 80% of all HCC cases.[25] Our results found the number of HCC cases were higher in HDV infected patients than in it's HBV monoinfected counterparts. These results are in line with what has already been concluded in other studies: although HDV infection leads to an earlier onset of cirrhosis (and associated complications, including death), it does not reduce the risk of developing HCC.[25] However, a more detailed analysis showed that in all cases of HCC in HDV-infected patients, cirrhosis and coinfection with HCV also co-existed. The same cannot be said for patients with HBV infection who later developed HCC. These two confounding factors call into question the veracity of the previous conclusion, since the oncogenic role of HDV has not yet been completely proven, as it is not integrated into the human genome.[26] This shines light into the need of further investigation about this matter.

Viral hepatitis is estimated to cause approximately 1,34 million deaths worldwide each year, yet the extent of HDV's contribution to these figures is not fully known.[21] The mortality rates in the HDV infection groups were higher and the individuals' age at death was remarkably inferior to those in HBV monoinfection groups, which might be related to HDV's accelerated disease progression.

HCC was the main cause of death in HDV infected and HBV monoinfected cases. Treatment of early-stage HCC is associated with 5-year survival rates of between 50-70%, but in advanced stages, survival does not reach one year.[27] Our observations highlight the need to closely monitor all HDV infected patients with advanced fibrosis or cirrhosis, with the EASL recommending a liver ultrasound with or without alpha-fetoprotein testing every 6 months.[12]

This study has limitations that are worth noting. Firstly, it is a retrospective study, which is a possible bias factor. Since the information was collected from medical records, a considerable number of cases had incomplete data. In addition, although the initial database was quite large, the numbers of patients in specific subgroups were very limited, as this was a single-center experience. Multicenter observational studies and with a larger number of patients are indispensable for more precise results.

Conclusion

Our data demonstrated HDV infection is associated with a worse prognosis, including death and end-stage liver disease. Measures to prevent HDV infection should always be applied and deserve greater recognition by both medical professionals and society. Our findings underscore a gap in adherence to current HDV infection guidelines. Implementation of anti-HDV testing in a methodical way is critical, as this diagnosis must be carried out as early as possible to avoid progression to unfavorable outcomes.

Appendix

Table I – Demographic and clinical characteristics of the population

	HBV monoinfection n=231	**HBV/HDV coinfection n=3**	**HBV/HDV superinfection n=7**	***p*-value**
Sex, M, n (%)	139 (60.17)	3 (100.00)	6 (85.71)	0.162
Age at last follow-up, years, median (IQR)	57.00 (46.00-65.00)	52.00	53.00 (50.00-58.00)	0.849
Portuguese natives, n (%)	207 (89.61)	3 (100.00)	6 (85.71)	1.000
Comorbidities				
Arterial hypertension, n (%)	45 (19.48)	2 (66.67)	4 (57.14)	**0.020**
DM2, n (%)	28 (12.12)	0	1 (14.30)	1.000
Dyslipidemia, n (%)	39 (16.88)	1 (33.33)	2 (28.57)	0.645
Mental health problems, n (%)	25 (10.82)	1 (33.33)	2 (28.57)	0.112
HIV infection, n (%)	1 (0.43)	0	0	1.000
HAV infection, n (%)	3 (1.30)	0	1 (14.30)	0.153
HCV infection, n (%)	6 (2.60)	3 (100.00)	5 (71.42)	**<0.001**
CKD, n (%)	16 (6.93)	0	2 (28.57)	0.105
Kidney transplantation, n (%)	10 (4.33)	0	0	1.000
Risk factors				
Alcohol consumption, n (%)	37 (16.02)	2 (66.67)	5 (71.43)	**<0.001**
Alcohol consumption, g/day, median (IQR)	40.00 (37.50-50.00)	-	-	-
Tabacco consumption, n (%)	18 (7.79)	0	1 (14.30)	1.000
Risk context				
Unknown, n (%)	213 (92.1)	2 (66.67)	4 (57.14)	**0.002**
IVDU, n (%)	2 (0.87)	1 (33.33)	3 (42.86)	**<0.001**
Unprotected sexual intercourse, n (%)	1 (0.43)	0	0	1.000
Immunosuppression, n (%)	11 (4.76)	0	0	1.000
Health care personnel, n (%)	2 (0.87)	0	0	1.000
Natives of endemic countries, n (%)	2 (0.87)	0	0	1.000
Follow-up, years, median (IQR)	14.50 (7.50-23.50)	4.50	14.50 (4.50-23.50)	0.749

Note: Categorical variables were described as absolute frequency and percentage. Quantitative data are depicted as median with interquartile ranges (IQR). The bold values are the statistically significant ones.

Abbreviations: CKD, Chronic kidney disease; DM2, Type 2 Diabetes Mellitus; HAV, Hepatitis A virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; IQR, Interquartile range; IVDU, Intravenous drug use; M, Male.

Table II – Comorbidities and risk factors

	HBV monoinfection n=231	**HBV/HDV coinfection n=3**	**HBV/HDV superinfection n=7**	***p-value***
HBV transmission				
Unknown, n (%)	162 (70.13)	2 (66.67)	3 (42.86)	0.327
Vertical, n (%)	41 (17.75)	0	0	1.000
Intrafamily, n (%)	18 (7.79)	0	0	1.000
IVDU, n (%)	4 (1.73)	1 (33.33)	2 (28.57)	**0.004**
Sexual, n (%)	3 (1.30)	0	2 (28.57)	**0.008**
Transfusional, n (%)	3 (1.30)	0	0	1.000
HBV genotype				
Unknown, n (%)	115 (49.78)	2 (66.67)	6 (85.71)	0.201
A, n (%)	22 (9.52)	1 (33.33)	0	0.282
B, n (%)	2 (0.87)	0	0	1.000
C, n (%)	1 (0.43)	0	0	1.000
D, n (%)	77 (33.33)	0	1 (14.30)	0.303
E, n (%)	6 (2.60)	0	0	1.000
F, n (%)	8 (3.46)	0	0	1.000
G, n (%)	0	0	0	1.000
H, n (%)	0	0	0	1.000
I, n (%)	0	0	0	1.000
J, n (%)	0	0	0	1.000
Anti-HBe positive, n (%)	198 (85.71)	2 (66.67)	7 (100.00)	0.287
HBV-DNA detectable, n (%)	139 (60.17)	0	3 (42.86)	0.056
HBV-DNA, IU/ml, median (IQR)	704.00 (155.00-13900.00)	NA	91.50	0.085
Antiviral therapy against HBV, n (%)	158 (68.40)	3 (100.00)	3 (42.86)	0.159
Last used antiviral therapy against HBV				
LAM, n (%)	6 (3.80)	0	0	1.000
ADEFO, n (%)	9 (5.70)	0	0	1.000
ENT, n (%)	62 (39.24)	0	1 (33.33)	0.566
TENO, n (%)	74 (46.84)	3 (100.00)	2 (66.67)	0.134
IFN/PEG, n (%)	5 (3.16)	0	0	1.000
COMB, n (%)	2 (1.27)	0	0	1.000
Duration of antiviral therapy against HBV, years, median (IQR)	12.00 (5.00-20.00)	3.00	12.00	0.121

Note: Categorical variables were described as absolute frequency and percentage. Quantitative data are depicted as median with interquartile ranges (IQR). The bold values are the statistically significant ones.

Abbreviations: ADEFO, Adefovir; Anti-HBe, Hepatitis B antigen antibody; COMB, Combination of antiviral treatments; DNA, Deoxyribonucleic acid; ENT, Entecavir; HBV, Hepatitis B virus; IFN/PEG, Pegylated interferon; IQR, Interquartile range; IVDU, Intravenous drug use; LAM, Lamivudine; TENO, Tenofovir.

Table III – Laboratory parameters

	HBV monoinfection n=231	**HBV/HDV coinfection n=3**	**HBV/HDV superinfection n=7**	***p-value***
AST, IU/L, median (IQR)	21.00 (17.00-29.00)	139.00	77.00 (16.00-80.00)	**0.007**
ALT, IU/L median (IQR)	21.00 (16.00-30.00)	89.00	28.00 (16.00-131.00)	**0.021**
Total bilirubin, mg/dL, median (IQR)	0.55 (0.40-0.73)	1.04	0.47 (0.43-1.53)	0.066
Albumin, g/dL, median (IQR)	4.58 (4.32-4.80)	3.95	4.24 (3.41-4.27)	**0.002**
PLT as 10^3/L, median (IQR)	212.50 (171.50-265.75)	58.00	204.00 (194.00-238.00)	**0.021**
INR, IU/L, median (IQR)	1.02 (0.98-1.10)	1.21	1.09 (0.97-1.51)	**0.042**
CAP, dB/m, median (IQR)	227.00 (179.50-272.50)	246.00	198.00 (60.72-275.00)	0.694
Liver Stiffness, kPa, median (IQR)	5.20 (4.30-7.92)	63.40	14,60 (7.95-100.15)	**0.041**

Note: Categorical variables were described as absolute frequency and percentage. Quantitative data are depicted as median with interquartile ranges (IQR). The bold values are the statistically significant ones.

Abbreviations: ALT, Alanine transaminase; AST, Aspartate aminotransferase; CAP, Controlled attenuation parameter; INR, International normalised ratio; IQR, interquartile range; PLT, platelets.

Table IV – Clinical Outcomes

	HBV monoinfection n=231	**HBV/HDV coinfection n=3**	**HBV/HDV superinfection n=7**	***p-value***
Endoscopic evaluation of portal hypertension, n (%)	60 (25.97)	2 (66.67)	4 (57.14)	0.052
Presence of endoscopic hypertension findings, n (%)	12 (20.00)	1 (50.00)	1 (25.00)	0.731
Endoscopic hypertension findings				
Hypertensive gastropathy, n (%)	1 (8.33)	0	0	1.000
Esophageal varices, n (%)	9 (75.00)	0	0	1.000
Both, n (%)	2 (16.67)	1 (100.00)	1 (100.00)	0.070
Cirrhosis, n (%)	32 (13.85)	3 (100.00)	5 (71.43)	**<0.001**
Child-Pugh score				
Child Pugh A, n (%)	19 (73.08)	2 (66.67)	2 (66.67)	1.000
Child Pugh B, n (%)	3 (11.54)	1 (33.33)	1 (33.33)	0.227
Child Pugh C, n (%)	4 (15.38)	0	0	1.000
MELD-Na, median (IQR)	10.00 (7.00-21.00)	9.00	18.00	0.563
HCC, n (%)	8 (3.46)	2 (66.67)	1 (14.29)	**0.010**
Liver transplantation, n (%)	1 (0.43)	0	0	1.000
Death, n (%)	13 (5.63)	2 (66.67)	2 (28.57)	**0.020**
Cause of death				
Unknown, n (%)	2 (15.38)	1 (50.00)	0	0.574
HCC, n (%)	6 (46.15)	1 (50.00)	1 (50.00)	1.000
Sepsis, n (%)	2 (15.38)	0	1 (50.00)	0.581
Other malignant tumors, n (%)	3 (23.08)	0	0	1.000
Age of death, years, median (IQR)	63.00 (54.50-72.50)	52.00	44.00	**0.031**
Time interval between diagnosis and death, years, median (IQR)	11.50 (2.00-14.50)	3.00	10.50	0.366

Note: Categorical variables were described as absolute frequency and percentage. Quantitative data are depicted as median with interquartile ranges (IQR). The bold values are the statistically significant ones.

Abbreviations: HCC, Hepatocellular carcinoma; IQR, interquartile range.

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