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Association of Hepatitis Delta Virus Infection and Hepatocellular Carcinoma, Hepatic Decompensation, All-cause and Liver-Related Death in a National Cohort

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Graphical Abstract

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Abstract:

Background: Hepatitis Delta Virus (HDV) infection is the most severe form of chronic hepatitis. However, studies on outcomes and causes of death in a United States-born population, with primarily horizontal transmission of HDV, are lacking. The aim of this study was to conduct a national study of patients with hepatitis D to understand the natural history and outcomes compared to patients with hepatitis B virus (HBV). infection.

Methods: In a national cohort of 4,817 HBV infected veterans tested for HDV (99.6% US-born, 3.3% HDV positive) over a 23-year period, we used multivariable models to identify the factors associated with a composite outcome of hepatocellular carcinoma (HCC), decompensation, and liver-related mortality (LRM), as well as all-cause mortality of patients with HDV compared to HBV mono-infection.

Results: HDV coinfection (vs. HBV monoinfection) was associated with a significantly higher incidence of composite liver-related outcomes at both 5 (23.84 vs. 7.98, p<0.001), and 10 years (19.14 vs. 10.18, p<0.001) respectively. The most common cause of death was liver-related (33.8% for HDV vs. 24.7% for HBV), followed by non-hepatic malignancies, (15.6 vs. 14.8%), cardiac (11.7 vs. 15.2%), and lung disease (5.2 vs. 3.7%). In multivariable models, HDV was associated with an increased risk of composite liver outcomes (aHR 2.57, 95% CI 1.87-3.52, p<0.001), and all-cause mortality (aHR 1.52, 95% CI 1.20-1.93, p<0.001).

Conclusion: In a predominantly U.S born cohort of Veterans, HDV co-infection was associated with an increased risk of liver-related outcomes and all-cause mortality. Our findings support widespread testing for early identification of HDV.

Introduction

Hepatitis D virus (HDV) co-infection occurs in 3.8-8.4% of patients with hepatitis B virus (HBV) infection, with an estimated worldwide prevalence of 15 million people.[1-4] The United States is not considered a country with high HDV prevalence, and most transmission is horizontal. There is limited data on the long-term outcomes of HDV in the United States.[5] Additionally, even among non-US studies, most studies that examine HDV-associated outcomes are from tertiary referral centers, which may be associated with selection bias by including patients who are sicker and may experience more severe outcomes.[6]

A better understanding of the contemporary epidemiology of HDV in the United States and the association between HDV infection and hepatocellular carcinoma (HCC), hepatic decompensation, and all-cause and liver-related mortality (LRM) will inform decisions about the cost-effectiveness of emerging HDV treatments.[5] Understanding the causes of death could also inform interventions to prevent morbidity and mortality in this population. Therefore, the primary aim of this study was to identify the causes and predictors of liver related outcomes (HCC, hepatic decompensation, and LRM) and all-cause mortality, in patients with HDV compared to those with HBV mono-infection in a primarily US-born population.

Methods

Study Design

This study was a retrospective analysis of the Veterans Analysis of Liver Disease (VALID) cohort that included 4.3 million well-characterized participants from the Veteran Health Administration (VHA).[1,7] The cohort included veterans with chronic liver disease (CLD), as well as non-CLD controls who were engaged with care and had at least one endoscopic procedure

within the VHA and included over 70,000 (out of 77,000 Veterans with a positive Hepatitis B surface antigen, Hepatitis B 'e' antigen or HBV DNA in the VHA). The data were sourced from the Veterans Health Administration (VHA) Corporate Data Warehouse (CDW) between January 2000 and December 2022, as outlined in prior studies.[8-10] The study was carried out in compliance with the Helsinki and Istanbul declarations, and the Institutional Review Boards at the Miami and Richmond Veterans Affairs (VA) Medical Centers approved the protocols and granted a waiver for the requirement of informed consent.

Patient Identification

We identified participants who were 18 years or older with at least one HDV test during the study period. Hepatitis D testing included the documentation of the testing of antibodies against HDV (anti-HDV), Hepatitis D RNA, or HDV antigen (HDV Ag). Patients were considered to have HDV infection if they were either anti-HDV or HDV PCR positive.[1] Patients who were anti-HDV positive and either HDV RNA-positive, untested or negative were considered HDV-positive. Patients who were only HDV Ag positive without a positive anti-HDV or HDV PCR, were not considered to have HDV.

We included all patients with evidence of Hepatitis B infection (positive Hepatitis B surface antigen, Hepatitis B 'e' antigen or HBV DNA) who underwent testing for HDV, defining the date of HDV testing as the baseline for HDV-negative patients, and the date of the first positive HDV test as the baseline for those with HDV. Patients who underwent HDV testing but without evidence of HBV infection on tests available within the VHA were excluded from the primary analysis but were included in the sensitivity analyses.

We obtained demographic data (age, sex, race/ethnicity, marital status, and country of birth) from the CDW. The following laboratory results were extracted: HCV RNA, HBeAg, HBV

DNA, HIV, ALT, AST, total bilirubin, platelet count, and INR. Elevated ALT level was defined as at least twice the upper normal limit (ALT ≥ 2 × 31 IU/ml).[11]

We used the International Classification of Diseases (ICD), ninth and tenth revisions (ICD-9 and ICD-10) to obtain information on hepatic decompensation, HCC, and substance use disorder.[12] Hepatic decompensation included new-onset ascites, variceal bleeding, or hepatic encephalopathy, captured using ICD 9/10 codes, as described in prior publications.[9,12] AUDIT-C is a screening test for risky alcohol use that is widely used in VHA.[13] AUDIT-C scores closest to the starting date and annual scores were collected. A high AUDIT-C was recognized as a score of 4 or more for men and 3 or more for women. Diabetes was diagnosed using a blend of ICD-9, ICD-10, medication, and laboratory results.[14] Fibrosis-4 (Fib-4) scores were calculated using baseline labs.

We set the criteria for a laboratory profile indicative of a high risk for HDV, marked by suppressed HBV DNA levels (below 2000 IU/ml) and an elevated ALT level (at least twice the upper normal limit), as defined by the AASLD guidelines.[11] We collected pharmacy data about the number and timing of oral nucleos(t)ide antiviral medications, including lamivudine, telbivudine, adefovir, entecavir, or tenofovir, and Interferon or Pegylated Interferon. A patient was considered to have received oral nucleos(t)ides or Interferon if they filled at least one outpatient prescription. We identified cirrhosis at baseline if the patient had two outpatient or one inpatient ICD 9/10 code for cirrhosis. Similarly, liver transplantation was identified using ICD 9/10 codes. We used a combination of the vital status file and the National Death Index (NDI) data, to identify any recorded deaths.[15] We identified the cause of death for each patient and defined liver-related death using ICD-10 codes from the NDI data.[15]

Sensitivity Analyses

Patients who underwent HDV testing but had missing evidence of HBV infection on tests available within the VHA were excluded from the primary analysis but included in the sensitivity analyses. Additional sensitivity analyses were performed, excluding participants who developed outcomes within 6 months of the baseline, doing a competing risk survival analysis of the full sample, restricting analysis to patients without cirrhosis at baseline, as well as examining association of HDV viremia with outcomes.

Statistical Analysis

We compared the descriptive statistics between HDV-positive and HDV-negative patients. For continuous variables, p-values were determined using the Kruskal-Wallis test, while Pearson Chi-squared tests were used for binary and categorical data.

Logistic regression analysis was employed to examine the factors associated with HDV-positive status using the adjusted odds ratios (aORs) of specific factors, including age, sex, race/ethnicity, diabetes, substance use disorder, high AUDIT-C, HIV coinfection, HCV RNA positivity, and high-risk laboratory profile. We assessed the goodness of fit of the logistic regression model using the Hosmer-Lemeshow test. We determined the incidence rates (instances per 1000 person-years), incidence rate ratios (IRR), and 5- and 10-year landmark analyses for outcomes of HCC, hepatic decompensation, all-cause mortality, and LRM. We also employed multivariable Cox proportional hazards models, with censoring at non-liver related death, to estimate the adjusted Hazard ratio (aHR) with 95% confidence levels, and cumulative incidence graphs for these outcomes. For a sensitivity analysis, we performed a multivariable analysis with non-liver related death as competing risk using the Fine-Gray method.

For each model, we adjusted for all potential variables that could be associated with the outcome in all models. For each outcome, we additionally excluded patients who already had that

outcome prior to diagnosis or testing for HDV. For example, patients tested for HDV after HCC diagnosis were excluded from the HCC models.

To test the proportional hazards assumption, we used Schoenfeld residuals. All statistical analyses were performed using Stata version 18, and a p-value <0.05 was considered statistically significant.

Results

Baseline Characteristics

A total of 4,817 patients with HBV were tested for HDV between 1/1/2000 and 12/31/2022; 158 (3.3%) were HDV-positive and 4,659 (96.7%) were HDV-negative (**Figure 1**). An additional 8,368 patients were tested for HDV but had no evidence of HBV infection based on testing in the VHA. Some of these patients likely had HBV diagnosed outside the VHA, but were excluded from the primary analysis but included in the sensitivity analysis.

The median age was 56.9 years (IQR 16.3), predominantly male (95.0%), and multi-racial, with 31.6% non-Hispanic white, 30.7% non-Hispanic Black, 14.2% Hispanic, and 9.8% Asian (**Table 1**). The majority of the participants were U.S.-born (99.6%), 19 (0.4%) were born in countries with a low prevalence of HDV, and one was born in a country with a high HDV prevalence. Most patients had a history of substance use disorders (n=2763, 57.4%).

Nature of HDV Testing

A cross tabulation of patients who underwent anti-HDV and HDV RNA testing revealed that 154 patients were anti-HDV positive, of whom 9 were HDV RNA positive, 20 were negative, and 125 were not tested for HDV RNA (**Table 1**). Additionally, one patient who was anti-HDV-negative and three patients who were not tested for anti-HDV but HDV RNA positive were considered to have HDV infection and were included in the analytic sample.

Comparison of HDV positive and negative baseline characteristics

HDV-positive participants were more likely to be male (98.7 vs. 94.9%, p=0.03), have a history of substance use disorder (76.0 vs. 56.7%, p<0.001), have an elevated ALT level that is twice the upper limit of normal (52.5 vs. 40.4%, p=0.002), have a high-risk laboratory profile of elevated ALT with suppressed HBV DNA (24.1 vs. 8.0%, p<0.001), be HCV RNA ever positive (26.0 vs. 12.0%, p<0.001), have higher aspartate aminotransferase (50.0 vs. 36.0, p<0.001), alanine aminotransferase (58.2 vs. 41.5, p=0.01), gamma glutamyl transferase (83.5 vs. 48.0, p=0.007), more likely to have cirrhosis (12.7 vs. 7.5%, p=0.02), and lower platelet count (165.0 vs. 198.0, p<0.001), and less likely to have diabetes mellitus (20.3 vs. 30.3%, p=0.007) at baseline than HBV patients without HDV. Less than 1% of the overall cohort underwent liver transplantation, which was more common among patients with HDV (3.2% vs. 0.8%, p=0.002). Only 12 out of 158 patients with HDV received Interferon or Peg-Interferon, all of which was part of hepatitis C treatment, and all preceded diagnosis of HDV. Bulevirtide is not FDA approved in the United States, and none of the patients in the study received it.

Predictors of HDV Positivity

The predictors of HDV infection included a history of substance use disorder (aOR 2.02, 95% CI 1.36-2.99, p<0.001), HCV RNA ever positive (aOR 1.94, 95% CI 1.32-2.86, p<0.001), and patients with a high-risk laboratory profile, defined as elevated ALT with suppressed HBV DNA (aOR 3.55, 95% CI 2.40-5.23, p<0.001) (**Table 2)**. There was no association between HDV positivity and other factors, such as age, sex, race/ethnicity, or HIV co-infection.

Incidence Rates of Liver-related Outcomes in HDV

The unadjusted incidence rates of composite liver outcomes were significantly higher among patients with HDV compared to HBV mono-infection (32.92 vs. 12.32 per 1000 P-Y, p<0.001), with an incidence rate ratio (IRR) of 2.67 (95% CI 2.23-3.18, **Table 3**). These differences were observed for each individual liver-related event, including HCC (18.65 vs. 4.84, p<0.01), hepatic decompensation (15.30 vs. 5.50, p<0.01), and LRM (15.65 vs. 7.84, p<0.01), as well as all-cause mortality (58.86 vs. 36.25, p<0.01). A landmark analysis at both 5 and 10 years confirmed similar trends.

Among participants who did not have cirrhosis at baseline (n=4447), the development of cirrhosis was more common among HDV positive vs. HDV negative patients at both 5 (15.2 vs. 7.1%, p<0.001) and 10 years (21.7 vs. 9.2%, p<0.001). Composite liver outcomes were higher in HDV vs. HBV at both 5 (23.84 vs. 7.98, p<0.001) and 10 years (29.14 vs. 10.18, p<0.001, **Figure 2** and Supplementary Table 1, http://links.lww.com/HEP/I664). This included HCC, which was higher in patients with HDV at 5 years (11.69 vs. 2.26%, p<0.001), and 10 years (14.29 vs. 3.22%, p<0.001) respectively.

Similarly, among patients without baseline hepatic decompensation, the incidence rates of decompensation were significantly higher among patients with HDV than among those with HBV mono-infection at both 5 (9.68 vs. 4.06%, p=0.001) and 10 years (12.26 vs. 5.0%, p<0.001).

Similarly, all-cause mortality was higher in HDV vs. HBV at both 5 (27.85 vs. 17.17%, p=0.01) and 10 years (37.34 vs. 24.32, p<0.001) respectively (**Figure 3** and Supplementary Table 1, http://links.lww.com/HEP/I664).

Association of Hepatitis D Infection and Composite Liver-related Outcomes

On multivariable analysis, HDV infection was associated with an increase in composite liver-related outcomes (adjusted Hazard Ratio [aHR] 2.57, 95% CI 1.87-3.52, p<0.001) after adjusting for age, sex, race/ethnicity, BMI, diabetes mellitus, substance use disorder, AUDIT-C, baseline cirrhosis, year of HDV testing, receipt of oral nucleoside/nucleotide therapy, HIV infection, HCV RNA ever positive, HDV DNA PCR, total bilirubin and INR (**Table 4 and Figure 3)**. Other predictors of liver related outcomes included older age (aHR per year increase 1.03, 95% CI 1.02-1.04, p<0.001), diabetes mellitus (aHR 1.33, 95% CI 1.10-1.61, p=0.003), substance use disorder (aHR 1.48, 95% CI 1.21-1.82, p<0.001),
receipt of oral nucleoside/nucleotide analogs (aHR 1.54, 95% CI 1.25-1.89, p<0.001),
HCV RNA PCR ever-positive (aHR 1.60, 95% CI 1.28-1.99, p<0.001), baseline cirrhosis aHR 4.24, 95% CI 3.24-5.56, p<0.001), and total bilirubin (aHR 1.05, 95% CI 1.05-1.06, p<0.001, **Table 4**).

Outcomes of HCC followed similar trends as liver-related outcomes. On multivariable analysis, HDV Infection was associated with a nearly four-fold elevation in HCC (adjusted Hazard Ratio [aHR] 3.61, 95% CI 2.35-5.54, p<0.001, Supplementary Table 2, http://links.lww.com/HEP/I664) after adjusting for age, sex, race/ethnicity, BMI, diabetes mellitus, substance use disorder, AUDIT-C, baseline cirrhosis, year of HDV testing, receipt of oral nucleoside/nucleotide therapy, HIV infection, HCV RNA ever positive, HDV DNA PCR, total bilirubin and INR. All variables associated with liver-related outcomes were also associated with increase in HCC, with the exception of total bilirubin (aHR 1.02, 95% CI 0.99-1.05, p=0.14).

On multivariable analysis, HDV infection was associated with an increased risk of hepatic decompensation (aHR 2.36, 95% CI 1.50-3.70, p<0.001, Supplementary Table 2,

http://links.lww.com/HEP/I664). Predictors of decompensation were the same as that for liver-related outcomes with the exception of HCV RNA PCR ever positive (aHR 1.27, 95% CI 0.94-1.73, p=0.12).

On multivariable analysis, HDV infection was associated with an increase in LRM (aHR 1.89, 95% CI 1.25-2.86, p=0.003, Supplementary Table 2, http://links.lww.com/HEP/I664). Predictors of LRM were similar to that of liver-related outcome, with the exception of the receipt of oral nucleoside/nucleotide analogues (aHR 1.22, 95% CI 0.96-1.54, p=0.10, Supplementary Table 2, http://links.lww.com/HEP/I664).

Association of Hepatitis D Infection and All-cause Mortality

A total of 1521 patients died during the follow-up period. All-cause mortality was higher in HDV-infected patients than in HBV mono-infected patients (58.86 vs. 36.25 per 1000 PY, p<0.001, **Figure 3** and Supplementary Table 1, http://links.lww.com/HEP/I664).

On multivariable analysis, HDV infection was associated with an increase in all-cause mortality (aHR 1.52, 95% CI 1.20-1.93, p<0.001, **Table 4 and Figure 3**) after adjusting for age, sex, race/ethnicity, BMI, diabetes mellitus, substance use disorder, AUDIT-C, baseline cirrhosis, year of HDV testing, receipt of oral nucleoside/nucleotide therapy, HIV infection, HCV RNA ever positive, HDV DNA PCR, total bilirubin and INR.

Predictors of all-cause mortality were similar to that of liver related outcomes with male sex (aHR 2.04, 95% CI 1.37-3.03, p<0.001), and being underweight (aHR 2.31, 95% CI 1.72-3.11, p<0.001) increasing the risk, and being overweight (aHR 0.86, 95% CI 0.76-0.98, p=0.02), and obese (aHR 0.82, 95% CI 0.72-0.94, p=0.004) associated with a decrease in all-cause mortality.

Causes of Death in Patients with HDV vs. HBV Infection

A total of 1521 patients died during the study (Supplementary Table 3, http://links.lww.com/HEP/I664). The most common cause of death was liver-related among patients with both HDV (n=26, 33.8%) and HBV monoinfection (n=357, 24.7%). The three most causes of non-liver related deaths in HDV and HBV infected patients were the same- namely, non-hepatic malignancies (14.8 and 15.6% respectively), followed by cardiac (11.7 vs. 15.2% respectively), and lung disease (5.2 vs. 3.7%). Although other causes of death in patients with HDV vs. HBV infection, such as HIV (1.3 vs. 2.1%) and accidental drug overdose (0.0 vs. 1.7%) were not directly caused by liver disease, they were associated with a shared risk factor (substance use disorder).

Sensitivity Analysis

For sensitivity analysis, we included an additional 8,368 patients who were tested for HDV but had no evidence of HBV infection based on testing in the VHA. This resulted in a total cohort of 13,185 patients. Similar trends in the primary analysis were observed. On multivariable analysis, HDV infection was associated with an increased risk of composite liver outcome (aHR 2.02, 95% CI 1.57-2.60, p<0.001), including HCC (aHR 2.52, 95% CI 1.72-3.70, p<0.001), hepatic decompensation (aHR 1.57, 95% CI 1.09-2.27, p=0.02), and LRM (aHR 1.94, 95% CI 1.40-2.69, p<0.001), as well as all-cause mortality (aHR 1.34, 95% CI 1.11-1.61, p=0.003), after adjusting for age, sex, race/ethnicity, BMI, diabetes mellitus, substance use disorder, AUDIT-C, baseline cirrhosis, year of HDV testing, receipt of oral nucleoside/nucleotide therapy, HIV infection, HCV RNA ever positive, HDV DNA PCR, total bilirubin and INR (Supplementary Table 4, http://links.lww.com/HEP/I664).

We performed a second sensitivity analysis, excluding patients who developed outcomes within 6 months of the baseline. The results showed similar or stronger associations between HDV and the outcomes of interest compared to the primary analysis.

On multivariable analysis, after adjusting for the same variables as in the primary analyses, HDV infection was associated with an increased risk of composite liver outcomes (aHR 2.58, 95% CI 1.82-3.65, p<0.001), including HCC (aHR 3.81, 95% CI 2.38-6.09, p<0.001), hepatic decompensation (aHR 2.77, 95% CI 1.70-4.52, p<0.001), and LRM (aHR 1.80, 95% CI 1.14-2.83, p=0.01), as well as all-cause mortality (aHR 1.43, 95% CI 1.11-1.84, p=0.001, Supplementary Table 5, http://links.lww.com/HEP/I664).

On a third sensitivity analyses, we performed a competing risk survival analysis to examine if a competing risk of death from non-liver-related causes influenced the associations observed on primary analysis. We found that the results were similar to the primary analysis. On multivariable analysis with non-liver related death as competing risk, patients with HDV had a higher LRM (aHR 1.89, 95% CI 1.22-2.91, p=0.004) after adjusting for the same variables as the primary analysis. (Supplementary Table 6, http://links.lww.com/HEP/I664).

A fourth sensitivity analysis was performed in patients without cirrhosis at baseline, and adding Fib-4 in the model, to adjust for possible association of baseline fibrosis on the outcome. On multivariable analysis, HDV infection was associated with an increased risk of composite liver outcome (aHR 2.51, 95% CI 1.79-3.52, p<0.001), including HCC (aHR 3.36, 95% CI 2.06-5.48, p<0.001), hepatic decompensation (aHR 2.48, 95% CI 1.50-4.09, p<0.001), and LRM (aHR 2.21, 95% CI 1.43-3.40, p<0.001), as well as all-cause mortality (aHR 1.55, 95% CI 1.21-1.99, p=0.001), after adjusting for after adjusting for the same variables as the primary analysis (Supplementary Table 7, http://links.lww.com/HEP/I664).

We evaluated the association of HDV RNA on outcomes, by using imputed values for HDV RNA and present this data in Supplementary Table 8, http://links.lww.com/HEP/I664. However, since only 29 of 158 patients had HDV RNA available, this association was not statistically significant.

We also performed a sensitivity analysis to compare outcomes between those with the categories of RNA+/RNA- and RNA unknown. Although HDV RNA positive patients had numerically higher events for the composite liver outcome, the sample was underpowered to detect statistical significance (Supplementary Table 9, http://links.lww.com/HEP/I664).

Discussion:

The current study analyzed liver-related outcomes in a national cohort of primarily U. S-born non-Asian patients with Hepatitis Delta, compared to Hepatitis B mono-infection, over a two-decade period. The main findings indicate an association between hepatitis delta and an increased risk of liver-related outcomes and all-cause mortality.

Because HDV ascertainment and testing is limited, our findings strongly support widespread HDV testing and consideration of universal reflex HDV testing, for early identification of patients with HDV.

Over a quarter of all deaths in the overall cohort and 35% of deaths in patients co-infected with HDV were directly related to liver complications, primarily HCC. However, it is important to note that non-hepatic malignancies and cardiac and lung diseases accounted for the next three common causes of death, corroborating that not all patients with HDV die from liver disease. Our findings highlight the need to examine liver-related death as an important outcome in future HDV studies. The association of HDV (vs. HBV) was highest in HCC, with a nearly four-fold

increase in HCC compared to HBV (aHR 3.70), followed by hepatic decompensation (aHR 2.08), LRM (aHR 1.91), and all-cause mortality (aHR 1.55). The higher association of HDV with LRD stands to reason, since most of the increase in deaths associated with HDV was liver-related.

Most risk factors associated with the outcomes in our study are consistent with those of prior studies, but some merit further discussion. For example, advanced age and diabetes have been associated with HCC, decompensation and mortality in patients with liver disease.[14] The finding of HCV with increased HCC/ decompensation and death is novel and has also not been well studied in most prior studies because triple infections of HBV/HCV and HDV are uncommon. Previous studies have shown that active HCV viremia may, in fact, have a suppressive effect on HBV/HDV.[16] However, most patients with HCV in this study achieved SVR during follow-up, and it is unknown whether SVR could then be associated with a rebound of HDV infection, similar to what has been described for HBV.[16] Additionally, it has been established that HCV is a strong risk factor for HDV infection in people with HBV.[17]

Another interesting finding is the association between weight and mortality. We found increased mortality in underweight patients and reduced mortality in obese and overweight patients. This could potentially be explained by the obesity paradox, where a low BMI has been shown to be associated with higher obesity and lower all-cause mortality.[18] The low BMI could be a surrogate marker of sarcopenia associated with a major systemic illness or malignancy. It is unlikely that sarcopenia was driven by liver disease since only a very small number of patients in our study had advanced liver disease or decompensation at baseline. This was further corroborated by the fact that there was no observed association between BMI categories and HCC, hepatic decompensation, or liver-related mortality. Additionally, active substance use disorder could also

be associated with malnutrition, leading to sarcopenia. We also observed an interesting inverse association between HIV infection and liver-related deaths. It is possible that HIV patients may have higher engagement with specialist care and are more likely to receive HCC surveillance and better management of HBV. Alternatively, there may be a competing risk of death from non-liver-related causes in patients with HIV, however, a formal analysis of HDV with LRM, with non-liver related mortality as competing risk showed similar associations with HIV. The association of oral nucleoside/nucleotide analog exposure with HCC, decompensation, and LRM, but not all-cause mortality, may be due to a higher HBV viral load with these liver-related outcomes. When we adjusted for viral load, this could represent residual confounding. The association between high AUDIT-C at baseline and decreased liver-related and all-cause mortality may be because alcohol represents a modifiable risk factor in patients with HBV or HDV infection, and these patients may be more likely to be referred for treatment of alcohol use disorder, with successful intervention at HBV or HDV diagnosis resulting in decreased mortality.

Our main findings of increased liver-related outcomes with HDV have been corroborated by other studies. In a U.S.-based Veteran cohort, Kushner et al. examined outcomes associated with HDV in the VHA prior to 2013.[5] Due to the shorter study period compared to this study, the sample size was smaller, and 73 patients with HDV were identified in that publication. Similar increases in outcomes were observed, with HDV being associated with a 2.9-fold increase in all-cause mortality and a 2.1-fold increase in HCC. Although the outcomes of hepatic decompensation or liver-related mortality were not examined, this study represents one of the first analyses of HDV outcomes in the United States.

Most other outcome studies of HDV have been conducted in Europe. In a study in Greece over 13 years, patients with HDV were more likely to have a liver-related event (20.0% *vs.* 8.5%,

p=0.01).[19] Other studies from Europe and Australia found an increased risk of liver-related outcomes associated with HDV.[20-24] In a comprehensive systematic review and meta-analysis of 12 studies, Gish et al. found that patients with HDV viremia were more likely to have outcomes of cirrhosis, hepatic decompensation, HCC, and LRM.[25]

We acknowledge the following limitations of our study: First, as in many prior studies, the majority of patients did not have HDV RNA or HDV antigen tested. In our cohort, this was likely because the study included patients throughout the health system, including those seen in the primary care setting. Because this was a retrospective study done across 130 VA medical centers, with no access to stored samples, testing of HDV RNA in stored samples was not feasible. We considered patients who were anti-HDV-positive but without documented HDV RNA to have HDV infection, which could lead to misclassification bias. Previous studies have demonstrated that approximately 60-70% of patients with anti-HDV are positive for HDV RNA.[26] This also highlights the need to improve provider education around HDV, and the need for universal HDV screening (including reflex testing) for all patients with HBV, and HDV RNA testing and specialist referrals for those who test positive for anti-HDV.[27]

Second, our cohort of primarily male veterans limits the generalizability to women and non-veterans. Third, only a minority of patients with HBV in the VHA have been tested for HDV.[1] Although our study may have missed undiagnosed/untested patients with HDV, our comparison group included patients with HBV who tested negative for HDV. Most patients did not have cirrhosis at baseline, but this study cohort likely included patients with more advanced disease when compared to a cohort in which universal HDV testing was implemented. We adjusted for baseline cirrhosis in all multivariable models and performed a sensitivity analysis in patients without cirrhosis, and adjusting for baseline fibrosis, using Fib-4 scores.[28]

As more guidelines and experts are now favoring universal HDV testing and reflex testing for all patients with HBV, HDV can hopefully be diagnosed earlier in the course of the disease in the future.[29-32]

Fourth, we defined baseline as the date of HDV positive test in those that are positive and, for the first negative test for those who are HDV negative. Therefore, when creating a cumulative incidence curve, the HDV negative group was defined as those who were HDV negative and remained negative in all future testing, which could lead to an 'immortal time bias' in that the HDV negative group was biased toward better outcomes. However, we believe that the implications of this bias are small given that only 3.3% tested positive for HDV at some point in time.

Despite these limitations, this study represents a multi-year follow-up of a national cohort of people with HDV, offering new insights into the natural history of this understudied virus. Moreover, the Veterans Health Administration is the largest integrated national system and provider of hepatitis care in the United States.[5,8] Consequently, veterans are highly retained within the VHA even as they relocate across states within the United States. This provides an advantage because many liver-related outcomes associated with HDV take several years to develop. The majority of these patients were U.S.-born and likely acquired the infection via horizontal transmission, giving insight into outcomes in a population that has been less well-represented in prior studies. The population described was drawn from a nationwide system without tertiary bias, and less than 1% of the patients underwent liver transplantation, offering a more representative picture of the natural history of the disease. We believe that the association between HDV and liver-related death demonstrated here and the description of the most common causes of death are novel and have not been well studied in prior studies.

Conclusion: In a predominantly U.S.-born cohort of Veterans, HDV infection was associated with an increased risk of liver-related outcomes and all-cause mortality. With the impending arrival of novel antivirals, these findings highlight the need for more widespread HDV testing so that patients with HDV can be identified for treatment.

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Figure 1: Study flow chart

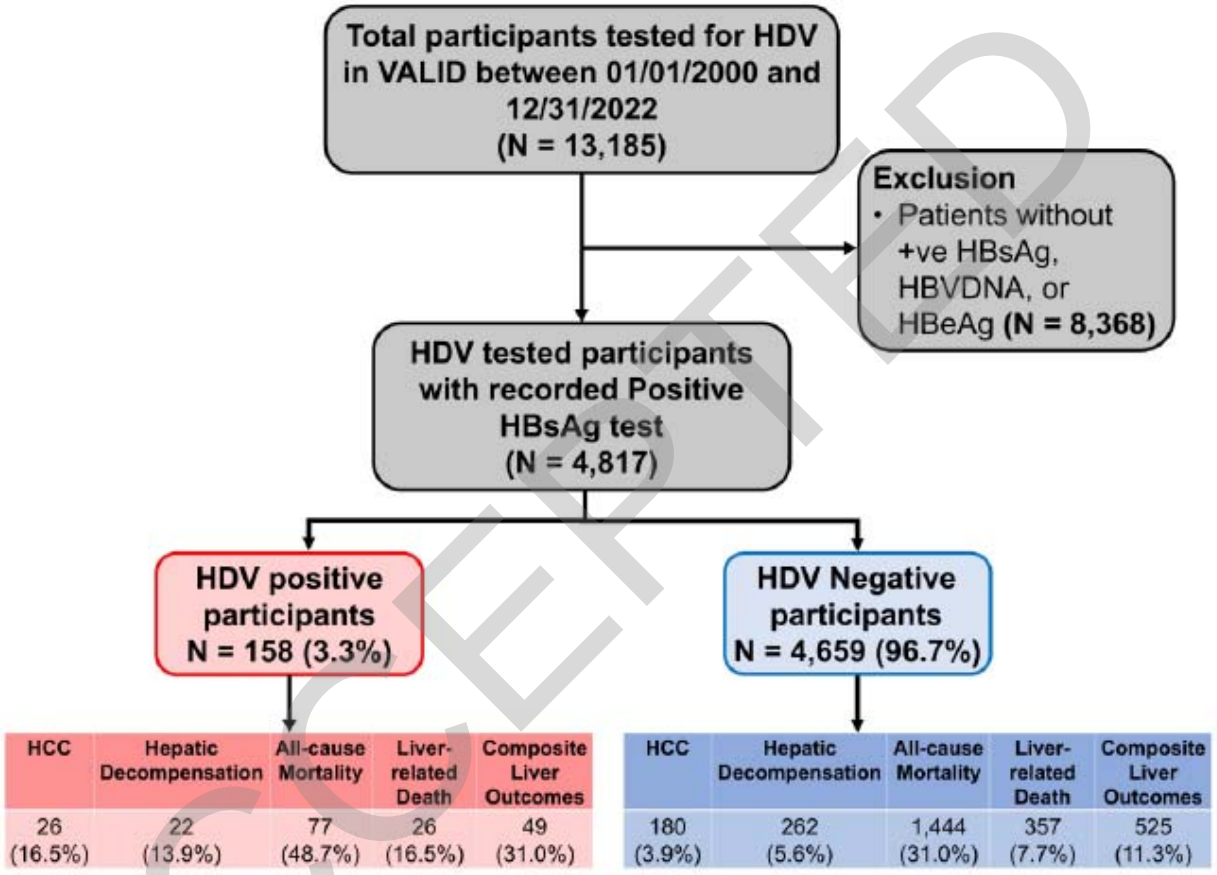

Figure 2: Landmark analysis of HCC, decompensation, liver-related mortality, all-cause mortality and composite liver outcomes (in %) at 5 and 10 years respectively for patients with Hepatitis D vs. Hepatitis B

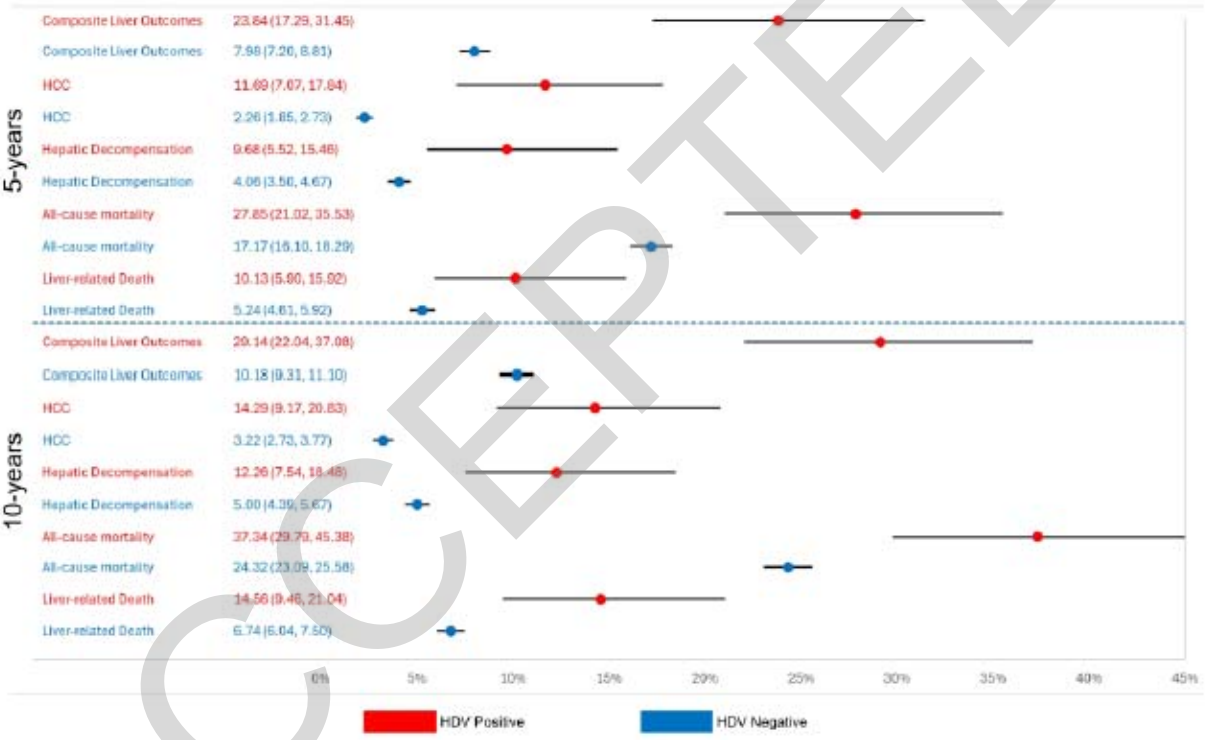

Figure 3: Cumulative Incidence frequency of Association of HDV and Composite Liver Outcomes (HCC, Hepatic Decompensation, and Liver-related Death) and All-cause Mortality

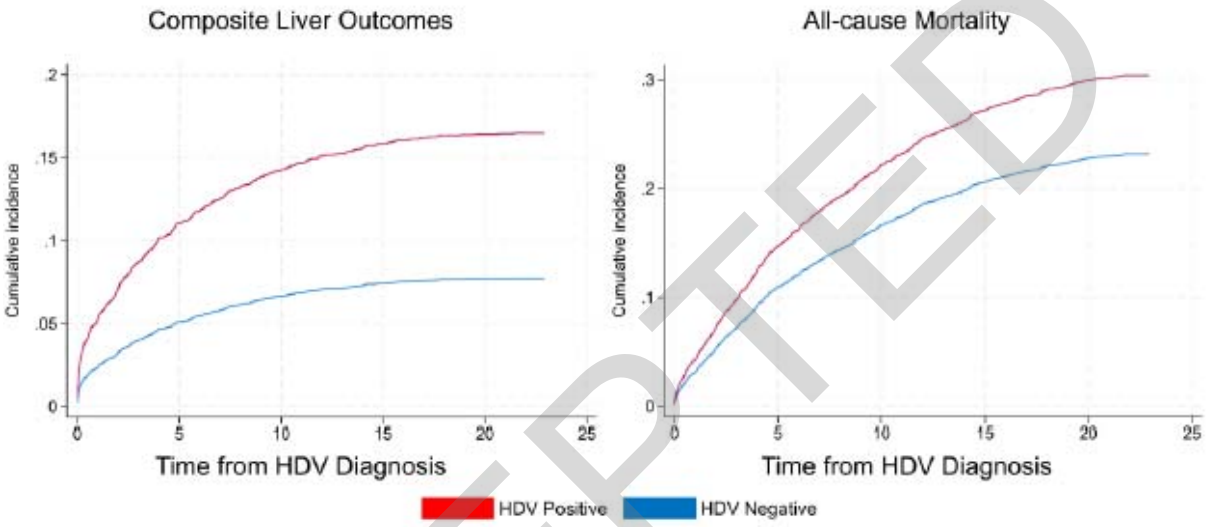

Table 1. Baseline Characteristics

Parameters	Total n = 4,817	HDV Positive n = 158 (3.3%)	HDV Negative n = 4,659 (96.7%)	*P Value*
Age, Median (IQR)	56.9 (16.3)	54.8 (17.2)	56.9 (16.3)	0.323
Sex, N (%)				
Female	239 (5.0%)	2 (1.3%)	237 (5.1%)	***0.030***
Male	4,578 (95.0%)	156 (98.7%)	4,422 (94.9%)	
Race / Ethnicity, N (%)				
White	1,521 (31.6%)	49 (31.0%)	1,472 (31.6%)	
Black	1,480 (30.7%)	52 (32.9%)	1,428 (30.7%)	
Hispanic/Latino	686 (14.2%)	23 (14.6%)	663 (14.2%)	0.300
Asians	470 (9.8%)	8 (5.1%)	462 (9.9%)	
Other	660 (13.7%)	26 (16.5%)	634 (13.6%)	

Marital Status, N (%)				
Single	1,062 (22.1%)	42 (26.6%)	1,020 (21.9%)	
Married	1,799 (37.4%)	52 (32.9%)	1,747 (37.5%)	0.496
Divorced/separated	1,713 (35.6%)	56 (35.4%)	1,657 (35.6%)	
Other	243 (5.0%)	8 (5.1%)	235 (5.0%)	
Country of Birth, N (%)				
United States	4,797 (99.6%)	158 (100%)	4,639 (99.6%)	
Low Endemic (non-US)	19 (0.4%)	0 (0%)	19 (0.4%)	0.711
High Endemic (non-US)	1 (0.0%)	0 (0%)	1 (0.0%)	
HDV Identification, N (%)				
HDVAb+ and HDVRNA unknown	125 (2.6%)	125 (79.1%)	0 (0%)	
HDVAb+ and HDVRNA-	20 (0.4%)	20 (12.7%)	0 (0%)	-
HDVAb+ and HDVRNA+	9 (0.2%)	9 (5.7%)	0 (0%)	
HDVAb- and HDVRNA+	4 (0.0%)	4 (2.5%)	0 (0%)	

Years of Follow-up, Median (IQR)	5.26 (7.90)	5.35 (8.02)	5.26 (7.90)	0.794
HDV Test Year, N (%)				
2000-2004	752 (15.6%)	47 (29.8%)	705 (15.1%)	
2005-2009	769 (16.0%)	19 (12.0%)	750 (16.1%)	
2010-2014	854 (17.7%)	21 (13.3%)	833 (17.9%)	***<0.001***
2015-2019	1,687 (35.0%)	59 (37.3%)	1,628 (34.9%)	
≥2020	755 (15.7%)	12 (7.6%)	743 (16.0%)	
Cirrhosis, N (%)	370 (7.7%)	20 (12.7%)	350 (7.5%)	***0.017***
Liver Transplantation, N (%)	440 (0.9%)	5 (3.2%)	39 (0.8%)	***0.002***
High AUDIT-C, N (%)	524 (10.9%)	14 (8.9%)	510 (11.0%)	0.408
Diabetes, N (%)	1,444 (30.0%)	32 (20.3%)	1,412 (30.3%)	***0.007***
Substance Use Disorder, N (%)	2,763 (57.4%)	120 (76.0%)	2,643 (56.7%)	***<0.001***

HIV Co-infection, N (%)	647 (13.4%)	22 (13.9%)	625 (13.4%)	0.854
HCV RNA Ever Positive, N (%)	598 (12.4%)	41 (26.0%)	557 (12.0%)	***<0.001***
Elevated ALT*, N (%)	1,965 (40.8%)	83 (52.5%)	1,882 (40.4%)	***0.002***
High Risk Laboratory Profile, N (%)**	409 (8.5%)	38 (24.1%)	371 (8.0%)	***<0.001***
HBeAg Ever Positive, N (%)	1,571 (32.6%)	40 (25.3%)	1,531 (32.9%)	***0.047***
HBV DNA Titer Group, N (%)				
<2000 IU/ml	2,205 (45.8%)	107 (67.7%)	2,098 (45.0%)	
2000-20000 IU/ml	691 (14.4%)	16 (10.1%)	675 (14.5%)	***<0.001***
>20000 IU/ml	1,431 (29.7%)	18 (11.4%)	1,413 (30.3%)	
Not Tested	490 (10.2%)	17 (10.8%)	473 (10.2%)	
ALT, Median (IQR)	42.0 (69.2)	58.2 (76.0)	41.5 (69.0)	***0.012***

AST, Median (IQR)	36.0 (56.0)	50.0 (63.0)	36.0 (56.5)	***<0.001***
GGT, Median (IQR)	49.0 (100.0)	83.5 (138.0)	48.0 (99.0)	***0.007***
Total Bilirubin, Median (IQR)	0.7 (0.6)	0.7 (0.5)	0.7 (0.6)	0.177
INR, Median (IQR)	1.1 (0.2)	1.1 (0.3)	1.1 (0.2)	***0.003***
PLT, Median (IQR)	197.0 (95.0)	165.0 (100.5)	198.0 (94.0)	***<0.001***
PLT				
<50,000	47 (1.0%)	4 (2.5%)	43 (0.9%)	
50,000-99,999	261 (5.4%)	15 (9.5%)	246 (5.3%)	
100,000-149,999	615 (12.8%)	34 (21.5%)	581 (12.5%)	***<0.001***
≥150,000	3,894 (80.8%)	105 (66.5%)	3,789 (81.3%)	
Fib-4, Median (IQR)	1.8 (2.6)	2.5 (3.7)	1.8 (2.5)	***0.001***

Abbreviations: AUDIT-C - Alcohol Use Disorders Identification Test-Concise; IQR - interquartile range; GGT – gamma-glutamyl transferase (Units/Liter). Elevated ALT* defined as ALT>=2*31 Upper limits. ** High Risk Laboratory Profile defined as ALT>=2*31 Upper limits and HBV DNA <2000 IU/ML. Other race included Native American / Alaska Native, Native Hawaiian, Pacific Islander, unknown, and two or more races. Statistical method/test applied: Kruskal-Wallis test for continuous variables. Pearson's test for categorical variables. Values in bold and italic denote statistical significance (p <0.05).

Table 2. Predictors of HDV Positive Status

	Univariable		Multivariable	
Parameters	aOR (95% CI)	*P Value*	aOR (95% CI)	*P Value*
Number of Patients=4,817 Number of HDV Positive=158				
Age at First HDV Testing	0.99 (0.98, 1.01)	0.310	1.00 (0.98, 1.01)	0.638
Sex				
Female	REF		REF	
Male	4.18 (1.03, 16.97)	***0.045***	3.30 (0.80, 13.50)	0.098
Race / Ethnicity		0.321		0.561
White	REF		REF	
Black	1.09 (0.74, 1.63)	0.658	1.26 (0.84, 1.89)	0.313
Hispanic	1.04 (0.63, 1.72)	0.872	1.20 (0.72, 2.00)	0.691
Asian	0.52 (0.24, 1.11)	0.090	0.75 (0.33, 1.68)	0.222
Other	1.23 (0.76, 2.00)	0.399	1.29 (0.79, 2.12)	0.333
Diabetes				
No	REF		REF	
Yes	0.58 (0.39, 0.87)	***0.007***	0.57 (0.38, 0.86)	***0.007***
Substance Use Disorder				
No	REF		REF	
Yes	2.41 (1.66, 3.49)	***<0.001***	2.02 (1.36, 2.99)	***<0.001***
AUDIT-C Score				
Low	REF		REF	
High	0.79 (0.45, 1.38)	0.409	0.67 (0.38, 1.19)	0.170
HIV Co-infection				
No	REF		REF	
Yes	1.04 (0.66, 1.65)	0.854	0.85 (0.53, 1.36)	0.498
HCV RNA Ever Positive				
No	REF		REF	

Yes	2.58 (1.79, 3.72)	***<0.001***	1.94 (1.32, 2.86)	***<0.001***
High Risk Laboratory Profile*				
No	REF		REF	
Yes	3.66 (2.50, 5.35)	***<0.001***	3.55 (2.40, 5.23)	***<0.001***

Abbreviations: AUDIT-C - Alcohol Use Disorders Identification Test-Concise; aOR – adjusted Odd Ratio; CI: Confidence interval.

Note: * Elevated ALT defined as ALT>=2*31 Upper limits. ** High Risk Laboratory Profile defined as ALT>=2*31 Upper limits and HBV DNA <2000 IU/ML. Other race included, Native American / Alaska Native, Native Hawaiian, Pacific Islander, unknown, and two or more races.

Statistical method/test applied: Logistic model. Values in bold denote statistical significance ($p<0.05$).

Table 3: Incidence Rates of Composite Liver Outcomes (HCC, Hepatic Decompensation, and Liver-related Death), HCC, Hepatic Decompensation, All-cause Mortality, and Liver-related Death at any Time During Follow Up, Stratified by HDV Status

	HDV Positive (N=158)	HDV Negative (N= 4,659)	IRR (95% CI) (HDV Positive versus HDV Negative)	P Value
Composite Liver Outcomes	32.92 (27.69, 38.86)	12.32 (11.72, 12.95)	2.67 (2.23, 3.18)	***<0.001***
HCC	18.65 (14.81, 23.18)	4.84 (4.47, 5.23)	3.86 (3.02, 4.87)	***<0.001***
Hepatic Decompensation	15.30 (11.86, 19.44)	5.50 (5.10, 5.92)	2.78 (2.13, 3.58)	***<0.001***
All-cause Mortality	58.86 (52.01, 66.36)	36.25 (35.23, 37.30)	1.62 (1.43, 1.83)	***<0.001***
Liver-related Death	15.65 (12.22, 19.74)	7.84 (7.37, 8.33)	2.00 (1.55, 2.54)	***<0.001***

Abbreviations: HCC=Hepatocellular Carcinoma; IRR= Incidence Rate Ratio; CI: Confidence interval.

Note: Incidence rates reported as cases per 1,000 person-years

Values in bold denote statistical significance ($p<0.05$).
Sample size for outcomes of composite liver outcomes (HCC, decompensation, and liver related death) includes 4,652 participants, HCC includes 4,751, hepatic decompensation includes 4,715, and all-cause mortality and liver-related death include 4,817.

Table 4: Multivariable Cox Model for Association of HDV and Composite Liver Outcomes and All-cause Mortality

	Composite Liver Outcomes	All-cause Mortality
Number of Patients	**4,652**	**4,817**
Number of Events Included in the Cox Model	**574**	**1,521**
Median time (in years) of follow-up (IQR)	**5.22 (8.02)**	**5.26 (7.90)**
Parameters	**aHR (95% CI)** ***P Value***	**aHR (95% CI)** ***P Value***
HDV Positive	2.63 (1.94, 3.56) ***<0.001***	1.52 (1.20, 1.93) ***<0.001***
Age at First HDV Testing	1.03 (1.02, 1.04)	1.06 (1.05, 1.06)
Sex (Ref: Female)	2.15 (1.06, 4.34)	2.04 (1.37, 3.03)
Race / Ethnicity (Ref: White)		
Global P-value	***<0.001***	***<0.001***
Black	0.63 (0.51, 0.79) ***<0.001***	0.82 (0.72, 0.93) ***0.002***
Hispanic	0.78 (0.59, 1.03) 0.082	0.72 (0.61, 0.86) ***<0.001***
Asian	0.84 (0.52, 1.34) 0.462	0.72 (0.52, 1.00) 0.053
Other	1.66 (1.33, 2.07) ***<0.001***	1.61 (1.40, 1.85) ***<0.001***
HDV Test Year (Ref: 2000-2004)		
Global P-value	***<0.001***	***0.010***
2005-2009	0.85 (0.68, 1.08) 0.179	0.90 (0.77, 1.04) 0.149
2010-2014	0.62 (0.47, 0.80) ***<0.001***	0.83 (0.70, 0.98) ***0.027***
2015-2019	0.44 (0.33, 0.59) ***<0.001***	0.74 (0.62, 0.89) ***0.001***
≥2020	0.28 (0.17, 0.48) ***<0.001***	0.62 (0.45, 0.87) ***0.005***
BMI (Ref: Normal (18.5-24.9))		
Global P-value	0.088	***<0.001***
Underweight (<18.5)	1.82 (1.05, 3.17) ***0.034***	2.31 (1.72, 3.11) ***<0.001***

Overweight (25-29.9)	0.97 (0.79, 1.19) 0.749	0.86 (0.76, 0.98) ***0.021***
Obese (>30)	0.89 (0.71, 1.11) 0.294	0.82 (0.72, 0.94) ***0.004***
Diabetes (Ref: No)	1.34 (1.11, 1.61) ***0.002***	1.35 (1.21, 1.51) ***<0.001***
Substance Use Disorder (Ref: No)	1.43 (1.18, 1.74) ***<0.001***	1.42 (1.26, 1.60) ***<0.001***
High AUDIT-C Score at Baseline (Ref: No)	0.96 (0.72, 1.29) 0.795	0.86 (0.72, 1.04) 0.124
Receipt of oral nucleoside/nucleotide analogues (Ref: No)	1.52 (1.24, 1.85) ***<0.001***	1.04 (0.92, 1.17) 0.569
HIV Co-infection (Ref: No)	0.78 (0.60, 1.01) 0.064	1.06 (0.90, 1.23) 0.492
HCV RNA Ever Positive (Ref: No)	1.64 (1.33, 2.03) ***<0.001***	1.36 (1.18, 1.57) ***<0.001***
HBV DNA Titer Group (Ref: <2000 IU/ml)		
Global P-value	0.132	***<0.001***
2000-20000 IU/ml	0.94 (0.70, 1.28) 0.707	0.95 (0.79, 1.16) 0.635
>20000 IU/ml	1.12 (0.91, 1.37) 0.294	1.25 (1.10, 1.43) ***0.001***
Not Tested	1.32 (1.02, 1.72) ***0.035***	1.72 (1.48, 2.00) ***<0.001***
Cirrhosis	4.23 (3.24, 5.51) ***<0.001***	2.16 (1.79, 2.60) ***<0.001***
Total Bilirubin	1.06 (1.04, 1.07) ***<0.001***	1.05 (1.04, 1.05) ***<0.001***
INR	1.00 (0.91, 1.10) 0.993	1.02 (0.99, 1.05) 0.249

Abbreviations: BMI - body mass index; AUDIT-C - Alcohol Use Disorders Identification Test-Concise; aHR – adjusted Hazard Ratio; CI: Confidence interval, Ref: Reference group.

Note: Other races included Native American / Alaska Native, Native Hawaiian, Pacific Islander, and two or more races.

Statistical method/test applied: Cox proportional-hazards model. Values in bold denote statistical significance ($p<0.05$)

Composite liver outcomes include HCC, decompensation, and liver-related death