Research Article
Viral Hepatitis

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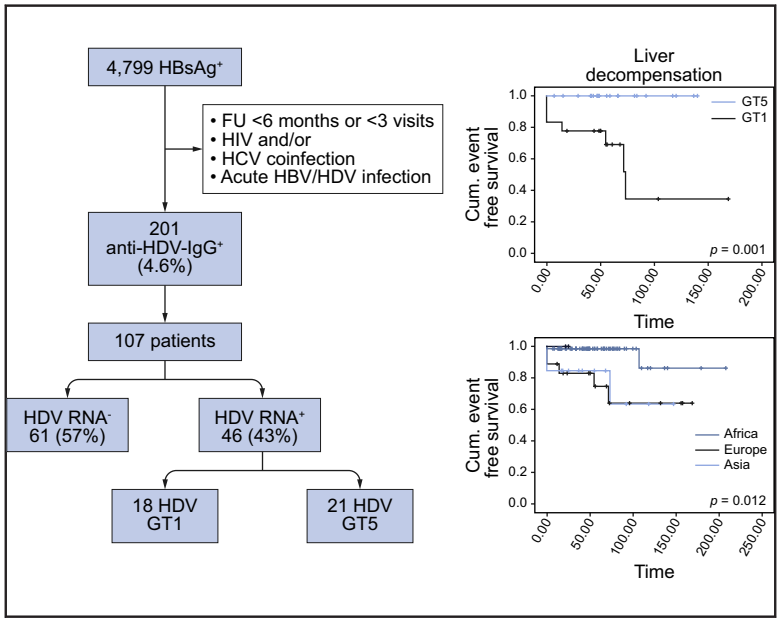
Hepatitis delta genotype 5 is associated with favourable disease outcome and better response to treatment compared to genotype 1

Graphical abstract



Highlights

- Patients of African origin who contract HDV less often have cirrhosis.
- Patients with HDV and detectable viral load have worse clinical outcomes.
- Patients with HDV genotype 5 less often develop hepatic decompensation.
- Patients with HDV genotype 5 seem to respond better to peg-IFN treatment.

Authors

Michelle Spaan, Ivana Carey, Matthew Bruce, …, Mary Horner, Geoff Dusheiko, Kosh Agarwal

Correspondence

m.spaan@erasmusmc.nl (M. Spaan).

Lay summary

Hepatitis delta is a virus that affects the liver. The virus is known to have different subtypes, called genotypes. With this research we discovered that hepatitis delta virus genotype 1 behaves differently than genotype 5 and causes faster development of liver disease. This is important for education of our patients and to determine how often we need to check our patients.

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Hepatitis delta genotype 5 is associated with favourable disease outcome and better response to treatment compared to genotype 1

Michelle Spaan[1,2,*], Ivana Carey[1], Matthew Bruce[1], Dazhuang Shang[1], Mary Horner[1], Geoff Dusheiko[1], Kosh Agarwal[1]

[1]*Institute of Liver Studies, King's College Hospital, London, United Kingdom;* [2]*Department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, the Netherlands*

Background & Aims: Coinfection with HDV causes rapid progression to liver cirrhosis and hepatic decompensation in patients with chronic hepatitis B. Factors that are associated with disease progression are poorly understood. In this study we aim to identify risk factors associated with disease progression and better characterise clinical differences and treatment response between HDV genotype 1 and 5.

Methods: In this retrospective study, all patients under our care between 2005 and 2016 with HBV/HDV coinfection (HBsAg+, anti-HDV antibodies positive) were analysed. Patients were excluded if follow-up was less than 6 months, if they had HCV and/or HIV coinfection or an acute HDV infection. Demographic data, stage of liver disease, development of liver complications and treatment response were recorded.

Results: One-hundred seven patients (mean age 36.0 years, 57% male) were followed for a median period of 4.4 years (range 0.6–28.1 years); 64% were of African origin and 17% were of European origin, with 28% of patients being cirrhotic at first visit; 43% patients had actively replicating HDV virus (anti-HDV-IgG+, anti-HDV-IgM+ or HDV RNA+) and 57% of patients were HDV exposed (anti-HDV-IgG+, HDV RNA-). Patients with actively replicating HDV more often developed liver complications than HDV-exposed patients ($p = 0.002$), but no differences in baseline characteristics were observed. Patients with HDV genotype 5 less often developed cirrhosis or hepatic decompensation compared to patients with HDV genotype 1. Twenty-four patients were treated with peg-IFN and post-treatment response was significantly better in patients infected with genotype 5 (10% GT1 *vs.* 64% GT5, $p = 0.013$).

Conclusion: Patients infected with HDV genotype 5 appear to have a better prognosis with fewer episodes of hepatic decompensation and better response to peg-IFN treatment than patients infected with HDV genotype 1.

Lay summary: Hepatitis delta is a virus that affects the liver. The virus is known to have different subtypes, called genotypes. With this research we discovered that hepatitis delta virus genotype 1 behaves differently than genotype 5 and causes faster development of liver disease. This is important for education of our patients and to determine how often we need to check our patients.

Introduction

Hepatitis delta virus (or HDV) is a single stranded RNA virus that infects around 15–20 million people worldwide.[1] It is underdiagnosed and is the severest form of viral hepatitis with no effective treatment currently available. The virus is dependent on HBsAg for packaging and propagation of its virions, although HDV replication independent of HBV has been shown in liver transplant patients.[2] HDV often leads to the accelerated progression to advanced hepatic fibrosis, increased risk of hepatocellular carcinoma and rapid decompensation.[3,4] The prevalence varies greatly between regions, with rates from 0% to 40% in HBsAg-positive patients. HDV is known to be endemic in Mediterranean countries, the Middle East, parts of Brazil, Mongolia and central Africa.[1,5] Due to the implementation of HBV vaccination programs, the incidence of HDV has significantly decreased in Europe. However, due to increased migration of people from highly endemic areas, this decline has recently reversed.[6–8] It is well known that patients with actively replicating delta *i.e.* those with detectable HDV RNA, have more severe liver disease than those who are anti-HDV-IgG alone. To diagnose an actively replicating HDV infection in places where HDV RNA testing is not available, anti-HDV-IgM can be used.[9,10] Little is known about the factors influencing spontaneous clearance of HDV RNA. Besides host factors, virus genotypic variability might be involved. Eight different genotypes were identified with 20–40% sequence divergence. Genotype 1 is the most prevalent and found worldwide. And while genotype 1 is the most prevalent in Africa, genotypes 5-8 are found exclusively in African patients and those who migrated to Europe.[11–13] A small number of studies have reported differences in clinical outcome between hepatitis delta genotypes.[14–17] The only available treatment option for HDV is pegylated interferon-alfa (peg-IFN), with only 15%–35% of patients achieving a sustained virologic response (SVR).[18–21] Baseline factors that predict clinical outcomes are poorly defined.

In this study we aim to better characterise disease progression and treatment response in a mixed cohort of patients of predominantly European and African origin, as well as to identify factors that predict disease progression and treatment response.

Keywords: Hepatitis B virus; HBsAg; Anti-HDV-IgG; HDV RNA; Liver decompensation.
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* Corresponding author. Address: Erasmus MC, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands; Tel.: +31-641414312.
E-mail address: m.spaan@erasmusmc.nl (M. Spaan).
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Patients and methods

Patient population

Between January 2005 and December 2016, all 4,977 HBsAg-positive patients attending the outpatient clinic in King's College Hospital, London were routinely screened for anti-HDV-IgG. Two-hundred and one patients (4.6%) were found to be positive and were further analysed for our study (Fig. 1). The following groups were excluded: 14 HIV coinfected patients, 4 patients with HCV coinfection (HCV RNA+) and 76 patients with follow-up for less than 6 months or less than 3 consecutive visits. In addition, 4 patients with HDV superinfection at diagnosis were excluded from the study. From 107 patients, clinical characteristics and liver-related endpoints were recorded. Cirrhosis was defined by liver biopsy (ISHAK score ≥F5), transient elastography (>12.5kPa on Fibroscan) or if patients had signs of cirrhosis via radiological criteria. This observational single centre study was conducted following the ethical principles of the Declaration of Helsinki and had ethical approval.

Virological testing

Serological markers of HBV, HCV and HIV were tested by commercial Chemiluminescent microparticle immunoassays (CMIAs) on Abbott ARCHITECT i2000 SR (Abbott Laboratories, North Chicago, IL). Anti-HDV-IgG, anti-HDV-IgM were tested using ETI-DELTA-IGMK-2 and ETI-AB-DELTAK-2 (Diasorin S.p.A 13040 Saluggia (vc), Italy). HDV RNA and HDV genotype were tested using an in-house quantitative HDV RNA assay with a lower limit of quantification of 640 IU/ml,[22] direct sequencing using ABI 3130×l genetic analyser (Life Technologies, Carslbad, CA) and phylogenetic tree analysis using neighbour-joining (NJ) distance analyses software (njplot, v 2.0). HBV DNA was tested using the Roche Cobas AmpliPrep/Cobas TaqMan assay with a lower limit of quantification of 20 IU/ml. HBV genotypes were determined by in-house nucleic acid amplification and direct sequencing using an ABI 3130×l genetic analyser (Life Technologies, Carslbad, CA). Since HDV RNA levels are known to fluctuate over time, patients were classified as having actively replicating HDV infection if HDV RNA was detected once during follow-up and/or anti-HDV-IgM was positive. For missing data, stored samples were retested using the assays above. Patients were classified as HDV exposed if HDV RNA was undetectable during follow-up for at least 3 repeated measured and anti-HDV-IgM was negative.

Statistical analyses

For normally distributed variables, unpaired *t* test was used for unpaired data. Paired continuous variables that were not normally distributed were assessed by Wilcoxon's rank-sum test and unpaired variables by the Mann-Whitney *U* test. Categorical data were compared using Fisher's exact test. Survival was calculated using Kaplan-Meier's method and compared using log-rank test. Spearman rank correlation test was used to calculate a correlation between 2 non-parametric values. Hazard ratios (HRs) were calculated using Cox proportional Hazard model. Statistical significance was considered at a p <0.05 level. All analyses were performed using SAS software (v9.4; SAS institute, Inc., Cary, NC.)

Results

Baseline demographics

A total of 107 patients were included in our study (mean age 36.0 years [range 16.5–61.7 years]). Patients' clinical and demographic characteristics are shown in Table 1. Interestingly, in contrast to other studies from Europe,[23–25] that mainly reported on patients born in Eastern Europe and Central Asia, our cohort consists of a large population born in (West or sub-Saharan) Africa (64.5%). There were 2 different patterns of HBV and HDV genotype distribution in our cohort. There was a strong correlation between HDV and HBV genotype (p <0.001) and origin (p <0.001); patients with HBV genotype D were mostly infected with HDV genotype 1 (10/10, 100%) in contrast to patients with HBV genotype E who were all infected with HDV genotype 5 (13/13, 100%). Forty-three (40.4%) patients had detectable HDV RNA levels during their follow-up and 33 (30.6%) patients had positive anti-HDV-IgM. All patients with detectable HDV RNA and/or anti-HDV-IgM were classified as having actively replicating HDV infection. As expected, patients who were only HDV exposed (undetectable HDV RNA and negative anti-HDV-IgM) had significantly less cirrhosis (p <0.001), and appeared to have less advanced liver disease with lower aspartate aminotransferase (p <0.001), alanine aminotransferase (ALT; p <0.001), bilirubin (p = 0.037), international normalized ratio (p = 0.008) and higher albumin (p = 0.042) compared to patients with actively replicating HDV (Table 1). In addition, 78% of HDV-exposed patients had normal ALT (<45 IU/ml) upon presentation. The baseline event-anticipation (BEA) score, designed to calculate the risk of developing a liver-related complication in 5 years, was calculated for all groups (Table 1). The presence of cirrhosis at first visit was significantly different between patients of African (n = 12, 17.4%), Asian (n = 5, 38.5%) and European origin (n = 9, 50%, p = 0.023). However, no differences were observed for age, time of follow-up, gender or HBV genotype between HDV-exposed patients and patients who had actively replicating HDV (Table 1). On the first visit, 11 patients (10%) were on nucleos(t)ide analog (NUC) treatment: 3 patients on tenofovir, 5 on entecavir, 1 on a combination of lamivudine and adefovir and 2 on peg-IFN/tenofovir. On the last visit 42 patients (39%) were on NUC treatment.

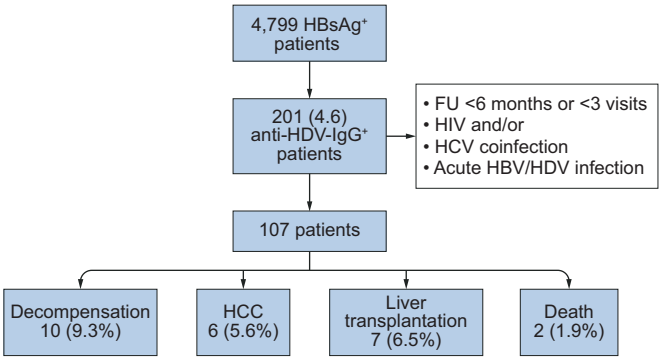

Fig. 1. Demographic characteristics and exclusion criteria of the patients at entry and development of liver-related endpoints along follow-up.

Disease progression

All patients with anti-HDV-IgG were followed for a median period of 4.4 years (range 0.6–28.1 years). Among the 77 patients that did not have cirrhosis at baseline, 2 patients progressed to cirrhosis. At baseline, 2 patients were already decompensated and 8 patients experienced at least 1 episode of liver decompensation during follow-up (Table S1). Liver decompensation throughout this study is defined as Child-Pugh >B7 or the

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Table 1. Baseline characteristics between HDV-exposed patients and those with actively replicating HDV.

	All patients	Exposed HDV undetectable HDV RNA	Actively replicating HDV detectable HDV RNA and/or anti-HDV-IgM	*p* value*
Number	107	61	46	
Age years, mean ± SD (range)	36.0 ± 10.4 (16.5–61.7)	36.6 ± 4.7 (17.3–61.7)	35.1 ± 10.4 (16.5–59.8)	0.493
Gender, male	57 (53.3)	30 (49.2)	27 (58.7)	0.218
Follow-up, years	4.4 (0.6–28.1)	2.0 (0.7–28.1)	4.1 (0.6–14.1)	0.485
Origin	18 Europe 13 Asia 69 Africa 2 N. America	8 Europe 7 Asia 43 Africa 1 N. America	10 Europe 6 Asia 26 Africa 1 N. America	0.567
HBV genotype	7 A 2 C 22 D 53 E	4 A 1 C 12 D 36 E	3 A 1 C 10 D 17 E	0.688
HBeAg positive	10 (9.4)	6 (9.8)	4 (8.9)	0.573
HBsAg level IU/ml	6.4×10^3 ($0.03–1.1\times10^5$)	6.8×10^3 ($0.03–2.7\times10^4$)	6.4×10^3 ($544–1.1\times10^5$)	0.695
HBV DNA IU/ml	50.1 ($0–1.8\times10^8$)	91.1 ($0–1.7\times10^8$)	32 ($0–1.8\times10^8$)	0.138
HDV genotype	18 GT1 21 GT5	61 unknown	18 GT1 21 GT5	
HDV RNA IU/ml	0 ($0–8.7\times10^8$)	0 (0–0)	4.2E4 ($0–8.7\times10^6$)	<0.001
Detectable HDV RNA	43 (40.2)	0	43 (93.4)	<0.001
Detectable anti-HDV-IgM	33 (31.4)	0	33 (73.3)	<0.001
Cirrhosis	30 (28)	7 (11.5)	23 (50)	<0.001
ALT IU/L	40.0 (2–573)	25.0 (2–185)	66.0 (14–573)	<0.001
AST IU/L	36.0 (13–372)	29.0 (13–342)	58.0 (24–372)	<0.001
Platelets × 10^9/L	192.0 (28–372)	197.0 (95–372)	171.5 (28–332)	0.045
Bilirubin μmol/L	10.0 (3–257)	9.0 (3–24)	11.0 (3–257)	0.037
Albumin mmol/L	44.0 (21–83)	44.0 (27–49)	43.0 (21–83)	0.042
INR	1.1 (0.9–2.7)	1.0 (0.9–1.4)	1.1 (0.9–2.7)	0.008
BEA scores A-B-C	71 A 31 B 3 C	45 A 14 B 2 C	26 A 17 B 1 C	0.057

Data presented as median (range), or n (%) unless stated otherwise.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline event-anticipation; GT, genotype; INR, international normalised ratio.
*Comparison made between patient groups with exposed and actively replicating HDV infection. For normally distributed variables (age), unpaired *t* test was used. For data that were not normally distributed, Mann-Whitney *U* test was used. Categorical data were compared using Fisher's exact test.

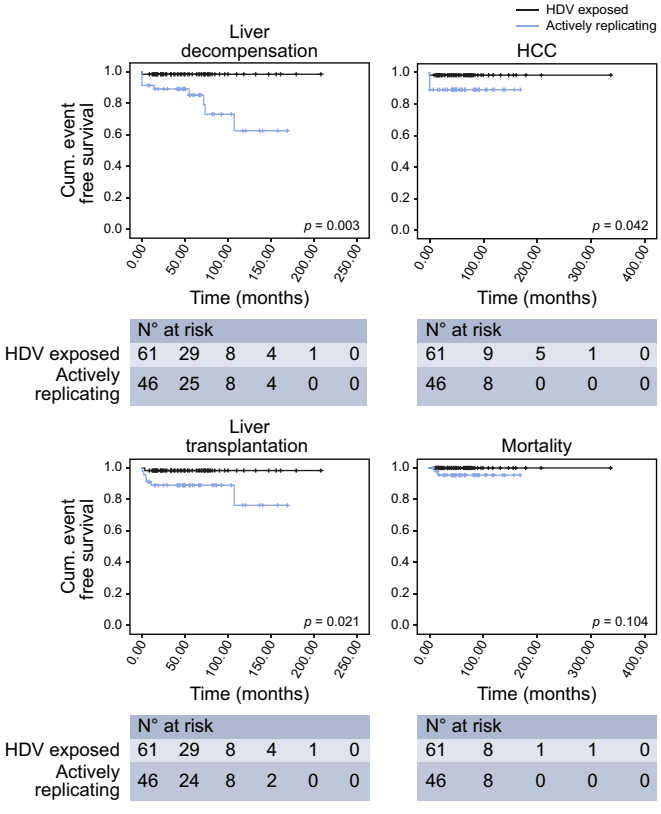

Fig. 2. Cumulative event-free survival in patients who are HDV exposed or have actively replicating HDV. Survival was calculated using Kaplan-Meier's method and compared using log-rank test. HCC, hepatocellular carcinoma.

presence of ascites or a variceal bleed. Nine patients developed ascites and 4 patients had a variceal bleed. Six patients developed hepatocellular carcinoma (HCC), all diagnosed within 3 months of the first visit. Seven patients required a liver transplant and 2 patients died of a liver-related event (Fig. 1, Table S1). Time to development of a liver-related event was strongly associated with the presence of cirrhosis (p <0.001), but not with HBeAg status (p = 0.443), HBV genotype (p = 0.157), having detectable HBV DNA (p = 0.459, all Kaplan Meier survival analysis) or age (r = −0.17; p = 0.079, Spearman correlation test).

Comparison between patients with actively replicating HDV or exposure to HDV

Patients with actively replicating HDV developed significantly more episodes of decompensation (p = 0.002), ascites (p = 0.005) variceal bleeding (p = 0.032) and more often received a liver transplant (p = 0.043) compared to HDV-exposed patients during follow-up (Table S1). Survival analysis showed that HDV-exposed patients had better liver event-free survival compared to patients with actively replicating HDV (Fig. 2). Because of the low event score, HRs were only calculated for composite clinical events (decompensation, HCC, liver transplantation and mortality). The HR for exposed *vs.* active HDV was 7.29 (95% CI 2.43–21.87; p = 0.0024). In line with the lower presence of cirrhosis, patients of African origin less frequently developed decompensation compared to patients of European or Asian origin in survival analyses (Fig. S1). No differences in baseline factors such as platelet count, alkaline phosphatase levels, fibroscan results, fibrosis-4 (FIB-4) or albumin-bilirubin (ALBI) scores were observed between African *vs.* non-African patients (results not shown).

Table 2. Patient characteristics of patients with HDV genotype 1 and 5.

	HDV genotype 1	HDV genotype 5	*p* value
Number	18	21	
Age, years	36.9 (16.5–59.8)	33.3 (17.4–43.6)	0.215
Gender, male	11 (61.1)	13 (61.9)	1.000
Follow-up, years	4.7 (1.2–14.1)	4.7 (0.6–11.7)	0.955
Origin	9 Europe 5 Asia 2 Africa 2 unknown	0 Europe 0 Asia 21 Africa	<0.001
HBV genotype	1 A 10 D 0 E 7 unknown	1 A 0 D 13 E 7 unknown	<0.001
HBeAg positive	1 (5.6)	3 (14.3)	0.609
HBsAg level IU/ml	3.9×10^3 ($544–2.9\times10^4$)	7.0×10^3 ($1.1\times10^3–1.1\times10^5$)	0.432
HBV DNA IU/ml	10.25 ($0–4.4\times10^6$)	36.5 ($0–1.8\times10^8$)	0.184
HDV RNA IU/ml	6.9×10^4 ($0–8.3\times10^5$)	2.7×10^5 ($944–8.7\times10^6$)	0.477
Detectable HDV RNA, %	100	100	1.000
Detectable anti-HDV-IgM	17 (94.4)	13 (61.9)	0.023
Cirrhosis	12 (66.7)	6 (28.6)	0.026
ALT IU/L	66.0 (39–126)	90.5 (32–573)	0.338
AST IU/L	61.0 (34–175)	55 (28–513)	0.693
Platelets *10^9/L	163.0 (48–332)	201.0 (28–321)	0.115
Bilirubin μmol/L	12.0 (5–63)	11.0 (3–33)	0.224
Albumin mmol/L	42.5 (21–50)	43.0 (25–83)	0.524
INR	1.1 (0.9–1.6)	1.1 (0.9–1.4)	0.489
FIB-4 scores	1.11 (0.3–7.4)	1.29 (0.4–6.8)	0.942
ALBI score	−2.97 (−3.3–0.6)	−2.97 (−6.4–1.8)	0.464
BEA scores A-B-C	8 A 8 B 1 C	15 A 6 B 0 C	0.299

Data presented as median (range), or n (%) unless stated otherwise.
ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline event-anticipation; FIB-4, fibrosis-4; INR, international normalised ratio.
*For normally distributed variables (age), unpaired T test was used. For data that were not normally distributed, Mann-Whitney *U* test was used. Categorical data were compared using Fisher's exact test.

HDV genotype 5 is associated with favourable disease progression compared to HDV genotype 1

Of 43 patients with actively replicating HDV, 39 patients had samples available for genotyping; 21 patients were found to have genotype 1 and 18 patients were infected with genotype 5 (Table 2). As expected, most patients with genotype 5 were of African origin (100%) and had HBV genotype E (93%) whereas patients with genotype 1 were predominantly of European origin (56%) and were infected with HBV genotype D (91%). Age, gender and median follow-up time were similar for both groups. Levels of HBV DNA were low and comparable in both groups and no differences were observed in levels of HDV RNA and HBsAg. The ALBI and the FIB-4 score were calculated and showed no differences. Interestingly, although patients of both genotypes had comparable liver function tests, the presence of liver cirrhosis at the time of diagnosis was more prevalent in patients with genotype 1 infection ($p = 0.026$, Table 2). At first visit, patients with HDV genotype 1 tended to have higher BEA-scores, but this did not reach statistical significance. During follow-up, patients with HDV genotype 1 were more likely to develop an episode of hepatic decompensation ($p = 0.001$), and to receive a liver transplant ($p = 0.025$, Fig. 3, Table S2). HR was calculated for composite clinical events (decompensation, HCC, liver transplantation and mortality). HR for genotype 5 *vs.* genotype 1 was 5.40 (95% CI 1.65–17.70) with a *p* value of 0.015. Focussing only on patients with cirrhosis, none of the 6 patients with genotype 5 developed hepatic decompensation during follow-up, while 7 out of 12 (58%) patients with genotype 1 developed liver decompensation ($p = 0.038$).

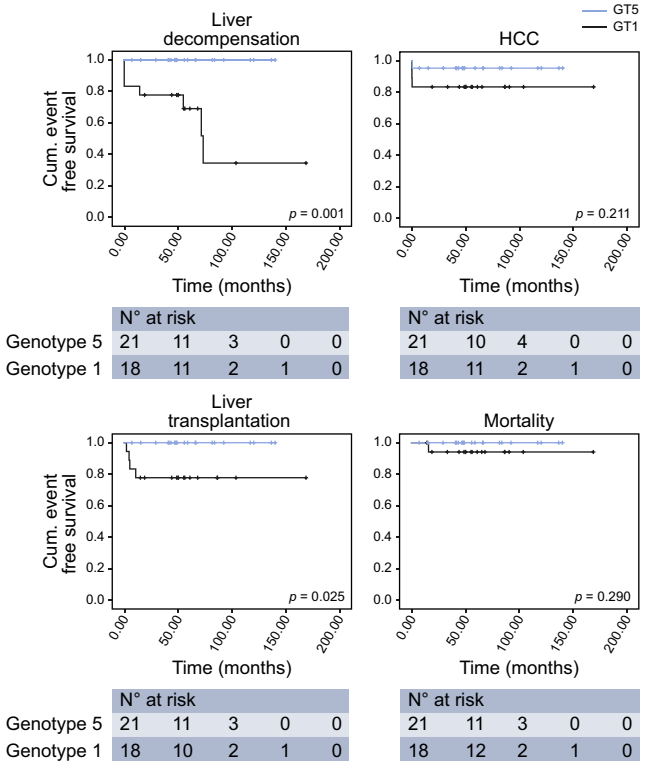

Fig. 3. Cumulative event free survival in patients who have HDV genotype 1 or HDV genotype 5. Survival was calculated using Kaplan-Meier's method and compared using log-rank test.

Table 3. Patients characteristics of patients that underwent peg-interferon treatment.

	All patients	Non-response	Response	*p* value*
Number	25	14	11	0.695
Age years	33.9 (17.8-52.3)	30.0 (17.8-50.1)	34.5 (22.1-52.3)	0.200
Gender, male	12 (48)	6 (43)	6 (55)	0.695
Cirrhosis	12 (48)	8 (57)	4 (36)	0.428
HBV genotype	2 A 5 D 11 E	0 A 5 D 4 E	2 A 0 D 7 E	0.032
HDV genotype	10 GT 1 14 GT 5	9 GT 1 5 GT 5	1 GT 1 9 GT 5	0.013
HIV	3 (12)	2 (14)	1 (9)	1
Origin	7 Europe 17 Africa	6 Europe 7 Africa	1 Europe 10 Africa	0.078
IFN-treatment weeks	48.0 (9-80)	48.0 (18-80)	48.0 (9-53)	0.267
Follow-up after treatment months	51.2 (6.4-116.5)	49.8 (25.8-116.5)	51.3 (6.4-112.4)	0.936
HDV RNA IU/ml	3.5×10^5 ($0\text{-}1.1\times10^8$)	5.9×10^5 ($2.9\times10^4\text{-}1.1\times10^8$)	1.8×10^4 ($0\text{-}2.1\times10^6$)	0.002
HBV DNA level IU/ml	41.8 ($0\text{-}1.9\times10^6$)	53.8 ($0\text{-}1.8\times10^5$)	0 ($0\text{-}1.9\times10^6$)	0.893
HBsAg level IU/ml	8.3×10^3 ($1.6\times10^3\text{-}4.6\times10^4$)	8.7×10^3 ($2.6\times10^3\text{-}4.6\times10^4$)	6.8×10^3 ($1.6\times10^3\text{-}2.0\times10^4$)	0.422
Detectable anti-HDV-IgM	16 (64)	10 (71)	6 (54.5)	0.673

Data presented as median (range), or n (%) unless stated otherwise.
GT, genotype; peg-IFN, pegylated interferon.
*Comparison made between patient groups with response and non-response to Peg-IFN therapy. For normally distributed variables (age), unpaired *t* test was used. For data that were not normally distributed, Mann-Whitney *U* test was used. Categorical data were compared using Fisher's exact test.

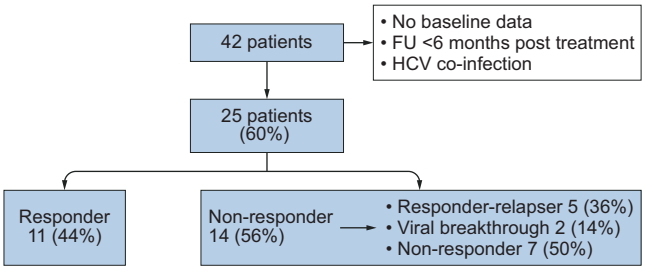

Fig. 4. Demographic characteristics and exclusion criteria of the patients who were treated with peg-IFN and their response to treatment. peg-IFN, pegylated interferon.

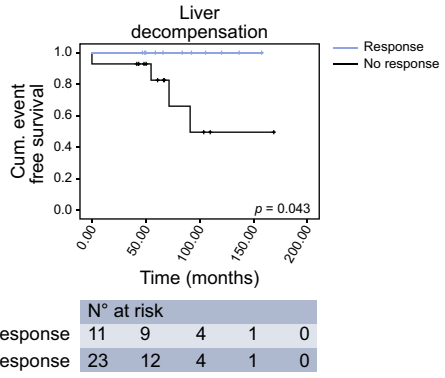

Fig. 5. Cumulative event free survival of any episode of liver decompensation in patients with or without a response to peg-IFN treatment. Survival was calculated using Kaplan-Meier's method and compared using log-rank test. peg-IFN, pegylated interferon.

Antiviral therapy

From our cohort of 201 patients with positive anti-HDV-IgG, 42 patients were treated with peg-IFN. Patients were excluded for this analysis if baseline data were not available (n = 15), if follow-up was less than 6 months post treatment (n = 1) or if patients were coinfected with HCV (n = 1). Twenty-five patients were included with a median age of 33.9 years (range 17.8–52.3 years). Patient characteristics at the start of antiviral therapy are shown in Table 3. None of the patients received peg-IFN therapy in the past. Median HDV RNA at the start of therapy was 10^5, comparable to levels at first visit. There was 1 patient with an exceptionally high HDV RNA level of 10^8 who eventually had a non-response. Ten (40%) patients were infected with HDV genotype 1 *vs.* 14 (56%) with genotype 5. We were not able to determine genotype in 1 patient. From 25 treated patients, 16 patients were HDV RNA negative at the end of therapy: 11 patients maintained HDV RNA status more than 6 months after completing therapy but 14 patients had a non-response; 5 patients relapsed – 4 within 6 months after stopping therapy and 1 patient relapsed after 22 months; 2 had a viral breakthrough on therapy and 7 patients were total non-responders (Fig. 4). HBsAg levels decreased during peg-IFN treatment but this was not statistically significant (Table 3) and no seroconversions were observed.

Baseline characteristics of both responders and non-responders did not differ by age, gender or presence of cirrhosis. Differences were found for HBV genotype (p = 0.032) and baseline HDV RNA level (p = 0.002, Table 3). Patients from Africa tended to respond better to peg-IFN, but this did not reach statistical significance (p = 0.078). Duration of peg-IFN treatment was significantly shorter in patients with genotype 5 compared to genotype 1 (median duration was 48 weeks for both genotypes, but mean duration was 38 weeks *vs.* 54 weeks, p = 0.007). Treatment was discontinued for various reasons including pregnancy, poor adherence and side-effects. Strikingly, although treatment duration was shorter in patients with HDV genotype 5, 6-month response rates after stopping therapy were higher in comparison to patients with genotype 1 infection (64% genotype 5 *vs.* 10% genotype 1, p = 0.013, Table 3). Survival analysis showed that treatment response was associated with improved clinical outcome at follow-up (p = 0.043, Fig. 5).

Discussion

This study evaluated the clinical outcomes of a diverse patient population with positive anti-HDV-IgG antibodies in the United Kingdom. The proportion of HBsAg-positive patients with positive anti-HDV total antibodies was 4.3%. Surprisingly, only 43% of

anti-HDV total positive patients had detectable HDV RNA. The presence of HDV RNA in serum is required to diagnose actively replicating HDV infection and is an important factor determining rapid development of cirrhosis and poor clinical outcome.[16,24] One explanation might be a relatively low sensitivity of the assay used and the possibility that low levels of HDV RNA were not detected. However, all patients with undetectable HDV RNA were tested on several occasions repeatedly and were also anti-HDV IgM negative. Patients in our cohort with undetectable HDV RNA and anti-HDV-IgM had stable disease and normal or mildly elevated liver enzymes, indicating they do not have actively replicating delta infection. Interestingly, none of the patients with undetectable HDV RNA had received peg-IFN previously and it is therefore likely that these patients spontaneously cleared their HDV virus after acute coinfection or superinfection. The proportion of patients with spontaneous HDV clearance may even be underestimated since patients who may have cleared HBsAg are not included in this cohort. To get more insight into mechanisms involved in clearance of HDV, we compared baseline characteristics of patients with actively replicating and exposed HDV, but no differences were observed. It is suspected that other factors might contribute to spontaneous viral clearance, namely mode of transmission, age at time of contraction and mode of infection (super *vs.* coinfection) and it would be of interest to focus future studies on these aspects. One explanation for the low proportion of patients with HDV RNA viremia is the relative high number of patients of African origin. Epidemiological studies from Africa have demonstrated that 35–62% of patients have detectable HDV RNA in serum,[5,26–28] which appears lower than studies from Europe and Asia that have shown that 70–93% of patients with anti-HDV-IgG have detectable HDV RNA levels.[16,25,29,30]

The ability to clear hepatitis delta is likely to be influenced by host and viral factors. A small number of studies have reported differences in clinical outcome between hepatitis delta genotypes. Independent of HBV genotype, infection with genotype 1 delta seems to be more damaging than genotype 2.[15,16] Genotype 3 has been associated with acute liver damage in an area of the Amazon[14] and genotype 4 seems to behave differently in various regions.[15,17] To our knowledge, this is the first study to fully characterise patients infected with HDV genotype 5 and compare its disease progression with patients infected with genotype 1. In this study we provide evidence that African patients have a milder course of disease compared to non-African HDV patients on 3 levels. i) African patients less often present with cirrhosis at first visit compared to non-African patients; ii) African patients less often developed the clinical hard endpoints such as decompensation compared to non-African patients and iii) Resolved HDV occurred more frequently in African patients.

One of the mechanisms that could clarify different outcomes between patients and HDV genotypes is variability in viral replication and virion assembly efficacy leading to a lower rate of HDV virion secretion and therefore slower infection of hepatocytes.[16,31,32] Host factors like race and single-nucleotide polymorphisms causing differences in entry receptors might also play a role.

Our cross-sectional, single-centre study has limitations as it focuses only on a small number of patients and is retrospective. However, this is a hepatitis virus that is poorly understood with little data on genotypic heterogeneity. It appears that patients of African origin are more likely to be exposed to HDV than have actively replicating HDV. To better understand whether this difference is due to the variation in HDV genotype, interactions between specific HBV and HDV genotypes or to the absence of HDV RNA in circulation in the exposed patients, more prospective, multicentre studies are needed. In addition, we were not able to separate the effect of HBV-related damage from HDV as high concordance between HDV and HBV genotypes was demonstrated.

In our patients with actively replicating HDV infection, 23 patients (50%) were cirrhotic at first presentation to our centre. Of the cirrhotic patients, 9 patients (39%) decompensated during a mean follow-up of 4.8 years, with an incidence rate of 8.0 per 100 person-years. The overall incidence rate of decompensation episodes was lower than in other studies.[24,25,29] Between these studies, patient characteristics varied greatly but it is possible that the low incidence of decompensation in our cohort reflects the high number of patients with genotype 5 who had a lower rate of decompensation than patients with HDV genotype 1.

Peg-IFN treatment has poor tolerability and factors predicting the outcome of peg-IFN are not well understood. Some studies have shown that neither cirrhosis nor liver biochemical tests at baseline affect the response to treatment,[18,33,34] while others appear to suggest that patients with cirrhosis or advanced disease respond less well.[21,35] Several studies have reported that after 6 months of therapy, a negative HDV-RNA was predictive of sustained response.[18,36] However, late-relapse often occurs and negative HDV-RNA 6 months post-treatment does not seem to predict response or prevent relapse.[34] In our cohort, 25 patients were treated with peg-IFN therapy for a median period of 48 weeks. Eleven patients (44%) had a treatment response, which is similar to the result of a recent large European trial.[20] Treatment response has been defined as undetectable HDV RNA at least 6 months post-treatment; however this does not seem to be a reliable end-point and late relapse occurs frequently;[34] only loss of HBsAg could be classed as sustained virological response in delta patients. Indeed, although most patients relapsed within 6 months post-treatment, 1 patient had a relapse 22 months post-treatment. Treatment responses are likely to be accurate in our cohort as there is long-term follow up. Of note, the patient with a relapse 22 months post-treatment had positive anti-HDV-IgM during and after treatment, which might suggest an ongoing antibody response to small amounts of virus that are still present in concentrations below the detection limit of our HDV RNA quantitative assay. In future studies anti-HDV-IgM might help to define treatment response, although in a previous study, anti-HDV IgM did not show a correlation with level of HDV replication but did show a correlation with disease activity.[10] We show that treatment response to peg-IFN is associated with lower disease progression, which is in line with results from Wranke *et al.*[30] Importantly, patients with genotype 5 appeared to respond better to peg-IFN treatment than patients with HDV genotype 1, while treatment duration was slightly shorter. This was not statistically shown in patients of African origin, most likely due to the presence of both genotype 1 and 5 in this patient population. Because of small numbers and retrospective design, prospective randomized clinical trials including patients with HDV genotype 5 are required to confirm this clinical observation.

In summary, this study demonstrates that disease progression and clinical outcomes are associated with HDV genotypic heterogeneity. We demonstrate that patients with HDV genotype 5,

predominantly of African origin, have a favourable disease outcome compared to patients with genotype 1 and appear to have a better treatment response to peg-IFN. Identifying the risk factors for decompensation is important for patient education, clinical management and to delineate patients who need meticulous follow-up.

Abbreviations

ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline event-anticipation; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; HRs, hazard ratios; INR, international normalised ratio; NJ, neighbour-joining; NUCs, nucleos(t)ide analogs; peg-IFN, pegylated interferon.

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Conflicts of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Michelle Spaan: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, statistical analysis. Ivana Carey: study concept and design, analysis and interpretation of data, critical revision of the manuscript. Matthew Bruce: acquisition of data, technical support. Dazhuang Shang: acquisition of data, technical support. Mary Horner: acquisition of data, technical support. Geoff Dusheiko: critical revision of the manuscript. Kosh Agarwal: study supervision, critical revision of the manuscript.

Supplementary data

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Author names in bold designate shared co-first authorship

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