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Hepatitis delta virus testing, epidemiology and management: A multicentre cross-sectional study of patients in London

Kate El Bouzidi[a,*], Wael Elamin[b], Katharina Kranzer[a], Dianne N. Irish[c], Bridget Ferns[a], Patrick Kennedy[d], William Rosenberg[e], Geoffrey Dusheiko[e], Caroline A. Sabin[f], Belinda C. Smith[g], Eleni Nastouli[a,**]

[a] University College London Hospitals NHS Foundation Trust, Department of Clinical Microbiology and Virology, 2nd Floor, 307 Euston Road, London NW1 3AD, UK
[b] Barts Health NHS Trust, Division of Infection, Pharmacy and Pathology Building, 80 Newark Street, London E1 2ES, UK
[c] Royal Free London NHS Foundation Trust, Department of Virology, Royal Free Hospital, Pond Street, London NW3 2QG, UK
[d] Barts Health NHS Trust, Department of Hepatology, The Royal London Hospital, Whitechapel Road, London E1 1BB, UK
[e] UCL Institute for Liver and Digestive Health, Royal Free Hospital, Pond Street, London NW3 2QG, UK
[f] UCL Research Department of Infection and Population Health Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK
[g] Imperial College Healthcare NHS Trust, Hepatology Research Unit, St. Mary's Hospital, Praed Street, London W2 1NY, UK

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ABSTRACT

Background: Hepatitis delta virus (HDV) testing is recommended for all patients with hepatitis B virus (HBV) infection. HDV infection is associated with severe liver disease and interferon is the only available treatment.
Objectives: To determine the rate of anti-HDV antibody testing in HBV patients; and to describe the epidemiology, clinical characteristics and management of HDV-infected patients at four hospitals in London.
Study design: The anti-HDV testing rate was estimated by reviewing clinical and laboratory data. Cross-sectional data collection identified HDV-infected patients who had attended the study centres between 2005 and 2012.
Results: At a centre with clinic-led anti-HDV testing, 40% (67/168) of HBV patients were tested. Recently diagnosed HBV patients were more likely to be screened than those under long-term follow-up (62% vs 36%, $P = 0.01$). At a centre with reflex laboratory testing, 99.4% (3543/3563) of first hepatitis B surface antigen positive samples were tested for anti-HDV. Across the four study centres there were 55 HDV-infected patients, of whom 50 (91%) had immigrated to the UK and 27 (49%) had evidence of cirrhosis. 31 patients received interferon therapy for HDV with an end of treatment virological response observed in 10 (32%).
Conclusions: The anti-HDV testing rate was low in a centre with clinic-led testing, but could not be evaluated in all centres. The HDV-infected patients were of diverse ethnicity, with extensive histological evidence of liver disease and poor therapeutic responses. Future recommendations include reflex laboratory testing algorithms and a prospective cohort study to optimise the investigation and management of these patients.

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1. Background

The worldwide prevalence of hepatitis delta virus (HDV) infection is estimated to be 5% of HBV-infected individuals. There are endemic areas in Eastern and Southern Europe, Central and Eastern Africa, the Amazon Basin, parts of Asia and the Middle East [1]. HDV infection was previously thought to be rare in Northern Europe and concentrated in high risk groups such as injection drug users. However, a study by Cross et al. in a South London liver unit

* Corresponding author at: Virology Academic Clinical Fellow, University College London. Tel.: +44 20344 79912; fax: +44 20344 79211.
** Corresponding author at: Virology Consultant, University College London Hospitals NHS Trust. Tel.: +44 20344 79912; fax: +44 20344 79211.
E-mail addresses: kate.elbouzidi@nhs.net (K. El Bouzidi), e.nastouli@ucl.ac.uk (E. Nastouli).

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suggested an increase in anti-HDV antibody (anti-HDV) prevalence, from 2.6% of HBV patients in the 1980s to 8.5% in the period from 2000 to 2006, and only 24% of HDV infections were associated with injection drug use [2]. Tong et al. reported a lower HDV seroprevalence of 2% from 2008 to 2010 in another London cohort of hepatitis B patients, and this study also highlighted the role of migration from endemic countries [3]. Sentinel surveillance by the Health Protection Agency in England found the seroprevalence to be 3.7% of all individuals who were tested for anti-HDV in 2011 [4]. The European Association for the Study of the Liver (EASL) guidelines recommend anti-HDV testing for all HBV-infected patients [5].

HDV infection is associated with higher rates of cirrhosis and hepatocellular carcinoma than HBV infection alone and persisting HDV replication is considered the most important predictor of mortality [5]. Interferon is the only available treatment for HDV infection but the optimal duration of therapy has not been established and virological response rates are low. Although significant morbidity is attributed to this virus, there is a paucity of current studies describing patients with active HDV infection, as evidenced by detectable HDV RNA.

2. Objectives

The aim of this study was two-fold: firstly, to determine the rate of anti-HDV testing in HBV patients at four tertiary hospitals in London; and secondly, to describe the epidemiology, clinical characteristics and treatment of HDV-infected patients across the study centres.

3. Study design

3.1. HDV testing

The rate of anti-HDV testing was estimated by dividing the number of HBV patients attending clinic in a 3-month period who had ever been tested for anti-HDV, by the total number of HBV patients attending clinic during the same period. In addition to the electronic results systems, clinic letters were reviewed to look for evidence of previous testing elsewhere. If clinic information was unavailable, then laboratory data were reviewed to determine the number of first hepatitis B surface antigen (HBsAg) positive samples that were tested for anti-HDV.

3.2. HDV-infected patients

A multicentre cross-sectional study was conducted to characterise patients with active HDV infection. Eligible patients were identified by searching laboratory results systems for positive HDV RNA results from the year 2000 onwards. The study included all adult HBV patients who had attended a hepatology clinic at one of the four centres between January 2005 and June 2012 and had at least one blood sample with detectable HDV RNA. Individuals with positive anti-HDV but no detectable HDV RNA were excluded as they could not be determined to have active infection with HDV viraemia. A data capture form was completed by members of the Delta Study Group using clinical and laboratory data extracted from the patients' case notes and electronic records.

3.3. Laboratory methods

Anti-HDV antibody testing was performed using the ETI-AB-DELTAK-2 assay (DiaSorin, Saluggia, Italy). HDV RNA quantitation was performed with a single-step quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) as previously described by Ferns et al. [6]. Available samples were genotyped using the following protocol, based on the method published by Le Gal et al. [7]. HDV RNA was extracted from 140 μl of plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Manchester, UK). The nucleic acid extract (20 μl) was amplified by a semi-nested PCR using HDV primers located in the R′1 and R0 regions. First round primers were 305s (5′ CTCCAGAGGACCCCTTCAGCGAAC 3′) [7] and XHO hdv (5′ GAAGGAAGGCCCTCSGAGAACAAG 3′) and second round primers were 305s and 1161as (5′ CCCGCGGGTTGGGGATGTGAACCC 3′) [7]. The first round 50 μl master mix contained 25 μl of 2× reaction mix buffer from the SuperScript® III One-Step RT-PCR System (Life technologies™), 0.8 μM of each first round primer, 10 μl of water and 1 μl of with Platinum® Taq High Fidelity. Conditions for amplification were 50 °C for 30 min, 95 °C for 15 min and then 2 cycles of 95 °C for 30 s, 50 °C for 45 s, 72 °C for 2 min and 38 cycles of 95 °C for 30 s, 60 °C for 45 s, 72 °C for 90 s with a 7 min extension at 72 °C. The Taq PCR master mix kit (Qiagen) was used for the second round with 25 μl Qiagen 2 × master mix, 0.8 μM of each primer, 21 μl of water and 2 μl of first round product. The second round conditions were 95 °C for 10 min, followed by 45 cycles of 95 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min with a final extension at 72 °C for 7 min. Products of 856 bp were bi-directionally sequenced using primers 305s and 1161as on an ABI 3130xl DNA analyzer. The HDV sequences were then referenced to the BLAST database (http://blast.ncbi.nlm.nih.gov) to determine the genotype.

3.4. Statistical analysis

The statistical analysis was performed using STATA version 11. Continuous variables were described by median and range or interquartile range (IQR), and categorical variables by frequency and percentage. Chi-square test was used to compare the rates of anti-HDV testing in patients with recent HBV diagnoses and those under long-term follow-up. Chi-square, Wilcoxon rank sum and Fisher's exact tests were used to compare the virological markers and treatment of patients with and without cirrhosis.

4. Results

4.1. HDV testing

Data on the number of HBV patients reviewed in clinic were available for one of the study centres (C1). At C1, HDV testing was only performed if requested by the clinician and, in the three-month period from June to August 2012, 168 HBV patients were seen in the C1 clinics and 67 (40%) had ever been tested for anti-HDV. Patients first diagnosed with HBV infection in the preceding six months were more likely to have had HDV investigations (16/26, 62%) than those under long-term HBV follow-up (51/142, 36%, $P = 0.01$, Chi-square test). The seroprevalence of those tested at C1 was 6% (4/67). Two seropositive patients were also HDV RNA positive. At a second centre, C2, the number of HBV patients seen in clinic was not recorded; however, reflex laboratory-led anti-HDV testing of all first HBsAg positive samples had been employed from 2001 to 2012. During this period, 99.4% (3543/3563) of first HBsAg positive samples were tested for anti-HDV and 4.5% (158/3543) were seropositive. Thirty-two of these 158 seropositive patients were also HDV RNA positive and so were included in the second part of the study. The two other centres, C3 and C4, used clinic-led testing but there were insufficient data to determine the rate of testing.

4.2. Patient characteristics

Across the four centres, 55 patients were identified as having had active HDV infection during the study period. The group had a median age of 40 years, IQR 31–51 years, and 33 (60%) were men.

Table 1
Country of origin and HDV genotype of HDV-infected patients.

Country of originby region	Number of patients(% of total)	HDV genotype if known[a](number of patients)
Asia	21 (38%)	
Afghanistan	4 (7.3%)	1 (*n* = 2)
Mongolia	4 (7.3%)	1 (*n* = 3)
Pakistan	4 (7.3%)	1 (*n* = 1)
Turkey	4 (7.3%)	1 (*n* = 1)
India	2 (3.6%)	–
Bangladesh	1 (1.8%)	–
Uzbekistan	1 (1.8%)	–
Vietnam	1 (1.8%)	–
Africa	11 (20%)	
Ghana	2 (3.6%)	5 (*n* = 1)
Nigeria	2 (3.6%)	1 (*n* = 1)
Sierra Leone	2 (3.6%)	–
Angola	1 (1.8%)	1 (*n* = 1)
Congo	1 (1.8%)	5 (*n* = 1)
The Gambia	1 (1.8%)	5 (*n* = 1)
Liberia	1 (1.8%)	5 (*n* = 1)
Africa (not specified)	1 (1.8%)	1 (*n* = 1)
Eastern Europe/Russia	14 (25%)	
Romania	7 (12.7%)	1 (*n* = 2)
Bulgaria	3 (5.5%)	–
Russia	3 (5.5%)	1 (*n* = 2)
Latvia	1 (1.8%)	1 (*n* = 1)
Rest of Europe	9 (16%)	
United Kingdom	5 (9.1%)	1 (*n* = 2); 5 (*n* = 2)
Italy	3 (5.5%)	1 (*n* = 2)
Portugal	1 (1.8%)	–

[a] - Denotes HDV genotyping not done.

Fifty patients (91%) had immigrated to the UK and they originated from 22 different countries (Table 1). The calendar years of both HBV and HDV diagnoses could be determined for 49 patients and, of these, 19 (39%) were diagnosed with both infections in the same year. The median difference in years from HBV to HDV diagnoses was 1 (range 0–25, IQR 0–8).

4.3. Virological markers

The majority of patients were hepatitis B e-antigen (HBeAg) negative (50/55 patients, 91%) and anti-HBe positive (44/55, 80%). The median HBV viral load was 0 IU/mL (IQR 0–519) for patients receiving HBV therapy and 66 IU/mL (IQR 0–483) for those who were not treated for HBV. The median HBsAg level was 2947 IU/mL (IQR 1216–10761) for the 21 patients in whom quantitation was performed. Quantitative HDV RNA results were determined for 19 of the 55 HDV RNA positive patients with a median viral load of 5 million copies/mL. HDV genotyping was performed in 25 cases: 19 (76%) had HDV-1 infection and six patients had HDV-5 (see Table 1 for geographical distribution).

4.4. Liver disease

Seventeen patients (31%) had a clinical diagnosis of cirrhosis, and a further three individuals had acute hepatic failure. Liver biopsies were performed in 37 patients (67%) and the histological staging ranged from mild to severe fibrosis (Table 2). Of the 19 patients with documented histological grades, 3 had minimal inflammation (grade 1–4), 12 mild (grade 5–8), 2 moderate (grade 9–12) and 2 had marked inflammation (grade 13–18).

4.5. Antiviral treatment

Overall, 31 patients (56%) received pegylated interferon alpha therapy for HDV at a dose of 180 microgrammes weekly via subcutaneous injection for between 1 and 2 years. Ten (10/31, 32%) had an end of treatment virological response (EOTVR), while the remainder failed therapy with detectable HDV RNA at the end of treatment. The most common duration of therapy was around one year (26/31 patients, 9/26 had an EOTVR, 1/9 also had HBsAg seroconversion). Five patients received longer courses of between 18 months and 2 years but only one of them experienced an EOTVR. This patient also had HBsAg seroconversion and the declining HBsAg appeared to be the reason for prolonging therapy in this case. No patients discontinued therapy prematurely. HDV treatment and outcomes were also examined for two subgroups: group 1 were individuals who did not have a clinical diagnosis of cirrhosis and had stage 1–4 disease on liver biopsy if performed, and group 2 comprised those with a clinical diagnosis of cirrhosis and/or stage 5–6 liver disease (Table 3).

Five of the patients who did not have an EOTVR following initial HDV treatment went on to have a second course of interferon but none cleared the HDV infection. None of the three patients with acute liver failure were treated for HDV infection but two

Table 2
Liver biopsy findings of HDV-infected patients.

Liver biopsy result	All patients*n* = 55	Treated HDV*n* = 31	Untreated HDV*n* = 24
Stages 1–2	8 (15%)	5 (16%)	3 (13%)
Stages 3–4	14 (25%)	10 (32%)	4 (17%)
Stages 5–6	15 (27%)	9 (29%)	6 (25%)
Biopsy not done	15 (27%)	5 (16%)	10 (42%)
Biopsy data missing/unknown	3 (5%)	2 (6%)	1 (4%)

Table 3
Comparison of HDV-infected patients with and without cirrhosis.

	Group 1: No cirrhosis[a] ($n = 26$)	Group 2: Cirrhosis ($n = 27$)	P value for comparison
Virological markers			
HBeAg positive (n, %)	1 (4%)	4 (15%)	0.35[b]
Anti-HBe positive (n, %)	23 (88%)	21 (78%)	0.30[c]
Median $\log_{10}$ HDV RNA (IQR)	8.1 (7.5–8.5)	6.2 (6.0–6.3)	0.007[d]
Antiviral treatment			
Received HBV treatment, n (% total)	5 (19%)	17 (63%)	0.001[c]
Received HDV treatment, n (% total)	14 (54%)	16 (59%)	0.69[c]
HDV EOTVR, n (% treated for HDV)	6 (43%)	3 (19%)	0.25[c]
Clinical outcome			
Died	0	1	–
Awaiting liver transplant	0	2	–
Decompensated disease,not suitable for transplant	0	1	–
Transferred care	1	1	–
Lost to follow-up	1	0	–

[a] There was insufficient information to categorise two of the HDV-infected patients into either group.
[b] Two-tailed P value from Fisher's exact test.
[c] Two-tailed P value from Chi-square test.
[d] Two-tailed P value from Wilcoxon rank sum test used to compare quantitative HDV results (10 patients in group 1 and 8 patients in group 2).

received nucleos(t)ide inhibitor therapy for HBV. With regard to HBV treatment, 9 of the 31 patients treated for HDV also commenced nucleos(t)ide inhibitor therapy for HBV, almost all starting after the course of interferon. Of the 24 patients who were not treated for HDV, 13 received therapy for HBV. Around half of the HBV-treated patients had tenofovir; other agents used included lamivudine, entecavir and adefovir.

5. Discussion

The rate of anti-HDV testing was low in a centre with clinic-led investigations. Recently diagnosed HBV patients were more likely to be tested than those under long term follow-up, but only represented a minority of outpatients at this site. In contrast, the centre with a reflex laboratory algorithm achieved anti-HDV testing of almost all first HBsAg positive samples over a 12-year period. A direct comparison of testing at these two centres was not possible due to a lack of information on HBV clinic attendees. The seroprevalence results of 6% and 4.5% were similar to the worldwide estimate of 5%, and higher than most London estimates.

This is one of the largest studies of patients with active HDV infection in the UK. We found a relatively young patient group, similar to other studies in London [2–4]. There was diverse ethnicity within our patient populations, reflecting the wide global distribution of HDV. The majority had emigrated from endemic areas, and this highlights the importance of screening high-risk groups for blood-borne viruses. HDV infection was diagnosed after HBV in over half of the patients and this needs further exploration to determine whether there is delayed diagnosis of HDV or if superinfection occurs subsequently. Most of the viruses sequenced in this study were HDV-1, which is the most prevalent of the eight delta genotypes found worldwide [1]. Genotyping of all HDV cases would allow a better understanding of the geographical distribution and permit comparison of virological responses between genotypes.

The study participants had a considerable burden of liver disease, with around half having either clinically diagnosed or biopsy proven cirrhosis. The true figure may be higher as one third of patients were not fully evaluated with a liver biopsy. Despite the hepatic pathology observed, interferon therapy was given to just over half of patients. The reasons for not treating were unclear but may reflect the contraindications and risk of adverse effects associated with interferon, as well as the low chance of success, deterring both patients and clinicians. Patients with cirrhosis were more likely to be treated for HBV than patients without cirrhosis but there did not appear to be a difference in the proportions treated for HDV. The patients with cirrhosis appeared to have lower pre-treatment levels of HDV viraemia than those without cirrhosis, however, only a minority of patients had quantitative RNA results available for analysis. The end of treatment RNA result was used to assess response as the concept of a "sustained" virological response in HDV infection has been brought into question by reports of patients who experienced late relapses following interferon therapy [9]. Only one third of individuals who received HDV treatment experienced a virological response, and this finding is consistent with the 25–40% rate cited by EASL [5]. There were insufficient numbers to demonstrate a significant difference in virological response between those with and without cirrhosis.

The limitations of this study include the use of retrospective data collection by several clinicians using different laboratory systems. Insufficient data management systems prohibited a comparison of HDV testing across the study centres. HBV patients were not always tested for anti-HDV and not all seropositive patients were tested for HDV RNA, therefore incomplete testing coverage makes it likely that some HDV-infected patients were not ascertained. Furthermore, selective HDV investigations may have biased the sample towards including those with more severe disease.

As a result of this study, future recommendations include reflex anti-HDV testing of HBV patients in all centres and standardised HDV RNA testing of seropositive patients using the WHO HDV standard (PEI code 7657/12, GenBank accession number HQ005371) [8]. The Delta Study Group has been established as a multicentre collaboration for the diagnosis, characterisation and management of HDV patients. A prospective cohort study and a comprehensive database are planned to further delineate the natural history and treatment outcomes of this disease. There is a clear need for more efficacious and tolerable treatment options and there has been encouraging progress in this field with the identification of the HBV receptor and the development of entry inhibitors [10]. A well-described cohort would facilitate clinical trials and enable clinicians to readily identify patients who could benefit from new therapeutic modalities.

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Competing interests

None declared.

Ethical approval

Ethical approval was obtained from the clinical governance committees of the participating hospitals.

On behalf of the Delta Study Group

Kate El Bouzidi, Wael Elamin, Katharina Kranzer, Dianne N. Irish, Bridget Ferns, Geoff Dusheiko, Tanzina Haque, Mike Jacobs, David Muir, Peter Karayiannis, Mir Mubariz Hussein, Paul Grant, Jeremy Garson, Deepak Suri, Patrick Kennedy, William Rosenberg, Emilie Sanchez, Belinda C. Smith, Eleni Nastouli.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2015.02.011.

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