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

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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.

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

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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.