Research Article

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Prevalence and clinical course of hepatitis delta infection in Greece: A 13-year prospective study

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Background & Aims: Hepatitis D virus (HDV) has decreased in Europe, but recent reports indicate a rising trend. We report the epidemiological changes, clinical progress, and effect of treatment on the natural course of HDV infection in Greece during the last 13 years.

Methods: Prospective data were extracted from the Hep-Net.Greece Cohort-Study.

Results: Since 1997, 4673 chronic HBV (CHB) cases (4527 adults, 146 children) have been followed prospectively. Two thousand one hundred thirty-seven patients were tested for anti-HDV [101 (4.7%) positive]. Anti-HDV testing in Greece decreased significantly (57.0% before 2003, 35.3% thereafter; p <0.001). Anti-HDV prevalence among HBsAg-positives was 4.2%; lower in native Greeks (2.8%) than in immigrants (7.5%) or in children (15.3%; p <0.001). Within 2.3 years of follow-up, HDV occurred in 11/2047 HBsAg-positive patients (2.2 new delta-infected adults and 8.7 children per 1000 HBsAg-positive annually). HDV-positive compared to CHB adults were younger (p = 0.035) and had more active and advanced disease at baseline, as indicated by laboratory indices and the higher prevalence of cirrhosis at younger age. During a 4.2-year median observation, significantly more anti-HDV-positive than CHB adults developed a liver-related first event (20.0% *vs.* 8.5%, $p_{\text{Log-rank}}$ = 0.014).Treatment was received by 46/90 (51.1%) patients, 40 of them interferon-based. In multivariable analysis, interferon significantly decreased disease progression in HDV-positive patients [HR = 0.14 (95% CI: 0.02–0.86; p = 0.033)].

Conclusions: In Greece, HDV serology is currently tested in only one-third of HBsAg-positive patients. HDV prevalence is lower in native Greeks compared to immigrants, who may contribute >50% of the HDV infection burden in Greece. Data show that HDV infection is a rapidly progressive disease, but interferon-based treatment may alter its course.

Keywords: Chronic HDV infection; HDV-epidemiology; HDV-clinical course; Treatment of HDV; Greece; Anti-HDV testing in HBsAg-positive patients.

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Abbreviations: ALT, alanine aminotransferase; CHBe-, HBeAg-negative chronic hepatitis B; CHBe+, HBeAg-positive chronic hepatitis B; CHD, chronic hepatitis D; CI, confidence interval; HBeAg, hepatitis B "e" antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B viral DNA; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus; HDV-PNALT, HDV-infected patients with persistently normal ALT; HDV RNA, hepatitis delta viral RNA; HIV, human immunodeficiency virus; IFN, alpha interferon (both recombinant or pegylated distinguished when necessary); IQR, interquartile range; IU/L, international units per liter; IU/ml, international units per milliliter; IVDU, illicit intravenous drug use(r); NA(s), nucleos(t)ide analogue(s).

Introduction

Following discovery of hepatitis D virus (HDV), anti-HDV prevalence was reported in ⩾25% of HBsAg carriers in many European countries including Greece [1,2]. In the subsequent two decades, anti-HDV prevalence decreased, mainly because of effective measures controlling hepatitis B virus (HBV) spreading [3,4], so in early 2000s, hepatitis delta was considered "a vanishing disease" in Europe [5]. However, more recent reports from several European sites indicated a rising prevalence of HDV infection attributed mainly to immigration from high prevalence areas and to local niches of intravenous drugs users (IVDU) [6–8]. At the same time, following introduction of effective treatments, the natural course of HBV infection has also changed [9] and changes in

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the underlying disease may have also indirectly affected the natural course of HDV infection.

In Greece, apart from the systematic longitudinal study of HDV infection in the Archangelos community and adjacent area in Rhodes island [2], no nationwide study has been undertaken to evaluate prevalence, clinical course, and treatment outcome of chronic hepatitis D (CHD) and the rising immigration may have changed the national profile of HDV infection. In the 2001 census, immigrants in Greece counted 762,191 people or 7% of the local population [10,11], but the true extent is probably well in excess of 1,200,000, because of the large number of illegal immigrants [12]. Most immigrants in Greece originate from northern Balkan countries (57.5% Albanians) [10] known to have high HDV prevalence [13,14]. We proceeded, therefore, to analyze data from the HepNet.Greece Cohort Study. This network, established in 2003 with the support of the Hellenic Center for Infectious Disease Control and Prevention (KEELPNO), collects data from 21 tertiary Hepatology Centers throughout Greece, evaluating current epidemiology, clinical course, longitudinal changes, and treatment effect on the natural course of viral hepatitis B, C and D in Greece.

Aim of this study was to evaluate the prevalence of HDV infection in native Greeks and immigrants, the clinical course and the effect of treatment on the progression of HDV-coinfection, as compared to HBV-monoinfection, in patients prospectively followed from 1997 to 2010.

Patients and methods

Eligible for enrolment were all HBsAg-positive adults (16–70 years old) and children, who initiated their follow-up from January 1997 till August 9th, 2010. Patients followed before January 1997 or those coinfected with hepatitis C or human immunodeficiency virus were excluded (Fig. 1).

A structured case report form (CRF) including all pertinent information and detailed therapy history was developed for data collection. Prior to network establishment (i.e., before 2003), data were collected retrospectively from medical records, but thereafter, follow-up was prospective and updated twice yearly. All CRFs were submitted to the Statistical and Data Management Center. After 2005, data were recorded electronically, the program automatically prohibiting submission if important information was missing. In this report, we analyze data for all HBV-monoinfected and HDV-coinfected patients at study entry, as well as those HBV-infected, who became anti-HDV-positive during follow-up.

HBV-monoinfected "inactive carriers" were classified according to current guidelines [15]. In HDV-coinfected patients, the lack of serum HDV-RNA measurements restricted the diagnosis of inactive disease to those with always normal ALT values throughout follow-up, absence of cirrhosis, and histological inactivity of liver disease, when a biopsy was available. Such patients were designated as "HDV-infected with persistently normal ALT" (HDV-PNALT).

The diagnosis of cirrhosis was histological (stage 5 or 6, by Ishak *et al.*) [16] or made by a consensus of clinical (ascites, flapping tremor), biochemical (liver synthetic capacity), endoscopic (varices, portal gastropathy), and ultrasound findings (hepatic parenchymal nodularity, splenomegaly). Liver elastography was not available. Cirrhosis was considered decompensated when esophageal or gastric varices, ascites, upper gastrointestinal bleeding or hepatic encephalopathy had developed. Criteria for liver failure included albumin <3.5 gr/dl; total bilirubin >3 mg/dl; prothrombin time >16 sec or INR >1.4. The criteria of HCC diagnosis were either histological or non-invasive [17].

Commercially available methods were applied to all HBV viral markers and anti-HDV determination. HBV DNA was tested by COBAS AMPLICOR HBV Monitor test (Roche Diagnostics, Branchburg, NJ; LLQ 250 copies/ml).

The study protocol was reviewed and approved by the Governing Board of KEELPNO.

Statistical analysis

Entry into the study was defined as the date of the first visit to the clinic of the respective participating center. Follow-up was considered the time interval between the study entry and the last available clinical information or until August 9th, 2010. Analysis time was the time interval between the study entry and diagnosis of a clinical event or the end of follow-up in the absence of an event. A primary end point or clinical event was defined as the development of cirrhosis, liver decompensation, liver failure, hepatocellular carcinoma (HCC), transplantation or liver-related death, whichever came first. Events were classified in two categories, those present at baseline and those that occurred during the follow-up. Categorical covariates are compared by Chi square or Fisher's exact test, as appropriate. For the continuous variables, medians and interquartile ranges (IQR) are given, while for comparisons between groups, the Mann-Whitney test is used. Prevalence of HDV at presentation and rates of new HDV cases during the follow-up are presented. The contribution of HDV coinfection to events already present at baseline was tested by logistic regression, adjusting for age, sex, total serum bilirubin, albumin, ALT, platelet count, HBeAg status, and alcohol use. In patients with active HBV infection, event-free at baseline, the effect of delta infection on the development of events at follow-up was tested by Kaplan-Meier curves and comparisons were performed using the log-rank test. Cox regression analysis was used to estimate the effect of HDV coinfection on liver-related survival. In these models, both HDV infection and treatment administration were handled as time-dependent variables. All analyses were conducted using the Stata 10.1 statistical software and the significance level (alpha) was set at 0.05.

Results

Baseline characteristics of HBsAg patients tested for HDV

The algorithm of sample selection of HDV-infected patients included in the HepNet.Greece Cohort Study is shown in Fig. 1 and the age-frequency in Fig. 2A. Out of 4673 evaluable CHB cases (4527 adults and 146 children) recorded since 1997, only 2137 (45.7%) were tested for anti-HDV (2078 adults and 59 children) at baseline. The group consisted of 1577 native Greeks, 365 Balkan immigrants (Albania 93.2%, Romania 2.2%, Bulgaria 3.0%, Moldavia 1.6%), 98 immigrants from Near East, Central Asia and Africa, and 97 HBsAg-positive patients of unknown origin.

Of the 2137 HBsAg-positive patients tested for HDV at baseline, 90 (4.2%) were anti-HDV-positive. The baseline patient characteristics by HDV status are presented in Table 1. The HDV-positive group included 81 adults (65.4% males) with a median age at presentation of 43.1 (31.4–53.1) years and 9 children (77.8% males) of 9.0 (6.2–11.3) years median age. Adult patients (n = 1997) and children (n = 50) with HBV-monoinfection at presentation had similar sex, but adult patients were significantly older compared to HDV-coinfected subjects (47.5 *vs.* 43.1, $p = 0.035$).

At baseline, a liver biopsy was performed in 37.2% of 774 adult patients [730/1997 (36.6%) HBV-monoinfected; 44/81 (54.3%) HDV-coinfected] and in 49.2% of 59 children [22/50 (44.0%) HBV-monoinfected; 7/9 (77.8%) HDV-coinfected]. Among adult patients, 47/138 (34.1%) with compensated cirrhosis during follow-up were diagnosed by liver biopsy.

The mode of infection was unknown in the majority (65.4%) of the HDV-coinfected adult cases. Reported possible sources of infection were family contact (19.8%), parenteral exposure to infected blood, including IVDU (13.6%) and sexual contact (1.2%) (data not shown). HBeAg prevalence did not differ in adults with or without HDV ($p = 0.316$) or in children ($p = 0.722$). At presentation, both adults and children with HDV-coinfection had more active disease compared to HBV-monoinfected patients.

Temporal changes in anti-HDV testing in Greece

Overall, testing for anti-HDV of the HBsAg-positive patients has decreased significantly in Greece lately, since 1280 of 2244 (57.0%) HBsAg-positive patients in the 1997–2003 period and