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EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

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.

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

Keywords: Chronic HDV infection; HDV-epidemiology; HDV-clinical course; Treatment of HDV; Greece; Anti-HDV testing in HBsAg-positive patients.
Received 3 December 2012; received in revised form 2 July 2013; accepted 3 July 2013
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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).

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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

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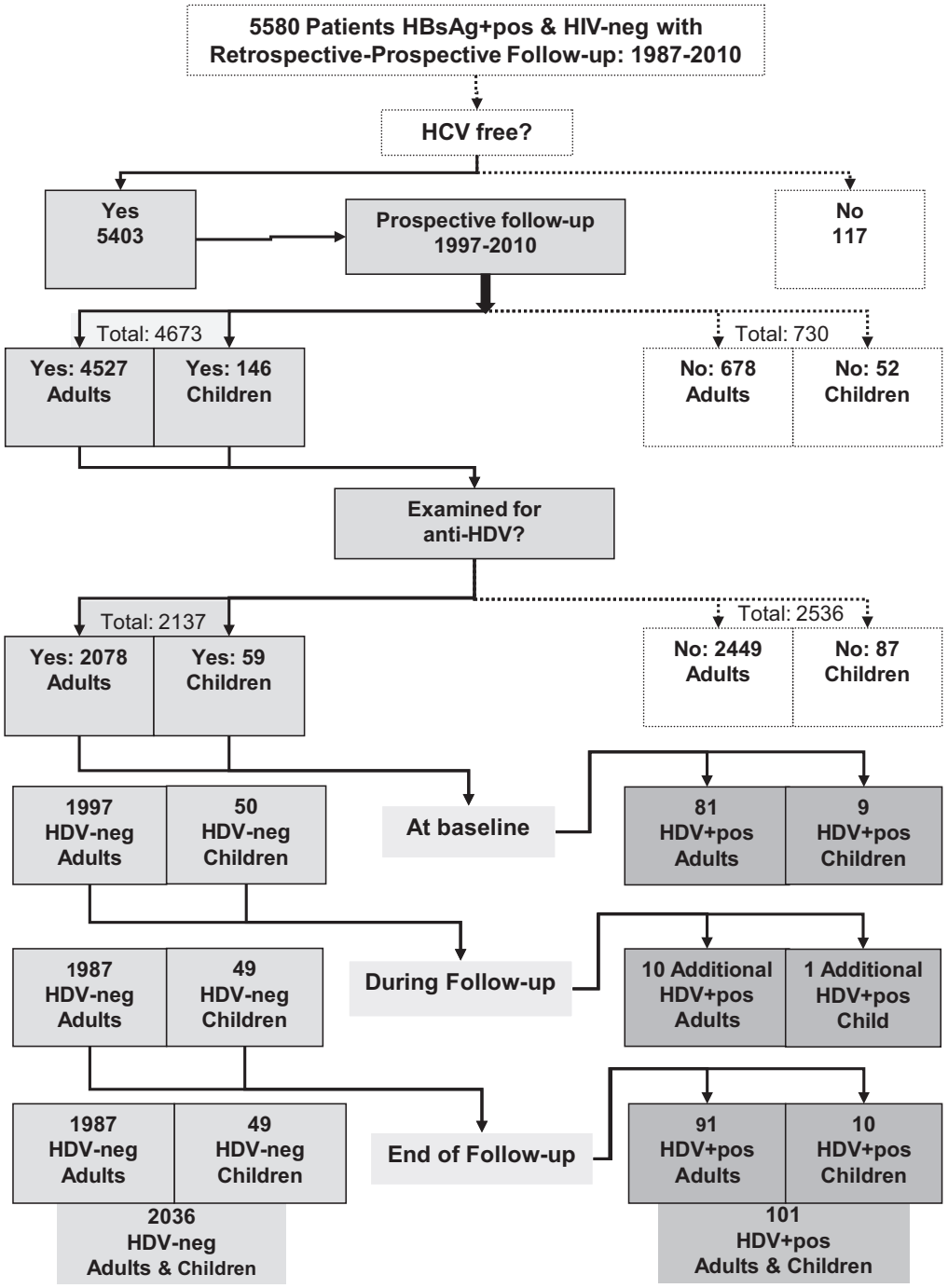

Fig. 1. Patient selection algorithm. The algorithm shows the selection process of the prospectively followed 2036 HBV-monoinfected and 101 HDV-coinfected adults and children out of a pool of 5580 HIV-negative and HBsAg-positive patients included in the HepNet.Greece Cohort Study database. Light-shaded boxes indicate HBV-infected cases and dark-shaded boxes HDV-coinfected patients. Solid arrow lines indicate patient groups further considered for inclusion and dotted arrow lines those excluded from further consideration.

only 857 of 2429 (35.3%) in the 2004–2010 period were tested for anti-HDV (p <0.001).

Trends of HDV prevalence in Greece

At presentation, the overall prevalence of HDV-infected cases in Greece was 4.2% and remained unchanged throughout the follow-up (4.1% *vs.* 4.4% for 1997–2003 and 2004–2010, respectively; p = 0.675). The prevalence of HDV infection among native Greek adults was significantly lower, compared to immigrants (2.8% *vs.* 7.5%, p <0.001). Immigrants from Balkan countries had HDV prevalence of 7.8% (26/333), while those from Eastern Europe, Central Asia or Africa had 6.5% (6/93).

The age-related prevalence of HDV infection is shown in Fig. 2B. In children, the overall prevalence was high (15.3%), unequally distributed among children of Greek (4.5%), Balkan

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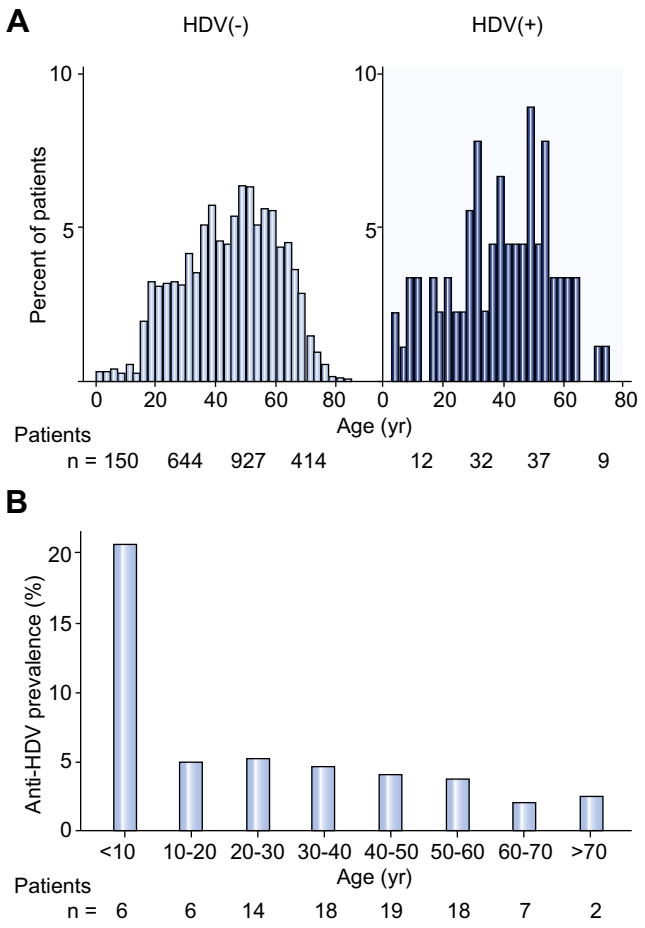

Fig. 2. Age-frequency distribution and anti-HDV prevalence of patient groups studied. (A) Age-frequency of HBV-monoinfected [HDV(−)] and HDV-coinfected [HDV(+)] patients. Note the bell-shaped pattern of age distribution in the HDV(−) patients and the more irregular pattern in the HDV(+) group. In both groups, the bulk of cases belongs to the 40–60-year group, while younger ages appear to prevail in the HDV(+) group. (B) Age prevalence in anti-HDV-positive patients. Please note the high anti-HDV prevalence in children <10 years old and the low prevalence among those of >70 years of age. However, the small numbers of HDV-coinfected cases, in children and older patients, may make the demonstrated age-frequencies unstable.

(15.6%) or Eastern European, Asian or African origin (60.0%) (p <0.001). More significantly, 8/9 anti-HDV-positive children came from immigrant families (5 from Albania and 3 from Russia).

Ten of the 1997 HBsAg-positive adults and 1 of 50 children, became anti-HDV-positive within 2.3 (95% CI 1.3–6.2) years of follow-up, giving an estimated incidence rate of 2.2 new HDV-coinfected adults and 8.7 in children per 1000 HBsAg-positive respective cases, annually. Seven of the 11 patients were Greeks, 3 Albanian immigrants, and 1 Moldavian. None of the 10 adult patients was an IVDU. The newly superinfected group was not significantly different from HDV-infected cases at baseline.

Natural course of delta infection in Greece. Comparison with HBV-monoinfection

The natural course of both conditions was compared, considering the number of liver-related events at presentation and during the years of follow-up. Events recorded at baseline, as well as those occurring at follow-up are shown in Table 2.

Events at baseline

HDV-coinfected compared to HBV-monoinfected adults had more advanced liver disease, with a higher number of events at baseline (p <0.001). In both groups, cirrhosis was the most frequent first event, most prevalent in HDV-coinfected than in HBV-monoinfected patients (p <0.001). In addition, HDV-coinfected patients had cirrhosis at a younger age (p <0.001). After adjusting for other significant factors (see Statistic analysis section), multivariable logistic regression showed that HDV infection had a marginally non-significant impact, increasing the odds of having an event at presentation by 2.58 times [95% confidence interval (CI) 0.97–6.85; p = 0.057]. Among children, only one from the HBV-monoinfected group demonstrated compensated cirrhosis at baseline.

Significantly fewer HDV-coinfected patients, compared to HBV-monoinfected, were HDV-PNALT [3 (4.2%) *vs.* 376 (22.6%); p <0.001].

Events during follow-up

Only one child developed a clinical event during follow-up. Therefore, the analysis that follows is restricted to adults with active HBV infection.

The median (95% CI) follow-up of adult HBV-monoinfected patients was 3.6 (3.3–3.9) *vs.* 4.2 (2.9–5.3) years for the HDV-coinfected group (p = 0.743). During that time, 13/65 (20.0%) HDV-coinfected and 159/1836 (8.7%) HBV-monoinfected patients, event-free at presentation, developed a clinical event, the difference being significant in univariable analysis ($p_{\text{Log-rank}}$ = 0.014; Fig. 3A). In a multivariable Cox proportional hazards model, including treatment as a covariate, factors with a significant effect on development of a first event were: presence of HDV infection [HR 2.15 (CI 1.10–4.17); p = 0.024], age [HR per 10 years 1.83 (CI 1.56–2.15); p <0.001] and baseline platelet count [HR 3.59 per 1000/mm^3 decrease (CI 2.31–5.58); p <0.001].

As at baseline, cirrhosis was again the most frequent first event, occurring significantly more often in HDV-coinfected than HBV-monoinfected patients ($p_{\text{Log-rank}}$ = 0.017; Fig. 3B). HCC followed, but with much lower frequency and without difference between the two groups. However, since compensated cirrhosis is usually the first event in progressive liver disease and may be followed by other more advanced events, we recorded all of them, subsequent to the first events. The total number of events during follow-up was 21 in HDV-coinfected and 258 in HBV-monoinfected patients and in decreasing sequence: compensated cirrhosis [10 *vs.* 139], liver failure [2 *vs.* 39], liver-related death [4 *vs.* 37], liver transplantation [1 *vs.* 2], HCC [2 *vs.* 36] and decompensated cirrhosis without other complications [2 *vs.* 5].

Treatment of CHD in Greece. Trends and efficacy

Forty-six HDV-infected patients [40 adults (49.4%), 6 children (66.7%)] received treatment, while the remaining 44 patients (41 adults, 3 children) did not. Five of the 11 patients that became HDV positive during follow-up had received treatment before becoming anti-HDV-positive. Forty of the 46 treated patients [34/40 adults and all 6 children] received standard or pegylated

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Table 1. Baseline characteristics of HBV-monoinfected and HDV-coinfected adults and adolescent/children.

Parameter	Adult patients (>16 yr) (n = 2078)			Adolescent/children (≤16 yr) (n = 59)		
	HBV-monoinfection (n = 1997)	HDV-co-infection (n = 81)	*p* value	HBV-monoinfection (n = 50)	HDV-co-infection (n = 9)	*p* value
Sex (male; n, %)	1243 (62.2)	53 (65.4)	0.640	32 (64.0)	7 (77.8)	0.704
Age (yr)†	47.5 (35.1-57.9)	43.1 (31.4-53.1)	0.035	10.6 (5.6-13.9)	9.0 (6.2-11.3)	0.393
Disease status (n*)	1664	72	<0.001	41	9	0.570
Active (n, %)	1288 (77.4)	69 (95.8)		36 (87.8)	9 (100.0)	
Inactive (n, %)††	376 (22.6)	3 (4.2)		5 (12.1)	0 ()	
Liver biopsy (n*)	730	44		22	7	
Grade†	6.0 (4.0-9.0)	9.0 (7.0-12.0)	<0.001	5 (2.5-7)	6 (4-11)	0.462
Stage†	2.0 (1.0-3.0)	3.0 (1.0-4.0)	0.007	1.5 (1-3)	3 (1-3)	0.477
Presence of cirrhosis (n*)	1997	81		50	9	0.999
Total cases (n, %)	145 (7.3)	16 (19.8)	<0.001	1 (2.0)	0 (0)	
Age at cirrhosis (yr)†	55.3 (48.5-63.9)	44.2 (36.6-51.9)	<0.001	-	-	
Alcohol consumption (n*)	1886	72		69	12	
Moderate or high (%)†††	250 (13.3)	7 (9.7)	0.747	-	-	
Serum HBeAg (n*)	1837	61		48	9	
Positive (n, %)	171 (7.1)	3 (3.4)	0.316	26 (54.2)	4 (44.4)	0.722
Serum ALT (IU/L)†	46 (26, 104)	83 (49, 199)	<0.001	51 (34-83)	103 (94-560)	0.045
Serum albumin (g/ml)†	4.50 (4.18, 4.80)	4.30 (4.08, 4.60)	0.033	4.5 (4.2-4.6)	4.3 (3.8-4.4)	0.123
INR**†	1.03 (0.98, 1.12)	1.20 (1.05, 1.33)	0.002	-	-	
Total serum bilirubin (mg/dl)†	0.70 (0.52, 0.96)	0.81 (0.60, 1.10)	0.033	0.63 (0.5-0.8)	1.60 (0.6-2.76)	0.090
WBC (n/mm^3)***†	6300 (5200, 7400)	5370 (4400, 6950)	0.006	7475 (5840, 9145)	6685 (6500, 7065)	0.478
Platelets (n/mm^3 x 10^3)†	200 (170-240)	170 (131-205)	<0.001	260 (230-310)	270 (240-310)	0.892
Serum HBV DNA (log_{10} copies/ml)†	4.491 (3.290, 6.075)	3.315 (2.505, 4.350)	0.001	7.602 (4.973, 7.627)	7.191 (7.138, 7.503)	0.879

† Median (interquartile range).
†† Normal ALT and HBV DNA <10^4 copies/ml throughout follow-up.
††† More than 20 g per day.
* Number of patients with measured parameter.
** International normalization ratio.
*** White blood cells.

interferon-alpha for a median duration of 11.9 (6.7–15.5) months, while 6 patients (15.0%) received lamivudine for 29.9 (16.7, 66.6) months. Twelve patients were still on treatment at the end of follow-up. Treated patients had more active disease, but comparable evidence of cirrhosis and indices of liver functional capacity, compared to untreated ones. The median (IQR) post-treatment follow-up was 4.4 (1.7–6.8) years.

Treatment was included as a time-dependent covariate in the multivariable Cox model for the time to a liver-related event. The effect of any treatment in all HBV-infected patients was favorable and associated with significant reduction of liver-related events [HR = 0.53, 95% CI (0.35–0.82); $p = 0.004$]. Inclusion of an interaction term in the final multivariable model showed no different treatment effect in HDV-coinfected compared to HBV monoinfected patients ($p_{interaction} = 0.180$). The adjusted effect of any treatment in the subgroup of HDV-infected patients was even more favorable, with HR = 0.12 (95% CI 0.02–0.75; $p = 0.023$).

In an attempt to distinguish the effect of interferon-alfa from that of nucleos(t)ide analogues (NAs), patients were classified into two categories, dependent on their therapy. For HDV-coinfected individuals, treatment with NA and no treatment were collapsed in one group due to small numbers. In the total sample (all HBV-infected patients irrespective of HDV-coinfection), the effect of interferon was protective but not significant at the nominal level of 5% (HR = 0.62; 95% CI (0.32–1.19), $p = 0.153$), while the effect of NAs was significantly prophylactic (HR = 0.54; 95% CI (0.34–0.87), $p = 0.011$). In the subgroup of HBV-monoinfected patients, results were completely analogous. By contrast, in HDV-coinfected patients, the effect of interferon was significant (HR = 0.14; 95% CI (0.02–0.86), $p = 0.033$), indicating a reduction of liver-related events in treated cases.

Discussion

This prospective multicenter study reveals several important findings. First, CHD prevalence in Greece has remained stable at 4.2% during the last 13 years. Second, anti-HDV prevalence is significantly lower in the native population compared to immigrants and inappropriately high in immigrant children. Third, HDV infection in Greece is under-reported, especially lately. Fourth, HDV aggravates the natural course of CHB, presenting as active hepatitis in childhood, progressing to cirrhosis and other advanced liver-events in young adults. Fifth, IFN improves the natural course of HDV-coinfected subjects, while HBV-monoinfected patients benefit only from NAs.

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Table 2. Clinical events at baseline and during follow-up of patients with chronic HBV or delta hepatitis.

First events recorded at baseline[1]

Events	HBsAg+ positive adults (n = 2078)			HBsAg+ positive children (n = 59)		
	HDV infection			HDV infection		
	No (n = 1997)	Yes (n = 81)*	*p* value	No (n = 50)	Yes (n = 9)	*p* value
Cirrhosis (n, %)	136 (6.8)	16 (19.8)		1 (2)	0 (0)	
Compensated	105 (5.3)	15 (18.5)		1 (2)	0 (0)	
Decompensated	31 (1.5)	1 (1.2)		0 (0)	0 (0)	
Liver failure (n, %)	14 (0.7)	0 (0)		0 (0)	0 (0)	
HCC[2] (n, %)	10 (0.5)	0 (0)		0 (0)	0 (0)	
Liver death (n, %)	1 (0.05)	0 (0)		0 (0)	0 (0)	
OLTx[3] (n, %)	0 (0)	0 (0)		0 (0)	0 (0)	
Total	161 (8.1)	16 (19.8)	<0.001	1 (2)	0 (0)	0.999

First events observed at follow-up (only patients event-free at baseline included)

Events	HBsAg+ positive adults (n = 1901)			HBsAg+ positive children (n = 58)		
	HDV infection			HDV infection		
	No (n = 1836)	Yes (n = 65)*	*p* value	No (n = 49)	Yes (n = 9)*	*p* value
Cirrhosis (n, %)	132 (7.2)	12 (18.5)		1 (1.3)	0 (0)	
Compensated	128 (7.0)	10 (15.4)		1 (1.3)	0 (0)	
Decompensated	4 (0.2)	2 (3.1)			0 (0)	
Liver failure (n, %)	8 (0.4)	0 (0)		0 (0)	0 (0)	
HCC[2] (n, %)	15 (0.8)	1 ()		0 (0)	0 (0)	
Liver death (n, %)	4 (0.2)	0 (0)		0 (0)	0 (0)	
OLTx[3] (n, %)	0 (0)	0 (0)		0 (0)	0 (0)	
Total	159 (8.7)	13 (20.0)	0.006	1 (1.3)	0 (0)	0.999
Follow-up (yr) median (95% CI)[4]	3.6 (3.3-3.9)	4.2 (2.9-5.3)	0.743	2.1 (1.6-3.8)	2.1 (0.7-6.4)	0.968

* Patients were anti HDV-negative at baseline and they became positive at follow-up.
[1] Events that occurred before or within 6 months of the 1st visit.
[2] Hepatocellular carcinoma.
[3] Liver transplantation.
[4] Median (95% CI) follow-up of patients without event 3.6 (2.0–8.0) years.

Greece, located at the southeast end of the European Union (EU) and surrounded by non-EU countries, has been a preferred place of mostly illegal immigration during the last decades [10,12]. Our study, although not population-based, confirms the existence of significant difference in HDV prevalence between the immigrant (7.5%) and native Greek (2.8%) population. The difference may be even wider, since illegal immigrants may have limited access to medical specialized care and thus have not been included in this study. Even so, extrapolating the observed difference to 10,452,554 native Greeks and 695,979 registered immigrants of known origin, numerical composition (2006 census data) [11,18,19] and ethnic HBsAg prevalence [11,12,20], it can be estimated that over 50% of the national HDV burden can be attributed to immigration. The real extent of HDV (and HBV) infection burden is probably much larger, considering that illegal refugees probably far exceed the number of the legal ones, originating from high HDV (and HBV) prevalence areas, as Albania, Pakistan, Kurdistan, Afghanistan, Iraq, and Somalia [11,12,20]. The situation in Greece is similar to that described in a recent multicenter survey in Italy [21], a country sharing with Greece a large burden of economic immigration. Greek and Italian data are somewhat different from those reported from Central Europe, where an overall increase in HDV prevalence is already present [7,22]. Increased HDV prevalence is probably a matter of time. Modes of wider HDV transmission in the community include prostitution, unprotected sex, IVDU and distribution by indigent immigrants and finally the inadverted mix of them with the native population.

The changing epidemiological pattern of HDV has probably not been realized by the medical community of our country since anti-HDV testing, already low in Greece, has further decreased lately. Indeed, before 2003, almost 1 in 2 HBsAg-positive patients was anti-HDV-tested (57.0%), but the rate dropped to 35.3% thereafter. Greece however, is not an exception, since anti-HDV testing has also been under-reported in other European countries [6]. Probable reasons are the belief that CHD is a rare condition, unlikely to be encountered in a medical practice and possible unawareness of the importance of diagnosis, given the frequent gravity of the disease.

The natural course of CHD, as seen in Greece, is that of a rapidly progressing disease [1,23]. It is true that delta infection is not always ominous [1,2]. However, in our study, all anti-HDV-positive children had active disease and among adults, the baseline histological and biochemical activity and indicators of liver failure were significantly increased compared to HBV-monoinfected patients. Cirrhosis was not observed in children, but it was significantly more frequent in HDV-coinfected adults and at a younger age compared to HBV-monoinfection. An additional indication of

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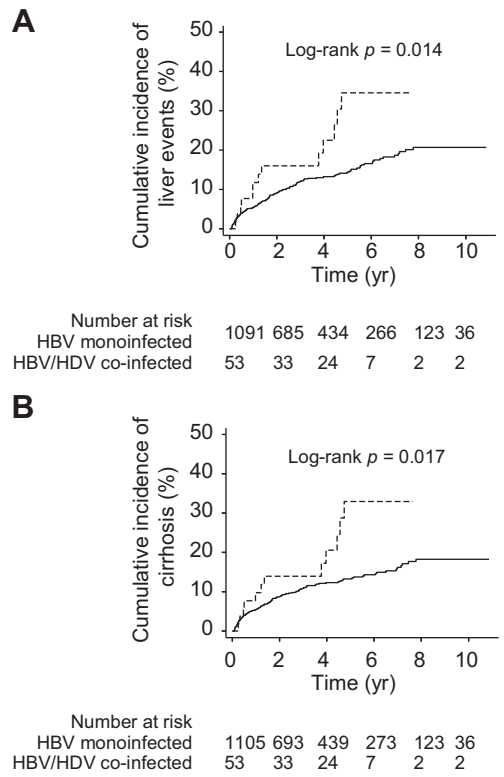

Fig. 3. Probability to develop any first liver-related event or cirrhosis of HBV-monoinfected and HDV-coinfected patients within 13 years of follow-up. (A) Hazard to develop any first liver-related event and (B) to develop cirrhosis as a first event of HBV-monoinfected (solid line) or HDV-coinfected patients (broken line) (Kaplan-Maier plot). Please note that both A and B diagrams are quite similar, since the most frequent liver-related event was cirrhosis.

worse course of HDV was the significantly lower number of HDV-PNALTs *vs.* HBV inactive carriers. However, since serum HDV-RNA or even IgM anti-HDV testing was not included in this study, we cannot precisely distinguish true HDV inactive carriers from smoldering CHD, or healed past HDV infection [23].

Worth of comment are the higher anti-HDV prevalence and incidence rate of new anti-HDV seroconversions among HBsAg-positive children, especially of immigrant families, compared to adults. Although the above estimates must be considered with caution due to the small numbers involved, if true, they indicate that HDV infection in Greece frequently occurs early in life and possibly heralds a future increase of delta-infection due to cohort effect. In 1998, universal HBV vaccination was introduced in Greece. Data, however show that immigrant children have significantly lower immunization coverage than native ones (63.7% *vs.* 72.6%, $p = 0.012$). Moreover, following universal immunization, HBsAg prevalence has decreased in native (6.5% *vs.* 1.7%, $p < 0.001$), but not in immigrant children (7.8% *vs.* 10.0%) [24]. The higher infectivity of HBeAg-positive children with or without HDV infection is probably an additional predisposing factor in this age group [25]. If no measures are taken, increasing HDV prevalence is expected in the next years due to the cohort effect.

Finally, the finding that interferon may change the natural course of untreated CHD is very interesting. Indeed, in the multivariable analysis, there was a beneficial effect of treatment (IFN with or without NAs *vs.* NAs-monotherapy) in all HBsAg-positive patients (including HDV-coinfected). The beneficial effect persisted even when "HDV infection" was added as an interaction term in the model, indicating that delta-infected patients responded overall in a similar way as HBV-monoinfected patients.

In order to separate the IFN effect, we grouped NA-treated/HDV-coinfected patients together with those never treated [27]. In that model, we found that only NAs had a significant beneficial effect in HBV-monoinfected patients, while only IFN altered the course of the disease in HDV-coinfected, associated with fewer liver-related events. So far, in CHD no treatment has been considered effective. NA suppress further the usually diminished HBV replication, but they do not affect HDV replication [26]. IFN is a more promising agent, but data originating from small series have been inadequate to show a significant effect so far, although almost always they do demonstrate a trend towards temporary and, occasionally, permanent improvement [26,27]. This study provides evidence for IFN efficacy in CHD, not based on biochemical or virological parameters, as in most previous studies, but on disease-progression-associated events. However, results of this study are based on small numbers of IFN-treated and untreated HDV-coinfected subjects and most importantly on small numbers of events in the subgroups of treatment type by coinfection status. Confirmation of these results in large prospective studies, including repeated follow-up endoscopy for presence and staging of varices [28,29], will be clinically very important.

In conclusion, we have described the current epidemiology of HDV-coinfection in Greece, including differences among the native and immigrant population, as well as its current under-reporting. Confusion of CHD with simple HBV-monoinfection may be least helpful to patients, since HDV-coinfection is an aggressive disease, and IFN treatment, as our data suggest, may alter its natural course. We believe that this study will improve the understanding of CHD and place it in its correct current perspective.

Financial support

This work was supported by a grant from the Greek National Center for Disease Control (KEELPNO).

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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