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Hepatitis Delta Virus Infection in Romania: Prevalence and Risk Factors

Liana Gheorghe[1], Irma Eva Csiki[2], Speranta Iacob[1], Cristian Gheorghe[1], Anca Trifan[3], Mircea Grigorescu[4], Adriana Motoc[5], Andra Suceveanu[6], Manuela Curescu[7], Florin Caruntu[8], Ioan Sporea[9], Ciprian Brisc[10], Ion Rogoveanu[11], Razvan Cerban[1], Letitia Tugui[1], Andrea Alexandrescu[12]

1) Center Digestive Diseases and Liver Transplantation, Fundeni Clinical Institute; 2) National Institute of Public Health, Bucharest; 3) Center Gastroenterology and Hepatology, Iasi; 4) Institute Gastroenterology and Hepatology Cluj Napoca; 5) Victor Babes Universitary Hospital of Infectious & Tropical Diseases, Bucharest; 6) Clinic Gastroenterology and Hepatology, University of Medicine and Pharmacy, Constanta; 7) University Hospital Infectious Diseases, Timisoara; 8) National Institute Infectious Diseases Matei Bals, Bucharest; 9) Center Gastroenterology and Hepatology, University of Medicine and Pharmacy, Timisoara; 10) Clinic Gastroenterology Hepatology, Oradea; 11) Clinic Gastroenterology Hepatology, University of Medicine and Pharmacy, Craiova; 12) Hoffmann La Roche, Bucharest, Romania

Address for correspondence:
Liana Gheorghe, MD, PhD
Center for Digestive Diseases and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania
drlgheorghe@gmail.com

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ABSTRACT

Background : Hepatitis delta virus (HDV) infection is associated with accelerated progression of fibrosis, early occurrence of hepatic decompensation and an increased risk for hepatocellular carcinoma. Epidemiological data on hepatitis delta virus (HDV) in Romania are still lacking. **Aim**: To assess the prevalence, virological, clinical and epidemiological features of HDV infection in Romanian patients.
Methods: We conducted a multicenter study in 10 centers. Data on sociodemographic characteristics and potential risk factors were collected through a questionnaire. Virological markers of HBV and HDV infection, biochemical and clinical features of liver disease were evaluated.
Results: The study population comprised 2,761 HBsAg(+) patients with a mean age of 43.8±13.8 years, out of whom 5.2% were HBeAg(+) and 55.7% were males. Liver cirrhosis was detected in 17.9% of patients, while 80.4% had chronic hepatitis. The prevalence of IgG anti-HDV(+) patients was 23.1%, out of whom 16.4% were HDV RNA positive. The highest prevalence of HDV infection was encountered in patients aged 50-59 years (28.9%) and patients aged ≥60 (24.8%) (p=0.0001). Seroprevalence of HDV was significantly higher in AgHBs(+) cirrhotics vs. noncirrhotics (43.4% vs 19.0%, p=0.0001). Risk factors for HDV infection were: occupational hazard, no HCV chronic infection, lack of anti-HBV vaccination, presence of blood transfusions, any previous surgery, frequent hospitalization or endoscopies, tattoos, body piercing, use of glass syringes, number of female sexual partners.
Conclusions: HBsAg(+) population in Romania is characterized by a high prevalence of HBeAg(-) HBV infection as well as HDV co-infection. A cohort phenomenon for HDV prevalence is also observed similar to that of HCV/HBV monoinfections.

Key words: hepatitis D (delta) virus – chronic hepatitis – prevalence – risk factors.

INTRODUCTION

Hepatitis D virus (HDV) is a defective RNA virus that requires the helper function of hepatitis B virus (HBV) for virion assembly and transmission. As a consequence, HDV can only infect HBsAg-positive individuals.

The HDV infection is endemic worldwide, but the prevalence rate varies in different geographical areas [1, 2]. A high prevalence of anti-HDV has been described in HBsAg-positive patients in the Mediterranean region, the Middle East, Central Africa, the northern part of South America, Russia and Eastern Europe. It is estimated that 15-20 million individuals (approximately 5% of all HBV carriers) are chronically co-infected with HDV worldwide, at a high risk of developing cirrhosis and hepatocellular carcinoma and potentially in need of liver transplantation [3-5].

The prevalence of HDV infection has significantly declined during the 1980s-1990s in some regions of the world such as Italy [6, 7], Spain [8], Taiwan [9], and Turkey [10], mainly as a result of vaccination against HBV, systematic screening of blood and blood products, implementation of safety protocols against blood borne infections in the healthcare system, the switch to disposable syringes, improved socio-economic conditions and the increased awareness and control on sexually transmissible agents [11]. However, the HDV prevalence stopped decreasing towards the end of the 1990s in Central and Western Europe. The recrudescence of HDV infection in European developed countries such as Italy [11], Great Britain [12], Germany [13, 14], or France [15] is mainly related to the increasing immigration of individuals from highly endemic

areas of Eastern Europe, ex-Soviet Union countries, Africa, Turkey, and the Middle East; other causes such as intravenous drug use, at-risk sexual practices and body modification procedures may also be involved. At tertiary referral centres in Europe, 10% to 16% of HBsAg-positive patients test positive also for anti-HDV antibodies. Thus, chronic HDV infection still represents a significant health burden in Europe [14, 16].

HDV infection is considered hyperendemic in Romania, but the real prevalence among HBsAg-positive individuals is not known. There is only very limited information from small cohorts of patients on the prevalence, virological and clinical characteristics of delta hepatitis. Clinical observations and some small investigational data indicate that chronic hepatitis D (CHD) is highly prevalent in Romania causing severe and progressive liver disease. Data reported from Romanian referral centers of gastroenterology and infectious diseases indicate a prevalence of anti-HDV antibodies between 20 and 28% [17-19]. Delta co-infection was reported in 37.9% and 51.2% of patients with HBsAg-positive chronic hepatitis and liver cirrhosis, respectively, in 56% of patients with end-stage liver disease on the waiting list for liver transplantation and in 48.7% of patients with hepatocellular carcinoma [18, 20].

In Romania, there is no national routine surveillance and registration system for HBV or HDV infections, and for viral related liver cancer. There is currently a lack of epidemiological data on HBV-HDV co-infection in Romania, and data on HDV infection prevalence were obtained from limited studies. Knowledge of real prevalence of HDV-HBV coinfection is essential for understanding the extent and seriousness of this health problem, for anticipating its future impact on the health system and for ensuring an adequate allocation of financial resources.

The aim of this study was to evaluate the prevalence and risk factors of HDV-HBV co-infection in the adult population in Romania.

STUDY DESIGN AND METHODOLOGY

This multicenter study was conducted among chronically HBV infected (HBsAg-positive) adult patients (age ≥18 years) in 10 Gastroenterology/Infectious Diseases centers covering approximately 80-90% of population from all geographical regions of our country. Each center has enrolled all consecutive HBV-positive patients addressed for routine monitoring or as new patients for further evaluation. Consecutive patients mean all patients addressed during the specified time period at the center, meeting the inclusion criteria. The enrollment of HBV infected patients has begun and has stopped in all centers at the same time.

Demographic data on age, gender, area of residence, ethnicity, marital status, education, employment, and data on risk factors for HBV and HDV infection had been collected by the participating investigators using a standard questionnaire. The questions regarding risk factors for HDV infection included: occupation with risk to exposure to blood products, family history of HBV, vaccinated against HBV, diagnosed with HCV, contact with a HBV infected person, blood transfusion, frequent hospitalization, surgery, emergency and dental surgery, frequent endoscopies, frequent injections at home or at outpatient unit, using glass syringes, serious accidents (work, traffic, domestic), injury from objects contaminated with blood, imprisonment, sharing toothbrushes, scissors, razors, acupuncture, tattoos, body piercing, beauty studio attendance (using cutting or other injuring instruments), injecting drug use, sexually transmitted diseases, sexual partner with chronic HBV infection, number of sexual partners, age at first sexual intercourse, alcohol consumption, and other potential risks for HDV infection.

For each patient, blood was collected and serum was separated and tested for the level of liver functional enzymes and complete blood counts. Ultrasound and Fibroscan (Echosens®, France) were performed in order to stage the disease and differentiate between chronic hepatitis and liver cirrhosis. For confirmation of HBV infection, ELISA technique designed for HBsAg, HBeAg, anti-HBeAb and anti-HBc IgG antibodies was used. In the next step, the HBV infected patients were assessed using the ELISA technique for the presence of anti-HDV IgG antibody. Semi-quantitative detection of HDV-RNA in serum was performed by single and nested PCR amplifications of a highly conserved region of the HDV genome, by using primers selected from genotype 1 of HDV [21]. The quantitative determination of HBV DNA was performed with HBV 2.0 Monitor (Roche Molecular Diagnostics). Apart from serum levels, detectable HDV viremia was assumed as indicative of an active HDV infection. For HBV-DNA, the cut-off levels of 2,000 UI/ml and 1,000 UI/ml were considered associated with active HBV infection in chronic hepatitis and liver cirrhosis, respectively.

The study was approved by the National Ethics Committee (ML25581/8/10/2010) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Statistical analysis and sample size estimation

For the sample size calculation, we assumed a hypothetical value for prevalence rate of 24.5% for HDV coinfection in HBV positive patients. The margin of error within which the estimated proportion is given was chosen to be 1.9%; therefore, a sample size of 2,700 patients was required for the 95%CI 22.7%-26.5%. This estimation was based on the following assumptions: 1) the prevalence of chronic HBV infection (HBsAg positivity) in adult population in Romania is 4.4%, which means an estimated number of 650,000 infected people; 2) Romania is categorized into a hyperendemic area of Southern and Eastern Europe, with a HDV infection figure of 20%-28%.

The crude prevalence of HDV co-infection in patients with chronic HBV infection by gender, age groups (18-29, 30-39, 40-49, 50-59, and over 60 years), residence (rural, urban), BMI groups (under 18.5 kg/m^2, 18.5-24.99 kg/m^2, 25-29.99 kg/m^2, and over 30 kg/m^2), ethnic groups, educational levels, marital, and social status and for all investigated risk factors was calculated. Categorical variables have been compared using the Chi-square test or Fisher's test, as appropriate. For comparison of quantitative variables (ALT and AST values) we used the Wilcoxon rank sum test.

The association between HDV infection and demographic characteristics of participants or risk factors was checked using an unconditional univariate logistic regression, calculating

rude odds ratio (OR) and the corresponding 95% CI for each investigated characteristic or risk factor. All statistical tests were two-sided and a level of $p \leq 0.05$ was considered statistically significant. Statistical analyses were done with Stata/MP 11.

RESULTS

From March 1, 2011, to September, 30, 2011, 2,784 patients signed the informed consent and completed the questionnaire; 23 patients did not meet all inclusion criteria; therefore the investigated sample consisted of 2,761 HBsAg-positive patients.

The mean age of patients was 43.8±13.8 years. Of the 2,761 HBsAg positive patients, 55.7% were males. Distribution by age was as follows: 21.4% patients between 18-29 years, 17.6% between 30-39 years, 17.7% between 40-49 years, 31.5% between 50 to 59 years and 11.8% were over 60 years old. Approximately 79% of the included patients live in urban areas.

The overall prevalence of anti-HDV IgG antibodies in HBsAg-positive patients was 23.1% (95%CI: 21.4-25.0%; N=639); 1.2% (N=32) of patients tested inconclusive for HDV IgG antibodies and 75.7% (N=2,090) tested negative. The prevalence of anti-HDV coinfection by different demographic characteristics is shown in Table I. A significantly higher delta co-infection rate was noted in the older population, in Romanians and Roma people compared to Hungarians, in patients with lower levels of education, widows, unemployed or retired. The female gender population had a higher prevalence of HDV positive antibodies as compared to males but this figure did not achieve statistical significance (24.6% vs 22.0%, p=0.103). There was a statistically significant difference of HDV prevalence by age groups, patients older than 50 showing highest rates of prevalence (p=0.0001, Fisher's test).

Patients with active HDV infection (positive HDV RNA) represented 16.4% (N=454) from the total HBsAg-positive cohort. Prevalence of positive HDV RNA was slightly higher among female patients (17.0% vs 16.0%, p=0.20). A significant difference by age group prevalence was observed for viremic HDV patients (p=0.0001).

There was no significant variation regarding prevalence of overall HDV IgG positive patients by residence – rural vs urban (23.8% vs 23.0%, p=0.669).

The median value of both AST (p=0.53) and ALT (p=0.78) was similar (Wilcoxon rank sum test) in HBV monoinfected and HBV-HDV coinfected patients.

In our cohort of 2,671 HBV positive patients, 17.9% of the patients had liver cirrhosis, 80.4% had chronic hepatitis and in 1.7% of patients the liver disease was not fully characterized. Only 5.2% of HBsAg-positive patients were HBeAg positive and 58.8% had anti-HBe Ab. A high proportion (36.0%) of patients had inconclusive HBeAg. There was a significantly higher percentage of patients with liver cirrhosis vs chronic hepatitis within the HBV-HDV coinfection group (43.4% vs 19.0%, p=0.0001). Within the group with HBV-HDV, patients with active delta infection (positive viral load) had a higher risk of liver cirrhosis (34.3% vs 12.7%, p=0.0001). Among patients with liver cirrhosis, HBV DNA level >1,000 IU/mL was encountered in 50.6% of HBV-HDV coinfected patients and in 49.4% of patients with HBV monoinfection (p=0.0001). More patients (41.8%) with liver cirrhosis and anti-HDV IgG antibodies/HDV RNA positivity had an HBV DNA >1,000 IU/mL compared to only 8.4% of HDV IgG positive/HDV RNA negativity (p=0.0001). On the contrary, in non-cirrhotic HBV/HDV population, active HBV viral replication >2,000 IU/mL was present in 0% of patients and in 81.8% of HBV monoinfection (p=0.011). HBV/HDV coinfected patients were less likely to have positive HBeAg compared to HBV monoinfected patients (14.0% vs 86.0%, p=0.0001).

Risk factors for HDV are shown in Table II. HDV seroprevalence was not significantly associated with imprisonment, family history or contact with a HBV infected person, injections at outpatient unit or at home, sharing syringes of any kind, dental surgery, serious accidents (work, traffic, domestic), intravenous drug users, beauty studio (salon) attendance, sharing tooth brushes, scissors, razors, sexually transmitted disease, age at first sexual intercourse, sexual partner with chronic HBV infection, number of male sexual partner in the last 3 years, alcohol consumption.

DISCUSSION

This is the first study having data at the national level to evaluate the prevalence and risk factors associated with HDV infection in Romania. The sample population of this referral-based survey consisted of an adult HBV infected population (≥18 years) referred to 10 tertiary centers covering all geographical regions of Romania over a 6 months period. Assuming the reported HBV prevalence of 4.4% [22] and an estimated HDV prevalence among HBsAg-positive individuals in hyperendemic areas of 20 to 28% [10, 19, 23], a sample consisting of 2,700 HBsAg-positive subjects was calculated. Out of 2,784 patients with chronic HBV addressed to 10 participating centers during the enrollment period, and who signed the informed consent, 2,761 had met the inclusion criteria and participated in the study.

Our four major findings were: 1) the seroprevalence (anti-HDV IgG positivity) was 23.1% (639 patients), while the prevalence of active HDV infection (HDV RNA positivity) was of 16.4% (454 patients, a percentage of 71.5% from anti-HDV positive subjects); 2) the prevalence of HDV infection was higher among patients older than 50, females and urban residents; 3) almost 18% of HDV-HBV infected patients were already cirrhotics; 4) the risk factors associated with HDV infection strongly suggest healthcare-associated transmission as a major route, followed by sexual and intra-familial transmission. These findings require the following comments.

First of all, our results confirmed that HDV infection is still highly endemic in Romania three decades later than the peak was reached in Southern Europe. The 23.1% rate of seroprevalence results in a national reservoir of more than 150,000 HDV-HBV infected individuals. Such a high prevalence rate is unique in Europe, being similar to the reports from Africa (10.7 up to 24.6% in Sudan, Nigeria, and Cameroon), South America (34.4% in Brazil), Central Asia or Turkey (11.3 up to 56.2%) [24-26]. Similar figures were reported in Italy until 1980s, with 23% of the HBV-infected population testing positive for anti-HDV [27]. At that time, intravenous drug use (IVDU) was the main source of delta infection in northern Italy, whereas intra-familial transmission was in

Table I. Prevalence of anti-HDV co-infection by demographic characteristics in chronically HBV infected patients

Variable	HDV+/Patients tested, N[a]	Prevalence, % (95%CI)	P[b]	Crude OR (95%CI)	P
All patients	639/2761	23.1 (21.4 – 25.0)		-	-
Gender			0.102		
Male	338/1539	22 (19.7 – 24.4)		1	
Female	301/1222	24.6 (21.9 – 27.6)		1.16 (0.97 - 1.38)	0.103
Age group (years)			0.0001		
18 – 29	110/592	18.6 (15.3 - 22.4)		1	
30 - 39	82/486	16.9 (13.4 - 21.0)		0.91 (0.66 - 1.24)	0.539
40 - 49	107/488	21.9 (18.0 - 26.5)		1.24 (0.92 - 1.67)	0.155
50 - 59	259/896	28.9 (25.5 - 32.6)		1.89 (1.47 - 2.43)	<0.0001
≥ 60	81/326	24.8 (19.7 - 30.9)		1.47 (1.06 - 2.03)	0.021
Residence			0.699		
Urban	500/2177	23 (21.0 - 25.0)		1	
Rural	139/584	23.8 (20.0 - 28.1)		1.04 (0.84 - 1.29)	0.724
BMI (kg/m^2)			0.062		
< 18.5	20/102	19.6 (12.0 - 30.3)		1	
18.5 - 24.99	303/1184	25.6 (23.3 - 28.3)		1.43 (0.86 - 2.37)	0.169
25 - 29.99	208/990	21.2 (18.7 - 23.8)		1.11 (0.66 - 1.85)	0.700
≥30	105/485	22.8 (19 - 26.6)		1.21 (0.71 - 2.06)	0.484
Ethnicity			0.002		
Romanian	593/2608	22.7 (20.9 - 24.6)			
Hungarian	8/55	14.5 (6.3 - 28.7)		0.58 (0.27 - 1.24)	0.161
Roma	18/47	38.3 (22.7 - 60.5)		2.08 (1.15 - 3.77)	0.016
Other	20/51	39.2 (23.9 - 60.6)		2.16 (1.22 - 3.82)	0.008
Educational level			0.0001		
No elementary school	12/30	40 (21.7 - 69.9)		1.67 (0.78 - 3.55)	0.184
Elementary school (4-8 yrs)	149/526	28.3 (23.9 - 33.3)		1	
High school (12yrs)	244/979	24.9 (21.9 – 28.3)		0.85 (0.67 - 1.08)	0.174
College	84/361	23.3 (18.6 – 28.8)		0.77 (0.57 - 1.05)	0.103
University	148/848	17.5 (14.8 - 20.5)		0.54 (0.41 - 0.70)	<0.0001
Marital status			0.0001		
Married/Consensual union	454/1943	23.4 (21.3 - 25.6)		1	
Unmarried	127/596	21.3 (17.8 - 25.4)		0.88 (0.7 - 1.10)	0.258
Divorced	13/101	12.9 (6.9 - 22.0)		0.5 (0.28 - 0.90)	0.022
Widowed	44/108	40.7 (29.6- 54.7)		2.25 (1.51 - 3.36)	<0.0001
Social status			0.0001		
Employer/Self employed	19/83	22.9 (13.8 - 35.7)		1	
Employee	203/1319	15.4 (13.3 - 17.7)		0.6 (0.35 - 1.03)	0.064
Unemployed	39/117	33.3 (23.7 - 45.6)		1.63 (0.86 - 3.10)	0.135
Retired/On welfare	292/873	33.4 (29.7 – 37.5)		1.67 (0.98 - 2.85)	0.059
Housewife	47/185	25.4 (18.7 - 33.8)		1.12 (0.61 - 2.06)	0.718
Others	30/135	22.2 (15.0 - 31.7)		0.93 (0.48 - 1.79)	0.834

CI: confidence interval; OR: odds ratio; a: Number of tested patients by categories may not sum to total because of missing data; b: Estimated by Fisher's exact test

southern Italy [28]. Longitudinal studies over the last 30 years have shown a decrease in HDV prevalence in some endemic areas, such as in Italy, Spain, Taiwan or Turkey. The decline in HDV in these regions was substantial in young subjects as a result of the introduction of anti-HBV vaccination programs, but additional factors including a general improvement in hygiene and socio-economic conditions, awareness of the virus and its mode of transmission and better implementation of preventive measures in healthcare system (change to disposable needles, syringes, and other medical equipment) have possibly

Table II. Prevalence of anti-HDV co-infection according to different risk factors in chronically HBV infected patients

Characteristics	Participants tested, N[a]	Anti-HDV positive, N (%)	P[b]	Crude OR (95%CI)	P
Occupation with risk to exposure to blood products			0.0424		
No	2,503	593 (23.7%)		1	
Yes	192	33 (17.2%)		0.68 (0.46 - 0.99)	0.047
Unreported	36	5 (13.9%)		0.51 (0.20 - 1.32)	0.167
Diagnosed with HCV			0.0001		
No	1,035	340 (32.9%)		1	
Yes	37	9 (24.3%)		0.64 (0.30 - 1.38)	0.254
Unreported	1,666	285 (17.1%)		0.42 (0.35 - 0.50)	< 0.0001
Vaccinated against HBV			0.0014		
No	2,275	559 (24.6%)		1	
Yes	133	20 (15.0%)		0.54 (0.34 - 0.89)	0.014
Unreported	330	57 (17.3%)		0.64 (0.47 - 0.87)	0.004
Contact with HBV infected person			0.038		
No	1,914	465 (24.29%)		1	
Yes	269	66 (24.54%)		1.01 (0.75 - 1.36)	0.952
Unreported	556	107 (19.24%)		0.75 (0.59 - 0.95)	0.016
Blood transfusion			0.0001		
No	2,265	498 (22.0%)		1	
Yes	420	132 (31.4%)		1.64 (1.30 - 2.06)	< 0.0001
Unreported	53	7 (13.2%)		0.53 (0.24 - 1.19)	0.123
Surgery			0.046		
No	1,172	249 (21.3%)		1	
Yes	1,388	350 (25.2%)		1.26 (1.05 - 1.52)	0.013
Unreported	5	1 (20.0%)		0.92 (0.10 - 8.25)	0.939
Emergency surgery			0.016	1	
No	677	201 (29.69%)			
Yes	426	96 (22.54%)		0.68 (0.52 - 0.91)	0.008
Unreported	10	1 (10%)		0.26 (0.03 - 2.05)	0.2
Frequent hospitalization			0.0001		
No	2,190	470 (21.5%)		1	
Yes	523	155 (29.6%)		1.54 (1.25 - 1.91)	< 0.0001
Unreported	14	2 (14.3%)		0.6 (0.13 - 2.69)	0.506
Frequent endoscopies			0.0001		
No	1,854	312 (16.8%)		1	
Yes	876	322 (36.8%)		2.87 (2.39 - 3.45)	< 0.0001
Unreported	13	0 (0%)		-	-
Injections at outpatient unit			0.189	1	
No	2,017	449 (22.26%)			
Yes	699	179 (25.61%)		1.21 (0.99 - 1.48)	0.064
Unreported	20	4 (20%)		0.86 (0.29 - 2.59)	0.79
Injections at home			0.257	1	
No	2,065	462 (22.37%)			
Yes	654	166 (25.38%)		1.18 (0.96 - 1.45)	0.109
Unreported	16	3 (18.75%)		0.79 (0.22 - 2.78)	0.712
Use of glass syringes			0.0001	1	
No	1,888	405 (21.5%)			
Yes	608	179 (29.4%)		1.54 (1.25 - 1.89)	< 0.0001
Unreported	234	48 (20.5%)		0.94 (0.67 - 1.31)	0.7

Table II (continued)					
Tattoos			0.0001		
No	2,555	570 (22.3%)		1	
Yes	189	66 (34.9%)		1.85 (1.36 - 2.54)	< 0.0001
Unreported	3	1 (33.3%)		1.71 (0.16 - 18.94)	0.66
Body piercing for earrings			0.0280		
No	1,638	351 (21.4%)		1	
Yes	1,103	284 (25.8%)		1.27 (1.06 - 1.52)	0.01
Unreported	5	1 (200%)		0.9 (0.10 - 8.09)	0.926
Body piercing - others			0.0001		
No	2,598	577 (22.2%)		1	
Yes	127	53 (41.7%)		2.47 (1.71 - 3.56)	< 0.0001
Unreported	15	5 (33.3%)		1.72 (0.59 - 5.06)	0.322
IDU (Injecting drug use)			0.277		
No	2,718	627 (23.07%)		1	
Yes	26	8 (30.77%)		1.46 (0.63 - 3.37)	0.376
Unreported	2	1 (50%)		3.28 (0.21 - 52.58)	0.401
Sexually transmitted disease			0.144		
No	2,591	598 (23.08%)			
Yes	86	27 (31.4%)		1.5 (0.94 - 2.39)	0.086
Unreported	69	13 (18.84%)		0.78 (0.42 - 1.43)	0.415
Sexual partner with chronic HBV infection			0.23		
No	2,232	506 (22.67%)		1	
Yes	120	34 (28.33%)		1.34 (0.89 - 2.02)	0.158
Unreported	383	96 (25.07%)		1.14 (0.89 - 1.47)	0.296
Number female sexual partner in the last 3 years			0.0001		
No	1,297	354 (27.3%)		1	
1	1,097	226 (20.6%)		0.69 (0.57 - 0.83)	< 0.0001
2-5	196	34 (17.4%)		0.56 (0.38 - 0.83)	0.004
>5	55	11 (200%)		0.67 (0.34 - 1.31)	0.242

CI: confidence interval; OR: odds ratio; a) number of tested patients by categories may not sum to total because of missing data; b) estimated by Fisher's exact test

contributed [29]. However, despite the introduction of universal anti-HBV vaccination of newborns in Romania beginning with 1995, due to the high prevalence of HBV chronic infection in the adult population (the highest figure in Europe) and the prolonged HDV autochthonous epidemic until 1990s, HDV-HBV infection is not yet controlled and a decline similar to that reported for Mediterranean countries such as Italy, Spain three decades ago cannot be observed in Romania. At the moment, the reservoir of HDV-positive patients in Romania consists of two pools: one represented by the survivors of the 1980s-1990s epidemic, showing already advanced liver disease and its complications (the vast majority, more than 80%) and the other one represented by younger patients with less severe disease due to more recent HDV acquisition by IDU, sexual or intra-familial route. Moreover, with more than 2 million people emigrated to Central and Western Europe, Romania might have contributed substantially to the recrudescence of HDV infection in countries such as Italy, Spain, France, Germany or the UK and to the liver-related morbidity, mortality and costs in these countries.

As in the case of Romania, the rates of HDV infection are generally high in areas where HBV is endemic, but there are exceptions from this rule such as in Vietnam, Indonesia or China, but also in neighboring countries such as Bulgaria, Ukraine or Moldova Republic [30-32] making the situation of Romania more similar with that of Mediterranean countries. It should be emphasized that a factor that may artificially alter epidemiological figures of HDV infection in Europe is the lack of awareness and testing for delta agent. Although European and national guidelines recommend that all HBsAg-positive patients should be tested routinely for anti-HDV antibodies, this is easily forgotten due to the optimistic perspective of an extinct disease and budget-constraints. The newly detected cases of anti-HDV positivity (N=639 patients) underline the importance of testing at least once in a lifetime for anti-HDV antibodies in HBsAg-positive subjects. Furthermore, the detection of HDV infection may have therapeutic implications since many of oral antivirals active for HBV seem to be ineffective for anti-HDV, while other drugs such as (pegylated) interferon may be useful.

Secondly, some interesting considerations result from the demographics analysis. On average, patients with HDV infection from our study tend to be older, more female and urban residents, and the virological profile of helper virus is HBeAg-negative with an undetectable/low HBV viral load. HDV prevalence in our study is increased in patients between 40 and 60 year-old with a peak in the age group of 50-59. This trend suggests a cumulative risk of HDV exposure over time, as well as a cohort phenomenon of HDV epidemic starting three decades ago. This data is similar to our previous reports on HCV and HBV infection [22, 33] and in accordance with other studies from the literature [34, 35]. As a consequence, in addition to the universal vaccination of newborns aimed to eliminate both HBV and HDV infection over a longer period of time, any short term strategy, including additional preventive measures such as HBV adult vaccination, at least in risk groups, and continuous improvement in healthcare conditions, socio-economic conditions and hygiene should be promoted as a national strategy. Statistically significant gender differences (prevalence rate higher in males than in females) were reported for HBsAg carriers in Romania [22], according to other studies [36]. On the contrary, in the current study the proportion of anti-HDV positivity and HDV RNA positivity was slightly higher in females (24.6% vs. 22.0% and 17.0% vs. 16.0%, respectively), although this figure did not reach statistical significance. This is in contrast with the data recently reported, stating no difference or a higher prevalence in males [37, 38], but is consonant with our previously reported data on HCV infection (significantly higher rates of prevalence in females), suggesting the high female exposure in late 1970s and 1980s due to illegal abortion procedures. The higher prevalence in urban residents might be a bias related to easier access to medical facilities and cultural discrepancies between people living in urban and rural areas in Romania.

A third important finding of our study was the severity of liver disease in HDV-HBV co-infected patients, with almost 1 in 5 patients being already cirrhotic and 12% of them already presenting hepatocellular carcinoma [20]. In general, liver disease induced by the dual HBV-HDV infection is more severe than that induced by HBV monoinfection; liver damage caused by HDV-HBV infection leads more frequently to cirrhosis and hepatocellular carcinoma, being an independent risk factor for mortality [3, 20, 39, 40]. HDV infection is a strong risk factor for hepatocarcinoma, which is more prominent in male patients. Therapeutic options for HDV infected patients are limited; the only approved therapy consists of high doses of standard interferon (5MU daily or 9MU three times per week) or peginterferon alfa for at least 12 months. Even so, the sustained virological response (undetectable HDV RNA after 6 months of therapy) is no higher than 20-25% [41-43] and is poorly maintained after discontinuation of therapy (HDV usually relapses if the patients remain HBsAg positive). Hence, the ideal endpoint of any treatment against HDV is not only the HDV clearance, but also clearance of the helper HBV virus, although the HBsAg clearance is seldom achieved. Our study showed that there was a significantly higher percentage of patients with liver cirrhosis than chronic hepatitis within the HBV-HDV coinfection group; also within the group with dual viral infection, patients with active delta infection (positive viral load) had a higher risk of liver cirrhosis.

Finally, with regard to the routes of HDV transmission, these are the same as for HBV, the most important being parenteral exposure and the transmucosal (sexual or intra-familial) spread; vertical transmission is rare. However, this multicenter survey performed in HBsAg-positive subjects shows that the high endemicity of HDV infection in Romania was generated by unsafe medical procedures in the past associated with blood transfusions, contaminated needles and reuse of medical or surgical instruments. Even frequent endoscopies represent a risk factor of delta infection. Previous endoscopy and colonoscopy procedures were reported as risk factors for HDV-HBV infection in only one other study from Iran [34]. As a consequence, proper disinfection of endoscopes and endoscopic devices, use of disposable needles and forceps is mandatory and should be checked on a regular basis by healthcare officials in endoscopy wards from hospitals and private practices.

Transmission of HBV through transfusion of blood products has been largely eliminated in most parts of the world by the screening of blood donors and implementing techniques that ensure viral inactivation of products prepared from blood [44]. However, in Romania, there is a positive correlation between previous blood transfusion, surgery and frequent hospitalization, and the presence of serum markers of HBV infection [22], similar to Bulgaria [45]. Our study has shown that blood transfusions were associated with HDV infection, raising the supposition that the majority of patients were superinfected with delta virus instead of having been coinfected with HDV. This is in agreement with the fact that most of the Romanian delta infected persons found out about their disease when advanced fibrosis or even when decompensated liver cirrhosis, instead of being diagnosed with acute viral infections. The HBV-HDV co-infection is usually self-limited with only 20% of cases progressing to cirrhosis, while chronic hepatitis D acquired as superinfection leads to cirrhosis in 70% of cases in up to 10 years [46]. Tattoos and body piercing were also found to be significant risk variables for HDV infection, probably accounting for the rather high prevalence of infection in young people. Recent studies provide increasing evidence that sexual transmission may be a more important factor in the spread of the disease than has previously been appreciated [46]. Between other risk factors for HDV seroprevalence, we found that only the number of female sexual partner in the last 3 years was statistically significant (Table II).

The results of this first multicenter study conducted in Romania on a sample of 2,761 HBsAg carriers contribute to the understanding of the actual epidemiology of HDV infection in Europe, classifying Romania as a highly endemic area. These data are critical for a better understanding of the burden of an infection that is a major public health problem in Romania, being the main indication of liver transplantation in a proportion similar to HCV on the national waiting list. We strongly recommend testing all HBV positive patients also for HDV, because HDV is far from being a disappearing disease. Universal screening would serve the important public health goal of allowing patients to be educated on their status and on the need for HDV-negative patients to protect themselves against superinfection and for HDV-infected patients to avoid transmission to others.

Our study has several strengths and limitations. As strengths we consider the large sample size, and new data from the Mediterranean area after an interval of three decades. Limitations are considered to be the referral-based study (hyper-addressability, catchment area, rural population under-represented) and the risk factors auto-reported in a structured questionnaire.

CONCLUSIONS

The present study holds important information for other European countries that are faced with the recrudescence of HDV infection. Two major consequences resulted from our analysis that might impact the healthcare systems in the following years. Firstly, we have to consider that over two million Romanian inhabitants who migrated to the Central and Western European countries might have led to the increasing prevalence, liver-related morbidity, mortality and costs in those areas. Second, as no dramatic changes in the management of HDV infection are anticipated, the total health care cost with HDV infected population will increase at a peak in the following 10 to 20 years, as a result of decompensated liver cirrhosis and hepatocellular carcinoma, which will account for about 50% of cases until 2030.

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