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Earlier liver cirrhosis onset in intrafamilial hepatitis Delta Virus transmission in Moldova

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ABSTRACT

Background and Objectives. Hepatitis delta, often considered a vanishing disease in Western Europe, remains widespread in the Republic of Moldova. However, so far, no first-hand study describing the hepatitis delta virus (HDV) - infected patients has been published in the country.

The aim of our study was to explore how intrafamilial transmission influences disease course.

Materials and Methods. In this comparative cross-sectional study, we describe the demographic, clinical and biological characteristics of 224 HDV-infected patients with chronic hepatitis or liver cirrhosis attending care in three Moldovan centers. These patients were compared with 100 hepatitis B virus (HBV) -mono-infected subjects.

Results. The age of liver cirrhosis onset was similar (48-49 years) in both HDV and HBV monoinfected patients. All delta-infected patients were anti-HBe and HBV DNA detectable much less frequently (28%) than in mono-infected ones (76-92%, P<1.0E-09). Most clinical and biological parameters were significantly worsened in the case of HDV infection. Familial transmission was much more prevalent in HDV than in HBV infection (39% vs 23%, P=0.0036). Amongst patients with HDV those with intrafamilial

contamination developed liver cirrhosis much earlier than others (40.5±9.3years vs 46.9±8.7years, P=0.053) and presented more frequently detectable HDV RNA in their plasma (98.7% vs 89.2%, P=0.0094). Patients from Southern Moldova were significantly more likely to have familial transmission than patients from other parts of the (39.2% vs 17.7%, P=0.0010).

Conclusions. Hepatitis delta is a significant health problem in Moldova. Familial transmission of HDV, especially prevalent in the South of the country, is responsible for the anticipation of the complications, so for this targeted population, an earlier identification of infection can provide precocious opportunities to improve linkage to care and treatment.

Keywords: hepatitis delta , viral transmission, intra-familial transmission

Abbreviations: HDV - hepatitis delta virus, HBV - hepatitis B virus, HCC - hepatocellular carcinoma

INTRODUCTION

HDV is considered to be responsible for the most severe form of viral hepatitis and represents, a significant medical issue in the field of liver diseases [1-4]. Chronic HDV-infected patients often progress toward liver cirrhosis with rapid decompensation as well as toward hepatocellular carcinoma (HCC) [2-4]. Moreover, antiviral treatments are notoriously ineffective against this virus [1-6].

In Europa, Romania is frequently cited as the primary target of Delta hepatitis infections, where HDV co-infection is considered underdiagnosed. According to a recent study with a 320,000 screened individuals across 24 counties, targeting socially disadvantaged detecting 6813 hepatitis B surface antigen (HBsAg)-positive individuals, HDV antibody prevalence was 4.87%, with active replication confirmed in 75.6% of HDV-positive cases [7]. Comparing to epidemiological changes in the last two decades showing an Anti-HDV detected in 20.4% of patients with HBV in 2006 in Bucharest [3]. Chronic liver diseases, including hepatitis B and C, in addition to the endemic pocket of delta hepatitis are widespread in Moldova, contributing to the highest incidence of liver cirrhosis and HCC in the European World Health Organization (WHO) region [8-10]. Considering the relatively small population size, it is noteworthy that Moldovan citizens are often mentioned in research studies on HDV published by Western European and North American investigators [11,12]. Our group reported that nearly half of Moldovan

HBV-infected patients with HCC are also carriers of HDV [10]. Despite this, there is a complete lack of in-depth data describing the clinical and biological status of Moldovan HDV-infected patients before the onset of neoplastic complications. Given the severity of HDV disease and the paucity of treatments, there is a great unmet need for handling with this disease.

MATERIALS AND METHODS

In this cross-sectional study, we collected data about 224 HDV-infected patients attending two community health centers and a tertiary care institution in Moldova in the last 5 years. Patient demographics and clinical characteristics, including age, sex, comorbidities, treatment for both HBV/HDV and HBV during the baseline period were captured. The anti-HDV testing rate was estimated by reviewing clinical and laboratory data. Patients were enrolled if they met the following criteria: (1) HBsAg-positive in serum, (2) serum positive HDV antibodies (IgG-anti-HDV) for at least 6 months, (3) chronic hepatitis or cirrhosis at histological and/or clinical evaluation, (4) no signs of current (RNA) or past (anti-HCV) co-infection with the hepatitis C virus. In parallel, 100 patients with chronic hepatitis B virus infection were randomly selected from the records of the same departments for comparison. This retrospective study was approved by the institutional review board of the Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova (No 35/24_ 12.02.2018).

Diagnosis

The diagnosis of Liver cirrhosis was based on clinical symptoms of portal hypertension, biochemical features of hepatic insufficiency and the grade of fibrosis (F4). Liver stiffness was measured by uni-dimensional transient elastography with Fibroscan 502TOUCH equipment (EchoSens, Paris, France). The liver and spleen dimensions were measured by Doppler abdominal ultrasonography on an AplioX6 apparatus (Toshiba, Tokyo, Japan)

Serological and molecular methods

Serological markers used to ascertain HDV infection were anti-HDV antibody (anti-HDV)-detected being indicative of chronic infection. All patients were screened for the presence of circulating viral genomes by real-time qPCR TaqMan assays (Cobas AmpliHep, and CobasTaqMan 48, Roche Diagnostics, Basel, Switzerland).

Statistical analyses

Statistical analyses were performed using a Prism 6.0d statistical package (GraphPad Software, Inc, La Jolla, CA, USA). Numerical variables were summarized by their median, mean and range according to their types of distribution (normal or non-normal).

They were compared either using a Student's T-test or by a Mann-Whitney test as appropriate. Categorical variables were summarized as frequencies that were compared either using Fisher's exact or the Chi square test. All tests were univariate and two-sided. The level of significance was set at $p<0.05$.

RESULTS

The age of patients was homogeneous (48-49 years), except for mono-infected patients with chronic hepatitis B, who were slightly younger (43.6 years). Additionally, the latter group was the only one exhibiting a strong male predilection, reflecting the increased susceptibility of males to HBV (M:F = 3:1).

To perform consistent comparisons, patients were stratified in four distinct subsets based on their infection Delta or B-only infection and disease progression status (ie liver cirrhosis or chonic hepatitis). As shown in Table 1, co-infected B-Delta and mono-infected B patients exhibited significant differences in a wide range of clinical and biological features. In all significant instances, the status of delta-infected patients was more severe than the corresponding feature in HBV mono-infected subjects.

Table 1: Demographic, Clinical, Viral and Biological features of the patients investigated

Features of the 324 patients	Cirrhosis			Chronic hepatitis		
	Anti-Delta(+)/HBsAg(+) n=125	Anti-Delta(-)/HBsAg(+) n=26	P values	Anti-Delta(+)/HBsAg(+) n=99	Anti-Delta(-)/HBsAg(+) n=74	P values
Age (years, mean±SD)	49.2±9.4	48.0±7.7	ns	48.5±9.6	43.6±10.9	**0.0022**
Sex ratio M:F	0.86 (58:67)	1.16 (14/12)	ns	0.94 (48:51)	3.1 (56:18)	**0.0020**
Disease course						
Age at chronic Hepatitis B diagnosis (years, mean±SD)	25.8±14.9	19.3±11.8	**0.0294**	25.	17.2±12.7	**4.6 E-08**
Age at Hepatitis D diagnosis (years, mean±SD)	38.7±12.8	na	na	38.	na	na
Age at liver cirrhosis diagnosis (years, mean±SD)	44.2±10.4	45.5±7.6	ns	na	na	na
Time frame separating Hep B diag from cirrhosis (years, mean±SD)	19.2±12.1	27.3±12.4	**0.0042**	na	na	na
Time frame separating Hep Delta diag from cirrhosis (years, mean±SD)	4.9±6.1	na	na	na	na	na
Viral features						
anti-HBe (%)	100.0	72.0	**1.7 E-06**	100.0	49.4	**2.4 E-17**
HBV DNA(+) (%)	28.0	92.3	**8.2 E-10**	28.8	76.0	**6.6 E-10**
HBV DNA loads (Log10 IU/mL, mean±SD)	2.8±1.2	3.7±1.2	**0.0024**	3.6±1.6	3.6±1.0	ns
HDV RNA(+) (%)	88.6	na	na	99.0	na	na
HDV RNA loads (Log10 IU/mL, mean±SD)	5.1±1.6	na	na	6.0±1.6	na	na
Clinical Features						
Decompensated cirrhosis (%)	51.1	42.3	ns	na		na
Ascites (%)	21.9	30.7	ns	0.0	0.0	ns
Encephalopathy (%)	15.4	0.0	**0.0451**	0.0	0.0	ns
Esophageal varices (%)	47.9	30.7	ns	0.0	0.0	ns
Decompensation (%)	51.6	42.3	ns	0.0	0.0	ns
Splenectomy (%)	13.7	0.0	**0.0444**	3.0	1.3	ns
mension (cm2, mean±SD)	114±29	129±12	**0.0075**	109±27	113±12	ns
dimension (cm2, mean±SD)	118±42	91±40	**2.0 E-04**	68±20	67±12	ns
Liver stiffness (Kpa, mean±SD)	23.2±10.2	18.2±6.0	*0.0511 (ns)*	10.2±3.7	8.3±1.6	**3.2 E-04**
Laboratory values						
Hemoglobin (SD)	122±15	116±17	*0.0941 (ns)*	134±16	137±14	ns
Leukocytes (±SD)	4.0±1.6	4.2±1.2	*0.509 (ns)*	4.7±1.5	5.7±1.2	**2.1 E-05**
Neutrophils (G/L, mean±SD)	2.0±0.7	2.2±0.9	*0.885 (ns)*	2.3±0.7	2.6±0.6	**0.0205**
Platelets (G/L, mean±SD)	111±65	157±63	**0.0006**	160±46	215±41	**1.6 E-13**
ALT (IU/mL, mean±SD)	71±52	69±36	*0.537 (ns)*	101±83	52±20	**2.4 E-06**
AST (IU/mL, mean±SD)	82±63	62±22	*0.453 (ns)*	78±66	47±16	**1.7 E-04**
GGT (IU/mL, mean±SD)	99±96	54±17	**0.0105**	76±75	50±32	**0.0099**
lirubin (mcromol/L, mean±SD)	34.5±53.9	14.7±5.5	*0.0638 (ns)*	15.4±8.1	11.9±2.9	**5.0 E-04**
esterase	5144±2486	5700±1368	*ns*	7553±2386	7535±1823	ns
n (g/L, mean±SD)	31.9±6.9	32.3±3.9	*ns*	37.7±3.8	38.9±2.5	**0.0039**
g/L, mean±SD)	39±229	48±202	*ns*	3.2±2.8	4.8±12.9	ns
INR (mean±SD)	1.4±0.2	1.4±0.1	*0.299 (ns)*	1.1±0.1	1.1±0.2	ns
Clinical scores and indices						
Child score	6.9±2.0	6.2±0.7	*0.0775 (ns)*	5.1±0.3	5.3±0.6	ns
King Score	82±97	46±60	**0.0131**	32±28	12±9	**9.4 E-08**
Fibrosis index	3.7±1.0	3.1±0.8	**0.0192**	2.6±0.7	1.9±0.4	**2.6 E-11**
GUCI	4.1±4.7	2.3±3.1	**0.0210**	1.6±1.5	0.7±0.4	**1.2 E-06**
FibroQ	14.0±20.6	5.9±5.4	**3.0 E-04**	3.9±3.5	2.3±1.1	**3.9 E-04**
de Ritis	1.3±0.7	0.9±0.1	**0.0299**	0.8±0.3	0.9±0.2	*0.058 (ns)*
CDS	7.1±1.5	6.8±1.3	*0.257 (ns)*	6.3±1.0	5.1±0.9	**5.1 E-11**
API	6.4±1.7	5.3±2.1	0.0052	5.2±1.8	3.1±1.8	**6.1 E-12**
Pohl (% of positive cases)	48.8	30.7	*0.120 (ns)*	8.1	1.3	ns
FIB4	6.4±6.2	3.3±3.9	**3.0 E-04**	2.5±1.5	1.4±0.6	**2.6 E-08**
APRI	2.7±2.8	1.5±2.1	**0.0038**	1.3±1.2	0.6±0.2	**7.9 E-07**
GPRI	1.2±1.3	0.5±0.35	**<0.0001**	0.5±0.5	0.2±0.1	**4.8 E-05**
PNI	23.5±18.9	17.3±10.3	*0.297 (ns)*	28.3±29.2	20.7±102.3	ns
PLR	71±69	163±61	**<0.0001**	113±142	214±553	ns
Risk Factors of hepatitis (%)						
Transfusion	17.3	12.5	ns	8.6	5.4	ns
Tatoo-Piercing	4.9	7.6	ns	5.5	13.5	ns
Sexual transmission	3.0	23.0	**0.0028**	6.6	8.1	ns
Intrafamilial infection	39.4	19.2	*0.068 (ns)*	40.3	16.2	**0.0010**
Unknown	47.7	50.0	ns	44.5	56.1	
Therapeutics						
Antiviral treatment (%)	24.3	25.0	ns	42.6	55.0	ns

To better understand the natural history of the disease in Moldova, we compared the milestones of infection in different settings. We observed that in the case of dual infection, age at diagnosis of B infection was always occurring later than in mono-infected patients (25 years versus 17-19 years). This difference is not well understood and may seem counterintuitive, but it is likely due to the more insidious course of the disease associated with the presence of HDV. Age at diagnosis of delta infection was similar both in cirrhosis and hepatitis groups and positioned 13 years later than inaugural persistent hepatitis B discovery. Surprisingly, the age of cirrhosis discovery was similar in dual and mono-infection (44-45 years). However, the time periods separating the discovery of hepatitis B and *bona fide* cirrhosis were very different and much shorter in case of dual infection (19.2±12.1 years vs 27.3±12.4 years, P=0.0042). This difference is likely related to the initial mean age difference of hepatitis B discovery between HBV-HDV and HBV-only infections. On average 5 years was separating the diagnosis of hepatitis delta and liver cirrhosis onset.

Viral parameters

Serological and viral parameters related to HBV were significantly different between dually and mono-infected patients. All delta-infected patient showed anti-HBe seroconversion both in hepatitis and in cirrhosis cases (eg in cirrhosis, 100.0% vs 72%, P=1.7 E-06). Therefore the presence of HBV DNA in the blood as detected by qPCR was much less frequent in delta infection (approximately 28.1% vs 76.0-92.0%). In presence of cirrhosis, when measurable, HBV DNA loads in dually infected subjects were one log lower than in mono-infected cases (2.8±1.2 log10 IU/mL vs 3.7±1.2 log10 IU/mL, P=0.0024). In these dually-infected cirrhotic patients, HBV DNA loads were also slightly lower than in delta hepatitis patients (P=0.0421). This situation likely reflects the greater loss of replication-competent hepatocytes in cirrhosis associated with HDV infection. Similarly, HDV RNA loads followed the same pattern. Delta-infected patients with liver cirrhosis were slightly, but significantly less often positive for HDV RNA than non-cirrhotic patients (88.6 vs 99.0%, OR=12.3, 95%CI=1.5-95.6, P=0.002) .In cirrhotic patients positive for HDV RNA, viral loads were lower than in non-cirrhotics patients (5.1±1.6 vs 6.0±1.6 log10 IU/mL, P=0.002).

Apparent impact of viral transmission pattern on the course of the disease

To better understand the causes of the endemic presence of HDV in Moldova, we compared risk factors of infections between the two categories of patients. Remarkably, when combining liver cirrhosis and chronic hepatitis cases, intra-familial transmission was the only risk factor differing significantly between HDV-infected and hepatitis B-only patients (39.8% in the case of HDV presence vs 23.0% in the case of HBV presence only, P=0.0036, OR=2.2, 95%CI=1.2-3.7). This difference was significant in chronic hepatitis settings (P=0.0010) and almost significant in the cirrhosis subgroup (P=0.068).

The implementation of preventive measures is essential to combat the endemic circulation of hepatitis viruses in the Republic of Moldova. Therefore, we tried to understand the peculiarities of these diseases in the country according to the patterns of transmission. So if we look at the risk factors for the acquisition of Delta viral infection, we will notice that the intrafamily transmission path prevails . (Figure 1)

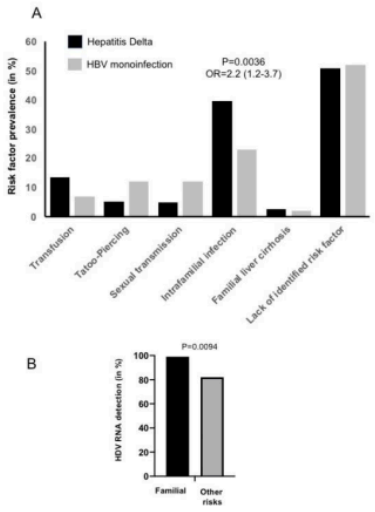

Figure 1. Data on sociodemographic characteristics on HDV/HBV infection and (A) - potential risk factors and (B) the level of HDV-RNA in familial transmission comparative to other risks

We then decided to examine in more detail the 80 patients with intra-familial transmission of the disease and to compare them with the 120 patients without known familial cases of chronic infection. Familial transmission was associated with significantly earlier discoveries of all disease milestones (ages at hepatitis B, hepatitis delta and liver cirrhosis diagnoses) in patients with cirrhosis but not in those who did not progress (Table 2).

Table 2: Disease milestones according to the mode of HDV transmission

Disease milestones in Hepatitis Delta virus-infected patients	Cirrhosis			Chronic hepatitis		
	Intra-familial n=43	other transmissions n=66	P values	Intra-familial n=37	other transmissions n=55	P values
Age at inclusion (years, mean±SD)	47.5±9.3	52.1±7.9	3.0 E-04	46.4±8.9	50.0±10.3	0.063 (ns)
Age at chronic Hepatitis B diagnosis (years, mean±SD)	20.9±11.4	30.3±14.4	5.1 E-04	27.0±13.7	24.5±13.4	0.395 (ns)
Age at Hepatitis D diagnosis (years, mean±SD)	34.0±13.0	42.6±11.5	5.7 E-04	36.8±10.4	39.5±13.3	0.302 (ns)
Age at liver cirrhosis diagnosis (years, mean±SD)	40.5±9.3	46.9±8.7	0.0053	na	na	na

These observations suggest that a subset of patients exposed to intrafamilial transmission is at risk of developing an early and severe form of the liver disease, presumably genetic susceptibility, environmental factors or both. Additionally, HDV RNA detection was slightly more frequent in patients with intra-familial -contamination than in others (98.7% vs 89.2%, P=0.0094). We observed that cases with familial transmission were more frequent among patients coming from Southern Moldova (39.2% vs 17.7%, OR=2.9, 95%CI=1.7-6.0, P=0.0010). Clinical and biological features were not

significantly different between familial-transmision cases and other cases of hepatitis delta infection.

We then wondered whether certain features might differentiate these HDV(+) patients from Southern Moldova from others. We observed that patients from this region were also more frequently positive for HBV DNA in the blood (42.1% vs 22.2%, OR=2.5, 95%CI: 1.3-5.0, P=0.004). When stratifying the subset of patients with familial contamination based on the identified member of the family at the origin of the disease (parents or siblings), variations in transmission patterns were apparent. The pattern of HDV transmission between siblings (brother or sister) was sighnficantly more prevalent in Southern Moldova (64.2% vs 20.0%, OR=4.6, 95%CI=2.0-10.7, P=6.2E-05). This observation suggests that household living conditins and not only circumstances surrounding childbirth are conducive to virus transmission in this part of the country. Additionaly, if a sibling was the putative virus transmitter, infected patients were more often male (64.0% vs 44.1%, OR=2.3, 95%CI=1.05-5.3, P=0.028).

We examine whether a similar pattern was observable in case of mono-infection with HBV but we did not notice any differences, except that HBV DNA burden was somewhat heavier in cases with a familial component of infection (4.2±0.7 log10 IU/mL vs 3.5±1.1 log10 IU/mL, P=0.023).(Figure 2).

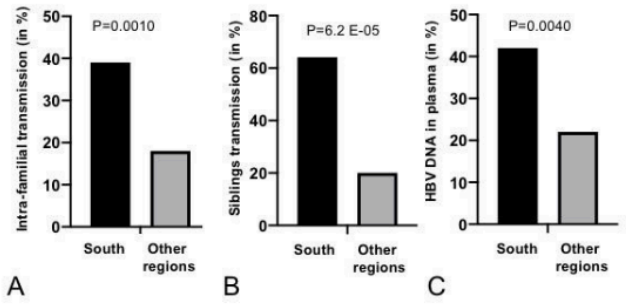

Figure 2. Epidemiological features of dually infection (A) Intra-familial transmission with a a statistically significant difference between inhabitants from southern areas and other regions, (B) Siblings transmission comparisions reffering to regions, (C) Virological parameters – with a HBV DNA more prevalent in South region.

DISCUSSION

The burden of viral hepatitis has historically been considered as worrisome in Moldova. Since the 1990s decade, HBsAg seroprevalence in Moldova has been reported regularly [13-16].

Since then, fortunately, a continuous decrease in the incidence of acute viral hepatitis B, C, and D has been observed in Moldova [14]. However, despite this apparent improvement, morbidity linked to severe forms of chronic viral hepatopathy, including liver cirrhosis and HCC, has increased concomitantly [10]. From 2000 to 2011, the prevalence of chronic hepatitis D in patients with chronic liver diseases reached 4.5%, set on a high background of persistent HBV infection (58.2%). In the same period, the multi-year morbidity from chronic viral hepatitis Delta increased from 12.6 to 38.6 cases per10^{-6}. The prevalence of liver cirrhosis caused by the delta virus doubled from 4.86 cases in 2000 to 10.22 cases per 10^{-6} residents in 2011. In parallel, HBV-related liver cirrhosis increased from 16.4 in 2000 to 57.9 per10^{-6} habitants in 2011. Most affected patients infected with HBV alone or with HDV were adults, a situation presumably due to the implementation of universal anti-hepatitis B immunization in 1994 and the time taken by these diseases to become symptomatic. In the local health system, only symptomatic patients are still currently registered, while HDV screening of HBsAg carriers remains at the discretion of the physician and thus remains relatively low. Furthermore, the lack of multicentre studies on Delta infection in the country prevents any clear national appraisal of the problem. Nevertheless, understanding the prevalence of HDV infection is essential to measure the extent of the problem, anticipate its future impact, and to secure adequate funding from the national health system. Nevertheless, data about the extent of hepatitis delta endemicity remain scarce just as molecular characterization of circulating strains [17].

The evolution of hepatitis delta is known to be remarkably heterogeneous and its determinants are still incompletely understood. Concerning the clinical presentation of the disease in Moldova, chronicity did not inevitably progress towards greater severity, as patients with chronic hepatitis and cirrhosis displayed roughly the same age (48 years). Furthermore, symptomatic Moldovan patients were older than patients from Eastern Turkey (36-44 years), from Germany (40 years) or from Greece (43 year) [18-20].

Regarding the biological features of Moldovan patients with a decompensated form of HDV-induced liver cirrhosis, they tended to be similar (Child score, Albumin, GGT, INR, Hb) or milder (bilirubin, ALT, AST) than those reported by Gheorghe *et al.* in Romania, very close to disease presentation described by Bahcecioglu et al. in Eastern

Turkey [21, 22]. Biological parameters were almost universally more deteriorated in case of dual infection compared to mono-infected.

With regards to viral parameters, PCR detection of HDV RNA was more frequent in the current series (88-99%) than in those from Eastern Turkey (37-61%) with no difference in detection rates between chronic hepatitis patients and cirrhotics [22]. This rate was also higher that reported in a German study, which included patients from Western and Eastern Europe, as well as Turkey (61-69%) (Heidrich, 2009, J Viral hepatitis). It was very similar to the rate reported recently in the global survey by Wranke et al. (85%) [12]. The replicative dominance of HDV over HBV, as seen in Romania or Greece, is observable at every stage of the chronic infection [20,23]. The detection rate of HBV DNA was much lower in Moldova (28%) than in Eastern Turkey (51%) or in the global survey (60%) but somewhat higher than in German patients (16%) [12,19]. This alines with the complete absence of HBeAg(+) patients in Moldova. HBV DNA loads were lower in Moldovan in anti-HDV(+) cirrhotics compared to their Turkish counterpart (2.8log10 IU/mL vs 3.6log10 IU/mL) and similar to those observed in Greece for all stages of chronic infection in adults (2.8log10 IU/mL) [22]. In contrast to Eastern Turkish liver cirrhotics, HBV DNA loads were lower in co-infected patients than in mono-infected ones, further emphasizing HDV dominance over HBV [22].

Most published studies in Moldova have included only a small number of cases with different medical conditions, resulting in patchy information. Additionally, there is national heterogeneity in HDV infection, with some areas being hyperendemic (the southern part) and others apparently less affected (the north of the country). As aresult, the transmission routes of HBV and HDV, which contribute to the current challenging situation, remain poorly defined in Moldova. Our work is the first attempt to dissect the contribution of various transmission modes. In contrast to the description of HDV-infected patients in Romania, the principal risk factor in our series was contact with an infected family member (around 40%). In Romania, however, risk factors were primarily tattoos/piercings (35-40%) and iatrogenic factors (transfusions, endoscopies, use of glass syringes, 29-37%) [24]. The conditions of HDV transmission in Moldova are, however, quite different from those observed in southeastern Turkey, where family transmission is overwhelming (79%), but rather similar to Greece, where the family transmission accounts for 57% of the routes identified [20,22]. Therefore, the Moldovan pattern appears to be a mix between endemic countries, where transmission is primarily familial, and non-endemic situations, where contamination occurs more frequently through iatrogenic accidents or intravenous drug use [25-28]. It is possible that the endemicity of HDV is currently evolving in Moldova, similar to what occurred in Italy, with

a higher proportion of older, indolent cases benefiting from the so-called “survival effect” [29].

CONCLUSION

Our analysis shows that in Moldova, hepatitis delta is primarily transmitted between members of the same family. This mode of transmission, which affects primarly the inhabitants of Southern Moldova, is associated with earlier development of liver cirrhosis. Appropriate measures should be taken urgently to prevent further contaminations within concerned families and to prevent disease progression in already infected individuals. Timely linkage to determination, care and treatment can reduce long-term liver-related morbidity and mortality.

CONFLICT OF INTEREST

None declared.

AUTHOR’S CONTRIBUTIONS

Conceptualization, A.T. and P.P.; methodology, A.T., P.P.; software, O.S., E.C.; validation, A.T., P.P. ; formal analysis, O.S.; investigation, A.T., O.S., E.C.; resources, E.C.; data curation, O.S.; writing—original draft preparation, A.T., O.S., E.C.; writing—review and editing, O.S., E.C; visualization, O.S.; supervision, A.T.; project administration, A.T. All authors have read and agreed to the published version of the manuscript.

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