Incidence and prevalence of acute hepatitis E virus infection in patients with suspected Drug-Induced Liver Injury in the Spanish DILI Registry

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List of abbreviations:

DILI: drug-induced liver injury

HEV: hepatitis E virus

Ig: immunoglobulinAg: antigen

ELISA: enzyme-linked immunosorbent assay

RT-PCR: reverse transcription polymerase chain reactionOR: odds ratio

AST: aspartate aminotransferaseHAV: hepatitis A virus

HBV: hepatitis B virusHCV: hepatitis C virus

ALT: alanine aminotransferase

ALP: alkaline phosphatase

TBL: total bilirubinINR: international normalization ratio

ULN: upper limit of normal

EASL: European association for the study of the liver

DILIN: drug-induced liver injury network

ALF: acute liver failure

Declaration of interests

All authors have no conflict of interests to disclose.

Ethics approval and patient consent statement

The use of samples for research was approved by the local Ethics Committee of the coordinating center at the Virgen de la Victoria University Hospital in Malaga. All patients gave written informed consent prior to collection of biological samples.

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ABSTRACT

Background and Aims: Drug-induced liver injury (DILI) presents with a wide phenotypic spectrum requiring an extensive differential diagnosis. Hepatitis E virus (HEV) is notsystematically ruled out during acute hepatitis assessment in Spain. The aims of this study were to establish the role of HEV infection and its phenotypic presentation in patients initially suspectedof DILI and to determine the anti-HEV seroprevalence rate.

Methods: An analysis of 265 patients with suspected DILI and considered for enrolment in the Spanish DILI Registry and 108 controls with normal liver profiles was undertaken. Anti-HEV Immunoglobulin (Ig) G antibodies were analyzed in serum from all subjects. In those with serum samples extracted within 6 months from liver damage onset (n=144), HEV antigen (Ag) and anti- HEV IgM antibodies were tested in duplicate by ELISA. In addition, RT-PCR was performed externally in 8 patients.

Results: Out of 144 patients, 12 (8%) were positive for anti-HEV IgM, mean age 61 years. Underlying hepatic diseases (OR=23.4, $p<0.001$) and AST peak >20 folds upper limit of normal (OR=10.9, $p=0.002$) were associated with the diagnosis of acute hepatitis E. The overall anti-HEVIgG seroprevalence rate was 35%, evenly distributed between patients with suspected DILI (34%),and controls (39%).

Conclusions: HEV seroprevalence and acute hepatitis E rates are relatively high in Spain. Asearch for active HEV infection is therefore advised in patients assessed for suspicion of DILI, particularly in patients with underlying liver diseases and high transaminase levels.

KEYWORDS: drug-induced liver injury, acute hepatitis assessment, acute hepatitis E virus infection, seroprevalence rate.

LAY SUMMARY

- HEV seroprevalence and acute hepatitis E rates are relatively high in Spain.
- A search for active HEV infection is therefore advised in patients assessed for suspected DILI.

INTRODUCTION

Drug-induced liver injury (DILI) can mimic any other liver disease in its clinical presentation. DILI therefore requires an extensive differential diagnosis to rule out other possible causes of liverinjury such as viral hepatitis A (HAV), B (HBV) and C (HCV), biliary obstruction, autoimmune hepatitis, among others, in addition to the presence of a compatible chronology between drug intake and onset of liver damage.

Hepatitis E virus (HEV) is not routinely ruled out during acute hepatitis assessment even though common symptoms are usually indistinguishable from other forms of viral hepatitis. In fact, there are many centers where serological tests for HEV are locally unavailable. In fact, HEV infection has been shown to masquerade as DILI in previous studies.[1,2]

HEV infection has traditionally been considered epidemic in developing countries, while in industrialized countries it is regarded as sporadic and usually related to traveling to endemic zones (imported cases).[3] However, over the last years a significant number of autochthonous cases have been described in developed countries and acute hepatitis E is now considered an emerging disease.[4] In some areas, for example Scotland, it is now considered a major cause of acute viral hepatitis.[5] In industrialized countries, acute hepatitis E is considered a zoonotic disease, andconsumption of raw or undercooked pork products is believed to be an important risk factor for acute hepatitis E in Europe.[6] Other routes of transmission include transfusion of infected blood products[7] and solid organ transplantations.[8]

The reported seroprevalence rate of anti-HEV immunoglobulin (Ig) G, indicating previous infection, varies between 0.03% and 52.2% in European countries, depending on geographical areas and diagnostic assays used.[9,10] In Spain, a seroprevalence rate of 20% has been reported in the general population (blood donors).[11]

In this study, we aimed to identify the rate of acute hepatitis E in Spanish patients initially suspected of DILI and to establish anti-HEV seroprevalence among these patients and controls.We also aimed to characterize the phenotypic presentation of suspected DILI patients having a final diagnosis of HEV infection.

METHODS

Two hundred and sixty-five patients with acute liver damage and suspected DILI submitted to the Spanish DILI Registry to be considered for enrolment as well as 108 controls with normal liver profiles were included in the study. Patients were identified prospectively for enrolment into the Spanish DILI Registry, and stored blood samples at the Biobank were used. The time of collection with regards to liver injury onset varied between the samples, as prior to 2016 they were mainly obtained for DNA analyses. All samples from cases identified between 2016 and 2018 were collected prospectively for the purpose of this study.

After evaluation by a panel of DILI experts, 193 patients were adjudicated as DILI and 72 were excluded as drug-related liver injury due to insufficient information to establish a diagnosis of DILI or not presenting a suggestive temporal relationship

between drug intake and onset on liver damage. As IgM antibodies are relatively short-lived,[2,12] only 144 patients with samples obtain within the first six months from liver injury detection were selected for HEV IgM and HEV Antigen (Ag) serological evaluation. This group consisted of 89 patients adjudicated as DILI and 55 patients excluded as having drug-related liver injury. The remaining 121 patients with liver profile elevations and samples obtained more than six months from liver injury recognition were only tested for anti-HEV IgG (Figure 1).

The use of samples for research was approved by the local Ethics Committee of the coordinating center at the Virgen de la Victoria University Hospital in Malaga. All patients gave written informed consent prior to collection of biological samples.

HEV serological evaluation

Serum samples were stored at -80ºC from the time of extraction until analyses. Anti-HEV IgG, anti-HEV IgM and HEV-Ag were tested in duplicate by ELISA (Wantai, Beijing Wantai Biological Pharmacy Enterprise Co., Ltd) following the manufacturer's instructions. All samples were tested for anti-HEV IgG and the samples obtained within the first six months from the onset of liver damage were also tested for anti-HEV IgM and HEV-Ag. Those positive for anti-HEVIgM were confirmed using immunoblot (recomBlot HEV IgM, Mikrogen Diagnostik GmbH). In addition, RT-PCR was performed externally in eight patients due to decisions made by thephysician in charge.

A diagnosis of acute hepatitis due to HEV was given to those cases with increased transaminases and fulfilling at least one of the following criteria: anti-HEV IgM positive (+), HEV-Ag positiveor HEV RNA positive in blood samples collected up to six months from the first detection of transaminase elevations.[2,13]

Severity

The severity of each case was assessed using the DILI severity index.[14] Cases were classified as,mild: elevated alanine transaminase (ALT)/alkaline phosphatase (ALP) with total bilirubin <2 mg/dL;moderate: elevated ALT/ALP with total bilirubin (TBL) ≥2 g/dL; severe: elevated ALT/ALP andone of the following: ascites, encephalopathy, international normalization ratio (INR) >1.5 and/orother organ failure considered to be due to DILI; fatal: death or liver transplantation due to DILI.

Pattern of liver injury

The pattern of liver injury (hepatocellular, cholestatic and mixed) was determined by calculating the ratio (R) of ALT to ALP from the first available blood analysis after DILI recognition, using multiples of the upper limit of normal (ULN) for both values.[14]

Statistical analyses

Variables were examined using descriptive statistics. Continuous variables were expressed as means or medians and were compared using the Student's t test or ANOVA. In those variables which did not follow a normal distribution, nonparametric analyses (Mann-Whitney U-test or Kruskal-Wallis) were used. Differences were reported as statistically significant if the *p* value was less than 0.05. Variables that were associated

with acute hepatitis E in univariate analyses were included as potential covariates in a multiple logistic regression model. Statistical analyses were performed using SPSS 19.0 (IBM Corp, Armonk, NY).

RESULTS

The 265 initially suspected DILI patients (193 adjudicated as DILI and 72 excluded as drug- related liver injury) and 108 controls were compared with regards to demographics and medical history. No significant differences were found between the three groups, except for the absence of underlying liver diseases in the control group (Table 1).

Determination of anti-HEV IgM and HEV-Ag

Of the 265 patients with acute liver damage, 144 had samples obtained within 6 months fromonset of the episode. Of these, 12 (8%) patients tested positive for anti-HEV IgM (ELISA (Wantai)) and were confirmed by immunoblot (recomBlot HEV IgM). There was no discrepancy between the two tests. Nine of the 12 patients were adjudicated as DILI patients, while the remaining three patients were adjudicated as having non-drug-related liver injury (Table 2). HEV-Ag was positive in three of the 144 patients analyzed (2.08 %), and RNA HEV was positive in two of the eight patients tested (25%).

In order to identify differences between DILI and acute hepatitis E, demographics and clinical parameters were compared between the two groups. No significant differences were found in age or gender between patients with acute hepatitis E (anti-HEV IgM+) and adjudicated DILI patients negative for anti-HEV IgM (anti-HEV IgM-). Concerning medical history, we found a higher percentage of patients with underlying hepatic diseases (42% vs 5%, p=0.009) and a previous solid organ transplant (27% vs 0%, p=0.001) in the group with acute hepatitis E in comparison with DILI patients. Laboratory tests showed that median peak values of ALT and aspartate aminotransferase (AST) expressed in ULN were higher in the former group (31 vs 11, p=0.018 and 30 vs 6, p=0.055 respectively). No differences in TBL or ALP values were found between the groups. Similarly, no differences in pattern of liver injury or severity were detected between the groups (Table 3). In a logistic regression model, underlying hepatic diseases and AST peak >20 x ULN were found to be associated with acute hepatitis E (OR=23.4; 95% CI 3.9-205, p<0.001 and OR=10.9; 95% CI 2.4-77, p=0.002, respectively).

Out of 12 patients with acute hepatitis E, 11 were Caucasians and one Arab. These patients had a mean age of 61 years with a slight predominance of males (58%). The majority of patients (58%) had major comorbidities including liver cirrhosis (two), alcoholic liver disease (one), prostate cancer (one) and other important medical conditions (two patients with medical history of liver transplantation and one patient with a previous lung transplantation, all in whom graft rejection was ruled out). Most of the patients (83%) were symptomatic, and only two patients had asymptomatic liver profile elevations. Clinical symptoms included abdominal pain, nausea, anorexia, asthenia, choluria, jaundice, pruritus, joint pain, diarrhea, myalgia, fever, malaise and acute kidney injury. According to severity, two (17%) were mild, seven (58%) were moderate, two (17%) were severe and one (8%) required a liver transplantation. Of the 12 patients tested

for HEV-Ag, only three (25%) were positive. HEV RNA was tested in eight patients, of whom two (25%) were found to be positive. Summarized information on demographics, clinical data and laboratory parameters are shown in Table 4.

Determination of anti-HEV IgG

Out of 265 suspected DILI patients, 89 (34%) were positive for anti-HEV IgG. Of the 108controls, 42 were positive for anti-HEV IgG, which results in a seroprevalence of 39% in this group. The overall seroprevalence rate in the total study cohort was 35%.

Comparing clinical characteristics according to the presence of anti-HEV IgG, patients positive for anti-HEV IgG were older than those being negative (mean age 59 vs 49, respectively, p<0.001). Furthermore, the group positive for anti-HEV IgG presented a higher proportion of patients with hypertension (32% vs 22%, *p*=0.040), dyslipidemia (20% vs 12%, *p*=0.015) and solid organ transplantation (3.1% vs 0%, *p*=0.015) compared with the anti-HEV IgG negative group (Table 5).

DISCUSSION

Hepatitis E can masquerade as DILI and is an increasingly recognized etiology of liver injury in Western countries. It is therefore a potential alternative cause when assessing DILI cases. Indeed, it is likely that many patients with HEV infection are taking medications at the time of the viral infection due to the common use of pharmacological treatments nowadays. Consequently, a HEV infection in conjunction with unassociated medical treatments prior to HEV infection symptoms can initially be mistaken as potential DILI.

The choice of method to detect HEV infection is an important factor in assessing incidence and seroprevalence. In our study we used commercial Wantai HEV ELISA assays to determine IgG, IgM and Ag based on sensitivity and specificity data found in the literature.[13,15,16] The Wantai commercial assays are the most frequently used assayas in published HEV studies as reported in a recent review.[16] Using these assays therefore allows us to make a better comparative evaluation with previously published results. In addition, we used a second method (immunoblot) for confirmatory testing. In the current study we determined the presence of HEV-Ag rather than HEV RNA as several studies have demonstrated good concordance between HEV-Ag and HEV RNA.[13,17] In addition, serology testing for HEV-Ag is recommended by the EASL clinical practical guidelines on hepatitis E virus infection.[18]

Although information with regard to incidence of acute hepatitis E in many countries is limited, as only certain countries have active surveillance, the number of reported cases in the European Union/European Economic Area (including countries with specific surveillance systems for HEV detection) has increased from 514 cases per year in 2005 to 5,617 in 2015, with most infections being locally acquired.[6]

In the present study, 8% of the patients with suspected DILI tested positive for anti-HEV IgM. We have considered these cases with positive anti-HEV IgM as recent hepatitis E despite the fact that some of them had negative HEV-Ag or RNA, because the samples were obtained up to six monthsfrom onset of liver injury. Hence, while some samples

were obtained shortly after clinical onset others were obtained several months after. Those samples obtained at a later phase may have passed the stage of viremia and antigenemia, despite being positive for HEV IgM. In fact, HEV RNA and Ag have been reported to only be detectable in blood up to four to eight weeks after clinical onset.[19] Thus, if a patient is sampled late in the symptomatic phase of illness, a negative HEV RNA or Ag result in the blood does not exclude a recent infection.

The proportion of anti-HEV IgM positive patients in the context of DILI varies among studies conducted to date. In a North American prospective cohort of patients included in the Drug- Induced Liver Injury Network (DILIN), 3% of patients had serological evidence of acute hepatitis E. On the other hand, a smaller retrospective study performed on a cohort of suspected DILI patients from UK found as much as 12.7% of the patients being positive for HEV.[1] Differences between HEV infection rates can be explained by variations in HEV distribution between demographic areas, but also by differences in HEV suspicion and subsequent search strategy for the infection. These findings support the need for acute HEV infection testing during DILI assessment. The current study supports that HEV should be ruled out even in cases with a high suspicion of DILI and the presence of a compatible temporal relationship between drug intake and liver injury in order to avoid misdiagnosis. In fact, it is advisable to test for HEV in all patients with acute hepatitis.

In our cohort of patients diagnosed with acute hepatitis E the mean age was 61 years, which is slightly lower than those of hepatitis E patients in cohorts from the US and UK with mean ages of 64 and 67 years, respectively.[1,2] Similarly, a previous study of HEV in Spain found that the patients had a mean age of 65 years at the time of the diagnosis.[20] Based on data from published cases to date in industrialized countries, acute hepatitis E seems to occur more frequently in older patients.

In the current cohort of patients with acute hepatitis E, we found a slight predominance of males (58%), which is similar to the proportion reported by Aspinall et al (61%-69%) among acute HEV patients from 17 European countries over the time period 2005-2015.[6] Regarding pattern of liver damage, our acute hepatitis E patients had predominantly hepatocellulartype of liver injury, presenting higher AST and ALT peak values than the adjudicated DILI patients. Additional studies have also found that patients diagnosed with acute hepatitis E present higher levels of ALT and ALT/ALP ratio compared with confirmed DILI cases.[1] Hence, acute HEV infection should be considered together with other forms of viral hepatitis, in suspected DILIpatients, particularly in the presence of important aminotransferase elevations.

Although most cases have a favorable outcome, HEV can lead to acute liver failure (ALF) as reported for up to 10% of hepatitis E patients in developed countries.[21,22] In our study we found one case that required liver transplantation (8%). This supports the importance of testing for hepatitis E in patients with severe liver injury, especially in cases with high level of transaminases,[23] and/or patients with major underlying medical conditions. Our findings suggest that dyslipidemia and solid organ transplantation comorbidities with their associated comedications can play a role in susceptibility to symptomatic hepatitis

E. Co-morbidities such as alcoholism, subclinical hepatic steatosis, fibrosis,[24] human immunodeficiency virus and chronic obstructive pulmonary disease, among others,[2] have also been proposed as possible risk factors for symptomatic acute HEV infection in previous studies. This is supported by our findings with 25% of the anti-HEV IgM positive patients having undergone previous organ transplantations, whereas in the anti-HEV IgM negative group there were no transplanted patients. Our Registry, unlike the DILIN, is not restricted to the inclusion of non-transplanted patients and this fact must be taken into consideration when comparing the prevalence of HEV infection across DILI Registries. Other studies consider underlying liver disease and immunosuppressed conditions to be prognosticfactors for HEV infection rather than risk factors.[25] Hence, such underlying conditions could increase the risk of having a more severe episode as well as a worst outcome. In fact, only one patient with acute hepatitis E in our cohort developed ALF that required a liver transplantation and this patient had also been previously transplanted. In a British/French study carried out in patients with decompensated chronic liver disease, 3.2% had acute hepatitis E, of whom 27% had a fatal outcome.[26] Similarly, in the largest Spanish cohort of acute hepatitis E reported to date, 20% of deaths were found among patients with underlying liver disease.[20] These studies as well as the current study support that patients with chronic liver disease seem to be more susceptible to developing clinical manifestations and a more severe clinical course of HEV infection. It istherefore important that HEV is ruled out in these patients when signs or symptoms of liver decompensation are present in order to provide optimal patient care.

In our study, we found similar anti-HEV IgG seroprevalence rates for patients with suspectedDILI and controls (34% and 39%, respectively). This high seroprevalence rate suggests that hepatitis E infection can present as an asymptomatic infection or with mild symptoms in many patients. Furthermore, the anti-HEV IgG seroprevalence rate in our study is higher than the last reported seroprevalence rate in Spain of 20% found in a large cohort of Catalonian blood donors in 2015.[11] However, it should be pointed out that the Catalan study included a larger cohort than in our study. Furthermore, the differences may also depend on the geographical location. Nevertheless, this cannot exclude a potential increase in anti-HEV IgG seroprevalence rate inSpain over the last years. In a neighboring country, France, where hepatitis E is considered endemic, the estimated anti-HEV IgG seroprevalence is 22.4%, ranging from 8% to 86% depending on demographic area.[27] The seroprevalence in Spain appears to be comparable with thatof France. Patients with positive anti-HEV IgG were older and presented a higher proportion of hypertension or dyslipidemia, which are typically age-related comorbidities. Confirmatory studies need to be performed in order to provide information not only on risk factors of hepatitis E infection, but also on anti-HEV IgG seroprevalence, incidence rate, transmission mechanisms and viral sources.

A limitation in our study is that we used a cut-off point of six months for analyzing the presence ofacute hepatitis E markers. It is well known that strong positive results of anti-HEV IgM are rare after 3-4 months from clinical onset.[12] In contrast, a very recent report describes positive anti-HEV IgM persisting more than one year after onset of symptoms

in a small percentage of patients,[28] so the prevalence of acute infection in our study could be underestimated.

In conclusion, HEV is nowadays highly present in Spain, and also in other developed countries, which argue strongly for the need of further studies to enhance the understanding of HEV transmission and subsequent prevention. Exclusion of acute hepatitis E should be considered in DILI causality assessment similarly to HAV, HBV and HCV infections and incorporated into clinical practice guidelines and recommendations. Patients with acute hepatitis E exhibit higher peak AST elevations compared with DILI patients. Immunosuppressed patients and patients with underlying liver disease appear to be more susceptible to acute hepatitis E.

Authorship Statement

Guarantor of the article: Dr Raúl J Andrade.

Author contributions: JSC, RSJ and MRD contributed to the concept and design of the study. MGC, IMC, MRD, AOA, JSC, MJP, RGG, RSJ and AGJ were responsible for the acquisition of data. RSJ conducted the laboratory analysis. JSC conducted the data analysis. MRD, CS, MIL and RJA supervised the study. JSC, RSJ, MRD, MIL, CS and RJA wrote the manuscript. MRD, CS, RJA, MIL critically revised the manuscript for important intellectual content. All authors have read, revised, and approved this version of the manuscript and are prepared to take public responsibility for its content.

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Figure 1. Flow chart of patients included in the study and serological test performed.

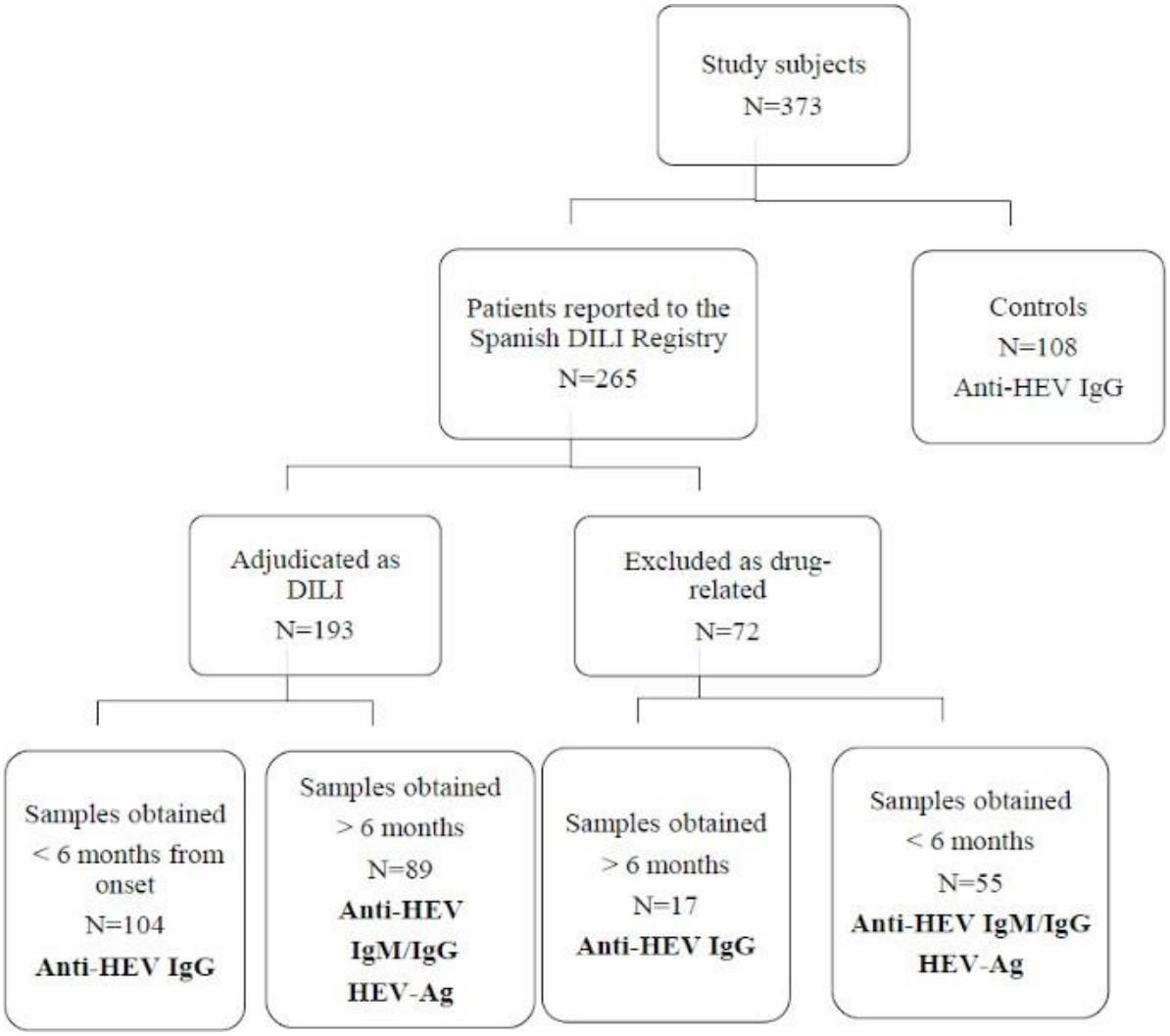

Table 1. Comparison of demographics and medical history among the different study groups: adjudicated as drug-induced liver injury (DILI), excluded as drug-related and controls.

	Adjudicated DILI (N=193)	**Excluded as drug-related** (N=72)	**Controls** (N=108)	**p value**
Age (y), mean ± SD	53 ± 18	54 ± 18	49 ± 17	0.090
Female, n (%)	86 (45)	41 (57)	60 (55)	0.091
Comorbidity, n (%)				
Arterial Hypertension	49 (28)	16 (22)	24 (22)	0.402
Diabetes	20 (10)	8 (11)	10 (9.3)	0.900
Dyslipidemia	30 (16)	9 (13)	15 (14)	0.805
Underlying hepatic diseases	16 (8.3)	11 (16)	0	<0.001
Chronic kidney disease	3 (1.6)	2 (2.8)	2 (1.9)	0.876
Autoimmune disease	16 (8.3)	9 (13)	4 (3.7)	0.090
Hypothyroidism	13 (6.7)	5 (6.9)	3 (2.8)	0.306
Neoplasia	11 (5.7)	4 (5.6)	4 (3.7)	0.727
Solid organ transplant	2 (1.0)	2 (2.8)	0	0.177

SD: standard deviation; y: years

Table 2. Acute hepatitis E virus (HEV) infection (positive anti-HEV IgM, Ag or HEV RNA) in 144 patients with samples obtained within 6 months after onset of the episode and anti-HEV IgG seroprevalence in 265 suspected DILI patients and 108 controls.

	Suspected DILI patients	**Controls**
Anti-HEV IgM +, n (%) (N=144)	12 (8.3)[†]	NA
HEV Ag +, n (%) (N=144)	3 (2.08)[‡]	NA
HEV RNA, n (%) (N=8)	2 (25)[§]	NA
Anti-HEV IgG +, n (%)	89 (34) N=265	42 (39) N=108

Ig: immunoglobulin; Ag: antigen; NA: not applicable

[†] 9 cases adjudicated as DILI and 3 cases excluded as drug-related

[‡] All cases adjudicated as DILI

[§] All cases adjudicated as DILI

Table 3. Comparison of demographics, clinical, and laboratory parameters between acute hepatitis E (IgM +) and adjudicated DILI patients IgM- with samples collected within 6 months from onset of liver injury.

	Acute hepatitis E IgM+ (N=12)	**Adjudicated DILI IgM-** (N=80)	**p value**
Age (y), mean ± SD	61 (16)	55 (19)	0.222
Female, n (%)	5 (42)	34 (43)	0.957
Comorbidity, n (%)			
Arterial hypertension	5 (42)	22 (29)	0.501
Diabetes	3 (25)	9 (11)	0.590
Dyslipidemia	4 (33)	8 (10)	0.047
Underlying hepatic diseases	5 (42)	4 (5.0)	0.009
Chronic renal disease	0	3 (3.8)	1.000
Autoimmune disease	1 (8.3)	2 (2.5)	0.324
Hypothyroidism	0	8 (10)	0.590
Neoplasia	1 (8.3)	6 (7.5)	1.000
Solid Organ transplant	3 (27)	0	0.001
Pattern of damage, n (%)			
Hepatocellular	8 (67)	39 (50)	0.654
Cholestatic	3 (25)	23 (29)	
Mixed	1 (8.3)	16 (21)	
Severity, n (%)			
Mild	2 (16)	21 (27)	0.103
Moderate	7 (58)	52 (66)	
Severe	2 (16)	6 (7.6)	
Fatal/Liver transplantation	1 (8.3)	0	
Laboratory parameters at peak, x ULN, median (IQR 25-75)			
TBL	8 (3-17)	8 (2-14)	0.943
ALT	31 (16-68)	11 (6-24)	0.018
AST	30 (3-53)	6 (3-19)	0.055
ALP	2 (2-3)	2 (1-2)	0.171

TBL; total bilirubin; ALF: acute liver failure; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; IQR: interquartile range; SD: standard deviation; ULN: upper limit of normal; y: years; Ig: Immunoglobulin

Table 4. Laboratory (onset) and clinical data of patients with acute hepatitis E.

Case	Age/Sex	Medical history	Suspected drug	Days from drug intake to onset	Treatment (days)	TBL (IU/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Liver injury pattern (liver biopsy findings)	Severity
1	49 / F	No relevant medical history	Paracetamol	1	1	3.1	1657	1063	378	Hep	Moderate
2	74 / M	DM, AH, acute myocardial infarction	Cefditoren	41	6	4.7	4191	2702	166	Hep (cholestasis and submassive necrosis)	Severe
3	26 / F	LTx due to Wilson disease, retransplantation due to liver ischemia	Dexketoprofen	5	7	13.4	1178	1561	29	Hep (liver explant, submassive necrosis, regeneration nodules and severe cholestasis)	Fatal/LTx
4	71 / F	AH, osteoarthrosis	Isoniazid	33	41	2.4	699	239	229	Hep	Moderate
5	35 / M	No relevant medical history	Erythromycin	30	3	5.8	2469	969	195	Hep	Moderate
6	75 / M	LTx due to alcoholic disease, AH, COPD	Amoxicillin	4	7	8.5	2517	1685	NA	Hep (cholestatic hepatitis)	Moderate
7	75 / M	AH, DM, DLP, COPD, AF, prostate cancer, cirrhosis or unknown etiology	Amoxicillin-clavulanate	77	10	16.8	668	1000	420	Chol	Severe
8	63 / M	AH, DM, DLP, obesity, moderate alcohol drinking	Herbal products	1	30	8.0	1004	339	286	Hep	Moderate
9	60 / M	No relevant medical history	Amoxicillin-clavulanate	30	10	7.9	3866	1832	276	Hep	Moderate
10	63 / M	Lung transplantation, alcohol liver disease	Voriconazole	5	15	NA	103	49	285	Chol	Mild
11	64 / F	Cirrhosis of unknown etiology	Amoxicillin-clavulanate	28	7	5.5	104	112	300	Chol	Moderate
12	71 / F	AF, DLP, hyperthyroidism	Carbimazole	203	211	0.9	386	125	339	Mix	Mild

M: Male; F: Female; DM: Diabetes mellitus; AH: Arterial Hypertension; COPD: Chronic obstructive pulmonary disease; DLP: Dyslipidemia; AF: Atrial fibrillation; LTx: Liver transplantation; Hep: Hepatocellular; Chol: Cholestatic; Mix: Mixed; NA: not available

Table 5. Comparison of demographics and clinical data from 373 subjects (265 suspected DILI patients and 108 controls) according to the presence or absence of anti-HEV IgG antibodies.

	Anti-HEV IgG+ (N=131)	**Anti-HEV IgG-** (N=242)	**p value**
Age (y), mean ± SD	59 ± 16	49 ± 18	<0.001
Female, n (%)	57 (44)	130 (54)	0.060
Comorbidity, n (%)			
Diabetes	17 (13)	21 (8.7)	0.182
Arterial hypertension	39 (32)	50 (22)	0.040
Neoplasia	7 (5.3)	12 (5.0)	0.872
Hypothyroidism	9 (6.9)	12 (5.0)	0.445
Underlying hepatic diseases	13 (10)	16 (6.6)	0.254
Chronic renal impairment	1 (0.8)	6 (2.5)	0.429
Autoimmune diseases	13 (10)	16 (6.6)	0.311
Dyslipidemia	26 (20)	28 (12)	0.015
Solid organ transplant	4 (3.1)	0	0.015

SD: standard deviation; y: years