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RESEARCH ARTICLE

Prevalence of Hepatitis E Virus in Patients with Chronic Hepatopathy in a Reference Center of Bahia State, Brazil

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ABSTRACT

Introduction: Hepatitis E virus infection can lead to severe liver disease and unregulated hepatitis in patients with a history of previous chronic liver disease of different etiologies.

Objective: To determine the seroprevalence of hepatitis E in untreated carriers of hepatitis C virus, hepatitis B virus, autoimmune hepatitis and in patients with drug-induced liver disease.

Materials and Methods: This is a cross-sectional study with a total sample of 301 outpatient hepatology volunteers. The detection of anti-HEV IgM and IgG antibodies was determined using the ELISA (RecomWell anti-HEV IgG and IgM, Mikrogen®, Germany), One-step real-time PCR was used for the detection of HEV-RNA (Taqman, Life Technologies TM, Foster City, CA, USA).

Results: The overall prevalence of anti-HEV IgG and IgM in the population studied was 12.95% and 2.3%, respectively. The prevalence by group were: anti-HEV IgG: hepatitis C virus with 13.2%, hepatitis B virus with 13%, autoimmune hepatitis with 8.1 and drug-induced liver disease with 21.1. Patients with HCV chronic hepatitis and positive HCV IgG serology showed an increase in transaminase levels in 66.7% (10/15) of the cases, while in the seronegative patients this increase was present in 42.4% (42/99) of the cases ($p<0.05$).

Conclusions: A high seroprevalence of HEV was observed. Among the patients with serology concomitantly positive for anti HEV IgG and IgM, 57.1% (4/7) had higher levels of TGO and TGP, suggesting acute HEV infection. A relationship between hepatitis C and E virus co-infection and elevated transaminase levels has been demonstrated. Future studies with evaluation of several clinical parameters are necessary.

Keywords: Hepatitis E, Hepatitis B, Hepatitis C, Drug-induced liver disease, Autoimmune hepatitis.

INTRODUCTION

The main cause of acute hepatitis in the world is the infection by hepatitis E virus (HEV) [1]. The World Health Organization (WHO) estimates that 20 million people become infected with HEV each year, with 3 million symptomatic cases and 56,600 cases of HEV-related deaths [2].

Brazil is considered a region with moderate endemicity, with seroprevalence rates ranging from 0.3 to 19.5 [3-12]. Published studies show a variety in prevalence, related to the diversity in the population and region studied in addition to the different methodologies used for diagnosis.

Hepatitis E was considered a curable, self-limiting, and acute infection. Although potentially serious in some situations, especially in pregnant women [13,14], there was no relationship with chronification and its risks [15]. However, published studies demonstrate the occurrence of chronic hepatitis E in different groups of patients, especially in individuals with some degree of immunosuppression [4,16-19]. Patients with autoimmune hepatitis (AIH) due to immunosuppressive treatment have an increased risk of developing a chronic E virus infection [20].

Studies demonstrate high rates of morbidity and mortality associated with HEV infection in patients with liver cirrhosis [9] and co-infected with other hepatotropic viruses [10,11]. The consequences of HEV infection in patients with chronic infection with other viruses and hepatic manifestations are not fully understood. Furthermore, in most countries there is no vaccination against HBV and hepatitis A (HAV) viruses, making patients susceptible to co-infection and worsening the clinical outcome of the disease. The objective of this study is to determine the prevalence of HEV in different groups of patients, and its relation to clinical, sociodemographic and lifestyle parameters

METHODOLOGY

STUDY DESIGN AND POPULATION

A cross-sectional study was conducted on a calculated sample of 301 volunteers. The sample size calculation was performed considering a prevalence of 9.8% (15), using the Epi Info TM software (CDC, Atlanta, USA -www.cdc.gov).

Volunteers were recruited at the outpatient clinic of University Hospital Professor Edgard Santos of Bahia University, Salvador City, between 2018 and 2019. All participants signed the Free and Informed Consent Form. The research protocol was previously approved by the HUPES ethics committee (Opinion Number: 2.368.68/CAAE: 61187416.7.3001.5029).

INCLUSION CRITERIA

The volunteers included in the study were: untreated patients diagnosed with chronic hepatitis C, chronic hepatitis B with HBeAg and anti-HBe positive and not on antiviral treatment, autoimmune hepatitis and drug-induced hepatitis (DILI).

EXCLUSION CRITERIA

Patients with HCV/HBV, HCV/HIV, HBV/HIV co-infection were excluded from the study.

SAMPLE COLLECTION

Blood samples were collected in 10 mL BD Vacutainer® (Becton, Dickinson and Company, Franklin Lakes, USA) tubes without anticoagulant. They were then centrifuged at 3,000 rpm for 15 minutes in a refrigerated centrifuge and later aliquoted and stored at -70°C until handling. Data on oxalacetic transaminase (TGO), pyruvic transaminase (TGP), gamma glutamyl transferase (GGT), alkaline phosphatase (FA) and histological evaluation were obtained at the time of collection of biological samples.

DETECTION OF ANTI-HEV IgG and IgM ANTIBODY

The presence of anti-HEV IgG was obtained using the enzyme immunoassay (anti-HEV IgM ELISA from Mikrogen®, Neuried, Germany) according to the manufacturer's instructions. Anti-HEV IgG positive samples were tested for the presence of anti-HEV IgM using the ELISA Kit (anti-HEV IgM from Mikrogen®, Neuried, Germany).

HEV-RNA DETECTION AND GENOTYPING

HEV-RNA was isolated from serum using 200 µL of the sample, using an extraction technique based on the commercial QIAmp® MinElute® Virus Spin kit (Qiagen®, Hilden Germany) according to the manufacturer's instructions. After extraction, the VHE-RNA will be transcribed immediately and amplified by the Nested-PCR technique using specific primers:

5'-AATTATGCC(T)CAGTAC(T)CGG(A)GTTG-3',
5' CCTTA(G)TCC(T)TGCTGA(C)GCATTCTC-3',
5'-GTT(A)ATGCTT(C)TGCATA(T)CATGGCT-3'
5'-AGCCGACGAAATCAATTCTGTC-3'

Amplified products were visualized under ultraviolet light after 1.5% agarose gel electrophoresis. The result was evidenced with specific bands.

All samples with positive anti-HEV IgG/IgM and with HEV-RNA undetectable by Nested-PCR were tested using a real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

methodology. Viral RNA was eluted in 60 µL of elution buffer and subjected to One-Step Real Time PCR amplification using the QuantiFast Pathogen + IC® Qiagen® RT-PCR Kit (Qiagen®, Hilden, Germany) and the primers and probe previously described TaqMan 21, which target the highly conserved ORF3 region. Serial dilutions (25,000, 2,500, 250 and 25 IU/ml) of the WHO international standard (6329/10, Paul Ehrlich Institute, Germany) quantified at 250,000 IU/ml were used as a standard curve. To assess the sensitivity of the assay, ten replicates of the different low concentration dilutions (125, 62.5, 31.3, 25 and 12.5 IU/ml) of the WHO international standard were tested and the sensitivity was set at 125 IU /ml. Samples were tested in triplicate with negative controls included in each run, in addition to serial dilutions of the reference standard for the HEV.

STATISTICAL ANALYSIS

The data obtained in the study were recorded and analyzed using Epi Info TM software (CDC, Atlanta, USA -www.cdc.gov). Statistical analyzes of data collected in the study were analyzed using SPSS software version 20.0 (SPSS Inc., 2011).

Descriptive analyzes were performed using absolute and relative frequencies, median and interquartile ranges, in order to identify the general characteristics of the sample studied. The normality of the distribution was verified by means of the Shapiro-Wilk test and the analysis of symmetry and flattening of the distribution.

The chi-square test and Fisher's exact test were used to identify the existence of associations between nominal variables. Continuous variables were compared using the Mann-Whitney test. An α level of 5% significance was established.

RESULTS

Samples from 301 volunteers from different groups of patients with liver disease were analyzed: 131 naïve chronic hepatitis B patients, 114 naïve chronic hepatitis C patients, 37 patients with autoimmune hepatitis (AIH) on immunosuppressant treatment (Azatipoprine and/or Prednisone) and 19 with drug-induced liver injury (DILLI).

The overall prevalence of the sample was: anti-HEV IgG in the serum: 12.95% and of IgM was 2.4%. When stratified by groups, the highest prevalence found in patients with DILI 21.1% with Ig G anti HEV and the lowest prevalence among patients with AIH 8.1% with Ig G anti HEV The seroprevalence among patients with hepatitis C and B were similar, 13.2 and 13%, respectively (Table 1).

Meanwhile, the prevalence of anti-HEV IgM found was 5.3% (1/19), 3.8% (5/131) and 0.9% (1/114) among patients with DILI, HBV and HCV, respectively, patients with AIH were not seropositive for anti-HEV IgM (Table 1). No statistically significant difference was observed between the seroprevalence of anti-HEV IgG and IgM in the studied groups.

Table 1 – Anti-HEV IgG and IgM seroprevalence in different groups of patients.

	Soro Prevalence % (n)			
Groups	**Anti-VHE IgG**	**Valor de p**	**Anti-VHE IgM**	**p Value**
VHC	13..2 (15/114)		0.9 (1/114)	
VHB	13 (17/131)		3.8 (5/131)	
HAI	8..1 (3/37)		0	
DILLI	21..1 (4/19)		5.3 (1/19)	
TOTAL	12.95 (39/301)	0.582	2.3 (7/301)	0.304

Source: Research data. Pearson's Chi-Square Test

Demographic and serological data are shown in Table 2. The mean age observed in the population was 52.1 (± 15.7). Mean age was higher among patients with the hepatitis C virus (HCV): 59 years (±14.7) and the difference was significant ($p < 0.001$). However, no statistically significant difference was observed between patients with positive and negative serology for anti-HEV IgG ($p = 0.662$).

Among the study participants, 52.1% (157/301) were female and 47.8% (144/301) were male. However, there was a predominance of males among patients with positive serology for HEV, observed in 61.5% (74/39) of the cases ($p < 0.001$).

The mulatto race was more prevalent in all groups, followed by black and white. While, among the volunteers with positive anti-HEV IgG, there was a predominance of the mulatto race with 53.8% (21/39), followed by the white race with 33.33% (13/39) and the black race with 12.82% (5/39), the difference was considered statistically significant ($p < 0.05$).

No significant statistical difference was observed between the marital status and family income of the research participants. High school was the most frequent level of schooling among the groups, followed by elementary and higher education. However, no significant difference was observed in the association between education and serology for HEV ($p = 0.87$).

Table 2 - Demographic and serological characteristics of patients

	Grupe				P Value	ELISA Anti-VHE IgG		P Value
Characteristic (N)	**VHC (114)**	**VHB (131)**	**HAI (37)**	**DILLI (19)**		**POSITIVE (39)**	**NEGATIVE (262)**	
Average Age (± DP)	58.9 (±13.1)	49.4 (± 16.1)	45.5 (±14.3)	41.8 (±14.7)	<0.001	53.1 (±15.7)	51.9 (±15.8)	0.662*
20 - 40 n(%)	12 (10.5)	46 (35.1)	12 (32.4)	8 (42.1)		3 (7.7)	34 (13)	
41 - 60 n(%)	41 (36)	42 (32.1)	21 (56.8)	8 (42.1)		8 (20.5)	33 (12.6)	
≥ 60 n(%)	61 (53.5)	43 (32.8)	4 (10.8)	3 (15.8)		4 (10.2)	50 (19.1)	
Sex n(%)					<0.001			<0.001*
Female	47 (41.2)	66 (50.4)	31 (83.8)	13 (68.4)		12 (30.8)	145 (55.3)	
Male	67 (58.8)	65 (49.6)	6 (16.2)	6 (31.6)		27 (69.2)	117 (44.7)	
Self identified race n(%)					0.31			<0.05*
White	31 (27.2)	30 (22.9)	3 (8.1)	3 (15.8)		13 (33.33)	54 (20.6)	
Mulatto	48 (42.1)	65 (49.6)	21 (56.8)	10 (52.6)		21 (53.8)	123 (46.9)	
Black	35 (30.7)	36 (27.5)	13 (35.1)	6 (31.6)		5 (12.8)	85 (32.4)	
Marital Status					0.82			0.25*
Single	35 (30.7)	48 (36.6)	15 (40.5)	5 (26.3)		16 (41)	87 (33.2)	
Married	57 (50)	55 (42)	16 (43.2)	10 (52.6)		19 (48.7)	119 (45.4)	
Widow/Separated	22 (19.3)	28 (21.4)	6 (16.25)	4 (21.1)		4 (10.3)	56 (21.4)	
Education (Level) n (%)					<0.05			0.87*
Basic	44 (38.6)	31 (23.7)	6 (16.2)	8 (42.1)		9 (23)	77 (29.4)	
High School	57 (50)	79 (60.3)	25 (67.6)	10 (52.6)		23 (59)	148 (56.5)	
Higher Education	13 (11.4)	21 (16)	6 (16.2)	1 (5.3)		7 (18)	37 (14.1)	
Multiple of minimum salary n(%)					0.397			0.804 #
< 1	31 (27.2)	34 (26)	11 (29.7)	6 (31.6)		10 (25.6)	72 (27.5)	
1 - 5	77 (67.5)	90 (68.7)	11 (70.3)	13 (68.4)		28 (71.8)	178 (67.9)	
> 5	6 (5.3)	7 (5.3)	0 (0)	0 (0)		1 (2.6)	12 (4.6)	

Source: Research data. * Pearson's Chi-square test. # Fisher's Exact Tests.

Regarding risk factors and behaviors among the volunteers studied, it was shown that the majority reported living in an urban area and having a sewage system and piped water. 69.2% of the volunteers with positive serology and 72.3% with negative serology, reported residing in urban areas, in the variable sewage system 71.8% of the volunteers with positive serology and 83.1% with negative serology reported have a sewage system in their homes. Piped water was a widely available variable, unrelated to the serological result (Table 3).

Contact with swine was reported by 26.3% and 20.5% of anti-HEV positive and negative patients, respectively. Meanwhile: 82.1% with anti-HEV IgG and 74.9% of anti-HEV IgG negative patients reported eating pork meat: Game meat consumption was reported by 50% of patients with positive serology and by 32.7% of patients with negative serology, presenting a statistical difference ($p < 0.05$). As for the consumption of raw shellfish, no significant difference was observed ($p = 0.324$), in which 64.1% and 55.7% of the volunteers had positive and negative serology for anti-HEV IgG, respectively.

Table 3 – Factors associated with the behavior and exposure of patients with serology for Anti-HEV IgG.

	ANTI-VHE IgG POSITIVE		ANTI-VHE IgG NEGATIVE	
VARIABLES	**N**	**%**	**N**	**%**
Zone				p = 0.69*
URBAN	27	69.2	188	72.3
RURAL	12	30.8	72	27.7
Has sewage network				p = 0.088*
YES	28	71.8	217	83.1
NO	11	28.2	44	16.9
Has running water				p = 0.92#
YES	38	97.4	255	97.7
NO	1	2.6	6	2.3
Contact with swine				p = 0.417*
YES	10	26.3	53	20.5
NO	28	73.7	205	79.5
Consumption of Pork				p = 0.332*
YES	32	82.1	188	74.9
NO	7	17.9	63	25.1
Consumption of Wild Game				p < 0.05*
YES	19	50	82	32.7
NO	19	50	169	67.3
Cosumptipon of Shellfish				p = 0.324*
YES	25	64.1	146	55.7
NO	14	35.9	116	44.3

Source: Research data. * Pearson's Chi-square test. # Fisher's Exact Tests.

The median values of the biochemical markers of TGO, TGP, GGT and FA do not show any important difference when considering seropositivity for anti-HEV IgG. However, a higher elevation is demonstrated in patients with DILI, unrelated to anti-HEV serology (Table 4). When considering HEV serology and its association with changes in liver enzyme levels, among patients with HCV 66.7% (10/15) with positive serology for HEV and 42.4% (42/99) with serology negative for HEV had an increase in transaminase levels ($p<0.05$). The same was not observed for GGT and FA (Table 4)

Patients with positive anti-HEV IgM showed an increase in TGO levels in 57.1% (4/7) of the cases, while TGP levels in 85.7% (6/7) Patients with negative anti-HEV IgM, elevation of TGO and TGP levels were observed in 36.1% (106/294) and 30.3% (89/294), this difference between both groups of serological profiles was statistically significant (TGO and TGP - $p < 0.05$). All patients with positive serology for IgM had positive serology for IgG.

Patients with HCV 84,2% (96/114) had genotype 1 and genotype 3: 15.8% (18/114). Patients with IgG HEV 80% (12/15) had genotype 1. While those that were IgG anti-HEV negative had genotype 1 in 84.8% (84/99) and genotype 3 in 15.2% (15/99), this could be because in State of Bahia the predominant HCV genotype is 1.

Table 4 – Laboratory and serological data by patient groups.

	ANTI-VHE IgG POSITIVE				ANTI-VHE IgG NEGATIVE			
DATA	**VHC (15)**	**VHB (17)**	**HAI (3)**	**DILI (4)**	**VHC (99)**	**VHB (114)**	**HAI (34)**	**DILLI (15)**
TGO					p<0.05			
Median (IQR)	46 (29-86.5)	33 (27-40)	37 (33-38)	559(301-679)	36 (28-57)	27 (23-37)	31 (24-49.8)	569 (466-1322)
Normal n (%)	5 (33.3)	14 (82.4)	3 (100)	4 (100)	57 (57.6)	91 (79.8)	21 (31.8)	0 (0)
Altered n (%)	10 (66.7)	3 (17.6)	0 (0)	0 (0)	42 (42.4)	23 (20.2)	13 (38.2)	15 (100)
TGP					p<0.05			
Median (IQR)	54 (22-137)	45 (32-75)	37 (27-56)	428(188-767)	47 (32-81)	31 (22-40)	30 (22-56)	704 (342-928)
Normal n (%)	10 (66.7)	10 (58.8)	2 (66.7)	4 (100)	59 (59.6)	98 (86)	26 (78.8)	0 (0)
Altered n (%)	5 (33.3)	7 (41.2)	1 (33.3)	0 (0)	40 (40.4)	16 (14)	7 (21.2)	15 (100)
GGT								
Median (IQR)	66 (35-103)	36 (19-47)	106 (32-227)	216 (98-602)	46 (32-74)	37 (22-65)	51 (26-105)	486 (303-530)
Normal n (%)	8 (53.3)	14 (82.4)	1 (33.3)	1 (25)	77 (77.8)	9 (79.6)	24 (70.6)	3 (20)
Altered n (%)	7 (46.7)	3 (17.6)	2 (66.7)	3 (75)	22 (22.2)	23 (20.4)	10 (29.4)	12 (80)
FA								
Median (IQR)	55 (40-97.7)	56 (24-72)	90 (75-125)	138(297-425)	52 (38-85)	50 (35-86)	47 (35.4-86)	123 (101-240)
Normal n (%)	13 (86.7)	17 (100)	2 (66.7)	1 (25)	94 (96.9)	101 (91)	31 (93.9)	8 (57.1)
Altered n (%)	2 (13.3)	0 (0)	1 (33.3)	3 (75)	3 (3.1)	10 (9)	2 (6.1)	6 (42.9)

Liver fibrosis stage was recorded in 44.9% (135/301) of the patient groups (Table 5). Although grade 2 and 3 fibrosis were present in 25% of patients with positive anti-HEV IgG and in only 18,9% and 13.5%, respectively, of anti-HEI IgG negative patients. While grade 4 fibrosis wasn't present in patients with positive anti-HEV IgG and was observed in 13,5% between anti-HEI gG negative patients. No statistically significant difference was observed between the groups (p = 0.248).

Table 5 – Characteristics of the patients' fibrosis stage.

DEGREE OF FIBROSIS						**p Value**
% (n)	**VHC**	**VHB**	**HAI**	**DILI**	**TOTAL**	
			Anti VHE IgG POSITIVE % (n=24)			
F0	33.3 (2/6)	33.3 (2/6)	16.7 (1/6)	16.7 (1/6)	25 (6)	
F1	33.3 (2/6)	50 (3/6)	16.7 (1/6)	0	25 (6)	
F2	50 (3/6)	33.3 (2/6)	0	16.7 (1/6)	25 (6)	
F3	33.3 (2/6)	33.3 (2/6)	16.7 (1/6)	16.7 (1/6)	25 (6)	
F4	0	0	0	0	0	
			Anti VHE IgG NEGATIVE (n=111)			
F0	33.3 (2/6)	33.3 (2/6)	16.7 (1/6)	16.7 (1/6)	5.4 (6)	
F1	42.6 (23/54)	29.6 (16/54)	24.1 (13/54)	3.7 (2/54)	48.7 (54)	
F2	47.6 (10/21)	38.1 (8/21)	14.3 (3/21)	0	18.9 (21)	
F3	40 (6/15)	46.7 (7/15)	13.3 (2/15)	0	13.5 (15)	
F4	46.7 (7/15)	46.7 (7/15)	6.6 (1/15)	0	13.5 (15)	0.248

Source: Research data. Fisher's Exact Test

None of the samples studied were detectable for HEV-RNA by PCR, demonstrating the absence of viremia for HEV in the studied group.

DISCUSSION

Studies on the seroprevalence of hepatitis E in the country demonstrate a wide variety of data and diversity of the studied population. Although some authors have correlated chronic liver disease and HEV infection, there is still limited data on clinical, epidemiological, etiological and demographic aspects related to the virus.

The prevalence of anti-HEV IgG observed in the population studied was 12.95%, studies that evaluated the prevalence in patients with previous liver disease, such as HCV [6], patients with and without cirrhosis [7], risk groups [12] and liver transplant recipients [22] obtained 10.2%, 13.2%, 19.5% and 8.2%, respectively. Tengan et al [23], in a meta-analysis, estimated a mean prevalence of HEV in Brazil of 6.0% (95% CI: 5.0–7.0), a high heterogeneity of data was observed, amounting to 86.7%, due to the diversity in the methodological strategies used in the studies included in the review such as different sample populations and the type of diagnostic test and region studied.

Published studies reveal the prevalence among different sample groups. In blood donors the values observed were 0.44% [14], 2% [25], 2.3% [28], 9.8% [29] and 10 % [5], while in representative groups of the general population occurrences ranging from 2.4 to 12.9% [31-33], were observed and hemodialysis patients from 0 to 6.2% [14, 24, 25]. Among pregnant women, a prevalence of 0 %[14], 1.0% was observed [23] and 19% [13]. Subjects immunosuppressed by HIV 6.4% [26] and 4.1% [30],2.5 kidney transplant recipients [26], 15% [4] and liver transplant recipients 8.2% [22].

The wide variation in seroprevalence rates observed in Brazil makes it difficult to interpret the magnitude of the infection in the country. Additionally, there is no public policy and the inclusion of epidemiological research in clinical practice. Therefore, studies focusing on epidemiological data contribute to understanding the distribution of HEV in the country.

In the present study, the global prevalence of anti-HEV IgM was found to be 2.4%. Data published in the literature reveal a prevalence of anti-HEV IgM ranging from 0.2 to 1.5% [3, 8, 12, 26-28]. The natural history of HEV infection demonstrates that anti-HEV IgM levels reach a peak elevation around the eighth week of exposure to the virus, however, after about 32 weeks it becomes undetectable in most patients [34]. Detection of anti-HEV IgM marker may be indicative of acute infection [35], acute hepatitis by HEV should be considered when anti-HEV IgM and/or IgG are present. In all the cases of our series, which presented positive anti-HEV IgM, there was concomitant seropositivity for anti-HEV IgG and the HEV-RNA was undetectable, suggesting a probable late detection of anti-HEV IgM, and a probable picture of hepatitis acute, despite negative viremia[35]. Additionally, these patients had higher levels of TGO in 57.1% (4/7) and TGP in 85.7% (6/7) of the cases. In the course of acute infection, the viremia and fecal elimination of HEV starts 1 to 2 weeks before the onset of symptoms and lasts for about 2 to 4 weeks, while ALT levels return to normal and the virus is cleared by most patients within 4 weeks of the onset of symptoms[34,35].

The presence of anti-HEV IgG was more common among males, in 69.2% (27/39). Previous studies corroborate our findings, Oliveira [27] et al. observed that 62.5% of the volunteers with positive serology for anti-HEV IgG were male. Hering et al. [4] obtained a distribution of 71% male in the studied population. Meanwhile, Trinta et al [24] demonstrated a prevalence in male blood donors of 4%, while in pregnant women it was 1%. These findings suggest that males are more susceptible to infection by the virus, possibly with greater exposure.

Our findings indicate that the prevalence of anti-HEV IgG was between 21.1% and 8.1% of the sample, the difference between the groups was not significant ($p = 0.582$). However, among patients with hepatitis C and anti HEV Ig G positive serology, elevations in serum levels of TGO and TGP were observed ($p < 0.05$). Previous studies indicate a relationship between HCV and HCV superinfection and worse prognosis [6, 37, 38]. Elhendawy et al. [37] in a prospective cohort studied villagers infected with HCV, anti-HEV IgG was positive in 71.4% of patients with hepatitis and in 96.1% of patients with advanced liver disease, suggesting a relationship between HCV and HCV and an effect of HEV on progression to late liver disease.

Bayram et al. [38] demonstrated the presence of anti-HEV IgG in 54% (94/174) of patients with chronic hepatitis C and in 13.7% (26/190) of patients with chronic hepatitis B. The presence of HEV infection was significantly higher in chronic carriers of the C virus, suggesting a relationship between HEV superinfection and greater severity of liver disease in patients with chronic hepatitis. Although there is currently no indication for serological investigation of HEV in patients with chronic hepatitis, based on our findings and previous studies, we can suggest the valuable

contribution of anti-HEV IgG in the follow-up of patients with chronic hepatitis, especially HCV whit epidemiological risk factors for HEV contagious.

Some authors discuss the possibility of the existence of similar transmission routes between HCV, HBV and HEV, such as sexual among men who have oral sex with men , and parenteral [6, 37, 38] but there is no general consensus on the type of transmission. sexual intercourse must be in a man who has oral sex with a man[39]. Furthermore, considering that there may be a greater severity of the hepatic condition associated with HEV superinfection, guidance on prophylactic measures is recommended, such as guidance on the consumption of water, seafood, pork products and hunting among patients with chronic hepatitis.

There is evidence in the literature on the association between the ingestion of raw or undercooked pork and HEV infection [38,40]. In the present study, no significant difference was observed between the consumption of swine and anti-HEV IgG. Data published by Bricks et al [6] showed no association between reactive anti-HEV IgG and pork consumption, although a relationship was observed between positivity of anti-HEV IgG and history of contact with pigs.

It is worth mentioning that, among the exposure variables, game consumption was significantly associated with positive serology for HEV. Previous studies suggest HEV contamination of several animals, such as wild boar [42], rabbits [43], deer and deer [44-46]. However, there are indications that contamination is more likely by the consumption of raw or undercooked meat and therefore the use of adequate heat treatment, above 72°C for at least 20 minutes, allows the inactivation of the HEV [46].

The present study has limitations, such as a relatively small number of samples, especially in the groups of patients with AIH and DILI, in addition to the absence of viremia detection, making the molecular characterization of HEV impossible. This difficulty in determining HEV-RNA may be associated with the use of in-house molecular techniques, with the absence of determination of the detection limit. One-step real-time PCR [21]. However, it is important to highlight that other studies were not successful in detecting HEV-RNA in our environment [3,12,] probably due to the natural history of HEV infection, considering that acute infection presents a short period of viremia, and viral clearance occurs soon after seroconversion [47].

CONCLUSION

In conclusion, our results show a high seroprevalence of HEV IgG in the State of Bahia, showing a possible relationship between HCV/HEV co-infection and higher levels of transaminases. Patients with both positive serological markers were shown to have elevated transaminases, suggesting acute hepatitis despite undetectable viremia. In the group of patients with DILI, a higher prevalence of anti-HEV IgG and IgM was observed, demonstrating the need to investigate HEV infection in this group of patients in clinical practice.

Additionally, our data indicate an association between the consumption of game animals and HEV infection. However, it is worth mentioning that future studies to evaluate the circulation of HEV among different animals, in our environment, and to investigate the relationship between bushmeat consumption and HEV infection are necessary to corroborate our findings. There is still a need to evaluate the circulation of HEV in rural regions, especially in and around pig farms.

CONFLICT OF INTEREST: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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