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Research article

Clinical profile and parameters of patients monoinfected with HBV and infected with HDV in Western Amazon

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ABSTRACT

Background: Hepatitis Delta represents a greater risk in the progression of advanced liver disease and HCC compared with HBV. The exact mechanism that determines the spontaneous clearance of delta virus or its progression to cirrhosis remains unknown. Therefore, this study aimed to analyze the clinical profile of HBV and HBV/HDV individuals in the Western Amazon.

Methods: The study was carried out at the Specialized Outpatient Clinic for Viral Hepatitis belonging to the Centro de Pesquisa em Medicina Tropical de Rondônia/CEPEM. 100 individuals were included, stratified into two groups: 50 with hepatitis B virus and 50 with hepatitis Delta virus.

Results: The overall mean age was 48 years. For the HBV and HDV groups, 66 % (33/50) and 54 % (27/50) were men and 56 % (28/50) and 58 % (29/50) were on antiviral treatment, respectively. Patients with detectable HDV-RNA demonstrated high levels of ALT and AST compared to individuals with undetectable HDV-RNA. Comparative analysis between HBV carriers and infected with HDV shows significant differences in terms of age, HBV-DNA levels, albumin, hepatomegaly and splenomegaly.

Conclusion: Several markers were important for differentiating HBV and HDV infections. HDV-RNA detectable showed significant changes in biomarkers compared to undetectable patients, suggesting a possible worse prognostic effect in this group.

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1. Background

Chronic hepatitis B is a public health problem that affects approximately 254 million people worldwide [1]. The natural history of chronicity by Hepatitis B virus is classified into four phases (Immunotolerant phase, Immunoreactive phase, Immunoreactive HBeAg-negative phase, and HBsAg loss phase) mediated by serum monitoring of biomarkers such as HBeAg, HBsAg, ALT, and viral DNA [2,3]. Complications may occur during the course of the disease, including fibrosis, cirrhosis, liver failure and hepatocarcinoma (HCC) [4,5].

Hepatitis Delta virus (HDV) infection affects more than 5 % of individuals infected with chronic hepatitis B, although its prevalence is still underestimated worldwide [6]. The clinical course of the disease is intrinsically at the time of infection in relation to HBV-mediated liver disease [7]. Co-infection consists of HDV infection simultaneously with acute HBV infection, while superinfection is characterized by HDV infectivity in individuals with chronic hepatitis B [8]. Hepatitis Delta represents an even greater risk in the progression of advanced liver disease compared with HBV monoinfection and is related to rapid progression to liver cirrhosis and HCC [7]. Furthermore, superinfected patients can develop cirrhosis within 5–10 years of diagnosis, corresponding to a threefold increase compared to HBV monoinfected patients [9].

Although the immune response plays an important role in pathogenesis, the exact mechanism that determines the spontaneous clearance of delta virus or its progression to fibrosis and cirrhosis remains unknown [10].

In the Brazilian Amazon, numerous outbreaks featuring severe cases of Delta Hepatitis have been documented [11–13]. Nevertheless, elucidating the intricate mechanisms inherent in the virological aspects associated with the clinical profiles of individuals enduring chronic infections of HBV and HDV remains a formidable challenge. Systematic investigation into these factors holds the potential to provide insights into the nuanced clinical profiles of individuals monoinfected with HBV and those co-infected with HDV in the Western Amazon region of Brazil.

The aim of this study was to analyze the clinical profile of individuals with HBV and HBV/HDV infection in the Western Amazon, to investigate clinical and laboratory markers in detail, in order to clarify the different clinical outcomes in the population.

2. Methods

2.1. Study site

This is a retrospective cross-sectional study carried out at the Specialized Outpatient Clinic for Viral Hepatitis belonging to the Centro de Pesquisa em Medicina Tropical de Rondônia/CEPEM - Brazil, a reference for the care and follow-up of chronic carriers of hepatitis B and Delta in the northern Amazon region.

2.2. Study population

Participants were recruited for the study according to the following criteria.

- **Inclusion criteria:** Patients of both sexes, aged between 18 and 65 years, chronically infected with HBV (total anti-HBc and HBsAg positive; anti-HBc IgM negative) and superinfected with HDV (anti-HDV and HBsAg positive; anti-HBc IgM negative).
- **Exclusion criteria:** Individuals with human immunodeficiency virus (HIV) infection, hepatitis C virus (HCV), autoimmune disease, malignant neoplasm, comorbidities such as hypertension and other chronic diseases.

Once included, the patients underwent a medical consultation, followed by the collection of biological samples for laboratory analysis.

2.3. Clinical analysis and patient screening

Following the Ministry of Health's protocol for chronic carriers of viral hepatitis [14], serological and biochemical tests were requested for HBV and HDV carriers.

Hematological tests (hemoglobin level, leukocyte count, platelets and prothrombin time), biochemical tests (level of ALT, AST, bilirubin, albumin, alkaline phosphatase and gamma glutamyl transferase); as well as imaging tests (upper abdominal ultrasound and upper digestive endoscopy); serological markers for HAV, HBV, HCV, HDV and HIV, according to international parameters.

For the diagnosis and definition of the chronic phase of HBV, the presence of the serological markers HBsAg, anti-HBs, anti-HBc Total, anti-HBc IgM, HBeAg and anti-HBe were tested using the commercial kit COBAS e601 (Roche). For the diagnosis of HDV infection, anti-HDV was tested using the ETI-DELTAK-2 kit (Diasorin).

2.4. Evaluation and classification of chronic liver disease

To assess and classify chronic liver disease, the patients' clinical profile, laboratory tests and imaging tests were analyzed. The clinical analysis included signs and symptoms suggestive of chronic liver disease, such as palmar erythema, hepatosplenomegaly, jaundice, ascites, lower limb edema, choluria, fecal acholia and other clinical findings. Laboratory tests included blood components capable of measuring hepatic inflammatory activity and the degree of fibrosis (AST, ALT, platelets) as well as markers related to liver

function (albumin, bilirubin and INR).

Imaging tests included upper abdominal ultrasound, hepatic elastography and upper digestive endoscopy only in patients with severe liver fibrosis and cirrhosis to screen for portal hypertension. Abdominal CT and abdominal MRI were performed on patients with suspected hepatocarcinoma. Non-invasive scores for assessing fibrosis such as the APRI (AST to Platelet Ratio Index) and FIB4 (Fibrosis-4) indices were applied to identify advanced fibrosis, in addition to imaging tests. The METAVIR score was used to assess the extent of inflammation and fibrosis.

2.5. Detection of HBV-DNA and HDV-RNA

The viral load for HBV was carried out by the Laboratório Central de Saúde Pública de Rondônia-Lacen/RO, using the commercial kit (Abbott RealTime HBV) on the ABI 7500 platform (Applied Biosystems, Foster City, CA, USA).

HDV-RNA was extracted using the magnetic beads method using the EXTRACTA 32 DNA and RNA extractor automated extraction platform (LOCCUS, São Paulo, Brazil), and purified with EXTRACTA KIT FAST - Viral DNA/RNA (MVXA-P016FAST) according to the manufacturer's instructions and eluted in 50 μL of Elution Buffer. The RT-qPCR procedure developed by Queiroz et al. (2023) was used to detect HDV-RNA [15].

2.6. Statistical analysis

Statistical analyses were carried out using R v 4.1.2 software. Frequency, measures of central tendency and dispersion were calculated for the descriptive statistics. Categorical variables were analyzed using Fisher's exact test and Odds Ratio. Student's t-test and Spearman's coefficient was used to compare the numerical variables. A p-value <0.05 was considered significant.

3. Results

A total of 100 patients were selected, 50 with chronic HBV infection and 50 superinfected with HDV. The overall average age of the study population was 48 ($\pm$10.38) years and 60 % (60) were male. Table 1 shows the general characteristics, clinical and serological data of the study population.

The results of the comparison between hepatitis delta carriers with detectable and undetectable HDV-RNA are shown in Table 2 and Fig. 1. It was possible to identify that among the carriers with detectable HDV-RNA, high levels of ALT and AST were observed ($p < 0.05$).

Table 3 and Fig. 2 shows a comparative analysis of laboratory data between patients with HBV and infected with HDV. There were significant differences in terms of age, HBV viral load levels and albumin levels ($p < 0.05$). In the study population, the Metavir F4 liver disease stage was more prevalent in the Delta hepatitis group and this finding was statistically significant. In patients with chronic hepatitis B, findings of portal hypertension such as esophageal varices and hypertensive gastropathy were identified in the upper digestive endoscopy examination in only one patient, while in patients with Delta hepatitis this finding occurred in three individuals of the sample.

HBV-DNA levels were compared with ALT and AST values in monoinfected HBV patients (Fig. 3A and B) where a positive and statistically significant relationship ($p < 0.05$) can be seen between viral load and transaminase values. For individuals infected with HDV (Fig. 3C and D), HBV-DNA values were reduced and no significant relationship ($p > 0.05$) was observed between viral load and ALT and AST levels.

Regarding clinical signs and symptoms, there was no significant difference in the data observed between HBV and HDV group (Fig. 4A). In terms of clinical findings, a higher percentage of hepatomegaly and splenomegaly was seen among HDV group (Fig. 4B).

4. Discussion

Our results support the notion that HDV represents a public health problem in many countries around the world, including in the western Amazon region of Brazil, where prevalence is high [16,17]. This study provided important information for a better

Table 1
Study population features.

Characteristics	HBV	HDV
Age, mean (DP)	45.4 (9.71)	50.56 (10.34)
Sex, male n (%)	33 (66)	27 (54)
Treatment, n (%)	28 (56)	29 (58)
Serology		
HBsAg, n (%)	50 (100)	50 (100)
HBeAg, n (%)	7 (14)	6 (12)
Anti-HBe, n (%)	43(86)	44 (88)
Anti-HBc Total, n (%)	50 (100)	50 (100)
Anti-HDV, n (%)	0 (0)	50 (100)
Anti-HAV IgG, n (%)	26 (52)	44 (88)

Table 2
- Epidemiological, laboratory and clinical characteristics of chronic HDV carriers with detectable and undetectable HDV-RNA.

Variable	HDV		OR (CI 95 %)	p value[a]
	RNA-HDV undetectable	RNA-HDV detectable		
	n = 21 (%)	n = 29 (%)		
Age group				
≤40	1 (4.8)	5 (17.2)	1	
41-50	7 (33.3)	12 (41.4)	0.342 (0.016–2.743)	0.370
51-60	8 (38.1)	8 (27.6)	0.200 (0.009–1.624)	0.181
≥60	5 (23.8)	4 (13.8)	0.160 (0.006–1.562)	0.153
Sex				
Female	11 (52.4)	12 (41.4)	1.544 (0.435–5.598)	0.567
Male	10 (47.6)	17 (58.6)		
Fibrosis stage				
F0/F1	9 (42.9)	9 (31)	1	
F2/F3	4 (19)	8 (27.6)	2 (0.439–9.096)	0.369
F4	8 (38.1)	12 (41.4)	1.499 (0.414–5.427)	0.536

[a] Fisher's exact test.

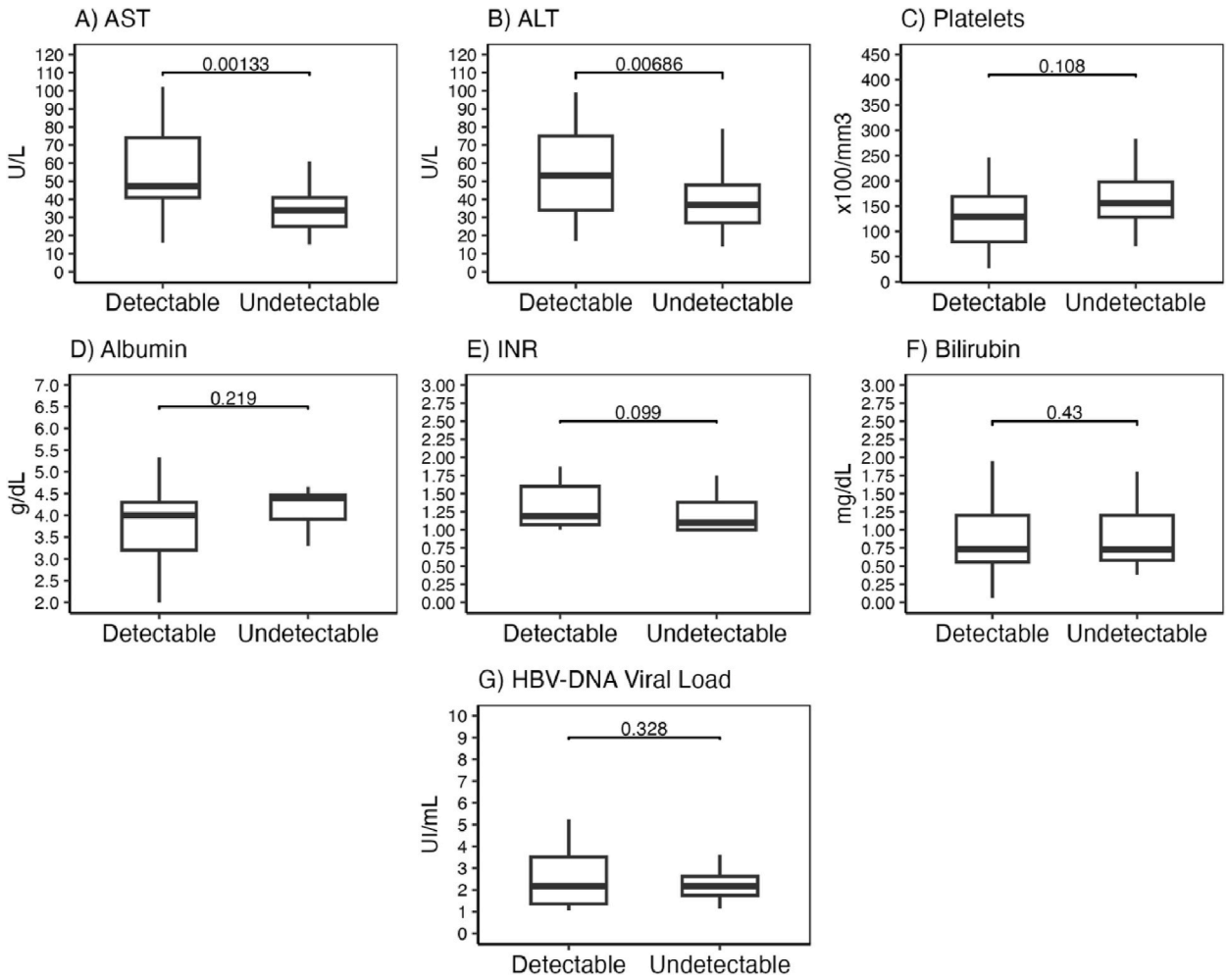

Fig. 1. Boxplot that compares the values of biochemical markers, viral load and platelets between individuals with detectable HDV-RNA and undetectable HDV-RNA. Student's t-test ($p < 0.05$).

Table 3
Comparative analysis of biomarkers in chronic carriers of HBV and HDV.

Variable	HBV	HDV	OR (CI 95 %)	p value[a]
	n = 50 (%)	n = 50 (%)		
Age group				
≤40	18 (36)	6 (12)	1	
41-50	16 (32)	19 (38)	3.562 (1.185–11.846)	**0.0287**
51-60	12 (24)	16 (32)	4 (1.264–13.969)	**0.0223**
≥60	4 (8)	9 (18)	6.750 (1.607–33.533)	**0.0124**
Sex				
Female	15 (30)	23 (46)	0.506 (0.203–1.234)	0.1488
Male	35 (70)	27 (54)		
Fibrosis stage				
F0/F1	27 (54)	18 (36)	1	
F2/F3	16 (32)	12 (24)	1.125 (0.427–2.935)	0.809
F4	7 (14)	20 (40)	4.285 (1.557–12.902)	**0.006452**

[a] Fisher's exact test.

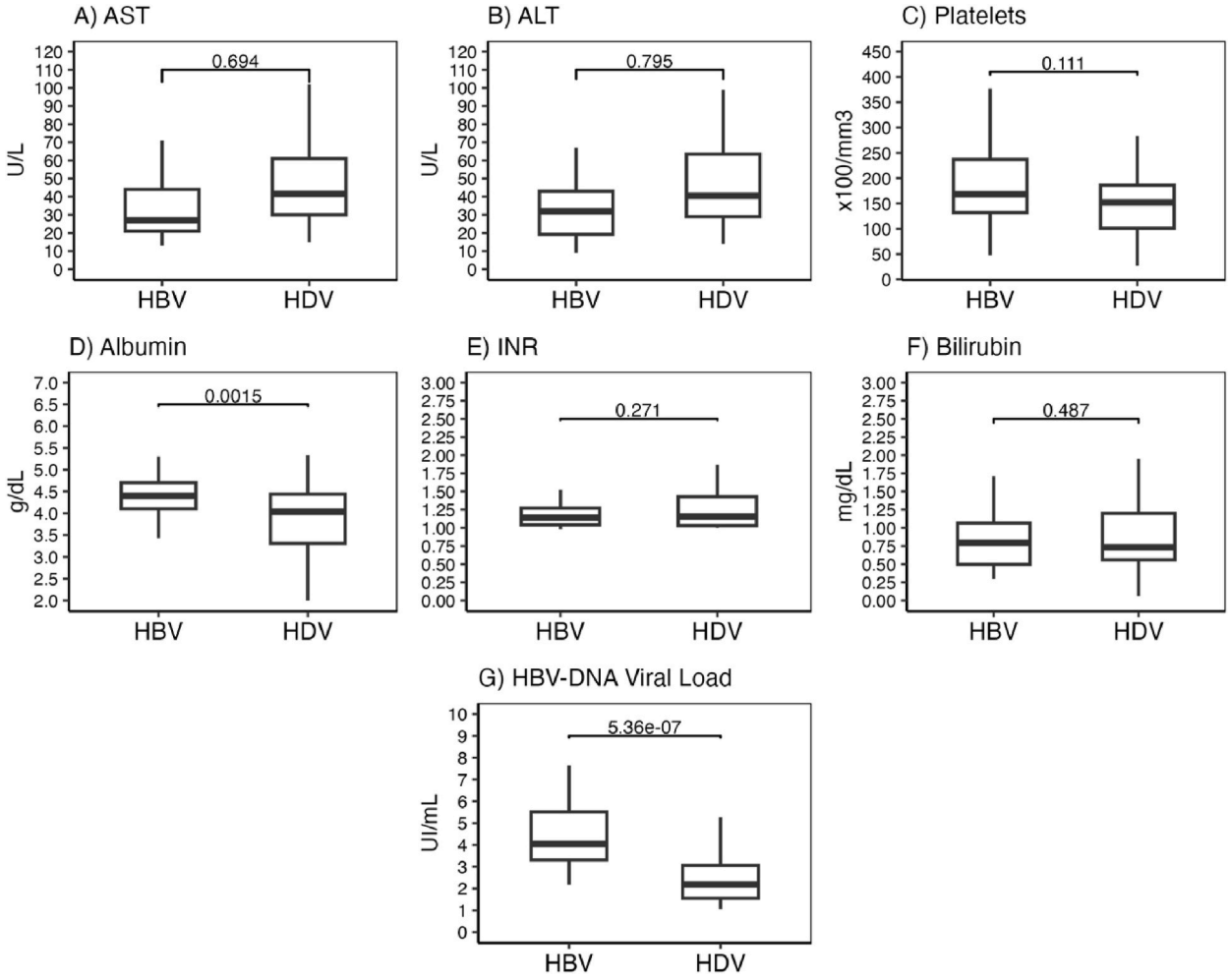

Fig. 2. Boxplot that compares the values of biochemical markers, viral load and platelets between HBV and HDV group. Student's t-test ($p < 0.05$).

understanding of the clinical outcome of patients monoinfected with HBV and super-infected with HDV.

When analyzing the biochemical markers in the study samples, alterations in the laboratory components (AST, ALT and albumin) possibly indicate a direct correlation in terms of progression, which is related to higher mortality in patients with chronic liver disease [18]. Our results revealed high levels of ALT/AST in the same group of individuals with detectable HDV-RNA. ALT/AST levels

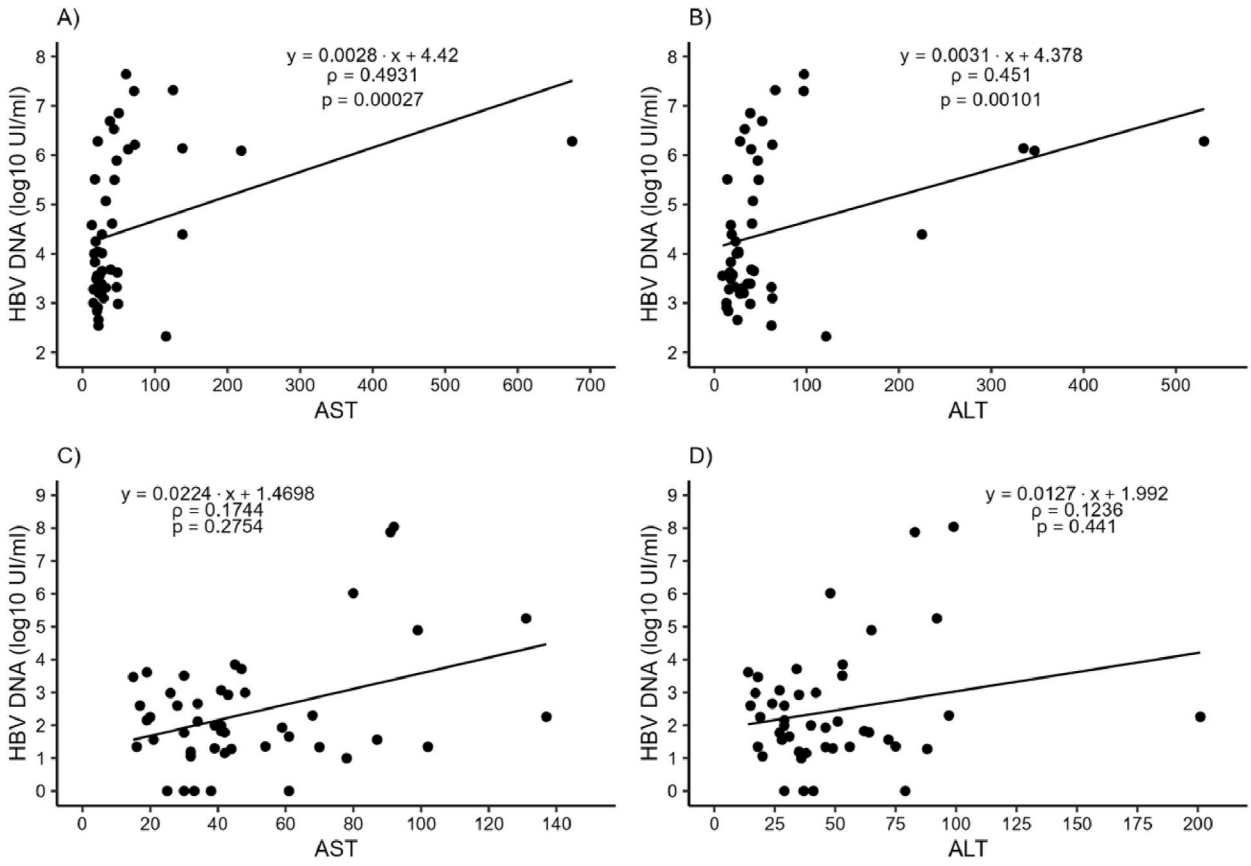

Fig. 3. Scatter plot showing the relationship of HBV-DNA viral load with AST and ALT levels in the HBV (A and B) and HDV group (C and D); AST = Aspartate Amino Transferase; ALT = Alanine Amino Transferase.

correlate with inflammatory activity and serve as a marker for assessing response to treatment [2,19].

In the present study, despite the detection of hypoalbuminemia in some individuals in the HBV group, this marker was predominantly found in individuals with detectable HDV-RNA. This is a relevant fact in HDV infection compared to HBV monoinfection in this population, since it estimates the functional reserve of the liver parenchyma and has important prognostic value and strongly associated with inflammation and is directly related to liver function disorders [20]. In the group analyzed, this marker was very similar to the results observed for ALT and AST. In a study of cirrhotic individuals with HBV, it was observed that changes in albumin levels were not significantly related to ALT levels, but rather to a reduction in viral replication, thus associating the improvement in hypoalbuminemia with the treatment applied to carriers [21].

HBV viral load levels in monoinfected individuals correlated with higher AST and ALT levels in this study. This association between viral load and high transaminase values has already been observed in previous studies; A study has already reported the relationship between HBV-DNA values and high ALT levels in 110 chronic HBeAg-negative individuals, however the same was not observed for AST [22]. An evaluation of 91 patients observed a greater sensitivity of AST for the detection of liver damage, which may suggest that AST dosage would be more indicated as a screening test for evaluation in these cases [23]. In a study by researchers in China with 11,738 treatment-naïve adult patients, fifty-five percent of patients with HBV-DNA $\geq$20,000 IU/mL had abnormal ALT and/or AST values, which was significantly higher than in patients with HBV-DNA levels below 20,000 IU/mL [22].

The mechanisms involved in HDV pathogenesis are not fully elucidated due to multifactorial mechanisms in addition to the complex dynamics of HBV/HDV interaction [24]. Our results showed significant changes in biochemical markers in individuals with HDV viremia, which suggests that replication may be influencing the poor prognosis of infected individuals. In agreement with this finding, longitudinal multicenter studies have shown that individuals who had persistent detectable viremia had a worse prognosis of the disease over the years [25,26]. It was observed that most patients had low HBV viral load values (<2000 IU/mL), possibly influenced by the recommended antiviral therapy. The course of HBV/HDV infection can be very dynamic, since fluctuating viral load profiles can be observed for both viruses throughout the infection; however, some studies have shown that HDV tends to suppress HBV replication [27–29].

The staging of liver fibrosis and the diagnosis of cirrhosis in the study participants was carried out using liver biopsy and elastography with Shear Wave technology, using the Metavir score for staging liver involvement. There was a higher prevalence of Metavir F4 individuals in the HDV group, which is in line with data in the literature which has shown that HDV infection is associated with

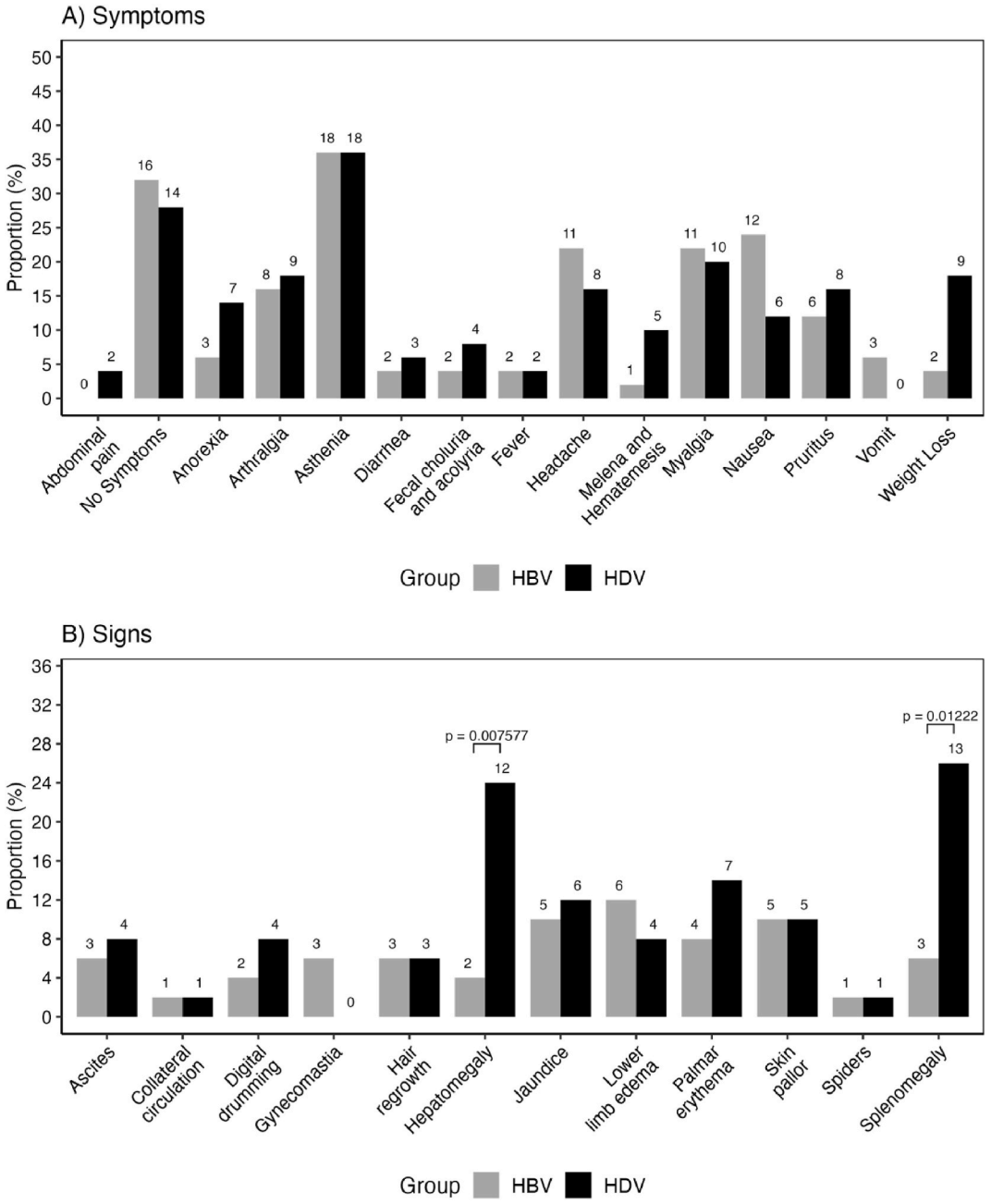

Fig. 4. Comparison of symptoms (A) and clinical signs (B) among hepatitis B and hepatitis Delta carriers.

more severe fibrosis and rapid progression to cirrhosis [30], which also supports other clinical findings found in our study, such as the high percentage of individuals with hepatomegaly and splenomegaly. It is important to note that the presence of liver cirrhosis in individuals represents a greater indirect risk for the development of hepatocarcinoma, especially in patients with HDV [31].

Otherwise, the sample size may have been a limiting factor in the study and our study population comprised individuals at different stages of the disease and some on recommended antiviral therapy, which did not allow us to reach more solid conclusions. In addition, a longitudinal study would be necessary to monitor the effect of the infection on clinical and laboratory parameters more clearly.

5. Conclusions

This study provided important clinical and laboratory findings in patients with chronic hepatitis B and hepatitis Delta. Hypoalbuminemia, hepatomegaly, splenomegaly and advanced fibrosis were more prevalent in individuals infected with HDV compared to

those monoinfected with HBV and may be important markers in differentiating the different infections. Patients with detectable HDV RNA also showed significant changes in biomarkers compared to undetectable patients, suggesting a possible worse prognostic effect in this group.

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Ethical approval statement

This study was approved by the research ethical committee from Centro de Pesquisa em Medicina Tropical de Rondônia – CEPEM/RO (CAAE Nº 17616019.1.0000.0011), and informed consent was obtained from all participants. The research procedures were carried out according to the ethical principles stipulated by the 1975 World Medical Assembly and the Brazilian Ministry of Health and follows the norms established in Resolution No. 466, December 12, 2012.

CRediT authorship contribution statement

Eugênia de Castro e Silva: Writing – original draft, Methodology, Data curation, Conceptualization. **Tárcio Peixoto Roca:** Writing – original draft, Methodology, Formal analysis. **Ana Maísa Passos-Silva:** Writing – original draft, Methodology, Data curation. **Lourdes Maria Pinheiro Borzacov:** Methodology, Data curation. **Adrhyan Araújo da Silva Oliveira:** Writing – original draft, Data curation. **Jackson Alves da Silva Queiroz:** Writing – original draft, Methodology. **Juan Miguel Villalobos Salcedo:** Writing – review & editing, Conceptualization. **Deusilene Vieira:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

ALT	Alanine Aminotransferase
APRI	Platelet Ratio Index
AST	Aspartate Aminotransferase
FIB4	Fibrosis-4
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDV	Hepatitis D Virus
HIV -	Human Immunodeficiency Virus
INR	International Normalized Ratio
ULN	Upper Limit of Normality

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