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Comparison of serologic and sonographic findings in patients with HBV/HDV and HBV/HCV Infections

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Abstract

Hepatitis B virus (HBV) and hepatitis C virus (HCV) pose significant public health challenges globally, being primary contributors to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Co-infections of HBV with HCV or HDV can present distinct sonographic and serologic findings that are valuable for diagnosis.
This study compares sonographic and serologic findings in patients with HBV/HCV and HBV/HDV infections.
The study involved 300 chronic hepatitis B patients, aged 13, in Birjand, Iran, who underwent blood tests and liver sonography. Statistical methods like Chi-square, Fisher's exact test, ANOVA, and Kruskal-Wallis tests were used for analysis.
A study involving 300 patients found that those with HBV/HDV co-infection exhibited significantly higher average levels of AST, ALT, total bilirubin, direct bilirubin, and prothrombin time compared to patients with HBV/HCV co-infection. This indicates more severe liver impairment in the HBV/HDV group (P = 0.01). The study found no significant difference in alpha-fetoprotein levels between HBV/HDV and HBV/HCV patients, and no significant differences in liver conditions, fibrosis, ascites, or cirrhosis among the two groups.
Overall, liver enzyme levels and prothrombin time were significantly elevated in patients with dual HBV and HDV infections compared to those with dual HBV and HCV infections. Furthermore, the former group exhibited significant sonographic findings indicative of disease progression and a poorer prognosis.

Keywords: Hepatitis B, Hepatitis D, Hepatitis C, Liver Enzymes, Serologic changes, Sonographic findings.

Introduction

Hepatitis B is one of the leading causes of chronic liver disease globally, with approximately 2 billion individuals infected and around 350 million suffering from chronic HBV infections at present (1). In patients with chronic hepatitis B, particularly during the extreme proliferation phase and without antiviral treatment, serious complications such as cirrhosis, hepatocellular carcinoma, liver failure, and death can arise. Key risk factors for developing cirrhosis and hepatocellular carcinoma include male gender, co-infection with hepatitis C or D, and HBV-DNA levels exceeding 10,000 copies/ml (2). The mortality rate associated with dual infections of hepatitis B and C can rise to 10%, largely due to an increased risk of cirrhosis, fulminant hepatitis, and cancer (3).

Hepatitis delta virus (HDV) is a defective RNA virus that relies on HBsAg for its transmission and replication. It is estimated that about 5% of patients infected with HBV also have HDV co-infection (HBV/HDV). HDV infection can lead to fulminant hepatitis and accelerate disease progression in individuals already infected with hepatitis B. Long-term co-infection with HBV and HDV is linked to a higher risk of cirrhosis and hepatocellular carcinoma. Although the rate of HBV/HDV superinfection is lower than that of HBV mono-infection, the prognosis is significantly worse due to a weaker response to treatment (4). In Africa and Southeast Asia, it is estimated that 4% of HBV infections are associated with HDV. Globally, there are approximately 350 million chronic carriers of HBV, leading to about 1 million deaths each year due to the disease. A study conducted on 697 patients with chronic hepatitis B in Colombia found that 5.68% tested positive for HDV antibodies, while HDV PCR was positive in 5.2% of cases (5).

Objectives

This study analyzes serologic and sonographic findings in patients with HBV/HDV and HBV/HCV co-infections. Clinicians should monitor and treat patients with co-infection more intensively, utilizing careful monitoring techniques, HBV antiviral drugs during HCV treatment to prevent reactivation, and decision-making based on serological indicators to improve patient management. Overall, this study provides insights that could enhance the treatment of HBV co-infection.

Methods

This cross-sectional descriptive-analytical study was conducted on 300 patients with chronic hepatitis B who were referred to the Birjand Hepatitis Clinic during 2013-2014. The study underwent a thorough ethical review process, approved by the Birjand University of Medical Sciences (BUMS), which ensured adherence to ethical standards and regulations for research involving human participants. Patient confidentiality was rigorously protected by anonymizing all collected data and ensuring identifiable information was not disclosed in any reports or publications. Informed consent was obtained from all participants, emphasizing their right to withdraw from the study at any time without any repercussions. Additionally, we implemented strict data security measures to safeguard participant information, aligning with national and international data protection laws. The patients were divided into four groups: those with co-infection of hepatitis B and C (HBV/HCV), those with co-infection of hepatitis B and D (HBV/HDV), those with co-infection of hepatitis B, C, and D (HBV/HDV/HCV), and a control group consisting of patients with chronic hepatitis B infection only (CHB). The following criteria were used to select participants:

1. Chronic Hepatitis B Diagnosis: Participants were required to have a confirmed diagnosis of chronic hepatitis B, as indicated by positive serologic markers (HBsAg).

2. Co-infection Status: We specifically included patients with documented co-infections of HBV/HCV or HBV/HDV. This was crucial for comparing the clinical findings between these two groups.

In our study, the control group consisted of patients diagnosed with chronic hepatitis B mono-infection (CHB) who met specific inclusion criteria. The selection process required patients to have a confirmed diagnosis of chronic HBV infection, indicated by positive serologic markers (e.g., HBsAg positivity) without any evidence of co-infection with other viruses such as HCV or HDV.

Initially, the purpose of the study was explained to the patients, and their participation was obtained through informed consent. The researcher completed checklists that included demographic information, modes of transmission, sources of information about the disease, risk factors, and results from physical examinations.

Laboratory tests were performed, including ALT, AST, total bilirubin (Bil-T), direct bilirubin (Bil-D), HBsAg, HBeAg, HBeAb, alpha-fetoprotein (AFP), HBV DNA, anti-HDV IgM antibodies, and HCV antibodies. HBeAg and HBeAb were measured using the radioimmunoassay method (Chicago Ausria II Corab M North), while HDV antibodies were detected using the Deltassay IgM radioimmunoassay (Cambridge Biotech, Dublin, Ireland). Anti-HCV and anti-HDV antibodies were identified using third-generation ELISA techniques. The results of these tests were communicated only to the patients.

Patients who tested positive for HCV antibodies underwent confirmatory PCR testing. PCR was also performed for patients with HDV infection, and antiviral treatment was initiated as necessary. A qualified sonographer conducted all ultrasound examinations. After collecting the results, the data were entered into SPSS (version 16) and analyzed using statistical tests such as chi-square, ANOVA, and Kruskal-Wallis ($p < 0.05$).

Results

The average age of patients in this study was 38.61 ± 11.98 years, with 54.7% being female. Among the participants, 3.7% had concurrent HBV/HCV infections, while 3.3% were co-infected with HBV/HDV. Only 0.6% of the patients exhibited triple infections involving HBV, HCV, and HDV.

Significantly higher average AST levels were observed in patients with HBV/HDV co-infection compared to those with chronic hepatitis B alone ($P = 0.002$). However, no significant difference was found when comparing AST levels in patients with HBV/HCV infections. The average ALT levels for patients with HBV/HDV, HBV/HCV, and chronic hepatitis B alone were 202.5, 50.6, and 44.6 IU/L, respectively, indicating a significant difference among these groups ($P = 0.01$).

Additionally, total bilirubin (Bil-T) and direct bilirubin (Bil-D) levels were significantly elevated in patients with HBV/HDV infection compared to those with HBV/HCV and CHB. Specifically, the average Bil-T values were 4.9±5.8, 1.82±2.8, and 1.29±2, while the average Bil-D values were 3.4±3/9, 1.0±2, and 0.8±1.5 for the HBV/HDV, HBV/HCV, and CHB, respectively.

The average prothrombin time (PT) for patients with HBV/HDV infection was significantly higher at 16.1 compared to 12.2 for those with HBV/HCV and 12.4 for CHB patients ($P < 0.001$). Although the average alpha-fetoprotein (AFP) level in patients with HBV/HDV infection was higher at 5.4 compared to 3 in those with HBV/HCV infection, this difference was not statistically significant (Table 1).

Among the patients with HBV/HCV infection, there were 5 cases that tested positive for PCR, while 7 patients with HBV/HDV infection also tested positive. Additionally, 5 cases in the HBV/HDV group were positive for HBeAg, which was

Table 1. AST, ALT, BIL (T, D), PT, AFD average comparison among 4 patient groups

Infection Biochemical tests	CHB Mean ± SD	hepatitis B and C Mean ± SD	hepatitis B and D Mean ± SD	hepatitis B, C, and D Mean ± SD	*P* value
AST	33.3±7.2	36.4±32.4	155±323.2	28±4.2	P=0.002
ALT	50.6±117.9	44.6±33.7	202.5±405.2	46±5.6	P=0.01
Bil (T)	1.29±2	1.82±2.8	4.9±5.8	2.6±2.6	P= 0
Bil (D)	0.8±1.5	1±2	3.4±3.9	1.6±1.9	P= 0
PT	12.4±1.3	12.2 ±0.6	16.1±5.5	14.5±3.5	P= 0
PTT	97±7.8	96±6	88.3±17.7	87±17.6	P= 0.09
AFP	3.3±3.1	3±2.5	5.4±2.5	5.1±4.2	P= 0.2

Table 2. Ultrasound changes frequency comparison among 4 patient groups

Infection Ultrasound changes		Chronic hepatitis B Frequency	Percent	Hepatitis B and C Frequency	Percent	Hepatitis B and D Frequency	Percent	Hepatitis B, C, and D Frequency	Percent	Chi-square Statistical test
Ascites	**Positive**	3	1.1	0	0	3	37.5	1	50	P= 0
	negative	278	98.9	9	100	5	62.5	1	50	
splenomegaly	**Positive**	4	1.4	0	0	2	75	1	0	P= 0
	negative	277	98.6	9	100	6	25	0	0	
fibrosis	**Positive**	3	1.1	0	0	3	37.5	1	50	P= 0
	negative	278	98.9	9	100	5	62.5	1	50	
cirrhosis	**Positive**	2	7	0	0	2	25	1	50	P= 0
	negative	279	99.3	9	100	6	75	1	50	
Fatty liver	**Positive**	6	2.1	1	11.1	0	0	0	0	P= 0.3
	negative	275	97.9	8	88.9	8	100	2	100	
hepatomegaly	**Positive**	2	7	0	0	0	0	0		P= 0.9
	negative	279	99.3	9	100	8	100	2	100	
normal	**Positive**	210	75	7	77.8	3	37.5	1	50	P= 0.09
	negative	70	25	2	22.2	5	62.5	1	50	

significantly higher compared to just 1 case in the HBV/HCV group (*P< 0.001*).

In terms of physical examinations, no significant findings were noted in patients with HBV/HCV infection. Specifically, jaundice was observed in 2 cases, while splenomegaly (*P* = 0.1), hepatomegaly (*P* = 0.08), ascites (*P* = 0.05), and liver shrinkage (*P* = 0.1) were each detected in only one case. In contrast, patients with HBV/HDV infection exhibited significant findings: 4 cases of jaundice (*P* = 0.01), 3 cases of splenomegaly (*P* = 0.01), 2 cases of liver shrinkage (*P* = 0.01), and 3 cases of ascites (*P< 0.001*).

Eighty-five percent of patients with HBV/HCV infection had normal ultrasound results. Changes indicative of fatty liver grade 1 and grade 2, liver fibrosis, and cirrhosis were each observed in one case. Conversely, among patients with HBV/HDV infection, 75% had normal ultrasound findings. Fatty liver changes were noted in one case, while fibrosis and cirrhosis were each identified in four cases, both of which were statistically significant (*P* = 0) (Table 2).

Discussion and Conclusion

In our study, we found that the average levels of AST, ALT, total bilirubin (Bil-T), direct bilirubin (Bil-D), prothrombin time (PT), and PTT activity were significantly higher in patients with HBV/HDV co-infection compared to those with HBV/HCV co-infection. However, this significant difference was not observed when comparing patients with HBV/HDV infection to those with CHB. Most existing studies have reported that patients with HDV antibodies tend to have significantly elevated ALT and AST levels compared to those without HDV antibodies (1-6). These findings indicate that HBV/HDV co-infection may worsen the condition of individuals with chronic hepatitis B. Our results align with these previous studies, suggesting that the presence of HDV exacerbates liver damage and leads to increased release of liver enzymes. The absence of a significant difference in liver parameters between the HBV/HDV co-infected group and the CHB group may suggest that chronic hepatitis B alone can lead to substantial liver damage. This finding is consistent with previous research indicating that chronic HBV infection itself is capable of causing significant hepatic impairment (7).

The timing of HDV co-infection with HBV infection remains unclear, but it may influence enzyme levels—either increasing or decreasing them (8). In cases of HBV/HCV co-infection, one virus may inhibit the replication of the other; for instance, a primary HBV infection could suppress newly introduced HCV, or vice versa. This dynamic differs in HBV/HDV co-infection, where both HDV and HBV can replicate without the suppression typically seen with HCV (9).

Our study also identified a significant correlation between jaundice and HBV/HCV co-infection, as well as between jaundice, splenomegaly, liver shrinkage, ascites, and HBV/HDV co-infection. Ultrasound examinations revealed significant correlations between splenomegaly, liver fibrosis, cirrhosis, and HBV/HDV infection.

In a study by Adam et al. (2005) (10), it was observed that patients with HBV co-infected by HDV experienced less steatorrhea compared to those co-infected with hepatitis C (60% versus 72%, P = 0.01). In a study conducted on patients with chronic hepatitis C, the prevalence of fatty liver was reported to range from 40% to 86%, indicating a significant association between chronic hepatitis C and the development of steatorrhea. This condition is attributed to both viral factors and metabolic disorders, particularly those related to obesity and insulin resistance. However, it did not correlate with the severity of liver lesions. This highlights the complex interplay between metabolic health and liver disease in individuals with chronic hepatitis C (11). Consistent with other research, our findings indicate that the progression of liver lesions in patients with HBV/HDV co-infection is more pronounced than in those co-infected with HCV. The accelerated progression of liver lesions in patients co-infected with HBV and HDV is driven by multiple factors. Unlike HBV, which is mainly non-cytopathic, HDV causes significant immune-mediated liver damage. Studies show that the adaptive immune response to HDV is often weak, particularly in CD8+ T cell responses, which are essential for controlling viral replication and reducing liver injury. This weakened immune response facilitates ongoing viral replication and heightened inflammation, resulting in more severe liver damage (12).

Overall, these results underscore the complex interactions between different hepatitis viruses and their impact on liver health. We suggest that future studies focus on the long-term outcomes of patients with HBV/HDV and HBV/HCV co-infections, particularly regarding progression to cirrhosis and hepatocellular carcinoma, as well as the efficacy of different antiviral therapies. Clinically, we recommend implementing stricter monitoring protocols for HBV/HDV co-infected patients due to their higher liver enzyme levels and prothrombin time and developing individualized treatment plans

based on the dominant virus and overall liver health.

Key limitations included the lack of longitudinal data, which restricts our ability to assess changes in liver health over time and the long-term outcomes of co-infections. Furthermore, the study is limited by its geographical focus, as it was conducted in Birjand, Iran. This may impact the generalizability of our findings to other regions with varying epidemiological profiles.

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