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Differences of Hepatitis B Serological Tests in Cirrhosis and Hepatocellular Carcinoma Patients

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ABSTRACT

Cirrhosis and hepatocellular carcinoma (HCC) are the most common complications of chronic hepatitis B. Hepatitis B Virus (HBV) reactivation occurs in the inactive phase, characterized by reappearance of HBsAg or negative HBsAg. The prevalence of occult hepatitis B infection in cirrhosis and HCC ranges from 20% to 60%. This study aimed to analyze the differences in hepatitis B serological tests in patients with cirrhosis and HCC for diagnosis of acute or chronic hepatitis B. The current research was analytical and descriptive with a cross-sectional study design. This study involved 177 patients, including 50 cirrhosis patients and 127 HCC patients. Hepatitis B serological tests (HBsAg, HBeAg, anti-HBe, total anti-HBc) were analyzed using VIDAS instruments by the Enzyme-Linked Fluorescent Immunoassay (ELFA) method. Hepatitis B serological test results were grouped based on the interpretation of serological test results. The data were processed with the statistical test Kolmogorov-Smirnov test, independent T-test, and Chi-Square, and results with $p<0.05$ were reported as significant. This study found that most males suffer from cirrhosis and HCC due to chronic HBV infection, with a mean age of 57. Chronic hepatitis was most common in patients with cirrhosis and HCC (71.2%). There was no significant difference in the interpretation of the hepatitis B serological test between patients with cirrhosis and HCC, with a p-value of 0.230 ($p > 0.05$). There was no significant difference in interpreting the hepatitis B serological test between cirrhosis and HCC. Both reactive HBsAg and non-reactive HBsAg can be obtained in cirrhosis and HCC.

Keywords: Serological tests, HBsAg, cirrhosis, HCC

INTRODUCTION

Hepatitis B is a disease caused by infection of the Hepatitis B Virus (HBV), which leads to destruction, necrosis, and autolysis of hepatocytes. Hepatitis B virus can be acute or chronic. The Hepatitis B virus is a member of the Hepadnavirus family, genus orthohepadna virus. The HBV genome is double-stranded deoxyribonucleic acid (dsDNA). Hepatitis B virus has various serological markers such as hepatitis B surface antigen (HBsAg), anti-HBs, anti-HBc IgM and IgG, and hepatitis B e antigen (HBeAg) and anti-HBe. According to the World Health Organization (WHO), around 240 million people worldwide have chronic HBV infection, and 686,000 people die each year from complications of hepatitis B, such as liver cirrhosis and hepatocellular carcinoma (HCC).[1-4]

Hepatitis B infection results from an interaction between the virus and the host's immune system. Pathophysiology of hepatitis B is divided into 5 phases; the first phase is immune-tolerant, characterized by the immune system inhibiting HBV replication and the presence of HBV DNA, HBeAg, and HBsAg in serum. The second phase is the reactive immune phase characterized by positive HBeAg, increased alanine transferase (ALT) levels, initial production of anti-HBc IgM, and increased HBV DNA, HBeAg, and HBsAg levels. Decreased viral replication, low HBV DNA, negative HBeAg, and the presence of HbsAg characterize the third phase. This phase is also known as the inactive carrier state, with a risk (10-20%) for viral reactivation. The fourth phase is characterized by negative HBeAg, a viral mutation in the pre-core, active replication of the promoter core region of the genome, leading to hepatic complications. Negative HBsAg characterizes the fifth phase, terminated viral virus replication, and potential risk of HBV transmission due to viral reactivation. Reactivation is characterized by the reappearance of HBsAg or the conversion of HBV DNA from negative to positive in patients with negative HBsAg, often called occult HBV Infection. In the inactive phase, the patient begins to experience liver cirrhosis and most likely develops into hepatocellular carcinoma (HCC).[5-8]

Chronic HBV infection can cause HCC, although the exact mechanism remains unknown. Evolution to HCC is thought to be a direct effect of the virus due to the integration of HBV DNA into hepatocytes or an indirect effect through inflammation, regeneration, and fibrosis processes. Approximately 10% of chronic hepatitis B due to fibrosis and cirrhosis can develop into HCC.[9,10]

Hepatocellular carcinoma is the most common primary liver malignancy and the third leading cause of cancer-related death worldwide. Hepatitis B infection is the main cause of HCC in Asia and Africa. Several studies conducted in several countries showed that hepatitis B infection was widely reported in patients with liver cirrhosis and HCC with negative HBsAg results (obscured Hepatitis B) found in Slovenes (1:7500), Poles (1:63,000), Japanese (1:107,000), and Ghana (1:6,000). Occult Hepatitis B infection is more common in patients with liver cirrhosis and HCC than with liver disease cases with minimum liver abnormality. The prevalence of occult Hepatitis B infection in chronic hepatitis is 5-50% and ranges from 20-60% in patients with liver cirrhosis and HCC.[10-12]

Based on the description above, the authors were interested in analyzing Hepatitis B serological tests in patients with liver cirrhosis and HCC at Dr. Wahidin Sudirohusodo Hospital, Makassar.

METHODS

This type of research was descriptive analysis using a cross-sectional study design. The research was conducted at Dr. Wahidin Sudirohodo Hospital, Makassar. Secondary data were taken at the Medical Record Installation and Hospital Information System at Dr. Wahidin Sudirohusodo Hospital, Makassar, with the recommendation of Ethics Approval with NO. UH22070396. The research was conducted from January 2019 to July 2022.

The research subjects comprised 177 patients, including all liver cirrhosis and hepatocellular carcinoma patients aged 18-91 years who had complete hepatitis B serology tests. Exclusion criteria in this study were patients with liver cirrhosis and hepatocellular carcinoma who did not have a full hepatitis B serology test.

The complete Hepatitis B serology test panel used in this study consisted of HBsAg, anti-HBs, HBeAg, anti-HBe, and total anti-HBc analyzed using the VIDAS instruments with the Enzyme-Linked Fluorescent Immunoassay (ELFA) method. Patients with liver cirrhosis and HCC were patients who had been diagnosed by a clinician supported by laboratory and radiological tests.

Data were analyzed using SPSS version 25. The Kolmogorov-Smirnov test assessed data normality; the Chi-Square test was used for categorical data; and the independent T-test was used for normally distributed numerical data. The statistical test results with p-value <0.05 were reported as significant.

RESULTS AND DISCUSSIONS

The results showed 139 (78.5%) males and 38 (21.5%) females with an age range of 29-91 years of 177 samples. The study sample was divided into 127 (71.8%) HCC patients and 50 (28.2%) liver cirrhosis patients. Interpretation of serological test results consisted of 24 (13.6%) acute hepatitis, 126 (71.2%) chronic hepatitis, and 27 (15.3%) serological gaps. Hepatitis serological tests on HCC showed reactive HBsAg in 102 patients, reactive HBeAg in 6 patients, reactive anti-HBe in 82 patients, and reactive total anti-HBc in 127 patients. Hepatitis serological tests in liver cirrhosis patients showed reactive HBsAg in 47 patients, reactive HBeAg in 6 patients, anti-HBe reactive in 36 patients, and total anti-HBc reactive in 49 patients (Table 1).

Independent T-test results showed no significant difference in the mean age between HCC and liver cirrhosis patients with a p-value of 0.332 ($p > 0.05$). Chi-square test results showed no significant difference in gender between HCC and liver cirrhosis patients with a p-value of 0.914 ($p > 0.05$) (Table 2).

Based on the interpretation of hepatitis B serological tests in HCC, 16 patients had acute hepatitis, 88 patients had chronic hepatitis, and 23 patients had serological gaps. Hepatitis serology test results on liver cirrhosis patients showed acute hepatitis in 8 patients, chronic hepatitis in 38 patients, and serological gap in 4 patients. Chi-Square test results showed no significant difference in interpretation of the hepatitis B serology test with a p-value of 0.230 ($p > 0.05$) (Table 3).

This study found that most males suffer from liver cirrhosis and HCC due to HBV infection, with a mean age of 57. Male:female ratio of HCC risk varies among populations worldwide, ranging from 2:1 to 4:1.[13] Several hypotheses explain that the effect of gender on the incidence of HCC is mediated by hormones, such as androgen hormones as cycle activators, estrogen hormones as protective, sex hormone activity on HBx HBV protein and stimulator of proinflammatory cytokine secretion.[14-16] However, this study found no significant differences in gender between HCC and liver cirrhosis patients because both groups had a higher number of males than females.

Table 1. Characteristics of research subjects

Criteria	n (%)	Mean ± SD	Median (Min-Max)
Gender			
Male	139 (78.5)		
Female	38 (21.5)		
Age		56.75 ± 11.1	57 (29 – 91)
Disease			
HCC	127 (71.8)		
Liver cirrhosis	50 (28.2)		
Interpretation of hepatitis B serological test			
Acute Hepatitis	24 (13.6)		
Chronic Hepatitis	126 (71.2)		
Serological gap	27 (15.3)		
Hepatitis B serological test results on HCC			
Reactive HBsAg	102		
Reactive HBeAg	6		
Reactive anti-HBe	82		
Reactive total anti-HBc	127		
Hepatitis B serological test results on liver cirrhosis			
Reactive HBsAg	47		
Reactive HBeAg	6		
Reactive Anti-HBe	36		
Reactive total Anti-HBc	49		

Table 2. Difference in characteristics of research group

Variable	HCC	Liver Cirrhosis	p
Gender			
Male	100	39	0.914*
Female	27	11	
Age			
Mean Mean±SD	58.30 ± 11.56	55.46 ± 8.96	0.332**
Median (min-max)	59 (32 - 91)	54.5 (33 – 72)	

*Chi-Square test

Table 3. Comparison of interpretation of hepatitis serological test

Variable	HCC	Liver Cirrhosis	p
Acute hepatitis	16	8	
Chronic hepatitis	88	38	0.230*
Serological gap	23	4	

*Chi-Square test

This study found no significant difference in interpreting hepatitis B serology tests between patients with liver cirrhosis and HCC. According to Liu *et al.*, reactive HBsAg and non-reactive HBsAg can be found in hepatoma and liver cirrhosis.[14] According to the interpretation of hepatitis B serological tests, chronic hepatitis was the most common result in patients with liver cirrhosis and HCC. This study followed a study by Chayanupatkul *et al.*, which obtained 8539 patients with chronic hepatitis B and 317 patients with hepatoma (HCC).[17,18] Weissberg *et al.* found 130 liver cirrhosis out of total 379 patients with chronic hepatitis B. Other interpretations of hepatitis B serological tests in this study were acute hepatitis and serological gaps. The interpretation for chronic hepatitis B was based on reactive HBsAg, non-reactive HBeAg, reactive anti-HBe, and anti-HBc. The acute hepatitis B group is characterized by the presence of HBsAg and total anti-HBc, whereas the presence of HBeAg indicates an early phase of hepatitis B infection. When HBsAg serology results are reactive, there are several potential differential diagnoses to consider, such as acute hepatitis B, exacerbation of chronic

hepatitis B, occult reactivation of hepatitis B, superinfection of HBV carrier with other hepatitis viruses, and acute exotoxic hepatitis in HBV carrier.[10]

The serological gap is characterized by total anti-HBc in the absence of reactive results on other serological tests such as HBsAg, HBeAg, and anti-HBe. This serological gap condition can occur in three different situations as follows: During the window period (window period) of acute hepatitis B, When anti-HBs fall to undetectable levels after recovery from acute hepatitis B; When HBsAg may be undetectable due to loss of HbsAg after years of chronic HBV infection.

The clinician should repeat serological tests for HBsAg, anti-HBs, anti-HBc, and anti-Hbe if the anti-HBc results are unclear. If the test is negative, anti-HBc IgM should be tested to exclude the window period of acute infection. A false positive may also be found in an anti-HBc detection test. HBV DNA determination may be considered to detect infection with low viral load.[10]

The study was a retrospective study that collected secondary data from medical records. However, the data were limited the interpretation of hepatitis B serological tests was unable to be confirmed. No results of anti-HBs test as a marker of protection against hepatitis B infection remained one of the limitations in this study.

CONCLUSIONS AND SUGGESTIONS

Both liver cirrhosis and HCC are the most common complications of chronic hepatitis B. There was no significant difference in the appearance of hepatitis B serological tests between liver cirrhosis and HCC. The results of this study can help clinicians consider HBV reactivation in cirrhotic and HCC patients.

Further research is still needed to investigate the status of HBV DNA on non-reactive HBsAg in larger populations and characteristics.

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