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Evaluation of HBV and HCV Seroprevalence in Serum Samples of Individuals Diagnosed with Hepatocellular Carcinoma

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ABSTRACT

Objectives: According to the data of the World Health Organization, hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related deaths. This study aimed to investigate the importance of hepatitis B virus (HBV) and hepatitis C virus (HCV) in the viral etiology of HCC. In this study, we evaluated HBsAg and anti-HCV test results in serum samples sent with the diagnosis of HCC to Virology Laboratory.

Methods: This study was planned as a record-based cross-sectional study. The patients with HCC who were analyzed HBsAg and anti-HCV antibody in serum specimens in Virology Laboratory between October 2016 and December 2018. HBsAg and anti-HCV were tested in serum samples with test parameters chemiluminescent micro-particular enzyme immunoassay method by Architect system.

Results: This study included 44 patients with HCC. The median age of the patients diagnosed with HCC was 64.0 (33.0-88.0) years. Thirty-six (81.8%) of the patients were male, and 8 (18.2%) were female. HBsAg seropositivity was found in 13 (29.5%) patients and anti-HCV seropositivity was found in 2 (4.6%). HBsAg seropositivity was found in 2 (25.0%) of female patients and 11 (30.6%) of male patients (p=0.755). Anti-HCV seropositivity was found in 2 (5.6%) male patients (p=0.666). The highest HBsAg rate was 35.3% in the age group of 50-69 years, and the highest anti-HCV rate was 14.3% in the age group of 70-88 years (p=0.415, p=0.407, respectively).

Conclusion: As a result, HBsAg seropositivity was found in 29.5%, and anti-HCV seropositivity was found in 4.6% of the patients diagnosed with HCC. HBV still keeps its importance in the etiology of HCC.

Keywords: Hepatitis B virus; hepatitis C; enzyme immunoassay; hepatocellular carcinoma.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignity and one of the leading causes of cancer-related deaths around the world.[1] HCC is the fifth most common cancer in men and seventh in women around the world and the second most common cause of cancer-related deaths.[2,3] Chronic viral hepatitis, cirrhosis, alcoholism and hepatosteatosis are among the risk factors of HCC.[4] Worldwide incidence of HCC varies according to the risk factors. Etiology of approximately 90% of HCC is known, and it is most commonly associated with chronic viral hepatitis B and C, alcohol consumption and aflatoxin exposure.[5] Eighty percent of HCC cases are seen in developing countries, such as Sub-Saharan Africa and Asia-Pacific regions, and the leading risk factor is the hepatitis B virus (HBV) infection. The leading risk factor in developed countries, such as the USA, Europe and Japan, is the hepatitis C virus (HCV) infection.[6] As it can be understood here, it was found

that HBV infections that were the main causes of HCC had been replaced by HCV infections in countries where neonatal HBV vaccination program was implemented, such as developed countries.

Approximately 54% of the cases worldwide are attributed to HBV infection and 31% to HCV infection.[5] HCC rates vary according to age, gender and race in different geographical regions. Moreover, it is also related to the differences in the prevalence of hepatitis virus and the age when the infection occurs.[3] Tumorigenesis may be seen in all the cirrhosis cases that arise from different etiological factors; however, the risk of tumorigenesis is higher in patients with chronic viral hepatitis. Generally, HCC occurs in one-third of the patients with cirrhosis throughout their life.[7] According to the long-term cohort studies, HCC occurs in patients with cirrhosis at a rate of 1-8% annually. This rate is 2% in patients with cirrhosis infected with HBV and 3-8% in patients with cirrhosis infected with HCV.[8,9]

HBV is a DNA virus belonging to the family Hepadnaviridae. HBV is transmitted by perinatal, parenteral and sexual routes. Perinatal exposure causes chronic infection in 90-95% of the cases and exposure in childhood causes chronic hepatitis B at a rate of 50%.[10] The mechanism responsible for carcinogenesis in HBV infection is chronic necroinflammation, followed by fibrous and hepatocyte proliferation.[11] HBV DNA is integrated with the host genome and causes changes in cellular signal transduction and hepatocyte proliferation.[12] HBV can also encode the oncogenic viral proteins. In addition to viral proteins, HBV genotype and viral load are associated with hepatocarcinogenesis.[10] HDV superinfection in HBsAg positive patients is responsible for cirrhosis and HCC occurrence at an earlier age.[13]

HCV belonging to the family *Flaviviridae* is a positive-sense single-stranded RNA virus. HCV is often transmitted parenterally. Approximately 80% of the patients infected with HCV progress to chronic hepatitis C.[14] HCV causes HCC by leading to an inflammatory, fibrogenic and carcinogenic tissue microenvironment in the liver.[15] Meta-analysis studies revealed that the occurrence risk of HCC increased in patients with HCV genotype 1b and genotype 3.[16,17] Occurrence rate of HCC is between 0.5% and 0.8% annually in patients with chronic HCV and between 1.4% and 2.5% in patients with cirrhosis.[18] HCC occurrence risk is higher in patients with coinfection than in patients with chronic HBV and HCV infections alone.[19]

In this study, we evaluated the results of HBsAg and anti-HCV tests in serum samples sent with the diagnosis of HCC to the Virology Laboratory and aimed to investigate the importance of HBV and HCV in the viral etiology of HCC.

METHOD

This study was planned as a record-based cross-sectional study. The patients with HCC who were analyzed HBsAg and anti-HCV antibody in serum specimens in Virology Laboratory between October 2016 and December 2018. HBsAg (Architect HBsAg Qualitative II Reagent Kit, Abbott, Germany) and anti-HCV (Architect Anti-HCV Reagent Kit, Abbott, Germany) test parameters in serum samples sent with the diagnosis of HCC to the Virology Laboratory between October 2016 and December 2018 were tested with Architect system (Architect i2000SR, Abbott, USA) and chemiluminescent microparticle enzyme immunoassay method.

The architect system calculates the test results using the Sample RLU/Cut-off RLU (S/Co) ratio for HBsAg and anti-HCV tests. S/Co value is accepted as reactive if the value is ≥1.

Based on the data collected in this study, SPSS 25 software (SPSS Inc, Chicago, IL, USA) was used for statistical evaluation. Continuous data were given as median and minimum-maximum value, while categorical data were given as freqeuncy and percentages. Compliance of the variables with the normal distribution was assessed by visual methods (histogram and probability graphs) and Kolmogorov-Smirnov test. Nonparametric results were compared using the Mann-Whitney U test. Pearson Chi-Square or Fisher exact tests were used in the comparison of the qualitative variables. The results with a p-value under 0.05 were accepted statistically significant.

RESULTS

This study included 44 patients with HCC. The median age of the patients diagnosed with HCC was 64.0 (33.0-88.0) years. Male/Female rate was 4.5/1. Median age of male patients was 63.5 (45.0-85.0) years and that of female patients was 65.5 (33.0-88.0) years (p=0.831). HBsAg seropositivity was found in 13 (29.5%) of the patients and anti-HCV was found in 2 (4.6%) of the patients. Median ages of HBsAg and anti-HCV positive patients were 63.0 (53.0-71.0) years and 63.5 (54.0-73.0) years, respectively. HBsAg seropositivity was found in 2 (25.0%) of female patients and 11 (30.6%) of male patients (p=0.755). Anti-HCV seropositivity was found in 2 (5.6%) males (p=0.666) (Table 1). The highest rate of HBsAg was 12 (35.3%) between the ages of 50 and 69 and the highest rate of anti-HCV was 1 (14.3%) between the ages of 70 and 88 (p=0.415, p=0.407, respectively) (Table 2).

DISCUSSION

The global distribution of HCC is associated with the prevalence of dominant risk factors. HBsAg prevalence around

Table 1. Demographic data of the patients diagnosed with hepatocellular carcinoma

Variable		Age (years)	p
Gender	**n (%)**	**Median (min-max)**	
Male	36 (81.8)	63.5 (45.0-85.0)	0.831*
Female	8 (18.2)	65.5 (33.0-88.0)	
Total	44 (100.0)	64.0 (33.0-88.0)	
Etiology	**Male (n=36)**	**Female (n=8)**	
HBsAg	**n (%)**	**n (%)**	
Positive	11 (30.6)	2 (25.0)	0.755**
Negative	25 (69.4)	6 (75.0)	
Anti-HCV	**n (%)**	**n (%)**	
Positive	2 (5.6)	0 (0.0)	0.666**
Negative	34 (94.4)	8 (100.0)	

*Mann-Whitney U test; ** Pearson Chi-Square test.

Table 2. HBsAg and anti-HCV seropositivity rates according to the age groups

	Age group (years) n (%)				
	33-49	**50-69**	**70-88**	**All ages**	**p**
HBsAg	0 (0.0)	12 (35.3)	1 (14.3)	13 (29.5)	0.415
Anti-HCV	0 (0.0)	1 (2.9)	1 (14.3)	2 (4.5)	0.407

Pearson Chi-Square test.

the world varies geographically. While the low prevalence of HBV infection is in North America, Australia, West and North Europe as <2%, it is 2-7% in middle endemic regions, such as East and South Europe and the Middle East. The highest prevalence of HBV infection is in China, Southeast Asia and Sub-Saharan Africa as >8%.[20,21] HBsAg seroprevalence in our country is reported as between 2% and 7%.[22]

HBsAg seroprevalence is quite variable among patients with HCC. HBV rates in HCC cases are reported as 13-15% in Europe, 9-45% in America, 22-41% in Asia and 27-45% in Africa.[5] In studies on patients diagnosed with HCC, HBsAg positivity rates were found between 22.5% and 68.0% in our country.[23–30] Additionally, when compared with positive results stated in different geographic regions around the world, this rate was found higher than the rates of European and Asian countries and similar to the rates of America and Africa. In our study, the HBsAg positivity rate was 29.5%. This rate found in patients diagnosed with HCC is one of the lowest HBsAg positivity rates compared to the other studies conducted in our country.

Implementations of vaccination against HBV have led to decrease in the prevalence of HBV carriers and contributed to the prevention of HCC.[31]

It is estimated that global HCV prevalence is 2.5% and this rate considerably varies among different regions. The rates are reported to be 1.8% in Europe, 1.3% in America, 2.8% in Asia, 2.7% in the Middle East and Africa and 1.8% in Australia.[32] Anti-HCV seroprevalence rates among the patients with HCC were found as 24-44% in Europe, 12-31% in America, 9-55% in Asia and 11-44% in Africa.[5] In studies on patients diagnosed with HCC, anti-HCV positivity rates were found between 5.1% and 28.6% in our country.[23–30] In our study, anti-HCV positivity rate was 4.6%. This rate found in patients diagnosed with HCC is the lowest anti-HCV positivity rate compared to the other studies conducted in our country. Although HCC risk is 4-8 times more in men than in women throughout the world, there was no significant difference in age distribution.[33,34] The prevalence of HCC is more in men has become a research subject for laboratory studies. It was found that chronic inflammation was a predisposing factor for liver malignities and IL-1, and IL-6 from inflammatory cytokines had an inducing role in HBV-related HCC occurrence.[32,34] It was revealed that 17ß estradiol in women suppressed IL-mediated inflammatory process in Kupffer cells and decreased hepatocyte proliferation.[35,36] Moreover, although IL-1 was ten times higher in neighbouring tissues of HCC than in normal tissues, this was not observed in women.[37] Sex hormones, such as androgens and oestrogens, show their biological functions by binding to the specific androgen and oestrogen receptors. It was revealed that androgen signalling pathways increased HBV replication. In addition, androgen signalling pathways induce HCC progression by suppressing p53 activity and DNA repair.[38] In this study, the highest HBsAg positivity and anti-HCV seropositivity in patients with HCC was found in male patients. Male/Female ratio was found as 4.5/1 in all patients. The predomination of the male gender was similarly reported in the studies conducted in our country.[23–30]

Advanced age is also a significant risk factor in HCC occurrence, as well as the presence of cirrhosis and gender. HCC incidence increases by age in all populations and reaches a peak at the age of 70 years. HCC is rarely seen in the first four decades of life except for populations in which HBV is hyperendemic. In Chinese and black African populations, the mean age of the patients with HCC is significantly younger.[3,39] Apart from these populations, while the occurrence risk of cirrhosis and HCC for chronic HCV infections is more significant worldwide in the ages above 60, this is not valid for chronic HBV infection. HCC risk is inversely correlated with the age when HBV infection occurs.[39,40] In epidemiologicalstudies carried out in patients with HCC in

Turkey between 2001 and 2014, it was observed that HCC progression occurred after the fourth decade.[23–30] In this study, which is consistent with other studies and literature in our country, the median age of HBsAg and anti-HCV positive patients with HCC was 63.5 years and 63 years, respectively.

The most important limitations of this study are the small size of the sample and difficulties in establishing a cause and effect relationship with cross-sectional studies. It is believed that the researchers should conduct at least a cohort or case-control studies with more samples in future studies on this subject. Because this study was conducted on the basis of hospital records, all clinical information about the patients could not be reached. Other risk factors (for example, chronic liver disease and cirrhosis) and exposure time that may cause HCC are unknown.

CONCLUSION

As a result, in this retrospective study, HBsAg seropositivity was found in 29.5% and anti-HCV seropositivity was found in 4.6% in the serum samples of the patients diagnosed with HCC. HBV still keeps its importance in the etiology of HCC. It is crucial to develop vaccine programs aimed for the protection of especially new generations against HBV infection and carryout awareness studies in order to inform the populations.

Disclosures

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Ethics Committee Approval: This study was performed with the approval of the Non-Interventional Clinical Research Ethical Committee of the University of Health Sciences Gulhane Training and Research Hospital (reference number: 26.02.2019/19/40).

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