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ORIGINAL ARTICLE

Hepatitis C virus-related hepatocellular carcinoma and liver cirrhosis

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Summary

Purpose: *The aim of this study was to compare patients with hepatocellular carcinoma (HCC) associated with hepatitis C virus (HCV) infection with patients with liver cirrhosis associated with HCV virus infection.*

Methods: *Forty-five patients were prospectively analyzed, all with HCV infection. Patients were divided into 2 groups. The first group consisted of 21 patients with histologically proven HCC and the second one consisted of 24 patients with liver cirrhosis without HCC. PCR was carried out in order to diagnose active HCV infection and HCV genotyping.*

Results: *There was no statistically significant difference in the structure of the compared groups of patients in relation to sex and age. In 76.19% of the patients with HCC cirrhosis preceded HCC, while 23.81% of the patients had chronic hepatitis. The prevalence of genotypes in the HCC group was 1a in 4.76%, 1b in 80.95% and 2a in 14.29%. In the group with liver cirrhosis 1a was detected in 20.83%, 1b in 45.83%, 2a in 12.50%, 2b in 4.17% and 3a in 16.67% of the patients. The prevalence of genotype 1b was significantly higher among HCV RNA positive patients with HCC compared to the group with liver cirrhosis and HCV RNA positive patients (x^2=4.48; p=0.034). In the group where cirrhosis preceded HCC, genotype 1b was found in 75% of the cases, genotype 2a in 18.75%, and genotype 1a in 6.25%. Genotype 1b was detected in 100% of patients with chronic hepatitis and HCC.*

Conclusion: *The role of HCV infection in the development of HCC has not been fully clarified. Most authors evaluate the role of individual genotypes in the pathogenesis of HCC. This study has shown that the dominant genotype found in patients with HCC is 1b.*

Key words: genotype, hepatitis C virus, hepatocellular carcinoma, liver cirrhosis

Introduction

Primary liver cancer is the 5th most common cancer in the world and the 3rd most common cause of cancer mortality [1]. In most countries, 75-90% of all liver cancers are HCCs [2], thus the trends in liver cancer incidence and mortality tend to reflect the trends in HCC incidence and mortality. Epidemiological studies have shown that the occurrence of HCC correlates with many factors such as viral infection, alcohol abuse and the toxic compound aflatoxin. Hepatitis B virus (HBV) and chronic infection with HCV in particular are the major contributors to HCC [3-7].

In the Western world, HCC arises almost exclusively as a late consequence of liver cirrhosis [8]. It has been shown that chronic HBV and chronic HCV infections are strong and independent risk factors associated with HCC development [9]. Moreover, an additive interaction on the risk of HCC development is observed in subjects with dual HBV/HCV infection [10]. HBV and HCV are independent predictors of HCC because they cause cirrhosis [11] and the great majority of HCCs is associated with cirrhosis. Chronic liver disease is characterized by varying degrees of inflammation and fibrosis [12]. The results of most authors show that the prevalence of HCV infection in HCC patients is higher than HBV infection [13]. While the very nature of HCV infection in the pathogenesis of HCC is not completely clear, most authors deal with the evaluation of the role of certain viral genotypes in the development of HCC. The distribution of HCV genotypes varies in different geographical areas. Some studies have shown that genotype 1b is the most aggressive and responsible for the development of HCC [14]. It is widely accepted that

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long-term chronic inflammation with necrosis and regeneration of hepatocytes is the molecular and cellular basis for tumor development, given that RNA viruses do not integrate into the host genome [15,16].

HCV infection has become a major health problem worldwide because of the potential natural course of this disease into cirrhosis and then HCC [17].

Methods

This was a comparative study of patients with HCC and active HCV infection (patients with HBV infection and alcoholism were excluded) and patients with cirrhosis caused by HCV infection but without HCC. The following parameters were analyzed: sex, age, HCV infection preceding HCC, and HCV genotype frequency. The study was performed at the Institute of Public Health (Department of Virology) and the Surgical Clinic, Clinic of Gastroenterology, Clinic of Oncology, and Laboratory of Immunology of the Clinical Centre in Nis. The diagnosis of primary HCC was established using computed tomography (CT), magnetic resonance imaging (MRI), liver ultrasound, and histological verification after surgical intervention.

Serological diagnosis of HCV and HBV infection was identified using the following tests: ELISA test generation II MONOLISA anti-HCV Plus Version 2 (BIO-RAD, Marnes- la-Coqette, France) for the detection of antibodies to the structural antigen C22-3 and non-structural antigen C33-c, and C100-3, HBsAg ELISA for the detection of HBs antigen of HBV-MONOLISA HBsAg ULTRA (BIO-RAD, Marnes-la-Coqette, France).

In order to diagnose active HCV infection and HCV genotype the PCR method was used (VERSANT HCV genotype assay/LiPa Bayer HealthCare, LCC Tarrytown, NY, USA).

Statistical analysis

For the description of the established groups, standard descriptive statistical parameters were used. The following statistical tests were applied: Student's *t*-test, x^2-test or Fisher's exact test, and Mann-Whitney U-test. SPSS for Windows (Version 10.0) and Epi Info 6 (version 6.04) were used for statistical data processing. Statistical significance was set to $p<0.05$.

Results

We analyzed a group of 21 patients (men 76.2%, women 23.8%, mean age 60.76±8.43 years, range 40-73) with histologically proven HCC and HCV infection in a period of 8 years. This group was compared with a group of 24 patients with liver cirrhosis and proven HCV infection without HCC. The latter group was composed of 79.2% men and 20.8% women, mean age 58.96±5.77 years (range 48-70) (Table 1). No statistically significant differences according to sex (Fisher's exact test: p = 1.00), and age (Student's t-test=0.645, p=0.415) were found between the groups.

In 76.19% of the patients with HCC cirrhosis preceded, while 23.81% of patients had chronic hepatitis (Table 2). The prevalence of viral genotypes in the group with HCC was: 1a in 4.76%, 1b in 80.95%, and 2a in 14.29%. In the group with liver cirrhosis it was: 1a in 20.83%, 1b in 45.83%, 2a in 12.50%, 2b in 4.17% and 3a in 16.67% of the patients (Table 3). The prevalence of

Table 1. Patient distribution with HCV-related HCC and liver cirrhosis according to age and sex

Characteristics	*HCC+HCV (n=21) n (%)*	*Cirrhosis+HCV (n=24) n (%)*
Males	16 (76.19)	19 (79.2)
Females	5 (23.81)	5 (20.8)
Age (years)		
41-50	3 (14.29)	2 (8.33)
51-60	6 (28.57)	12 (50.0)
61-70	10 (47.62)	10 (41.67)
> 70	2 (9.52)	–
Mean ± SD (years)	60.76±8.43	58.96±5.77
Range	40-73	48-70

HCV: hepatitis C virus, HCC: hepatocellular carcinoma, SD: standard deviation

Table 2. Patient distribution according to liver disease preceding HCC

Disease before the appearance of HCC	*Patients, n*	*%*
Chronic hepatitis C	5	23.81
Cirrhosis	16	76.19
Total	21	100.0

HCC: hepatocellular carcinoma

Table 3. Distribution of HCV genotypes in patients with HCC and liver cirrhosis

HCV genotype	*HCC+HCV (n=21)*		*Cirrhosis+HCV (n=24)*	
	No.	*%*	*No.*	*%*
1a	1	4.78	5	20.83
1b*	17	80.95	11	45.83
2a	3	14.29	3	12.50
2b	–	–	1	4.17
3a	–	–	4	16.67

*x^2=4.48, p=0.034
HCV: hepatitis C virus, HCC: hepatocellular carcinoma

genotype 1b was significantly higher among HCV RNA positive patients with HCC compared with the group with liver cirrhosis and HCV RNA positive patients (x^2=4.48, p=0.034). In the patient group where cirrhosis preceded genotype 1b was found in 75%, genotype 2a in 18.75%, and genotype 1a in 6.25% of the patients. Genotype 1b was detected in 100% of the patients with chronic hepatitis and HCC (Table 4). There was no statistically significant difference in the prevalence of genotype 1b and other genotypes in the patients where cirrhosis preceded HCC in comparison to those with chronic hepatitis and HCC (Fisher's exact test: p=0.532).

Table 4. Distribution of HCV genotypes in liver disease preceding HCC

HCV genotype	*Chronic hepatitis (n=5)*		*Cirrhosis (n=16)*	
	n	*%*	*n*	*%*
1a	–	–	1	6.25
1b	5	100.00	12	75.00
2a	–	–	3	18.75
2b	–	–	–	–
3a	–	–	–	–

HCV: hepatitis C virus, HCC: hepatocellular carcinoma

Discussion

This study showed that HCC associated with HCV infection often occurs in elderly men. Studies have shown that the incidence of HCC among patients with cirrhosis caused by HCV infection is higher in men older than 50 years [18]. A comparative analysis of patients with HCC associated with HCV and patients with cirrhosis and HCV conducted by Sanchez et al. [19] confirmed that the majority of the patients were older men. Men comprised 61% of the patients with HCC and 69% of the patients with cirrhosis, while the mean patient age was 60.1 and 68.9 years, respectively.

Many authors stated that the first contact with HCV causes acute infection, usually with mild clinical course. Approximately 55-85% of persons who develop acute hepatitis C will remain with chronic HCV infection. Among these individuals, 5-20% are reported to develop cirrhosis over a period of 20-25 years [20]. The evolution of chronic hepatitis C can lead to either cirrhosis or directly into primary HCC [21]. According to recent literature data, in 70-80% of the patients with HCC associated with HCV infection, cirrhosis precedes cancer. In these patients, cancer usually develops in cirrhotic nodules induced by cirrhosis and in old age [22-24]. Saeian and Reddy [25] showed that the rate of development of HCC in patients with liver cirrhosis caused by HCV infection is 1-7% per year. The incidence of HCC in that study was 1.4% per year, with an increase of 4-7% during a period of 3-5 years. Twenty-three percent of patients with liver cirrhosis caused by HCV eventually developed HCC. Degos et al. [17] also concluded that the incidence of HCC and its course correlated with the severity of the previous liver disease. Davcev et al. [26] in their work state that 26.2% of their patients had liver cirrhosis associated with HCV infection before the appearance of HCC.

The role of HCV infection in the pathogenesis of HCC has not been fully clarified. Most authors deal with the evaluation of the role of individual viral genotypes in the development of HCC. The distribution of HCV genotypes varies in different geographical areas. According to Simmonds et al. [27], in North America genotype 1a predominated, followed by 1b, 2a, 2b and 3a. In Europe, the dominant genotype was 1b, followed by 2a, 2b, 2c and 3a [27]. Genotypes 4 and 5 appear exclusively in Africa [27]. However, a challenging question is whether HCV genotypes play a role in the progression of chronic liver disease and then to HCC. Different views are to be found in the relevant literature, although results of several studies suggest that there is a correlation between HCV genotypes and the development of HCC [28-31]. Our results show that 1b is the dominant genotype in patients with HCC associated with HCV infection. The prevalence of genotype 1b in published studies [28-31] ranged from 60-80% and was significantly higher than in patients with cirrhosis associated with HCV infection or chronic hepatitis. Patients infected with genotype 1b carry 2-fold higher risk of HCC compared to those infected with other genotypes [28]. In a prospective study that observed patients with cirrhosis associated with HCV infection, the results showed that HCC occurs more frequently in patients infected with genotype 1b [31,32]. Reid and his associates [33] compared the distribution of HCV genotypes in patients with HCC and those with cirrhosis without HCC. The results showed a high prevalence of genotype 1b in both groups. The prevalence of genotype 1b in patients with cancer was higher than in the group of patients with cirrhosis but without statistical significance (41 vs. 24%; p=0.24) [33]. Several studies suggest that HCV genotypes have no significant impact on the degree of severity and outcome of liver disease in patients with chronic HCV infection [33-36].

Why the genotype 1b is commonly associated with the development of HCC? It is widely accepted that long-term chronic inflammation with necrosis and regeneration of hepatocytes is the molecular and cellular basis for the development of tumors [15,16]. As a result, one can hypothesize that genotype 1b has increased cytopathogenic effect that creates its ability to

induce progressive liver disease with the possibility of cancer development. Several authors have proven the high cytopathogenic effect of this genotype. First, HCV genotype 1b infection is rarely detected in patients with minimal liver damage, which is associated with persistently normal ALT values and slow disease progression [37]. Second, reactivation of HCV infection in a patient with liver transplantation is much more frequent and accompanied by a faster disease progression if the patient is infected with genotype 1b than with other genotypes [38]. Third, a weaker response to interferon therapy is found in patients infected with genotype 1b [39,40]. Fourth, genotype 1b infection is usually accompanied by a higher viral titre in blood, than infection with other genotypes [36]. Patients with cirrhosis infected with HCV genotype 1b had a significantly higher risk of developing HCC compared to those in whom the cirrhosis was caused by other genotypes. Sixty-two percent of patients in whom cirrhosis preceded HCC had genotype 1b [31]. Genotype 1b was detected in all patients with HCC and previous chronic hepatitis [30]. Our study has shown the same results. Kobayashi et al. had also found a statistically significant prevalence of genotype 1b in comparison to other genotypes in patients with HCC which was preceded by chronic hepatitis [41]. The fact that HCC occurs in the absence of cirrhosis suggests that HCV may possess a direct oncogenic effect. How can such a direct oncogenic effect of the virus be explained? In contrast to HBV infection, in which the development of HCC is associated with chromosomal integration of DNA genome in the genome of hepatocytes, HCV-RNA genome is not integrated into cellular DNA. However, the viral gene products may be responsible for the induction of hepatocyte proliferation. Recent studies have focused on the role of HCV core protein, NS3 and NS5a protein [42-45].

In conclusion, about 85% of the patients infected with HCV develop chronic infection. Chronic HCV infection is often asymptomatic but can lead to liver cirrhosis and the development of HCC. HCV is a major cause of chronic liver disease in Japan, Europe and USA. The prevalence of HCV markers in patients with HCC varies in different geographic areas. In north Europe it averages 30%, while in Italy and Spain ranges between 60-70% [46].

Antibodies to HCV were found in 76% of HCC patients in Japan [47]. The role of HCV infection in the pathogenesis of HCC has not been fully clarified. Most authors deal with the evaluation of the role of individual genotypes in the development of HCC. The distribution of HCV genotypes varies in different geographical areas. This study has shown that the dominant genotype is 1b in patients with HCC associated with HCV infection.

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