

Hepatitis virus and hepatocellular carcinoma in Brazil: a report from the State of Espírito Santo

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ABSTRACT

Introduction: Few studies have examined hepatocellular carcinoma (HCC) in Brazil, and the incidence and risk factors for this type of malignancy vary greatly geographically. In this paper, we report several risk factors associated with HCC diagnosed at the University Hospital in Vitória, ES, Brazil. **Methods:** We reviewed 274 cases of HCC (January 1993 to December 2011) in which hepatitis B (HBV) and C (HCV) virus infection and chronic alcoholism were investigated. A diagnosis of hepatocellular carcinoma was confirmed by histology or by the presence of a characteristic pattern on imaging. **Results:** HCC with associated liver cirrhosis was noted in 85.4% of cases. The mean ages of men and women were 56.6 years and 57.5 years, respectively. The male-to-female ratio was 5.8:1. Associated risk factors included the following: HBV, 37.6% (alone, 23.4%; associated with chronic alcoholism, 14.2%); HCV, 22.6% (alone, 13.5%; associated with chronic alcoholism, 9.1%), chronic alcoholism, 17.1%, non-alcoholic steatohepatitis, 2.6% and cryptogenic, 19.3%. The male-to-female ratio was higher in cases associated with HBV or chronic alcoholism compared with HCV-associated or cryptogenic cases. In 40 cases without associated cirrhosis, the male-to-female ratio and mean age were lower than those in cirrhosis-associated cases. **Conclusions:** These results demonstrate that the main risk factor associated with HCC in the State of Espírito Santo is HBV. Chronic alcoholism is an important etiological factor, alone or in association with HBV or HCV infection.

Keywords: Hepatocellular carcinoma. Hepatitis B virus. Hepatitis C virus. Alcoholism.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the seventh most common malignancy worldwide and accounts for nearly 750,000 new cases and 700,000 deaths per year. The incidence of HCC varies greatly around the world, depending on a person's exposure to different risk factors. The incidence is high in Africa and Southeast Asia (13.9 per 100,000 persons in Zimbabwe, 5.1 to 21.0 in China, and 26.9 to 38.1 in Korea) areas in which hepatitis B virus (HBV) is endemic. In contrast, incidence is low in more developed countries such as the United States (from 2.2 to 3.4 in white Americans) and in most European countries (0.6 to 2.2 in the United Kingdom and Netherlands, and 1.6 to 10.9 in Austria, Belgium, France, Spain and Germany), with the exception of some countries in Southern Europe (2.8 to 25.0 in Italy) and of Japan (11.4 to 30.4), where

the tumor has a high incidence, with hepatitis C virus (HCV) being the main associated risk factor¹⁻³.

Studies on the incidence of HCC in Brazil are lacking, and the information that does exist originates from four cancer registers in Cuiabá, Goiânia, Brasília (Central west Region) and São Paulo (Southeast Region)³. The higher incidence rate was reported in São Paulo: 2.4 and 0.6 cases/100,000, respectively, for men and women³. The incidence rates registered in Brazil are similar to those observed in other countries in South and Central America (from 0.6 to 2.1/100,000 in men)³.

Approximately 70 to 90% of patients with HCC also have chronic liver disease and cirrhosis^{4,5}. The main causes of liver cirrhosis in patients with HCC include infection with hepatitis B or C virus, abusive ingestion of alcohol and non-alcoholic steatohepatitis (NASH). Less frequent causes include hereditary hemochromatosis, alpha-1 antitrypsin deficiency, autoimmune hepatitis and porphyrias^{6,7}. Hepatitis B and C viruses are responsible for approximately 75% of HCC cases⁸.

Risk factors associated with this form of cancer were reported in two national surveys, sponsored by the Brazilian Society of Hepatology, that were conducted in 1999⁹ and 2010^{10,11}, as well as in a series of cases from Minas Gerais (MG)¹². The results of the first survey indicated that HBV was a common factor associated with HCC in the North and Northeast regions, whereas HCV was the main risk factor overall, especially in metropolitan areas in the South and Southeast regions⁹.

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The second survey yielded different results: HCV was found to be the main associated risk factor in all regions, but a high frequency of HBV-associated cases in the North, Central West and Northeast regions was also noted^{10,11}. In a series of HCC cases that were diagnosed in Belo Horizonte, MG, the main associated factor was found to be HCV (44% of cases), while HBV infection was observed in 23.5% of cases¹². Associated liver cirrhosis has been reported at different frequencies in HCC cases in Brazil, ranging from 87% in the first national survey⁹ to 98% in the second survey^{10,11}. Because Brazil is a large country and the environmental risk factors for HCC may vary across regions, we chose to examine the main associated risk factors in 274 cases of hepatocellular carcinoma diagnosed at the University Hospital in Vitória; we thus investigated HBV and HCV infections and chronic alcoholism in all cases.

METHODS

The medical records of 274 consecutive patients who were diagnosed with hepatocellular carcinoma at the University Hospital Cassiano A Morais (HUCAM) between 1993 and 2011 were examined. Age, sex, skin color, and whether cirrhosis or any of the main associated etiological factors presented were recorded for each patient. In addition, medical records were examined for evidence of HBV and HCV infection or chronic alcoholism.

In all cases, documentation of alcohol abuse (up to 80g per day for over 10 years) and of the presence of hepatitis B surface antigen (HBsAg) was sought, as were anti-HCV antibody levels, as measured by enzyme-linked immunosorbent assay (ELISA) in the clinical laboratory at HUCAM. All anti-HCV-positive cases were confirmed by the detection of viral ribonucleic acid (RNA) in serum.

The diagnosis of hepatocellular carcinoma was confirmed either by histopathology or by imaging (ultrasonography, computerized tomography or magnetic resonance imaging) in conjunction with alpha-fetoprotein measurements. Barcelona's diagnostic criteria¹³ were used when the diagnosis was established using non-invasive methods. The diagnosis of cirrhosis was based on clinical and laboratory data and was confirmed by histology (biopsy or necropsy), imaging methods and endoscopic evidence of esophageal varices. The diagnosis of NASH non-alcoholic steatohepatitis was established when information in the patient's medical record met the diagnostic criteria recently established for this disease¹⁴. Cases that did not fulfill the criteria for diagnosis of persistent HBV or HCV infection, chronic alcoholism or NASH were considered cryptogenic.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 15.0 IBM, Chicago, IL, USA). Comparisons of means, medians and frequencies were performed using either parametric or nonparametric tests, depending on the types and distributions of the variables. A p-value less than 0.05 was considered statistically significant.

Ethical considerations

The Ethics Committee of *Centro de Ciências da Saúde da Universidade Federal do Espírito Santo* approved this research.

RESULTS

Patient age and gender distributions are summarized in **Figure 1**. The patients were predominantly male (85.4% of cases), with a male-to-female ratio of 5.8:1. Most (71.5%) cases occurred between the fifth and eighth decades of life, with a mean age of 56.6 ± 14.4 years and a median age of 57.5 years (53.4 ± 13.4 years, median 57 years in men; 58.1 ± 19.1 years, median 62 years in women).

The main risk factors found to be associated with HCC are shown in **Table 1**. The three most common associated risk factors were the following: persistent HBV infection, in 37.6% (23.4% isolated infections; 14.2% associated with alcoholism); HCV infection, in 22.6% (13.5% alone; 9.1% associated with chronic alcoholism); and abuse of alcohol alone, in 17.1%. Non-alcoholic steatohepatitis was found in 2.6% of cases, and 19.3% of cases were considered cryptogenic.

The median age and the male-to-female ratio varied with the associated risk factor (**Table 1**). The male-to-female ratio was higher in cases associated with HBV and abuse of alcohol than in cases with other etiologies or in cases that were cryptogenic.

In 85.4% of cases, the malignancy was associated with liver cirrhosis. The cases of HCC without associated liver cirrhosis exhibited several differences from cases in which cirrhosis was present. A significantly greater proportion of cases of HCC without cirrhosis were women, compared with cases of HCC with associated cirrhosis (13/40 and 27/234, respectively, $p < 0.001$; male-to-female ratio 2.1:1 and 7.6:1, respectively, $p < 0.001$). Cryptogenic cases were more prevalent among cases without liver cirrhosis than among those with associated liver cirrhosis (21/40 and 32/234, respectively, $p < 0.001$). The mean age was 52.6 ± 19.1 years in non-cirrhotic cases and 57.4 ± 13.2 years in cases associated with cirrhosis ($p < 0.05$). The age distribution showed a bimodal pattern (**Figure 2**).

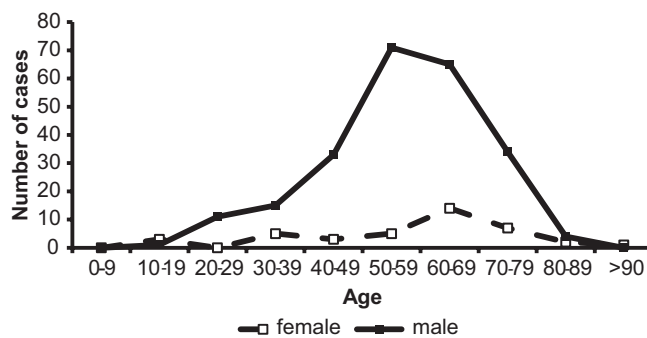
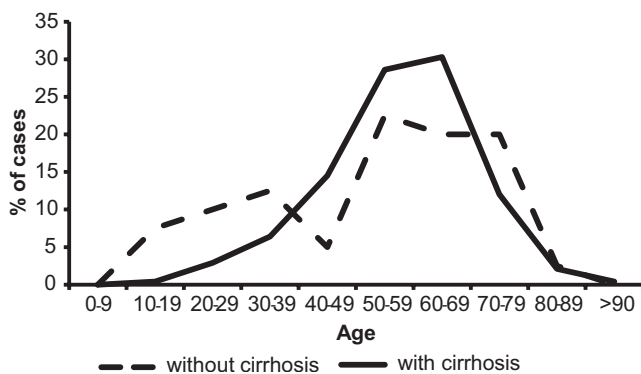


FIGURE 1 - Age and gender distributions of 274 cases of hepatocellular carcinoma diagnosed in Vitória, State of Espírito Santo, Brazil.

TABLE 1 - Risk factors associated with 274 cases of hepatocellular carcinoma diagnosed in Vitória, State of Espírito Santo, Brazil.

Risk factor*	Number	Percentage	Male-to-female ratio	Age (years; mean \pm SD)	Median
HBV	64	23.4 ^a	60/4 (15:1) ^a	53.3 \pm 16.0	57.5
HBV plus alcoholism [#]	39	14.2	39/0 (39:0) ^a	54.1 \pm 11.4	54.0
HCV	37	15.5	26/11 (2.4:1)	59.1 \pm 9.8	60.0
HCV plus alcoholism [#]	25	9.1	25/0 (25:0) ^a	54.6 \pm 8.5	52.0
Chronic alcoholism	47	17.1	46/1 (46:1) ^a	61.0 \pm 10.3	61.0
Cryptogenic	53	19.3	34/19 (1.7:1)	65.0 \pm 11.6	62.0
NASH	7	2.6	2/5 (0.4:1)	56.5 \pm 20.2	60.0

HBV: hepatitis B virus; **HCV:** hepatitis C virus; **NASH:** non-alcoholic steatohepatitis. *One case had HBV and HCV infection, and another case had chronic alcoholism and both HBV and HCV infections. ^aComparisons of male-to-female ratios: $p < 0.05$ in relation to HCV, cryptogenic and NASH. [#]Comparisons of ages: HBV x HBV plus alcoholism, $p = 0.779$; HCV x HCV plus alcoholism, $p = 0.074$.

**FIGURE 2 - Age distribution of hepatocellular carcinoma with or without associated liver cirrhosis diagnosed in Vitória, State of Espírito Santo, Brazil.**

HBV or HCV infection associated with chronic alcoholism was significantly less frequent in cases without cirrhosis ($p < 0.05$). However, non-significant differences between the two groups were found for the frequencies of isolated HCV or HBV infection, chronic alcoholism alone and NASH (Table 2).

DISCUSSION

Age at the time of diagnosis and the male-to-female ratio of HCC cases reported here are similar to those reported in the results of two national surveys^{9,10} and in São Paulo¹¹ and Minas Gerais¹². The age at the time of diagnosis is also close to the mean ages reported for areas of intermediate or high incidence of HCC, where this type of cancer is much more commonly observed in men and the mean of age at diagnosis age is between 55 and 59 years (review in reference 6). The ages at the time of diagnosis and the male-to-female ratio observed in cases reported here and in other Brazilian regions suggest that the incidence of HCC in Brazil may be higher than suggested by the low incidence rates reported by the few cancer registries from the Central West and Southeast regions³.

The mean age observed in these cases may be related to risk factors, namely, the high prevalence of hepatitis B and chronic alcoholism, alone or associated with HBV or HCV. In fact, HCC that is associated with HBV occurs at younger ages, and it is more frequent in men, especially in areas of high prevalence of

TABLE 2 - Risk factors in hepatocellular carcinoma with or without liver cirrhosis, diagnosed in Vitória, State of Espírito Santo, Brazil.

Risk factor	HCC with cirrhosis (n=234)		HCC without cirrhosis (n=40)	
	n	%	n	%
HBV (n=64)*	54	23.0	10	25.0
HBV plus alcoholism (n=39) [#]	38	16.2	1	2.5
HCV (n=37)*	34	14.5	3	7.5
HCV plus alcoholism (n=25) [#]	25	10.6	0	0.0
Chronic alcoholism (n=47)*	43	18.3	4	10.0
Cryptogenic (n=53) [#]	32	13.6	21	52.5
NASH (n=7)*	6	2.5	1	2.5

[#] $p < 0.05$. **HCC:** hepatocellular carcinoma; **HBV:** hepatitis B virus; **HCV:** hepatitis C virus; **NASH:** non-alcoholic steatohepatitis. Comparison between HCC with cirrhosis and HCC without cirrhosis: * $p > 0.05$.

the virus, where vertical transmission is frequent. In the State of Espírito Santo, where the prevalence of HBV infection is moderate^{15,16}, there is indirect evidence of vertical transmission, as more than one case of HBsAg-positive chronic liver disease is frequently observed in the same family at the Gastroenterology Unit of HUCAM (data not shown). On the other hand, when associated with hepatitis virus infection, chronic alcoholism, which is more frequent in men, accelerates the progression of liver lesions related to viral infection, especially those associated with HCV^{5,17,18}. This accelerated progression, manifested by a younger age at the time of diagnosis was not observed in the cases reported here. Although lower median ages were observed in HCV and HBV cases associated with chronic alcoholism, the differences did not reach statistical significance.

While the results presented in this paper confirmed the large variation in the etiological factors of HCC that is observed worldwide, these findings nonetheless differ in several respects from those reported for most countries and for other regions of Brazil. In a recent review by El Seragh et al¹⁹, HCV was noted to be the most common risk factor in Japan, Italy and the United States, whereas in sub-Saharan Africa and Southeast Asia, the main risk factor is HBV.

HCV infection was present in 22.6% of HCC cases, which is lower than the 54%, 44% and 38% of cases reported in recent surveys in Brazil¹¹ and case series from Minas Gerais¹² and Latin America²⁰, respectively. This variability reflects differences in exposure to risk factors for chronic liver disease in our county, where alcoholism and chronic infection with hepatitis B virus are more prevalent than in other regions of Brazil or Latin America²¹. The high frequency of HBV associated with HCC reported here (37.6% of the cases) is noteworthy. This prevalence is higher than the 16% reported in a recent nationwide survey of Brazil^{10,11} and the 23.5% in Minas Gerais¹². This difference is in agreement with the moderate prevalence of hepatitis B virus infection in the State of Espírito Santo, where there are areas in which the prevalence of HBsAg ranges from 1.2 to 6.7%^{15,16}. Moreover, these results are consistent with previous reports of a high frequency of HBsAg-positive hepatocellular carcinomas diagnosed at the University Hospital in Vitória during a period of time before the cases reported here were diagnosed²².

Another important feature of the data presented here is the high frequency with which chronic alcoholism was observed in HCC cases; chronic alcoholism was present in 40.4% of cases (17.1% as the only associated factor; 23.3% associated with HBV or HCV infection). These data reflect the high prevalence of alcohol abuse in metropolitan Vitória (27.8% in men; 10.8% in women)²³ and the high frequency of chronic alcoholism in the etiology of liver cirrhosis in the State of Espírito Santo²¹. In addition, this observation confirms the importance of the abusive use of alcohol in the development of HCC^{6,17,18,24}. A recent study reported changes in the etiology of HCC in Italy, with an increasing number of cases associated with abusive alcohol ingestion in the last ten years²⁵.

Non-alcoholic steatohepatitis was identified in 2.6% of cases, a prevalence similar to that reported in the last national survey^{10,11}. An increased number of NASH-associated HCC

has also been observed in more developed countries²⁶, and it is possible that some cryptogenic cases may be related to unidentified NASH, instead, as has been hypothesized previously^{5,27,28}. Cryptogenic cases accounted for 19.3% of our sample, which is a greater frequency than that reported for the most recent national survey in Brazil. The high frequency of cryptogenic cases may result from the under-diagnosis of NASH but may also be due to other environmental, etiological factors such as food contamination by aflatoxins, which has been reported in Brazil²⁹. However, we do not know what the impact of these mycotoxins may be on the etiology of HCC in Brazil. A mutation at codon 249 of TP53 occurred in 14.6% of 41 cases of HCC diagnosed in Vitória³⁰, an indirect indication of moderate exposure to aflatoxin in our county. It is also possible that occult HBV infection may have been present in some HBsAg-negative cases³¹⁻³³ and that it could thus help to explain the greater frequency of cryptogenic cases; however, this possibility was not investigated for the cases reported here.

The proportion of cases associated with liver cirrhosis was 85.4%, which is consistent with that reported in the literature (cirrhosis has been found to occur in 80 to 90% of cases of HCC^{5,19}). However, the frequency of cirrhosis was lower than that reported for the second national survey^{10,11}, in which cirrhosis was associated with 98% of cases. In case series of HCC from Minas Gerais¹², no information on the frequency of associated liver cirrhosis is reported. We do not have a definitive explanation for this difference and hypothesize that it may be related to the quality of the samples studied; in health units that are specialized in the care of cirrhotic patients, the frequency of HCC with associated cirrhosis may be greater than that in a general hospital with a gastroenterology unit that admits patients with all types of liver disease.

Reported cases of HCC in the non-cirrhotic liver have exhibited peculiar characteristics with respect to age group, gender and etiology, although the reported findings have been inconsistent^{5,34,35}. For the 40 cases of hepatocellular carcinoma without liver cirrhosis in our sample, the male-to-female ratio was lower than for cases that were associated with liver cirrhosis. Moreover, the age distribution was different from that observed for cases associated with cirrhosis, with a lower mean age, a bimodal distribution, and a higher proportion of cases diagnosed before the fifth decade. Cryptogenic cases were more frequent among non-cirrhotic HCC cases. The occurrence of three cases of HCV-positive HCC in non-cirrhotic patients confirms that, infrequently, HCV may induce this type of cancer without any accompanying liver cirrhosis, as has been reported by other authors³⁶⁻³⁸.

In conclusion, the results reported here demonstrate that the main risk factor associated with HCC in the State of Espírito Santo was infection with HBV. Additionally, this risk factor was observed more frequently in patients in this study than in patients studied in São Paulo and Minas Gerais, confirming the substantial geographic variation in risk factors associated with this malignancy in Brazil. Furthermore, our results demonstrate that chronic alcoholism was an important risk factor, alone or in association with HBV or HCV infection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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