

## A case-control study on the association of hepatitis B virus infection and hepatocellular carcinoma in Northeast Brazil\*

### *Estudo caso-controle da associação entre a infecção por vírus B da hepatite e carcinoma hepatocelular em uma área no nordeste brasileiro*

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COTRIM, H. et al. A case-control study on the association of hepatitis B virus infection and hepatocellular carcinoma in Northeast Brazil. *Rev. Saúde públ.*, S.Paulo, 26: 301-5, 1992. Hepatitis B virus (HBV) serological markers were investigated in 40 incident cases of hepatocellular carcinoma (HCC) and in two age and sex matched control groups, comprising 40 patients with other cancers and 80 healthy individuals, resident in Bahia, Brazil. Serologic tests were done by radioimmunoassay. The study observed high proportion of seropositivity to HBsAg (42.5%) and of those presenting HBsAg or antiHBc (65.0%) among HCC cases, higher in men than women and in those aged 17 to 30 years old. HBsAg seropositivity among HCC patients was greater than in the control group with other cancers (7.5%) and in healthy controls (2.5%), corresponding to odds ratio estimates of 15.0 (95% CI 3.29, 68.30) and 33.0 (95% CI 9.13, 119.28), both statistically significant. HBeAg was not observed and antiHBe was present in 41.2% of cases, suggesting the absence of viral replication, possibly with viral DNA integration into the hepatocyte genome. The presence of cirrhosis was associated with HBsAg seropositivity among HCC cases. A history of chronic alcoholism is shown to be more frequently related to those cases with cirrhosis. This study highlights the relevant association between HCC and HBV in Northeast Brazil, particularly for young individuals, and the high risk of development of HCC for HBsAg carriers.

**Keywords:** Hepatitis B, complications. Hepatoma, complications. Risk factors. Liver neoplasms, complications.

## Introduction

Hepatocellular carcinoma is one of the ten leading neoplasms worldwide. It is estimated that nearly one million new cases are registered every year, most of them in African and Asian countries<sup>4</sup>. Various studies have described a concurrent distribution of HCC incidence and the endemic level of the hepatitis B surface antigen (HBsAg). The adult population of China and other Asian countries are believed to have HBsAg seropositivity rates of up to 10 to 15% and incidence rates of HCC varying from 10 to 20 cases per

100,000 inhabitants per year. In contrast, in areas where less than 1% of the population are HBsAg carriers, HCC incidence rates of one to five cases per 100,000 inhabitants are observed<sup>17</sup>.

Published data in Brazil, gathered through histopathological information have reported 1,849 cases of liver cancer, representing 0.5% of the total number of patients with neoplasms registered from 1976 to 1980<sup>3</sup>. Some 59.3% were males and 51.2% classified as HCC. This source shows only 42 HCC cases from the state of Bahia during the same period, which is heavily underrepresented. Previous surveys carried out in the city of Salvador, Bahia, revealed prevalence rates of 4.2% of HBsAg<sup>5</sup> and 30.0% of hepatitis B antibody surface antigen (antiHBs) among blood donors<sup>9</sup>. However, controlled studies for the estimation of the frequency of HBsAg carriers according to the risk of development of hepatocellular carcinoma are not yet available in Brazil.

The present study was conducted for the purpose of evaluating the magnitude of

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the association between HBV infection and HCC, and the relative importance of some risk factors, in Salvador, Bahia, Brazil.

## Methods

This age and sex matched case-control study was carried out in Salvador, a city located in the coastal area of Northeast Brazil. Incident cases of hepatocarcinoma included those referred by some city hospitals and physicians during the period 1982-1987. A total of 40 patients were studied. Participation criteria included histologic confirmation of HCC through blind percutaneous biopsy, laparoscopic biopsy or autopsy. History of alcoholism was recorded in all cases, and chronic alcoholism defined as the regular ingestion of alcoholic beverages over eight or more years and a mean daily consumption level of 80 or more grams of ethanol.

Two control groups were defined. One was formed of 40 individuals, chosen from among patients newly admitted to city hospitals who presented other primary neoplasms, with normal levels of hepatic biochemical parameters, and the other of 80 healthy individuals, presenting normal transaminase levels (Reitman-Frankel method), selected from among individuals attending private clinics for periodic examinations as part of job requirements. Participation criteria included the absence of history of blood transfusion, jaundice and hepatitis. Homosexuals, health professionals and those referring the usage of intravenous drugs were excluded.

Participants of all three groups had blood samples collected by venipuncture using disposable materials and serological determination of HBV markers. HCC cases had exams for HBsAg, antiHBs, antibody to hepatitis B to core antigen (antiHBc), hepatitis B and antigen (HBeAg) and antibody to hepatitis B and antigen (antiHBe), and controls had exams for HBsAg, antiHBs and antiHBc. All serological tests for HBV markers were performed by radioimmunoassay using Abbott kits (North Chigago, USA).

The presence of HBsAg and HBcAg in samples of liver tissues was also detected in 26 cases of HCC using the immunoperoxidase technique.

**Table 1.** Frequency of hepatitis B virus (HBV) serological markers in 40 cases of hepatocellular carcinoma and control groups of cancer patients (40) and healthy subjects (80).

HBV serological markers	Cases		Cancer controls		Healthy controls	
	n	%	n	%	n	%
HBsAg	17	42.5	3	7.5	2	2.5
antiHBc	9	22.5	2	5.0	5	6.3
HBsAg or antiHBc	26	65.0	5	12.5	7	8.8
antiHBs*	2	8.7	11	29.7	17	21.8
HBeAg**	0	-	-	-	-	-
antiHBe**	7	41.2	-	-	-	-

\* Percentages calculated for HBsAg negatives.

\*\* Tested for 17 HCC HBsAg positives.

The samples were examined for histological findings using standard procedures.

## Results

Overall proportion of HBsAg seropositivity in the group of cases of hepatocellular carcinoma was 42.5% (Table 1). In this group cumulative percentages of HBV serological markers were 65.0% for those presenting HBsAg or antiHBc. The absence of HBeAg positivity in contrast to an antiHBe positivity rate of 41.2% was observed.

The evaluation of HBV markers in samples of liver tissue demonstrated that out of 26 HCC cases examined 19.2% were positive for HBsAg, all of them showing also HBsAg seropositivity. Only one case had liver tissue positive for HBcAg.

The mean age of HCC patients was  $49.3 \pm 15.3$ , varying from 17 to 82 years. Table 2 shows that cases were found more often in the age group 51 years and older. The 17 to 30 year-old age group presented the highest frequency of seropositivity to

**Table 2.** Age distribution of seropositivity to hepatitis B virus serological markers in 40 cases of hepatocellular carcinoma.

Age (years)	Number observed	HBsAg positivity %	HBsAg or antiHBc positivity %
17-30	6	66.7	83.3
31-50	13	38.5	53.8
51+	21	38.1	66.7
Total	40	42.5	65.0

**Table 3.** Odds ratio (OR) estimates and confidence intervals (CI) of hepatocellular carcinoma comparing cases to cancer controls and healthy controls.

Cases	Cancer controls			Healthy controls		
	OR	95% CI		OR	95% CI	
Overall	15.0*	3.29,	68.30	33.0**	9.13,	119.28
Males	10.6	2.05,	54.96	93.5	5.20,	-
Females	7.0	0.70,	69.49	7.0	1.17,	42.0
Age < 30	23.4	0.89,	613.02	45.0	1.80,	-
Age 31-50	3.4	0.53,	22.43	7.5	1.21,	46.51
Age 51+	12.3	1.37,	110.31	53.5	2.90,	989.54

Sex and age strata: independent comparisons.

\*  $\chi^2$  (1 d.f.) = 12.25;  $p$  = 0.000465 (Mantel-Haenszel Matched Chi-square Test)

\*\*  $\chi^2$  (1 d.f.) = 28.44;  $p$  < 0.00001 (Mantel-Haenszel Matched Chi-square Test)

HBsAg. Some 25 (62.5%) cases were male. HBsAg was present in 48.0% of males and in 33.3% of female patients, a difference not statistically significant. Among cancer controls, seropositivity was found to be 8.0% (2/25) and 6.7% (1/15) for men and women, respectively, and for healthy controls the only two HBsAg positives were women.

The comparison of the frequency of HBV serological markers as between HCC cases and controls showed that cases had a proportion of HBsAg seropositivity nearly six times greater than the cancer controls and 17 times greater than healthy controls. Odds ratio estimates for HBsAg positivity were 15.0 (95% CI 3.29, 68.30) as compared to cancer controls and 33.0 (95% CI 9.13, 119.28) when compared to healthy controls, both of them statistically significant (Table 3). Moreover, the risk of HCC for those who were exposed (HBsAg positives) was higher for males than females, and also for individuals aged less than 30 years old and for those 51 years and over.

Cirrhosis (histologic diagnosis) was present in 30.0% (12/40) of HCC cases. Some 75.0% (9/12) of cases showing cirrhosis were HBsAg seropositive in contrast to 28.6% (8/28) of HBsAg seropositivity among those not showing cirrhosis, a statistically significant difference ( $\chi^2=7.410$ ;  $p=0.006$ ).

Chronic alcoholism was registered in 42.5% (17/40) of HCC cases, and was not significantly associated to HBsAg seropositivity or cirrhosis. In fact, the proportion of HBsAg positivity among cases who referred chronic alcoholism was 41.2% (7/17) and for those who did not refer it

was 43.5% (10/23). Moreover, seven out of 12 cases with cirrhosis (58.3%) presented chronic alcoholism in contrast to 10 out of 28 cases (35.7%) without cirrhosis ( $\chi^2=1.759$ ;  $p=0.185$ ). In addition, HBsAg seropositivity was related to cirrhosis in the presence of chronic alcoholism: 71.4% (5/7) of HBsAg positivity for cases showing cirrhosis in contrast to 20.0% (2/10) for those without cirrhosis (Fisher's Exact Test=0.0584). Among HCC cases without chronic alcoholism 80.0% (4/5) with cirrhosis tested positive to HBsAg while only 33.3% (6/18) of those without cirrhosis presented a positive test to HBsAg (Fischer's Exact Test=0.0886).

## Discussion

The overall proportion of seropositivity to HBsAg among cases of hepatocellular carcinoma observed in this study was comparable to one described in Japan, but lower than those from hyperendemic areas of Asia and Africa<sup>8,10,15</sup>. Sakuma et al.<sup>12</sup> observed in Japan a relative risk of HCC for seropositives of 29.4 while in New York City the risk was of 9.7<sup>11</sup>, lower than that described by Beasley et al.<sup>2</sup> in Taiwan, an area of high endemicity, where a relative risk of 233 of HCC development was found. In the group studied a significant estimate of risk was observed although differences in methods should be allowed for in comparing findings related to the magnitude of the association between HBV and HCC. Moreover, the present study suggests that under comparable exposure conditions HCC cases presented a higher frequency of seropositivity to HBV markers

than did controls with other cancers, indicating the relative specificity of this association.

Although strongly associated with the presence of HBsAg, the overall proportion of 30.0% of cirrhosis is much lower than reported frequencies of 60% to 80%<sup>7,13,18</sup>. This may be due to the fact that histological diagnosis followed percutaneous liver biopsies in the majority of cases. In addition, the macroscopic findings on laparoscopy or autopsy, this latter performed in only three cases, were not taken into consideration.

The role of chronic alcoholism was only evaluated among cases. However, the data indicated that a history of alcoholism was more frequently related to HCC cases with cirrhosis than to those without it, and that the HBsAg association with cirrhosis was found among those reporting alcoholism.

It is noteworthy that few (8.7%) HCC patients were seropositive to antiHBs, in contrast to 29.7% for cancer controls and to 21.8% for healthy controls. Schafritz et al.<sup>13</sup> demonstrated DNA-HBV integration to hepatic cells in antiHBs positive HCC patients detected by a recombinant-DNA technique. It suggests that even in the presence of antiHBs, liver carcinoma could be related to HBV infection. Among HCC patients, however, only two aged 61 and 82 years old, had antiHBs in serum without other HBV markers, perhaps indicating the role of other etiologies. It seems likely that young HBsAg carriers present a higher risk of HCC, possibly acquiring the infection early in life through vertical transmission, as has already been observed in the population studied<sup>14</sup>. It might also be speculated that male individuals are more susceptible to infection and to the development of liver disease.

Positivity to HBeAg was not observed. Seropositivity to antiHBe in this study may indicate the absence of viral replication and possible integration of viral DNA to the liver cell genome<sup>6,13</sup>. More recent studies have suggested that positivity to antiHBe in HBsAg carriers may represent a new viral strain due to mutation in its pre-core region, affecting HBeAg synthesis<sup>1,16</sup>. However, in order to test this hypothesis it would be necessary to identify other serological markers of viral replication and to conduct studies in molecular biology. In

this study, the observation of antiHBe in serum with nearly absent HBcAg in liver tissue suggests that carcinogenesis was initiated following viral integration to hepatic cells.

The results presented make it clear that the HBsAg carrier state is an important risk factor of hepatocellular carcinoma in Northeast Brazil.

COTRIM, H. et al. Estudo caso-controle da associação entre a infecção por vírus B da hepatite e carcinoma hepatocelular em uma área no nordeste brasileiro. *Rev. Saúde públ.*, S. Paulo, 26: 301-5, 1992. Estudou-se a associação entre carcinoma hepatocelular (CHC) e a infecção pelo vírus B da hepatite (VHB) em Salvador, Bahia (Brasil), utilizando-se o desenho caso-controle, pareado por idade e sexo, comparando-se a frequência de positividade aos marcadores virais de um grupo de 40 casos de CHC com dois grupos controles: 40 indivíduos com outras neoplasias e 80 indivíduos sadios. Utilizou-se a técnica de radioimunoensaio para os testes sorológicos. Foram observadas altas proporções de indivíduos positivos para AgHBs (42,5%) e daqueles com AgHBs ou antiHBs (65,0%) entre os casos de CHC. A proporção de positivos para AgHBs entre os casos foi maior do que no grupo controle com outras neoplasias (7,5%) e no grupo de indivíduos sadios (2,5%), equivalente a estimativas de risco de 15,0 (Intervalo de Confiança (IC) a 95% de 3,29, 68,30) e de 33,0 (IC a 95% de 9,13, 119,28), ambos estatisticamente significantes. Não foi observada positividade ao AgHBe AntiHBe estava presente em 41,2% dos casos, sugerindo a ausência de replicação viral, com integração do DNA do vírus ao genoma hepático. Entre os casos de CHC houve associação entre cirrose e AgHBs. Foi referida história de alcoolismo crônico mais frequentemente por aqueles com cirrose. O estudo demonstra a relevante associação entre CHC e VHB, principalmente em indivíduos jovens.

**Descritores:** Hepatite B, complicações. Hepatoma, complicações. Fatores de risco. Neoplasias hepáticas, complicações.

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