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Hepatitis B and C virus and hepatocellular carcinoma

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Abstract

Antibody to hepatitis C virus (anti-HCV) was detected in 18·7% of patients with hepatocellular carcinoma (HCC) and in 10·9% of controls ($P<0·001$). The corresponding prevalences of hepatitis B surface antigen (HBsAg) were 59·3% and 50·0% ($P<0·001$). Using patients with non-hepatic disease as controls, stepwise logistic regression analysis indicated that both anti-HCV (odds ratio 6·88%; 95% confidence interval [CI] 1·63–9·77) and HBsAg (odds ratio 6·46; 95% CI 1·68–18·13) were independent risk factors for HCC. Calculation of the incremental odds ratio indicated no interaction between hepatitis B virus (HBV) and HCV. Blood transfusion was a significant risk factor for acquiring HCV infection with odds ratios of 5·48 (95% CI 1·07–29·0) and 2·86 (95% CI 1·31–22·72) for HCC cases and controls, respectively. The mean age of HCC cases with HBsAg and anti-HCV was lower than that of HCC patients with anti-HCV alone ($P<0·01$). It is concluded that there is a high rate of HBV infection and a low rate of HCV infection, among Nigerian patients with HCC. However, HBV and HCV are independent risk factors for the development of HCC, with HBV having an effect more rapidly. Screening of blood products for transfusion might minimize the risk of HCV transmission.

Keywords: hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, Nigeria

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent cancers among Africans, and in Nigeria by 1970 the estimated rate was 6·6 per 100 000 population per annum (DOLL *et al.*, 1970). It is now the commonest malignant tumour seen on medical wards (OLUBUYIDE *et al.*, 1986) and is the commonest cause of death from cancer in the middle aged (JUNAID, 1979) and elderly population (OLUBUYIDE & SOLANKE, 1990). There has been no recent report of the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) in patients with HCC in Nigeria. To evaluate the role of these viruses in the development of HCC in Nigerians, we measured hepatitis B surface antigen (HBsAg) and anti-HCV antibody in serum samples from patients with HCC and non-hepatic disease(s) in a case–control study, using a newly established third-generation enzyme immunoassay for anti-HCV. Moreover, we assessed the independent and interactive effects of these viruses in the development of HCC, and examined the age of the patients with these viral infections.

Materials and Methods

Study population

The case–control study was conducted at the Gastroenterology Unit, University College Hospital, Ibadan, Oyo State, Nigeria between January and September 1995. During this period, 90 patients with an initial diagnosis of HCC were referred to the unit. The diagnosis of HCC was based on clinical features, liver tests and echographic suspicion. All patients came from Ibadan local government areas and the various local government areas within Oyo State. The 90 control subjects were patients admitted to the hospital during the same period for reasons other than neoplasm or liver disease. They were frequency-matched with HCC cases according to age (±5 years), gender and place of residence, since the prevalence of risk factors probably varies in different areas. The main reasons for admission were gastroduodenal ulcer (64%), diabetes mellitus (15%), chronic obstructive airways disease (9%), osteoarthritis (8%), and anaemia (4%).

Questionnaire

A questionnaire was used to collect demographic and clinical data including demographic characteristics, past medical history (previous anti-cancer therapy, blood transfusion, use of intravenous drugs, history of jaundice, traditional surgery such as circumcision, uvulectomy, dental extraction and scarification). The alcohol consumption rate was determined as previously described (OLUBUYIDE & BAMGBOYE, 1989).

The HCC cases were then clinically examined and subjected to needle liver biopsy; only the 64 (71%) histologically diagnosed as having HCC were used for subsequent analysis.

Biochemical tests and serology

Blood samples were taken from the HCC cases and controls and serum was separated and stored at −20°C. Routine serum biochemical tests were carried using a Hitachi 717 Analyzer (Boehringer Mannheim, Germany). HBsAg and anti-HCV were assayed by commercially available enzyme immunoassay kits (Murex Diagnostics Ltd, Dartford, UK). The Murex anti-HCV (version III) enzyme immunoassay uses antigens from the putative core (C, structural), protease/helicase (NS3, non-structural), NS4 (non-structural), and replicase (NS5, non-structural) regions of the virus to provide a sensitive diagnostic test.

Statistical analysis

Student's t test or the χ^2 test were used as appropriate to assess the significance of differences in sociodemographic characteristics, age, and prevalence of HBsAg and anti-HCV. Odds ratios with 95% confidence intervals (95% CI) were used to determine the risk of acquiring HCV by blood transfusion and possible causal relationships between HBV and HCV. Stepwise logistic regression was used for multivariate analysis. Interaction between the viral infections was assessed by the incremental odds ratio. A level of $\alpha=0·05$ was used as the indicator of statistical significance.

Results

The commonest symptoms among the HCC cases included abdominal swelling, abdominal pain, weight loss, and anorexia. A triad of hepatomegaly, ascites and jaundice constituted the commonest presenting feature, in agreement with a previous observation at this hospital (OLUBUYIDE, 1992). A previous history of jaundice was reported by 21 cases (32·8%), but by none of the controls.

All controls had serum transaminase concentrations within normal limits; 28 HCC cases had serum transaminase levels within the normal range, but the level was raised in the other 36.

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Table 1. Sociodemographic characteristics of hepatocellular carcinoma cases and control subjects

	Cases	Controls
No.	64	64
Male/female ratio	42/22	42/22
Age group (years)		
<45	18	20
45–54	12	10
≥55	34	34
Mean age (years)[a]	52·4(15·8)	52·3(15·7)
Place of residence		
Ibadan	39	39
Elsewhere in Oyo State	25	25
Occupation[b]		
Civil Servant	23	20
Farming	17	18
Production	4	5
Army	1	0
Trade	22	24
None	5	3
Medical history		
Previous blood transfusion	11	9
Use of intravenous drugs	0	0
Traditional surgical procedures[b]	75	79
Scarification marks	48	46

[a]Standard deviation shown in parentheses.
[b]Some subjects had more than one occupation or had undergone more than one traditional surgical procedure.

Table 2. Prevalence of anti-hepatitis C virus antibody and hepatitis B surface antigen in hepatocellular carcinoma cases and in control subjects

	Cases		Controls	
	Anti-HCV[a]		Anti-HCV[a]	
	Yes	No	Yes	No
HBsAg[b]				
Yes	7(18·4%)	31(81·6%)	4(12·5%)	28(87·5%)
No	5(19·2%)	21(80·8%)	3(9·4%)	29(90·6%)

[a]Antibody to hepatitis C virus.
[b]Hepatitis B surface antigen.

(18·7%) and 7 controls (10·9%) were anti-HCV positive ($P<0·001$). The prevalence of HBsAg was significantly higher ($P<0·01$) than that of anti-HCV in both groups.

Transfusion was a significant risk factor for acquiring HCV infection, with an odds ratio of 5·48 (95% CI 1·07–29·0) and 2·86 (95% CI 1·31–22·72) among cases and controls, respectively. This was not the case with HBV infection.

The prevalence of anti-HCV was 18·4% (7/38) in the HCC cases who also had HBsAg and 19·2% (5/26) in the HCC cases who were HBsAg negative. The difference was not significant, indicating that HCV may play an important role in the development of HCC.

Table 3 shows a comparison by univariate and multivariate analysis of the risk of developing HCC in persons with HBV or HCV infection. The risk for developing HCC increased with the presence of anti-HCV (odds ratio=6·88). After adjusting for the confounding effect caused by age and gender by multivariate analysis, there was still an association between HCV infection and development of HCC. The risk for developing HCC was also strongly associated with HBV infection. Therefore anti-HCV or HBsAg must be a risk factor for the development of HCC.

Dual HBV and HCV infection was noted in 7 cases (10·9%) and 4 controls (6·2%). By using cases who were negative for both HBsAg and anti-HCV (21/64; 32·8%) as a reference group and the patients with non-hepatic disease as controls (29/64; 45·3%), the odds ratio for developing HCC in patients with dual infection (2·42; 95% CI 1·4–3·52) was lower than that of cases who had anti-HCV alone (6·88; 95% CI 1·63–9·77). The calculated incremental odds ratio was 0·7 (95% CI 0·5–2·3), indicating that there was no interaction between HBV and HCV.

The mean age of the subjects with anti-HCV but no HBsAg, 64·8 years (SD=6·9), was significantly higher than that of the subjects with HBsAg only, 46·7 years (SD=16·4), and that of those with neither anti-HCV nor HBsAg, 55·7 years (SD=12·8) ($P<0·01$).

Discussion

Many publications on the aetiology of HCC worldwide failed to include control groups, and when they were included the subjects were generally poorly defined. In the present study, we matched cases and controls by age and gender to diminish possible bias, and potential differential access to hospital was balanced by matching for place of residence. The good comparability of sociodemographic characteristics among cases and controls seemed to indicate that the source populations for both groups were indeed comparable.

Table 3. Risk for hepatocellular carcinoma related to hepatitis B and hepatitis C virus infection

	Cases	Controls	Odds ratio[a]	Adjusted odds ratio[a]
HBsAg[b]				
Yes	38	32	1·46(0·68–18·13)	1·02(0·5–8·1)
No	26	32	1·0	1·0
Anti-HCV[c]				
Yes	12	7	1·88(0·63–9·77)	1·14(0·4–7·8)
No	52	57	1·0	1·0

[a]95% confidence interval in parentheses; 'adjusted' refers to adjustment for age and gender by multivariate analysis.
[b]Hepatitis B surface antigen.
[c]Antibody to hepatitis C virus.

No attempt was made to classify the HCC patients into groups with or without liver cirrhosis.

There was no statistically significant difference between the HCC cases and controls in sociodemographic characteristics (Table 1).

Only one case had an alcohol consumption level above 80g daily.

Table 2 shows the prevalence of HBsAg and anti-HCV in the cases with HGC and controls. Of the 64 cases with HCC, 38 (59·3%) were HBsAg positive compared with 32 (50%) of the controls ($P<0·001$); 12 cases

The lack of specificity and sensitivity of first generation tests has been invoked to explain the discrepancies in previous seroepidemiological studies on the prevalence of HCV in patients with HCC in tropical communities (TIBBS *et al.*, 1991). In the present study, anti-HCV antibodies were determined by a more reliable third generation enzyme immunoassay.

Table 4. World prevalence of hepatitis B surface antigen and antibody to hepatitis C virus in hepatocellular carcinoma cases

	No.of cases	Percentage with HBsAg[a]	Anti-HCV[b]	Reference
Spain	96	9·4	75·0	BRUIX *et al.* (1989)
France	74	ND	33·4	DUCREUX *et al.* (1990)
South Africa (Blacks)	380	48·4	28·9	KEW *et al.* (1990)
Mozambique	189	66·1	36·5	DAZZA *et al.* (1990)
Japan	148	25·7	69·6	YUKI *et al.* (1992)
Taiwan	127	86·6	11·0	LEE *et al.* (1992)[c]
India	53	28·3	15·1[c]	RAMESH *et al.* (1992)

[a]Hepatitis B surface antigen (ND=no data given).
[b]Antibody to hepatitis C virus.
[c]Measured by second generation anti-hepatitis C virus immunosassay.

The present results confirmed our previous reports (see OLUBUYIDE, 1992) of the high prevalence of HBsAg in patients with HCC, and showed HBV to be an independent risk factor for HCC. In the present study, 48% of patients with HCC were HBsAg positive and anti-HCV negative. On the other hand, only 7·8% of patients with HCC were HBsAg negative and anti-HCV positive. Our observation of an association between HCV infection and HCC has not previously been reported in Nigeria, but agrees with reports from elsewhere (DE-MITRI *et al.*, 1995) which showed that HCV is an independent risk factor for HCC.

It is interesting that only 10·9% of patients with HCC were positive for both anti-HCV and HBsAg. It is not known to what extent a common source of transmission, e.g. maternal–neonatal spread, might be responsible for co-infection; it is also not clear whether such dual infection eventually expedites hepatocarcinogenesis. Although the odds ratio for patients with dual infection was low (2·42), the 95% CI (1·4–3·52) was nevertheless narrow. This may have been due to the small number of persons with dual viral infection (7 of the HCC group and 4 of the others). There is a possibility that an incremental effect may exist if the number of individuals with dual infection increases. At present, the results indicate that HBV and HCV have independent effects in the pathogenesis of HCC, agreeing with the reports of other workers (GORITSAS *et al.*, 1995). In contrast to our finding, other case–control studies have found an interactive effect between the 2 viruses (HADZIYANNIS *et al.*, 1995).

The negative correlation between anti-HCV and HBsAg status in the current study is consistent with reports from countries where HBV is endemic (LEVRERO *et al.*, 1991), as well as from other areas of low endemicity (DI BISCEGLIE *et al.*, 1991). This negative correlation further agrees with the proposition that the viral agents operate independently in the causation of HCC. It also suggests that the main modes of transmission of the 2 viruses are probably different. While it is probable that the high prevalence of HBV infection in this environment is due to horizontal transmission during childhood (OLUBUYIDE, 1989), we have shown that blood transfusion is a significant risk factor for acquiring HCV infection only. This corroborates other reports from Nigeria (MUTIMER *et al.*, 1994) and the USA (RESNICK, 1991). This is not surprising as the screening of blood products in Nigeria is routinely done for HBV only.

In several recent studies, prevalence of anti-HCV can be classified as extremely high (60–70% or more), as in Spain (BRUIX *et al.*, 1989) and Japan (YUKI *et al.*, 1992), moderately high (30–40%), as in South Africa (KEW *et al.*, 1990), Mozambique (DAZZA *et al.*, 1990) and France (DUCREUX *et al.*, 1990), and low (<20%), as in Taiwan (LEE *et al.*, 1992) and India (RAMESH *et al.*, 1992). The prevalence of anti-HCV in patients with HCC in Nigeria reported here is one of the lowest in the world (Table 4), whereas the prevalence of HBsAg and the incidence of HCC are among the highest. The comparative incidences of these viruses in different areas may be an important clue to the marked geographical variation in the incidence of HCC throughout the world and the variability in the biology and natural history of this deadly disease.

The lower mean age of HBsAg positive patients with HCC compared with those with anti-HCV alone, which we found, agrees with previous reports (KEW *et al.*, 1990; BILE *et al.*, 1993) and suggests that HCV infection may result in HCC only after a longer period than is needed for HBV infection (BILE *et al.*, 1993).

Neither HBsAg nor anti-HCV was detected in 21 (32·8%) of the 64 patients with HCC. It is possible that other (non-viral) factors outside the scope of this study may have contributed to the development of HCC. It has been previously shown, in a larger series from this hospital, that there was no significant association between the drinking habits and risk of HCC among patients with chronic liver disease (OLUBUYIDE, 1992). The present study confirmed these observations. However, in Nigeria—a warm humid country near the Atlantic sea—contamination of food by aflatoxin is a serious problem. This chemical carcinogen has been associated with the development of HCC (OLUBUYIDE *et al.*, 1993) and could be aetiologically related to HCC in this group of patients. No information was available about the presence of cirrhosis, and its role in the development of HCC could not be assessed.

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References

Bile, K., Aden, C. & Norder, H. (1993). Important role of hepatitis C virus infection as a cause of chronic liver disease in Somalia. *Scandinavian Journal of Infectious Diseases*, 25, 559–564.

Bruix, J., Barrera, J. M., Calvet, X., Ercilla, G., Costa, J. & Bru, C. (1989). Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet*, ii, 1004–1006.

Dazza, M. C., Meneses, L. V., Girard, P. M., Villaroel, C., Brechot, C. & Larouze, C. (1990). Hepatitis C virus antibody and hepatocellular carcinoma. *Lancet*, 335, 1216.

De-Mitri, M. S., Poussin, K., Baccarini, P., Simon, N., Brechot, C. & Paterliini, P. (1995). Hepatitis C virus associated liver cancer without cirrhosis. *Lancet*, 345, 413–415.

Di Bisceglie, A. M., Order, S. E., Klein, J. L., Waggoner, J. G., Sjogren, M. H., Kuo, G., Houghton, M., Choo, Q.-L. & Hoofnagle, J. H. (1991). The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. *American Journal of Gastroenterology*, 86, 335–338.

Doll, R., Muir, C. & Waterhouse, J. (1970). *Cancer Incidence in Five Continents*. New York: Springer Verlag (for Union Internationale contre le Cancer), pp. 110–111.

Ducreux, M., Buffet, C., Dussaix, E., Pelletier, G., Jacques, L. & Etienne, J. P. (1990). Antibody to hepatitis C virus in hepatocellular carcinoma. *Lancet*, 335, 301.

Goritsas, C. P., Athanasiadou, A., Arvanti, A. & Lampropoulou-Karatza, C. (1995). The leading role of hepatitis B and C viruses as risk factors for the development of hepatocellular carcinoma. *Journal of Clinical Gastroenterology*, 20, 220–224.

Hadzivannis, S., Tabor, E., Kaklamani, E., Mueller, N. & Trichopoulos, D. (1995). A case–control study of hepatitis B and C virus infections in the aetiology of hepatocellular carcinoma. *International Journal of Cancer*, 60, 627–631.

Junaid, T. A. (1979). Mortality in middle-aged Nigerians—an autopsy study. *Tropical and Geographical Medicine*, 31, 389–394.

Kew, M. C., Houghton, M., Choo, Q.-L. & Kuo, G. (1990). Hepatitis C virus antibodies in Southern Africans with hepatocellular carcinoma. *Lancet*, 335, 873–874.

Lee, S. D., Lee, F. Y., Wu, J. C., Hwang, S.-J., Wang, S. S. & Lo, K. J. (1992). The prevalence of anti-hepatitis C virus among Chinese patients with hepatocellular carcinoma.

Cancer, 69, 342–345.
Levrero, M., Tagger, A., Balsano, C., Diop, D., Villa, E., Diodati, G. & Alberti, A. (1991). Antibodies to hepatitis C virus in patients with hepatocellular carcinoma. *Journal of Hepatology*, 12, 60–63.
Mutimer, D. J., Olomu, A., Skidmore, S., Olomu, N., Gunson, B. K. & Elias, E. (1994). Viral hepatitis in Nigeria—sickle cell disease and commercial blood donors. *Quarterly Journal of Medicine*, 87, 407–411.
Olubuyide, I. O. (1989). Hepatitis B virus infection in the tropics today. *Tropical Gastroenterology*, 10, 89–95.
Olubuyide, I. O. (1992). The natural history of primary liver cell carcinoma: a study of 89 untreated adult Nigerians. *Central African Journal of Medicine*, 38, 25–30.
Olubuyide, I. O. & Bamgboye, E. A. (1989). A case–control study of the current role of cigarette smoking and alcohol consumption in primary liver cell carcinoma in Nigeria. *African Journal of Medicine and Medical Sciences*, 19, 191–194.
Olubuyide, I. O. & Solanke, T. F. (1990). The causes of death in an elderly African population. *Journal of Tropical Medicine and Hygiene*, 93, 270–274.
Olubuyide, I. O., Ayoola, E. A. & Atoba, M. A. (1986). Hepatobiliary diseases in tropical Africa—the Ibadan experience. *Tropical Gastroenterology*, 7, 54–64.
Olubuyide, I. O., Maxwell, S. M., Hood, N., Neal, G. E. & Hendrickse, R. G. (1993). HBsAg, aflatoxins and primary hepatocellular carcinoma. *African Journal of Medicine and Medical Sciences*, 22, 89–91.
Ramesh, R., Munshi, A. & Panda, S. K. (1992). Prevalence of hepatitis C virus antibodies in chronic liver disease and hepatocellular carcinoma patients in India. *Journal of Gastroenterology and Hepatology*, 7, 393–395.
Resnick, R. H., Stone, K. & Antonioli, D. (1991). Hepatitis C-related hepatocellular carcinoma. *New England Journal of Medicine*, 324, 126–127.
Tibbs, C. J., Palmer, S. J., Coker, R., Hojvat, S., Peterson, D. & Banatvala, J. E. (1991). Prevalence of HCV in tropical communities: the importance of confirmatory assays. *Journal of Medical Virology*, 34, 143–147.
Yuki, N., Hayashi, N., Kasahara, A., Katayama, K., Fusamoto, H. & Kamada, T. (1992). Hepatitis B virus markers and antibodies to hepatitis C virus in Japanese patients with hepatocellular carcinoma. *Digestive Diseases and Sciences*, 37, 65–72.

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