

# The Gambia Liver Cancer Study: Infection With Hepatitis B and C and the Risk of Hepatocellular Carcinoma in West Africa

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**Hepatocellular carcinoma (HCC) is the most common cancer in The Gambia. Hepatitis B virus (HBV) infection is endemic, with 15% to 20% of the population being chronic carriers, whereas hepatitis C virus (HCV) prevalence is low. We recruited 216 incident cases of HCC and 408 controls from three sites. HBV carriage was present in 61% (129/211) of HCC patients and 16% (64/402) of controls, whereas 19% (36/191) of HCC patients were HCV seropositive compared with 3% (11/382) of controls. HCC patients with HCV were notably older and were more likely to be female than those with HBV. Increased HCC risk was strongly associated with chronic HBV (odds ratio, 16.7; 95% CI, 9.7–28.7), HCV (16.7; 6.9–40.1), and dual infection (35.3; 3.9–323). We interpret the additive nature of risk with coinfection as representative of HBV and HCV acting primarily through shared steps in the multistage process of hepatocarcinogenesis. HCV infection was not observed among younger participants, suggesting a possible cohort effect. Reasons for the striking age and gender differences in HCC associated with HBV compared with HCV are unclear, but transmission patterns and age at exposure may be factors. In conclusion, in a standardized evaluation of well-characterized study participants from The Gambia, most cases of HCC are attributable to HBV (57%), but HCV adds a significant fraction (20%), especially among older patients and females. If HCV transmission is not perpetuated in future cohorts, focusing available resources on HB vaccination efforts could greatly ameliorate a major cause of cancer death in sub-Saharan Africa. (HEPATOLOGY 2004;39:211–219.)**

*Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; GLCS, Gambia Liver Cancer Study; RVH, Royal Victoria Hospital, Banjul; MRC, Medical Research Council Hospital, Fajara; BSG, Bansang Hospital, Bansang; AFP,  $\alpha$ -fetoprotein; HBeAg, hepatitis B "e" antigen; anti-HCV, antibody to HCV; OR, odds ratio; CI, confidence intervals.*

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**H**epatitis B virus (HBV) and hepatitis C virus (HCV) infections are established causes of hepatocellular carcinoma (HCC).<sup>1</sup> Despite decades of experimental and epidemiologic investigation and widespread acceptance of their carcinogenicity, the specific mechanisms by which they lead to HCC and the effect of coinfection with HBV and HCV remain poorly understood.

Geography plays an important role, whereby variation in the epidemiologic patterns of infection and the corresponding HBV and HCV prevalence crudely reflects HCC incidence patterns.<sup>2,3</sup> The greatest burden of HCC is in sub-Saharan Africa and parts of Asia, where HCC is the most frequent cause of cancer death among men.<sup>2</sup> Chronic HBV infection is highly prevalent and is the predominant risk factor for HCC in these high-incidence regions.<sup>2–4</sup> The United States and Europe have much lower HCC rates, with increases in recent years attributed to HCV infection.<sup>5,6</sup> The age of onset of HCC is much younger in Africa and Asia, with a median of 40 to 50 years compared with 55 to 65 years in the United States (see Fig. 1). The male-to-female ratio of HCC cases is

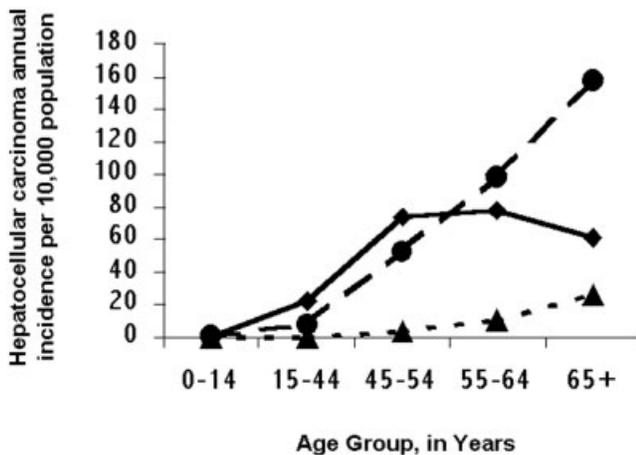


Fig. 1. Comparison of age-specific, annual incidence rates of hepatocellular carcinoma from different geographical regions (The Gambia (diamond), Hong Kong (solid circle), and the United States (triangle)). Compiled from international cancer registry data adapted from Globocan 2000 (<http://www-dep.iarc.fr/globocan/globocan.html>).

approximately 5 to 1 in HBV-endemic regions compared with 2 to 1 in the United States or Europe.<sup>2,3</sup>

Some of the heterogeneity in age-specific HCC rates is attributable to differences in the age at primary HBV infection. The probability of HBV chronicity is much higher with pediatric, and especially neonatal, infection, which predominates in less developed countries and results in 15% to 20% of adults in some populations being chronic carriers of HBV surface antigen (HBsAg).<sup>7</sup> In the United States, where HBV is primarily acquired through sexual contact or injection drug use among adults, less than 1% of those infected become chronic HBsAg carriers.<sup>8</sup>

In contrast to HBV, most people who are infected with HCV become chronic carriers, although correlates of HCV chronicity are not clearly defined. Parenteral injection accounts for most incident HCV infections worldwide, but other methods of transmission are not well defined, especially in Africa.<sup>9,10</sup> Sub-Saharan Africa has an HCV prevalence estimated at 3%,<sup>11,12</sup> but the burden of liver disease and HCC resulting from chronic HCV infection in this area is not well documented.

Estimates of the relative risk for HCC associated with HBV infection have ranged from 5 to 20 in case-control studies and from 5 to 100 in cohort studies.<sup>1,3</sup> Risk estimates for HCC with HCV range from 6 to 60.<sup>1,3</sup> The combined effect of HBV and HCV coinfection on HCC risk is even less clear, with risk estimates ranging from subadditive<sup>13,14</sup> to beyond additive<sup>15,16</sup> to multiplicative.<sup>17</sup>

To clarify the roles of HBV and HCV on the risk for HCC, we conducted a case-control study in The Gambia, a small country in West Africa, where HCC is the most com-

mon cancer among men and the second most common among women.<sup>18</sup> Approximately 15% of Gambian adults are chronic carriers of HBsAg because of horizontal transmission of HBV occurring in young childhood.<sup>19-22</sup> Prior data on HCV infection in The Gambia are limited but suggest a low prevalence of approximately 1% to 2%.<sup>23</sup> Our aim was to evaluate the risk for HCC from HBV or HCV infection alone, to assess the proportion of HCC attributable to each infection, and to estimate the HCC risk from HCV coinfection in this HBV-endemic region.

## Patients and Methods

**Study Population.** The Gambia Liver Cancer Study (GLCS) was conducted in conjunction with The Gambia Hepatitis Intervention Study, an international collaborative project designed to evaluate the efficacy of phased introduction of hepatitis B vaccine into The Gambia's national immunization program in preventing chronic HBV infection, chronic liver disease, and HCC.<sup>24</sup> The GLCS recruited subjects from September 1997 through January 2001 to determine the fraction of HCC resulting from HBV, HCV, or aflatoxin before any significant influence from nationwide HB vaccination. The GLCS recruited subjects from liver-disease referral clinics at each of The Gambia's three tertiary care hospitals (Royal Victoria Hospital [RVH], Banjul; Medical Research Council Hospital [MRC], Fajara; and Bansang Hospital [BSG], Bansang). Incident HCC patients were identified from among patients with suspected liver disease referred by local physicians or identified through active surveillance of the wards and clinics by study field staff. All patients with suspected liver disease underwent a standardized ultrasound examination. All study participants underwent a standardized clinical examination, collection of biologic specimens, and a structured interview that assessed socio-demographic, lifestyle, and medical history information.

Of the 831 potential cases observed in the liver disease clinics, 54 participants were not fully evaluable and 379 were excluded because they did not have a primary liver disease; the most common alternative diagnoses were diseases of cardiac, biliary, hematologic, or renal systems, other intra-abdominal tumors, and abdominal tuberculosis. An additional 121 patients, not included in the current analysis, had ultrasound findings of cirrhosis but without focal liver lesions. Subsequently, 277 participants had space-occupying hepatic lesions on ultrasonography characteristic of HCC. Pathologic specimens from 61 suspected HCC patients that underwent liver biopsy or aspiration were evaluated at the RVH Department of Pathology as the gold standard for HCC diagnosis. HCC was pathologically confirmed in 54 cases, whereas seven

suspected cases were excluded because of a diagnosis of metastatic disease. A subset of 19 histologic specimens was reviewed independently by two collaborating pathologists from France with 100% agreement as to the presence or absence of HCC. Of the pathologically confirmed cases, 20 had a serum  $\alpha$ -fetoprotein (AFP) level of less than 100 ng/mL, 33 had AFP levels of 100 ng/mL or higher, and one did not have AFP testing performed. To assure high specificity, final HCC case status for participants without a pathologic diagnosis required ultrasound findings of one or more space-occupying lesions compatible with HCC and a serum AFP level of 100 ng/mL or more. Sensitivity analysis performed with inclusion of all suspected cases, only patients with AFP >20 ng/mL, only patients with AFP >100 ng/mL, or only patients with AFP >400 ng/mL did not significantly alter the findings. However, the results presented include only those confirmed HCC patients (n = 216) that met the case definition of either pathologic confirmation (n = 54; 25.0%) or an AFP >100 ng/mL with ultrasound lesions present (n = 162; 75.0%). Most HCC patients (69%) were recruited from among patients admitted to the wards, whereas the remainder (31%) attended the outpatient clinics. Control participants (n = 408) without clinical evidence of liver disease were recruited from the outpatient clinics of the same hospital sites, frequency matched by age (within 10-year groupings) and gender, and determined to have normal AFP levels. Local and international scientific and ethical review committees approved the study protocol, and informed consent was obtained from each participant before inclusion in the study.

**Laboratory Testing.** Blood specimens collected at the RVH and MRC sites were transported on ice to the MRC serology lab for processing. BSG specimens were first separated at BSG hospital then transported on ice to the MRC serology lab. Storage of specimens was at either  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$ , depending on the testing planned for each aliquot. AFP was detected and quantified by standard radiometric assay methods (DiaSorin SA, Sallugia, Italy). HBsAg was determined as a marker of chronic HBV carriage by reverse passive hemagglutination assay (Murex Diagnostics Limited, Dartford, UK) with radioimmunoassay testing of negative samples (Sorin Biomedica Diagnostics, Vercelli, Italy). Participants positive for HBsAg were tested for HBV "e" antigenemia (HBeAg) as a surrogate marker of active replication using a radioimmunoassay kit (DiaSorin). HCV antibody status (anti-HCV) was determined by third generation enzyme-linked immunosorbent assay (ELISA; ORTHO Clinical Diagnostics, Neckargemund, Germany) and ELISA reactives were confirmed by recombi-

**Table 1. Demographic Characteristics of HCC Patients and Controls**

Variable	Controls		HCC Cases	
	No.	%	No.	%
Age (yrs), mean (SD)	44.8 (15.2)		48.1 (15.2)	
Age group				
<35	129	31.6	45	20.8
35-44	77	18.9	40	18.5
45-54	72	17.7	56	25.9
55-64	80	19.6	39	18.1
$\geq 65$	50	12.3	36	16.7
Gender				
Males	292	71.6	173	80.1
Females	116	28.4	43	19.9
Site				
RVH	109	26.7	85	39.4
MRC	106	26.0	68	31.5
BSG	193	47.3	63	29.2
Recruitment timing				
November-January	101	24.8	54	25.0
February-April	91	22.3	59	27.3
May-July	88	21.6	50	23.2
August-October	128	31.4	53	24.5
Ethnicity				
Mandinka	132	32.7	57	27.0
Fula	84	20.8	51	24.2
Wollof	61	15.1	44	20.9
Other	127	31.4	59	28.0
Education				
Ever attended school	360	89.1	166	78.7
None	44	10.9	45	21.3
Earth floor house				
Yes	200	49.4	123	58.9
No	205	50.6	86	41.2

NOTE. Study groups included 216 HCC patients and 408 control participants. Deviations from these totals are the result of missing data on ethnicity or socioeconomic status variables for some participants.

Abbreviations: HCC, hepatocellular carcinoma; RVH, Royal Victoria Hospital, Banjul; MRC, Medical Research Council Hospital, Fajara; BSG, Bansang Hospital, Bansang.

nant immunoblot assay (RIBA HCV 3.0 SIA; CHIRON, Emeryville, CA).

**Statistical Analysis.** Univariate analysis evaluated variables associated with HCC using Pearson's chi-square and Fisher's exact tests. Multivariable unconditional logistic regression was performed on frequency-matched participants with estimation of odds ratios (OR) and 95% CI as a measure of association. Despite frequency matching by age, gender, and site, recruited controls were more likely to be younger, female, and recruited from the BSG site (Table 1). Adjusted models presented herein include age, gender, recruitment site, and recruitment date; variables identified in exploratory analysis as associated with case status (ethnic group and socioeconomic status, including education level and living in an earthen floor house); and the primary variables of interest (HBV and HCV status). Ethnicity was determined based on self-reported ethnic group of the participant's father. Other

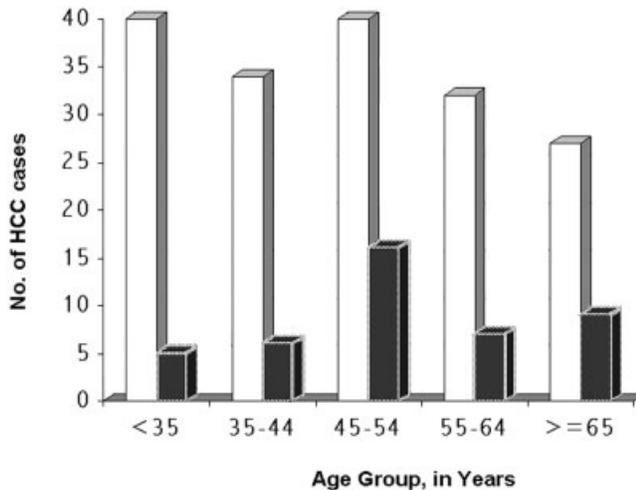


Fig. 2. Age and gender (males, open square; females, solid square) distribution of HCC patients recruited in the Gambia Liver Cancer Study. Males are more commonly affected throughout the entire age range, but the ratio is more pronounced in younger ages, with up to eight males per each female case younger than 35 years of age.

variables evaluated but that failed to alter the predictive value of the model significantly included lifestyle (alcohol and tobacco use) and other sociodemographic variables (living in thatched roof housing, household crowding, place of residence). Analyses stratified by site, gender, age  $\geq 45$  years, and by viral cause of HCC also were performed. Estimates of the population attributable risk with each virus were calculated by using the control prevalence as surrogates for the population. All analyses were performed using Stata statistical software (College Station, TX).

## Results

**Characteristics of Study Participants.** Demographic information on 216 HCC patients and 408 controls is presented in Table 1. HCC patients tended to be older, to be more commonly male, and to be recruited

more frequently from the two urban hospitals compared with the control group. The median age of HCC patients was 47 years and ranged from 17 to 87 years. Males predominated among HCC patients, with an overall male-to-female ratio of 4 to 1. This overrepresentation of males was observed throughout all age groups, although it was more notable among younger patients (Fig. 2). Ethnic variation between HCC patients and controls was observed, with more HCC patients reporting Fula and Woloff ethnicity compared with controls (Table 1), differences that persisted after stratification by recruitment site (data not shown). HCC patients also were of generally lower socioeconomic status, evidenced by a higher degree of participants with no previous education or living in dwellings with earthen floors.

**HBV and HCV Status and HCC Risk.** Carriage of HBsAg was observed in 61% (129/211) of HCC patients compared with 16% (64/402) of controls ( $P < .001$ ; Table 2). Among HCC patients, 11% (23/211) were HBeAg positive compared with only 0.5% (2/402) of controls. HCV seropositivity was confirmed in 19% (36/191) of HCC patients and 3% (11/382) of controls ( $P < .001$ ). Each of the viruses was associated with significantly increased risk for HCC, with adjusted ORs for HBsAg or anti-HCV positivity of 13.5 (95% CI, 7.8–23.2) and 14.7 (95% CI, 6.3–34.4), respectively (Table 2). HCC risk among HBeAg-positive subjects was increased 88.8-fold (95% CI, 18.3–430) compared with HBsAg-negative subjects and 7.1-fold (95% CI, 1.4–35.3) compared with HBsAg carriers who lacked HBeAg.

**Age and Gender Differences in HBV and HCV Infections.** Differing patterns of HBV and HCV infection were observed by age and gender. HBsAg positivity waned with increasing age among patients and especially among controls. Among participants 45 years or older, HBeAg positivity was observed exclusively among HCC patients (7.8%; 10/128) compared with none of 198 control participants ( $P < .01$ ). Conversely, anti-HCV was

Table 2. HBV and HCV Infection in HCC Patients and Controls and Estimates of HCC Risk

Variable	Controls		HCC Cases		Unadjusted HCC Risk		Adjusted HCC Risk	
	No.	%	No.	%	OR	(95% CI)	OR	(95% CI)
HBV status								
HBsAg negative	338	84.1	82	38.9	1	—	1	—
HBsAg positive	62	15.4	106	50.2	7.0	(4.7–10.5)	13.5	(7.8–23.2)
HBeAg positive	2	0.5	23	10.9	47.4	(11.0–205)	88.8	(18.3–430)
Anti-HCV status								
Negative	371	97.1	155	81.2	1	—	1	—
Positive	11	2.9	36	18.9	7.8	(3.9–15.8)	14.7	(6.3–34.4)

NOTE. Adjusted analysis represents multivariable model including age, gender, recruitment site and date, socioeconomic status, and HBV and HCV variables.

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B "e" antigen; anti-HCV, antibody to HCV; OR, odds ratio.

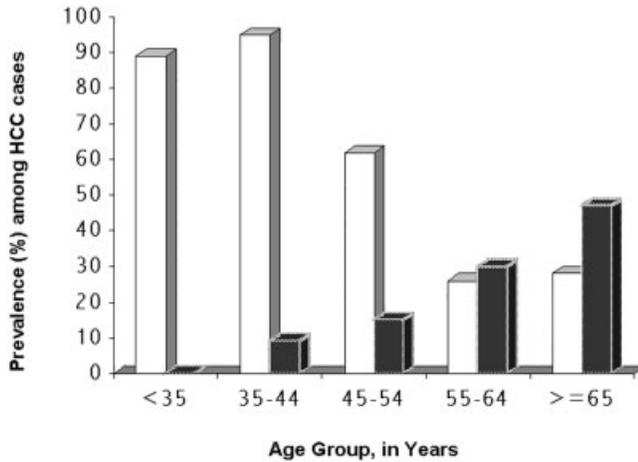


Fig. 3. Viral hepatitis markers in HCC patients by age group. HBV infection (open square; diagnosed as HBsAg positive) predominates in the younger age groups, whereas HCV infection (solid square; anti-HCV positive) increases in importance in the older age groups.

uncommon in younger participants: only 4% (3/74) of HCC patients and 0.5% (1/192) of controls younger than 45 years of age were anti-HCV positive ( $P = .03$ ). No study participant younger than 40 years of age tested positive for anti-HCV. HBV was the predominant virus among younger HCC patients, whereas HCV was predominant among older HCC patients (Fig. 3). The median age at diagnosis was 60 years for HCV-related HCC, compared with 40 years for HBV-related HCC.

The higher burden of HCC in males was accentuated with HBV infection, with the male-to-female ratio more than 5 to 1 for HBV-related HCC, whereas it was approximately 5 to 2 for HCV-related HCC. The prevalence of HBsAg was higher among male than female control participants (18% vs. 10%, respectively;  $P = .05$ ), with a similar trend among HCC patients (64% vs. 51%, respectively;  $P = .15$ ). We found no gender difference in the HCV prevalence among controls (2.6% in males, 3.6% in females;  $P = .57$ ). Among HCC patients, however, a larger proportion of HCV-related HCC was observed among females (11/34; 32%) than in males (25/157; 16%;  $P = .03$ ). Stratified by virus, male gender was associated significantly with increased risk for HBV-related HCC (OR, 2.6; 95% CI, 1.5–4.6), but not for HCV-related HCC (OR, 0.9; 95% CI, 0.4–2.4).

**Joint effect of HBV and HCV on HCC risk.** Coinfection with HBV and HCV was uncommon in both HCC patients (7/186; 3.8%) and controls (1/370; 0.3%; Table 3). No subject in the study had both HBeAg and anti-HCV. In a multivariable logistic regression model, the HCC risk was similar (OR, 16.7), with only HBsAg or with only anti-HCV (Table 3). HCC risk with dual HBsAg and anti-HCV (OR, 35.3) was nearly equal to

that expected with an additive statistical interaction ( $16.7 + 16.7 - 1 = 32.4$ ), but did not approach that expected with a multiplicative interaction ( $16.7 \times 16.7 = 279$ ).

**Estimates of the Population Attributable Risk for HCC With HBV and HCV.** Because causality was well established for each infection, estimates of the population attributable risk for HCC associated with HBV, with HCV, and with coinfection may be interpreted as the proportion of cases etiologically related to the exposure. Our estimate of the etiologic fraction of HCC related to HBV is 57.2%, related to HCV is 19.9%, and infection with both HBV and HCV is 3.5%.

## Discussion

We present data from a hospital-based case-control study of 216 incident HCC patients and 408 frequency-matched control participants from The Gambia, West Africa, a population characterized by high HCC incidence, endemic HBV infection, and low HCV prevalence. The GLCS is one of the larger and better characterized investigations of HCC from sub-Saharan Africa and reinforces the HCC risk associated with HBV and HCV infections.

Striking age differences were observed in the patterns of HBV and HCV infections and in the burden of HCC associated with each infection. HBV predominated in younger individuals, whereas HCV was found exclusively in older study participants (Fig. 3). HBV-related HCC occurred at a median age of only 40 years, which was 20 years younger than HCV-related HCC. Some 80% of HCC patients younger than 45 years of age were HBV related, whereas 65% of patients 45 years or older were HCV related. As has been described previously in HBV-endemic populations, HBsAg levels and detection rates progressively waned with increasing age among both control and HCC participants, with downward trends seen

Table 3. Coinfection With HBV and HCV and Risk of HCC

Variable	Controls		HCC Cases		Adjusted HCC Risk	
	No.	%	No.	%	OR	95% CI
HBV and HCV status						
HBsAg (-)/anti-HCV (-)	308	81.5	41	22.0	1	—
HBsAg (+)/anti-HCV (-)	59	15.6	110	59.1	16.7	(9.7–28.7)
HBsAg (-)/anti-HCV (+)	10	2.7	28	15.1	16.7	(6.9–40.1)
HBsAg (+)/anti-HCV (+)	1	0.3	7	3.8	35.3	(3.9–323)

NOTE. Adjusted analysis represents multivariable model including age, gender, recruitment site and date, socioeconomic status, and HBV/HCV status as single variable.

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; OR, odds ratio; HBsAg, hepatitis B surface antigen; anti-HCV, antibody to HCV.

by the third to fourth decade.<sup>25</sup> However, anti-HCV positivity was not detected in any study participant younger than 40 years of age.

Age at the time of primary infection is likely the main determinant in the earlier occurrence of HBV-related HCC. There is considerable evidence that in The Gambia,<sup>19,21,22,26</sup> predominantly horizontal transmission from close intrafamilial and intrahousehold contact in young children results in 15% to 20% of adults having chronic HBV infection. Estimates of the median latency period from initial HBV infection in childhood until development of HCC range from 30 to 50 years.<sup>27,28</sup> Our data are highly consistent with this latency period, with a mean age of 40 years at diagnosis of HBV-related HCC.

Natural history data on HCV infection and outcomes in sub-Saharan Africa are limited.<sup>11</sup> Cohort studies in North America and Europe have yielded estimates of the latency period from HCV infection to cirrhosis or HCC of 20 to 30 years.<sup>29,30</sup> If this latency period holds in The Gambia, HCV infection would have occurred at approximately 30 to 40 years of age for HCV-related HCC to develop at a median of 60 years. In actuality, the age at acquisition and the method of transmission for HCV is unknown in The Gambia. Neither history of blood transfusion nor intravenous drug use was significantly associated with HCV infection or HCV-related HCC in our study (data not shown).

The absence of HCV infection in participants younger than 40 years of age raises the possibility of an exposure occurring only in later ages, of transmission occurring after cumulative exposures, or of a cohort effect. From a similarly designed HCC case-control study conducted in 1988, control subjects from two of the same hospital sites had an HCV seroprevalence of 3.3% among controls younger than 50 years of age and 6.3% among older controls (Hall AJ, unpublished data, 1998). We observed a lower HCV seroprevalence of <1% among controls younger than 50 years of age but a similar prevalence of 5.7% among older controls. The observed decrease in prevalence 12 to 15 years later in the younger age group supports the possibility of an HCV cohort effect occurring in this population, perhaps reflecting the historical role of unsterile injections postulated as the major contributor to HCV transmission in Egypt and sub-Saharan Africa.<sup>9,31</sup>

In our study, the presence of HBV "e" antigenemia was associated with a sevenfold incremental increase in HCC risk beyond that risk associated with HBV surface antigenemia (Table 2). Persistence of HBeAg positivity among HBV carriers was much more common among HCC patients (18%) than controls (3%). Among participants over age 45, HBeAg was detected in no controls,

compared with 18% (8/45) of HCC patients who carried HBsAg. These data suggest that HBeAg in an older person reflects not only persistent HBV replication, but also a high risk for HCC. Similar findings from a cohort study in Taiwan<sup>32</sup> question whether monitoring and early intervention for HCC should be especially aggressive for HBsAg carriers who are positive for HBeAg. Unfortunately, HBeAg was positive in only 12% of our HCC patients at diagnosis and in only 29% of those in Taiwan at cohort inception.<sup>32</sup>

New molecular approaches may improve identification of those at highest HCC risk. First, quantitation of HBV DNA viral load has been demonstrated to predict progression from cirrhosis to HCC.<sup>33</sup> Second, stop codon mutations in the HBV precore gene region may account for HBeAg negativity despite active HBV replication.<sup>34</sup> However, geographic differences, at least between Africa and Asia, in HBV genotypes and replication dynamics must be considered. A high prevalence of precore gene mutations have been identified in HBV genotypes B and C, the most common in Asia, whereas most HBV infections in The Gambia are genotype E and precore mutations are less common.<sup>34,35</sup> HBV DNA and HBeAg wane more rapidly, and thus disappear at younger ages, in Africa compared with Asia, making it difficult to compare their value in prediction of HCC risk.<sup>25</sup> In general, despite similar HBsAg carriage rates, Asian populations have notably higher HCC incidence rates, especially at older ages (Fig. 1), and these rates parallel differences in HBV DNA persistence with increasing age.<sup>25</sup>

The two- to fivefold higher risk of HCC for males than females is not fully understood. Males may be more likely to experience chronic HBV carriage, and some<sup>36,37</sup> but not all<sup>33,38</sup> studies suggest that this may account for their higher incidence of chronic liver disease and HCC. In our study, the prevalence of HBsAg was significantly higher among male than female control participants, but this difference was reduced when adjusting for HBV exposure (using antibodies to HBV core; data not shown) and was not significant among HCC patients. The presence of HBeAg was detected at comparable rates by gender among HCC patients. These limited data suggest that the higher HCC risk for males reflects differential exposure or initial response to HBV rather than gender differences in persistence of HBV viral replication. It has been suggested that males with chronic active hepatitis may have higher liver DNA synthesis<sup>39</sup> or may have higher liver cell proliferation<sup>40</sup> than females, with resulting increased progression to HCC. In each example, differences by age, duration of HBV infection, level of HBV replication, and the presence of other HCC risk factors may explain the observed variation by gender. Further investigation of

gender differences in the natural history of HBV infection are needed.

The joint effects of HBV and HCV on the risk of HCC remain unclear. A meta-analysis of 21 case-control studies placed the combined effect of coinfection somewhere between additive and multiplicative.<sup>41</sup> This meta-analysis identified data on only eight HBV and HCV coinfecting control participants from seven studies, thereby making precise estimates of the combined effect difficult. In the South African study,<sup>16</sup> which was included in the meta-analysis, the effect of coinfection on HCC risk was reported to be beyond additive based on 1 control and 17 coinfecting HCC patients. Similarly, in our study, we had only one coinfecting control participant. The change in ORs we observed from 17 for HBV or HCV independently to 35 with coinfection approximates an additive model of interaction. When evaluating interaction of two factors, an additive interaction suggests that the factors are acting through the same steps in a shared causal pathway, whereas a subadditive model would represent antagonism.

Several studies suggest reciprocal inhibition of replication by HBV and HCV.<sup>42,43</sup> However, in another study with liver histology review, Zarski et al.<sup>44</sup> found that despite this inhibition of replication, the severity of liver histologic changes may be accelerated with coinfection. Prolonged hepatocellular injury mediated by either chronic HBV or HCV may stimulate regenerative processes that favor malignant transformation. Although HBV can integrate into cellular DNA and subsequently may stimulate genomic instability, a direct mechanism for HCC development with HCV infection has not been demonstrated repeatedly.<sup>45</sup> Therefore, most HCC develops after persistent chronic active hepatitis with immune-mediated hepatocyte destruction and regeneration leading to the development of liver fibrosis, cirrhosis, and ultimately, cancer. In this model, by using the same pathway, HBV and HCV coinfection would incite immune-mediated hepatic responses proportional to their independent infections, resulting in an observed OR approximating the sum of the individual effect ORs.

In comparison of GLCS data with a Gambian community-based study conducted in 1981–1982,<sup>46</sup> the mean age of HCC patients (48 years) and the proportion of HCC attributable to HBV (60% vs. 53%, respectively) has remained stable over the past two decades. Previous data from the Gambia Hepatitis Intervention Study has established a 93% efficacy of childhood vaccination in preventing chronic HBV carriage at up to 9 years of age among Gambians.<sup>47</sup> Using these data, we can estimate that HB vaccination should prevent approximately 56% of HCC patients in the vaccinated cohort. For our pop-

ulation in The Gambia, we estimate the fraction of HCC attributable to HCV to be 23%. This burden of HCC resulting from HCV approaches that attributable to HCV in some more developed countries<sup>4</sup> and highlights the much higher HBV burden, rather than an absence of HCV infection, in less developed regions. Although we estimate that 81% of HCC patients are attributable to infection with viral hepatitis, some of the remaining 19% likely represent misclassification of HBV-infected HCC patients that have lost HBsAg, in addition to those cases resulting from other etiologic factors, such as aflatoxin exposure. In the best-case scenario, the effect of HCV will diminish with declining HCV prevalence in younger cohorts, and HB vaccination will prevent HBV infection and carriage. Unfortunately, until very recently, only one country other than The Gambia in sub-Saharan Africa routinely included HB vaccine in their national immunization programs.<sup>48</sup> After support from the Global Alliance for Vaccines and Immunizations, delivery of HB vaccine to sub-Saharan Africa is increasing, and it is hoped that these concerted international efforts will be effective and sustainable.<sup>49</sup> Then, even if HBV is prevented and HCV passes with the current cohort, there are currently some 32 million HCV-infected<sup>12</sup> and 65 million HBV-infected persons<sup>50</sup> in sub-Saharan Africa alone, all sharing a 15- to 20-fold increased risk of HCC developing. Although preventive efforts should be maximized, improved methods for identifying and treating those HBV- and HCV-infected persons at highest risk for HCC in this limited resource setting should be research priorities.

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