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Research Article

Viral Hepatitis Markers in Liver Tissue in Relation to Serostatus in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) incidence is increasing in the United States. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major causes of HCC. Hepatitis infection in patients with HCC is generally diagnosed by serology, which is not always consistent with the presence of HBV and HCV in the liver. The relationship of liver viral status to serostatus in hepatocarcinogenesis is not fully understood.

Methods: HBV and HCV were evaluated in formalin-fixed, paraffin-embedded liver tissue specimens in a retrospective study of 61 U.S. HCC cases of known serologic status. HBV DNA and HCV RNA were detected by PCR, reverse transcription PCR (RT-PCR), and pyrosequencing, and HBsAg and HBcAg were evaluated by immunohistochemistry.

Results: Viral markers were detected in the liver tissue of 25 of 61 (41%) HCC cases. Tissue viral and serologic status were discordant in 27 (44%) cases, including those with apparent "occult" infection. Specifically, HBV DNA was detected in tissue of 4 of 39 (10%) serum HBsAg (−) cases, including 1 anti-HCV(+) case; and HCV RNA was detected in tissue of 3 of 42 (7%) anti-HCV seronegative cases, including two with serologic evidence of HBV.

Conclusions: Viral hepatitis, including HBV-HCV coinfection, may be unrecognized in up to 17% of patients with HCC when based on serology alone. Further research is needed to understand the clinical significance of viral makers in liver tissue of patients with HCC in the absence of serologic indices.

Impact: The contribution of HBV and HCV to the increasing incidence of HCC in the United States may be underestimated. *Cancer Epidemiol Biomarkers Prev; 22(11); 2016–23. ©2013 AACR.*

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most frequent cause of cancer-related death in the world (1). Approximately 80% of HCC cases occur in developing regions including sub-Saharan Africa and Southeast Asia (1). HCC has been increasing in areas of historically lower incidence including the United States (2–5). From 1975 to 2009, the age-adjusted incidence of HCC in the United States tripled from 2.6 to 7.8 per 100,000 (3, 4, 6). Today, HCC is one of the most rapidly increasing causes of cancer mortality in the country for both men and women (7). Historically, hepatitis B virus (HBV), hepatitis C virus (HCV), and excessive alcohol have been the major risk factors for HCC in the United States (4, 8–11). The increasing incidence of HCC in the United States, in part, is attributed to increases in chronic HCV among individuals infected through transfusions with contaminated blood and blood products and other iatrogenic exposures as well as intravenous drug use (12, 13). Nonetheless, up to half of HCC cases in the United States do not have a viral or alcohol etiology (4, 9–11, 14–17). There is growing recognition that the increasing prevalence of obesity and diabetes in the country may also be contributing to the increasing rates of HCC (18, 19).

In the clinical setting, the viral status of patients with HCC is primarily based on serologic testing rather than detection in liver tissue. Serologic status, however, is not always consistent with the presence of HBV and HCV in the liver. Occult infection, defined as detection of HBV DNA in the liver tissue of individuals seronegative for HBsAg (20, 21), has been recognized in HCC. Primary occult infection, which occurs in the absence of anti-HBc and anti-HBs, is distinct from seropositive occult HBV, which results from loss of HBsAg following self-limited acute hepatitis or after prolonged chronic viral carriage (21). Less is known about occult HCV or the presence of HCV RNA in the liver of individuals without HCV antibodies in serum (22–24). As with HBV, primary occult HCV is distinguished from anti-HCV (+) occult HCV, which is characterized by the presence of residual virus in

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the liver following resolution of clinically apparent infection occurring spontaneously or after antiviral therapy (23).

The clinical significance of HBV and HCV detection in liver tissue of patients with HCC in the context of serology is not fully understood. In particular, few studies have examined the role of occult HBV and occult HCV in HCC in the United States (25, 26). The liver viral status of most patients with HCC is unknown as testing of tissue is not standard in diagnosis and treatment. Furthermore, there has been a lack of agreement on the definitions and laboratory standards for establishing occult viral infection (20, 22, 23).

Materials and Methods

The presence of HBV and HCV in liver tissue was evaluated in a retrospective study of de-identified HCC cases of known serologic status. The study was approved by the University of Hawaii Committee on Human Studies.

HCC cases, tissue specimens, and data sources

A total of 61 HCC cases diagnosed in Hawaii in 1990–2010 were selected on the basis of the availability of both liver tissue and serologic data. HCC was defined by topographic (C22.0) and morphologic (8170–8175) codes according to the International Classification of Diseases for Oncology version 3 (27). Formalin-fixed, paraffin-embedded (FFPE) liver tissue specimens were obtained through the National Cancer Institute's (NCI) Surveillance, Epidemiology, and End-Results (SEER) Residual Tissue Repository (RTR) of the Hawaii Tumor Registry, a de-identified collection of FFPE tissue specimens from patients with cancer diagnosed within the state of Hawaii (28).

HCC cases were annotated with deidentified data from the Hawaii Tumor Registry and a clinical database. Data included demographic, clinical, treatment, and pathologic information as well as survival, cause of death, and serologic status. As blood specimens were not available for this retrospective study population, HBsAg, anti-HBc, and anti-HCV serologic status was derived from clinical laboratory testing conducted as part of patients' diagnoses and/or prior medical history. Data on serum antibody to hepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg), and serum HBV DNA and HCV RNA were not available for most cases. The 61 HCC cases included 19 HBsAg (+), 1 anti-HBc (+), 16 anti-HCV (+),1 HBsAg (+)/anti-HCV(+), 2 anti-HBc(+)/anti-HCV (+), and 22 viral negative cases.

Laboratory analysis

HBV DNA and HCV RNA were detected by PCR, reverse transcription PCR (RT-PCR), and pyrosequencing, and HBsAg and HBcAg were evaluated by immunohistochemistry. FFPE tissue from HCC cases was used to construct a tissue microarray (TMA). For each case, four 0.6-mm cores of tumor and, where available, 2 cores of adjacent nontumor tissue, were arrayed from representative areas selected by a pathologist (D. Horio). HBsAg and HBcAg were evaluated in the TMA via immunohistochemistry using commercial monoclonal antibodies (Leica Microsystems, Inc.) following protocols provided by the manufacturer. Slides were read by a pathologist (O. Chan) who was blinded to the clinical history of cases. HBsAg and HBcAg were each assessed by intensity (none, weak, moderate, strong), location (cytoplasmic, nuclear, membrane), and cell distribution (few cells, patchy, diffuse). Tissue specimens with any positive staining for HBsAg or HBcAg were considered to be positive regardless of the location and pattern of staining. Positive and negative control tissues for HBsAg and HBcAg were included to ensure proper staining specificity. Lack of a suitable antibody for hepatitis C viral proteins precluded evaluation by immunohistochemistry.

DNA and RNA were extracted from tumor and, where available, non-tumor tissue. HBV DNA status was assessed using extracted DNA in two different PCR assays targeting different regions of the viral genome. The first targeted a 228 bp region of the HBV X gene (5′-CTGGATCCTGCGCGGGACGTCCTT-3′ and 5′-GTTCACGGTGGTCTCCAT-3′; refs. 29, 30). The second assay was a nested PCR designed to amplify a 239 bp sequence of a highly conserved region of the HBV polymerase gene (outer primers: 5′-AGG TAT GTT GCC CGT TTG TCC TC-3′ and 5′-AAT TCT TTG ACA TAC TTT CCA ATC AAT-3′; inner primers 5′-AYT GCA CYT GTA TTC CCA TCC CAT-3′ and 5′-biotin-TYA AAT GTA TAC CCA AAG ACA AAAGAA A-3′, Y = C or T; ref. 31). Positive and negative controls were included in each PCR run. All samples were PCR tested for the human beta-globin gene as an internal control for sample sufficiency. PCR products were visualized on an ethidium bromide–stained 2% agarose gel. Specimens that were positive for HBV DNA were genotyped via pyrosequencing using the nested PCR amplicon and a sequencing primer (5′-AGT GGG CCT CAG TCC GTT TC-3′; ref. 31).

HCV RNA status was evaluated using isolated RNA in a RT-PCR assay targeting the highly conserved 5′-untranslated region of the hepatitis C genome (32). In the first round PCR, a 142-bp region was amplified with primers (5′-GGCGACACTCCACCATAGATCA-3′ and 5′-GGTTCCGCAGACCACTATGGC-3′). This was followed by second round of amplification of an 82-bp region (5′-CTCCCCTGTGAGGAACTAC-3′ and 5′-GGTCCTGGAGGCTGCACA-3′; ref. 33). Positive and negative controls were included in PCR and pyrosequencing runs. All samples were PCR tested for β-actin as an internal control for sample sufficiency. PCR amplicons were visualized on an ethidium bromide–stained 2% agarose gel. Pyrosequencing of HCV RNA–positive specimens targeted the untranslated region of the HCV genome (forward primer: 5′-TGCGGAACCGGTGAGTACA-3′ and reverse primer: 5′-biotin-CCTTTCGCRACCCAACRCT-3′) to amplify a 130-bp sequence within the 5′-UTR. PCR products were assayed in one-step RT-PCR to generate single-stranded

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DNA and evaluated with a sequencing primer (5′-TGCGGAACCGGTGAGTACA-3′). Pyrosequencing was conducted using a PyroMark Q24 Pyrosequencer (Qiagen Inc.). HBV genotypes and HCV genotypes were determined by comparison with known sequences available from the National Center for Biotechnology Information and other public databases (34).

Statistical analyses

All analyses were conducted with SAS version 9.2 (SAS Institute Inc.). HBV-positive liver tissue was defined as the detection of HBV DNA, HBsAg, and/or HBcAg in liver tissue. HCV-positive liver tissue was based on the detection of HCV RNA. Demographic and clinical characteristics were compared across groups using the χ^2 test. For all statistical measures, $P < 0.05$ was considered to be significant.

Results

Viral markers were detected in liver tissue of 25 of 61 (41%) HCC cases: 18 were positive for HBV alone, 5 were positive for HCV alone, and 2 were positive for both HBV and HCV. The 18 cases solely positive for HBV included 2 HBV DNA(+), 7 HBsAg(+), 3 HBV DNA(+)/HBsAg(+), 1 HBsAg(+)/HBcAg(+), and 5 HBV DNA(+)/HBsAg(+)/HBcAg(+). Of the 2 HBV-HCV positive cases, 1 was HBV DNA(+)/HCV RNA(+) and 1 was HBsAg(+)/HCV RNA(+). Viral genotypes were ascertained in tissue of 17 viral positive cases. HBV genotype A was detected in 4 cases, genotype D in 3, and genotype B in 3. HCV RNA genotype 1a was detected in 4 cases, type 3a in 2, and 1 case was positive for both 1a and 1b.

Overall, liver tissue viral status was consistent with serologic status for 56% (34 of 61) of HCC cases (Table 1), including 18 cases who were viral negative based on both tissue and serology. Thirteen cases were HBV positive based on both tissue and serology and 3 were HCV positive in both tissue and serology.

Tissue viral status was discordant with serologic status in 27 of 61 (44%) HCC cases. Twenty of these discrepant cases were serologically positive and tissue negative for the corresponding virus: 4 were HBsAg (+), 1 anti-HBc(+), 12 anti-HCV(+), and 1 anti-HBc(+) anti-HCV(+). In addition, in 2 cases serologically positive for both HBV and HCV, liver tissue was positive for HBsAg(+) but HCV RNA was not detected.

The remaining 7 discordant cases were serologically negative but tissue positive, which is consistent with the standard definition of primary occult HBV (20, 21) and HCV infection (22–24). Overall, HBV DNA was detected in liver tissue of 4 of 39 (10%) HBsAg seronegative cases, and HCV RNA was detected in tissue of 3 of 42 (7%) anti-HCV seronegative patients.

The 4 "occult" HBV cases, that is, HBV DNA+ tissue positive/HBV serum negative, included 1 case that was anti-HCV(+) seropositive. HBV DNA was detected in the

Table 1. HBV and HCV serologic and liver tissue status of HCC cases ($n = 61$)

Serostatus vs. tissue viral status	Serologic markers			Tissue viral markers[a]				No. of cases
	HBsAg	Anti-HBc	Anti-HCV	HBV DNA	HBsAg	HBcAg	HCV RNA	
Concordant	−	−	−	−	−	−	−	18
Discordant ("occult" HCV)	−	−	−	−	−	−	+	1
Discordant ("occult" HBV)	−	−	−	+	+	+	−	1
Discordant ("occult" HBV)	−	−	−	+	−	−	−	1
Discordant ("occult" HBV)	−	−	−	+	+	−	−	1
Concordant	+	−	−	+	−	−	−	1
Concordant	+	−	−	+	+	−	−	2
Concordant	+	−	−	−	+	−	−	3
Concordant	+	−	−	−	+	+	−	1
Concordant	+	−	−	+	+	+	−	5
Discordant	+	−	−	−	−	−	−	4
Discordant ("occult" HCV)	+	−	−	−	+	−	+	1
Concordant	+	−	−	−	−	+	−	1
Discordant	−	+	−	−	−	−	−	1
Discordant ("occult" HCV)	−	+	−	−	−	−	+	1
Concordant	−	−	+	−	−	−	+	3
Discordant ("occult" HBV)	−	−	+	+	−	−	+	1
Discordant	−	−	+	−	−	−	−	12
Discordant	+	−	+	−	+	−	−	1
Discordant	−	+	+	−	−	−	−	1
Discordant	−	+	+	−	+	−	−	1

[a]Includes viral markers in tumor and/or non-tumor tissue.

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tissue of all 4 cases, HBsAg was additionally detected in 1 case, HBsAg and HBcAg in 1 case, and HCV RNA in 1 case. The 4 "occult" HBV cases were positive for genotypes A, B (2 cases), and D.

The 3 "occult" HCV cases, that is, HCV tissue positive/HCV seronegative cases, included 1 who was serum HBsAg(+) and 1 who was serum anti-HBc(+). In addition to HCV RNA detection in the tissue of all 3 cases, HBV DNA was additionally detected in 1 case. Notably, for 2 of these 3 cases, HCV RNA was detected in the adjacent non-tumor tissue but not in tumor tissue (data not shown). The 3 "occult" HCV cases were positive for genotypes 1a and 3a; genotype could not be ascertained for 1 case.

Characteristics of HCC cases were compared by liver tissue viral status (combining tumor and non-tumor status; Table 2). Patients with HBV-positive liver tissue were more likely to be Asian or Pacific Islander ($P = 0.04$) and born in Asia or the Pacific Islands ($P = 0.002$). There were no differences by sex, age, stage, presence of cirrhosis, or treatment. Patients with HCV-positive liver tissue were more likely to be diagnosed after 1999 ($P = 0.03$). There were no differences in demographic or clinical characteristics by HCV status.

The 4 "occult" HBV cases included 3 males and 1 female of Asian or Pacific Island ancestry. Three were U.S.-born (Hawaii) and 1 was Asian-born. Three cases had localized tumors and 1 was unstaged. Liver cirrhosis was present in 1 of the 3 "occult" HBV cases; information on cirrhosis was missing for 1 case. Survival time ranged from 12 to 71 months (mean, 33.0 ± 27.1). The 3 "occult" HCV cases were composed of 2 males and 1 female, all of Asian ancestry. Two cases had localized tumors and 1 had regional metastasis. Liver cirrhosis was present in 2 of the 3 "occult" HCV cases. All 3 cases had a survival time of less than 12 months (mean, 7.7 ± 0.58).

Discussion

In this limited sample of U.S. HCC cases, HBV and/or HCV was detected in liver tissue of fewer than half of cases, which is substantially lower than the nearly two-

Table 2. Characteristics of HCC cases by HBV and HCV liver tissue status ($n = 61$)

Characteristics	HBV+ ($n = 20$) *n* (%)	HBV− ($n = 41$) *n* (%)	*P*	HCV+ ($n = 7$) *n* (%)	HCV− ($n = 54$) *n* (%)	*P*
Sex						
Male	15 (75)	28 (68)	0.59	5 (71)	38 (70)	0.95
Female	5 (25)	13 (32)		2 (29)	16 (30)	
Age, y						
<60	13 (65)	23 (56)	0.51	3 (43)	33 (61)	0.36
≥60	7 (35)	18 (44)		4 (57)	21 (39)	
Race[a]						
White	0 —	10 (24)	0.04	1 (14)	9 (17)	0.16
Asian/Pacific Islander	20 (100)	31 (76)		6 (86)	45 (83)	
Birthplace[b]						
United States	15 (79)	11 (33)	0.002	3 (50)	23 (50)	1.00
Asia and Pacific Island	4 (21)	22 (67)		3 (50)	23 (50)	
Diagnosis year						
1990–1999	7 (35)	15 (37)	0.90	0 –	22 (41)	0.03
2000–2010	13 (65)	26 (63)		7 (100)	32 (59)	
Stage						
Localized	14 (70)	29 (71)	0.87	4 (57)	39 (72)	0.21
Regional/Distant	5 (25)	11 (27)		2 (29)	14 (26)	
Unstaged/Unknown	1 (5)	1 (2)		1 (14)	1 (2)	
Cirrhosis[c]						
No	6 (35)	16 (43)	0.58	2 (29)	20 (43)	0.48
Yes	11 (65)	21 (57)		5 (71)	27 (57)	
First course of treatment						
Surgery[d]	17 (85)	31 (76)	0.40	6 (86)	42 (78)	0.63
Other/None	3 (15)	10 (24)		1 (14)	12 (22)	

NOTE: Based on the detection of HBV (HBV DNA, HBsAg, and/or HBcAg) in tumor and/or non-tumor liver tissue and on the detection of HCV RNA in tumor and/or non-tumor liver tissue.

[a]Excludes race other than white, Asian, and Pacific Islander.

[b]Excludes birthplace other than United States, Asia, and Pacific Islands; United States includes U.S. mainland and Hawaii only.

[c]Excludes 7 cases with no information on cirrhosis status.

[d]Surgical resection or transplantation.

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thirds of cases attributed to viral hepatitis based on serology. Nonetheless, viral markers were detected in liver tissue of 17% of serologically negative HCC cases. Our results provide a proxy estimate for the prevalence of occult hepatitis infection in patients with HCC. The findings imply that occult infection may account for underestimation of the role of both HBV and HCV in the increasing incidence of HCC over the past 3 decades. Notably, 3 of the 7 "occult" HCC cases had markers of both HBV and HCV infection in tissue and/or sera. This is evidence that, in particular, HBV-HCV coinfection in HCC may be underestimated as based on serology alone.

The 4 primary "occult" HBV cases were characterized by the presence of HBV DNA in liver tissue in the absence of HBsAg and anti-HBc in serum, which is consistent with most definitions of occult infection (20, 21). Moreover, HBV DNA in liver tissue was measured on the basis of PCR assays amplifying two distinct areas of the viral genome, which meets the criteria for the detection of occult infection by some standards (25, 35–42). The 3 primary "occult" HCV cases were also consistent with current definitions, which are based on the presence of HCV RNA in liver tissue in anti-HCV seronegative individuals (22–24). However, we did not have information on serum HCV RNA, which would have afforded a more thorough assessment of HCV status (23).

There were a number of limitations to our establishment of occult infection HCC cases. As patient blood specimens were not available, HBV and HCV serologic status was based on testing associated with patients' cancer diagnoses and/or prior medical history. Although testing was conducted at CLIA-certified clinical laboratories, this retrospective study had no information on the specific laboratory assays, nor the sensitivity and specificity of assays as patients were diagnosed at multiple medical facilities over various time periods. Moreover, we did not have information on other HBV serum markers including anti-HBs, HBeAg, and HBV DNA, which collectively would have provided a more complete characterization of HBV status (20). The PCR assays were nonquantitative such that were unable to correlate HBV and HCV viral load with occult status. Moreover, the sensitivity and specificity of the HBV X gene PCR and HCV RT-PCR assays as well as the immunohistochemical assays for surface and core HBV antigen have not been established. An exception was the nested PCR assay for the HBV polymerase gene for which the sensitivity had been previously established as approximately 1 HBV DNA copy (31). The specificities of the PCR and immunohistochemical assays, however, were maintained by the use of positive and negative controls. In addition, housekeeping genes were included as internal controls for all PCR and RT-PCR assays.

Occult viral hepatitis infection has not been widely evaluated in U.S. HCC cases. Our results indicate that occult HBV infection may be prevalent in up to 10% of patients with HCC who have no detectable serum HBsAg. This is close to the 11% prevalence of occult HBV observed in a retrospective study using fresh frozen liver tissue of U.S. patients with HCC with chronic HCV infection (25). Our estimates were lower than that of another retrospective study using FFPE tissue from a single U.S. institution, which observed occult HBV infection in 3 of 19 (16%) of HCC HBsAg (−) cases (26). To our knowledge, the prevalence of occult HCV infection in patients with HCC in the United States has not been previously reported. Our findings indicate that occult HCV infection may affect 7% of HCC cases who are seronegative for HCV.

Overall, concordance of liver viral status with serology was better for HBV than for HCV. More than two-thirds of HBV seropositive cases were positive for intrahepatic HBV. In contrast, only one-fifth of HCV seropositive cases had intrahepatic HCV. HBV and HCV were detected in both tumor and adjacent non-cancerous components of liver tissue. Interestingly, HCV RNA was frequently detected in non-tumor liver tissue including cases where no virus was detected in the tumor component. Of note, our observed "occult" HCV cases included those where HCV RNA was only detected in non-tumor tissue. Our ability to detect intrahepatic HBV was enhanced by targeting both viral antigen markers and viral DNA. In contrast, the detection of HCV was limited to HCV RNA and this may have resulted in underestimation of HCV-positive liver tissue. In particular, obtaining usable RNA from FFPE specimens can pose a unique challenge. Accordingly, the prevalence of occult HCV infection may have been underestimated in the present study. Furthermore, as our assays were not quantitative, we were unable to evaluate HBV and HCV viral load in liver tissue.

"Occult" viral hepatitis cases comprised only one-fourth of HCC cases with discordant tissue serologic status. The majority of discordant cases were those with serologic indices of viral hepatitis infection with viral negative tissue. These cases likely reflect resolution of past infection. This underscores that although virally associated HCC generally develops in the context of chronic infection by these hepatotrophic viruses, continual presence of virus in liver tissue may not be required for hepatocarcinogenesis. Moreover, in some cases, viral infection may be only an initiating or "hit –and-run" event in HCC development.

There is strong evidence that the relationship of obesity with HCC risk is mediated by insulin resistance. Excess adipose tissue induces a state of systemic and hepatic insulin resistance (43). There is evidence that the risk of obesity-associated HCC is most pronounced in HCV-infected individuals (44). HCV infection dysregulates host lipid and glucose metabolism leading to hepatic and extrahepatic insulin resistance (45–49).Thus, it is possible that HCV and obesity may be contributing to the increasing rates of HCC in the United States both as independent risk factors and acting in synergy.

Our study population, however, is not representative of HCC cases in the United States. In particular, our study likely included a greater proportion of HCC cases

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etiologically associated with HBV. It is well-established that U.S. Asians and Pacific Islanders, many of whom emigrate from HBV endemic regions, are disproportionately affected by HCC, particularly by HBV-related HCC as based on serology (13, 50, 51). The state of Hawaii, with its sizeable Asian and Pacific Islander population (52), consistently has among the highest incidence rates of HCC in the United States (3, 53). We observed intrahepatic HBV more frequently in patients of Asian or Pacific Islander ancestry.

Occult HBV likely reflects the persistence of replication-competent virus with suppressed replication (21). Viral load in occult HBV is lower than that in overt chronic HBV (40). Nonetheless, the virus maintains the same pro-oncogenic and other transforming properties of overt HBV infection (21). There is inconsistent evidence that occult HBV may be a risk factor for HCC development in patients chronically infected with HCV (21, 25, 41). Little is known of the role of occult HCV infection in the development and progression of HCC. HCV can persist at low levels in the liver of patients considered to be clinically cleared of infection (23, 54). Moreover, there is evidence that individuals with serologic clearance of HCV may progress to HCC (23, 55). It is not known to what extent viral infection of liver tissue of patients with seronegative HCC increases the risk of HCC recurrence. Recurrent HCC with detectable HBV DNA in the liver has been reported 10 years after clearance of HBsAg (56).

Our study results have important epidemiologic and clinical implications. Notably, our results suggest that the contribution of HBV and HCV, including coinfection, to the increasing incidence of HCC in the United States may be underestimated. Our study results imply that for some patients with HCC, serologic testing alone may result in failure to identify and treat underlying viral hepatitis, which can potentially affect disease recurrence. Specifically, testing of liver tissue for HBV and HCV may be warranted in patients with negative serology. Moreover, if liver tissue is positive for HBV or HCV, anti-viral treatment following resection may be prudent along with close monitoring of serum HBV DNA and HCV RNA levels. Testing of liver tissue for HBV may be particularly important for seronegative Asians and Pacific Islander patients with HCC. Notably, in the present study, all HCC cases with occult HBV and HCV infection were of Asian/Pacific Islander ancestry.

Our results require confirmation in larger studies of patients with HCC with liver tissue and serologic information. In particular, further research is needed to understand the clinical significance of HBV and HCV in liver tissue in the absence of serologic indices on the development, progression, and recurrence of HCC. Research is also needed on the immunology of occult viral hepatitis as this may elucidate immunologic approaches for averting viral-related HCC pathogenesis.

Disclosure of Potential Conflicts of Interest

L.L. Wong has honoraria from the speakers' bureau of Bayer Healthcare. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: B.Y. Hernandez, S.A. Kwee, K.A. McGlynn
Development of methodology: B.Y. Hernandez, X. Zhu, G. Okimoto
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.Y. Hernandez, X. Zhu, O. Chan, D. Horio, S.F. Altekruse, L.L. Wong
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.Y. Hernandez, X. Zhu, S.A. Kwee, G. Okimoto, K.A. McGlynn, S.F. Altekruse
Writing, review, and/or revision of the manuscript: B.Y. Hernandez, S.A. Kwee, O. Chan, G. Okimoto, K.A. McGlynn, S.F. Altekruse, L.L. Wong
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.Y. Hernandez, X. Zhu, D. Horio

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372–80.
3. Reis LAG, Melbert D, Krapcho M, Stinchcomb DG, Howlader N, Horner MJ, et al. SEER Cancer Statistics Review, 1975–2005. 2008; [cited 2013 Jan.] Available from: http://seer.cancer.gov/csr/1975_2005/.
4. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–23.
5. Eheman C, Henley SJ, Ballard-Barbash R, Jacobs EJ, Schymura MJ, Noone AM, et al. Annual Report to the Nation on the status of cancer, 1975–2008, featuring cancers associated with excess weight and lack of sufficient physical activity. Cancer 2012;118:2338–66.
6. Ries LAG, Melbert D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, et al. SEER Cancer Statistics Review, 1975–2004. 2007; [cited 2013 Jan.] Available from: http://seer.cancer.gov/csr/1975_2004/.
7. Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, et al. Annual Report to the Nation on the Status of Cancer, 1975–2009, featuring the burden and trends in human papillomavirus(HPV)-associated cancers and HPV vaccination coverage levels. J Natl Cancer Inst 2013;105:175–201.
8. Kulkarni K, Barcak E, El-Serag H, Goodgame R. The impact of immigration on the increasing incidence of hepatocellular carcinoma in the United States. Aliment Pharmacol Ther 2004;20:445–50.
9. Di Bisceglie AM, Lyra AC, Schwartz M, Reddy RK, Martin P, Gores G, et al. Hepatitis C-related hepatocellular carcinoma in the United States: influence of ethnic status. Am J Gastroenterol 2003;98:2060–3.

Published OnlineFirst August 27, 2013; DOI: 10.1158/1055-9965.EPI-13-0397

10. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999; 29:664–9.
11. Raza SA, Clifford GM, Franceschi S. Worldwide variation in the relative importance of hepatitis B and hepatitis C viruses in hepatocellular carcinoma: a systematic review. Br J Cancer 2007;96: 1127–34.
12. Seeff LB, Hoofnagle JH. Epidemiology of hepatocellular carcinoma in areas of low hepatitis B and hepatitis C endemicity. Oncogene 2006; 25:3771–7.
13. El-Serag HB. Hepatocellular carcinoma: an epidemiologic view. J Clin Gastroenterol 2002;35:S72–8.
14. Hassan MM, Frome A, Patt YZ, El-Serag HB. Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. J Clin Gastroenterol 2002;35:266–9.
15. El-Serag HB, Mason AC. Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 2000;160: 3227–30.
16. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002;36: 1349–54.
17. El-Serag HB. Epidemiology of hepatocellular carcinoma in USA. Hepatol Res 2007;37 Suppl 2:S88–94.
18. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.
19. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 2005;54:533–9.
20. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol 2008;49:652–7.
21. Raimondo G, Caccamo G, Filomia R, Pollicino T. Occult HBV infection. Semin Immunopathol 2013;35:39–52.
22. Carreno V, Bartolome J, Castillo I, Quiroga JA. New perspectives in occult hepatitis C virus infection. World J Gastroenterol 2012;18: 2887–94.
23. Pham TN, Coffin CS, Michalak TI. Occult hepatitis C virus infection: what does it mean? Liver Int 2010;30:502–11.
24. Castillo I, Pardo M, Bartolome J, Ortiz-Movilla N, Rodriguez-Inigo E, de Lucas S, et al. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. J Infect Dis 2004;189:7–14.
25. Lok AS, Everhart JE, Di Bisceglie AM, Kim HY, Hussain M, Morgan TR. Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in United States patients with chronic hepatitis C. Hepatology 2011;54:434–42.
26. Kannangai R, Molmenti E, Arrazola L, Klein A, Choti M, Thomas DL, et al. Occult hepatitis B viral DNA in liver carcinomas from a region with a low prevalence of chronic hepatitis B infection. J Viral Hepat 2004; 11:297–301.
27. Fritz A, Percy C, Shanmugaratnam K, Sobin L, Parkin DM, Whelan, et al. The international classification of diseases for oncology. 3rd edition. Geneva, Switzerland: World Health Organization; 2000.
28. Goodman MT, Hernandez BY, Hewitt S, Lynch CF, Cote TR, Frierson HF Jr, et al. Tissues from population-based cancer registries: a novel approach to increasing research potential. Hum Pathol 2005;36:812–20.
29. Moon EJ, Jeong CH, Jeong JW, Kim KR, Yu DY, Murakami S, et al. Hepatitis B virus X protein induces angiogenesis by stabilizing hypoxia-inducible factor-1alpha. FASEB J 2004;18:382–4.
30. Akhter S, Liu H, Prabhu R, DeLucca C, Bastian F, Garry RF, et al. Epstein-Barr virus and human hepatocellular carcinoma. Cancer Lett 2003;192:49–57.
31. Lindstrom A, Odeberg J, Albert J. Pyrosequencing for detection of lamivudine-resistant hepatitis B virus. J Clin Microbiol 2004;42: 4788–95.
32. Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, et al. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci U S A 1990;87:9524–8.
33. Svoboda-Newman SM, Greenson JK, Singleton TP, Sun R, Frank TS. Detection of hepatitis C by RT-PCR in formalin-fixed paraffin-embedded tissue from liver transplant patients. Diagn Mol Pathol 1997;6: 123–9.
34. Kuiken C, Yusim K, Boykin L, Richardson R. The Los Alamos hepatitis C sequence database. Bioinformatics 2005;21:379–84.
35. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? Hepatology 2001;34:194–203.
36. Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? Hepatology 2001;34:204–6.
37. Branco F, Mattos AA, Coral GP, Vanderborght B, Santos DE, Franca P, et al. Occult hepatitis B virus infection in patients with chronic liver disease due to hepatitis C virus and hepatocellular carcinoma in Brazil. Arq Gastroenterol 2007;44:58–63.
38. Shetty K, Hussain M, Nei L, Reddy KR, Lok AS. Prevalence and significance of occult hepatitis B in a liver transplant population with chronic hepatitis C. Liver Transpl 2008;14:534–40.
39. Hassan ZK, Hafez MM, Mansor TM, Zekri AR. Occult HBV infection among Egyptian hepatocellular carcinoma patients. Virol J 2011; 8:90.
40. Wong DK, Huang FY, Lai CL, Poon RT, Seto WK, Fung J, et al. Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. Hepatology 2011; 54:829–36.
41. Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, Chen Z. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int 2012;32:231–40.
42. Momosaki S, Nakashima Y, Kojiro M, Tabor E. HBsAg-negative hepatitis B virus infections in hepatitis C virus-associated hepatocellular carcinoma. J Viral Hepat 2005;12:325–9.
43. McArdle MA, Finucane OM, Connaughton RM, McMorrow AM, Roche HM. Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. Front Endocrinol 2013;4:52.
44. Chen Y, Wang X, Wang J, Yan Z, Luo J. Excess body weight and the risk of primary liver cancer: an updated meta-analysis of prospective studies. Eur J Cancer 2012;48:2137–45.
45. Moucari R, Asselah T, Cazals-Hatem D, Voitot H, Boyer N, Ripault MP, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology 2008;134:416–23.
46. Bugianesi E, Salamone F, Negro F. The interaction of metabolic factors with HCV infection: does it matter? J Hepatol 2012;56 Suppl 1:S56–65.
47. El-Zayadi AR, Anis M. Hepatitis C virus induced insulin resistance impairs response to anti viral therapy. World J Gastroenterol 2012; 18:212–24.
48. Syed GH, Amako Y, Siddiqui A. Hepatitis C virus hijacks host lipid metabolism. Trends Endocrinol Metabol 2010;21:33–40.
49. Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, et al. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 2008;135: 111–21.
50. Howlader N NA, Krapcho M, Neyman N, Aminou R, Waldron W, et al., editors. National Cancer Institute. Bethesda, MD: SEER Cancer Statistics Review, 1975-2008. Available from: http://seer.cancer.gov/csr/1975_2008/, based on November 2010 SEER data submission, posted to the SEER web site, 2011.
51. Miller BA, Chu KC, Hankey BF, Ries LA. Cancer incidence and mortality patterns among specific Asian and Pacific Islander populations in the U.S. Cancer Causes Control 2008;19:227–56.
52. U.S. Census Bureau, 2011 American Community Survey. [cited 2013 Jan.] Available from: http://factfinder2.census.gov/faces/tableservices/jsf/pages/productview.xhtml?src=bkmk
53. Davila JA, Petersena NJ, Nelson HA, El-Serag HB. Geographic variation within the United States in the incidence of hepatocellular carcinoma. J Clin Epidemiol 2003;56:487–93.

Published OnlineFirst August 27, 2013; DOI: 10.1158/1055-9965.EPI-13-0397

54. Castillo I, Rodriguez-Inigo E, Lopez-Alcorocho JM, Pardo M, Bartolome J, Carreno V. Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. Clin Infect Dis 2006;43:1277–83.
55. Makiyama A, Itoh Y, Kasahara A, Imai Y, Kawata S, Yoshioka K, et al. Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. Cancer 2004;101:1616–22.
56. Shinkawa H, Nakai T, Tamori A, Tanaka H, Takemura S, Ohba K, et al. Hepatocellular carcinoma (HCC) recurring 10 years after clearance of hepatitis B surface antigen and 20 years after resection of hepatitis B virus-related HCC. Int J Clin Oncol 2008;13:562–6.

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