J. RADIAT. RES., 42, 117–130 (2001)

Liver Cancer in Atomic-bomb Survivors: Histological Characteristics and Relationships to Radiation and Hepatitis B and C Viruses

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(*Received on October 25, 2000*)
(*Revision received on February 16, 2001*)
(*Accepted on March 2, 2001*)

Hepatocellular carcinoma/Cholangiocarcinoma/Epidemiology/Radiation

Histological features of primary liver cancer among atomic-bomb survivors and their relationship to hepatitis B (HBV) and C viral (HCV) infections are of special interest because of the increased risk of liver cancer in persons exposed to ionizing radiation and the high and increasing liver cancer rates in Japan and elsewhere. We conducted a pathology review of liver cancers occurring from 1958 to 1987 among subjects in the 120,321 member cohort of 1945 Hiroshima and Nagasaki residents. A panel of pathologists classified tumor histological types and defined accompanying cirrhotic changes of the liver. Archival tissue samples were assessed for HBV using pathology stains and PCR. Reverse transcriptase (RT) PCR was used to determine HCV status. We used unconditional logistic regression to

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Abbreviations: CC, cholangiocarcinoma; CI, confidence interval; HbsAg, hepatitis-B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LET, linear-energy transfer; LSS, Life Span Study; OR, odds ratio; RBE, relative biologic effectiveness; RERF, Radiation Effects Research Foundation; Sv, Sievert

compare 302 hepatocellular carcinoma (HCC) cases to 53 cholangiocarcinoma (CC) cases, adjusting for age, year of diagnosis, sex and viral status.

Cirrhotic changes occurred significantly more often among HCC than CC cases (76% in HCC and 6% in CC). Compared to CC cases, HCC cases were 10.9 times more likely to be HBV-positive (95% confidence interval: 2.1–83.2) and 4.3 times more likely to be HCV-positive (95% confidence interval: 1.1–20.5). No significant differences were found between HCC and CC cases in radiation exposures. The predominance of HCC in the atomic-bomb survivors follows the background liver cancer pattern in Japan. Our findings suggest that HBV and HCV are involved in the pathogenesis of HCC with or without cirrhosis and are significantly less important in that of CC.

INTRODUCTION

Follow-up data from the Life Span Study (LSS) cohort of atomic-bomb survivors in Japan recently have shown a significant dose response between radiation exposure and liver cancer, indicating that low-linear-energy-transfer (LET) radiation is capable of causing liver cancer[1–2]. The LSS follow-up consists of mortality and cancer incidence data. Cancer incidence data from the Hiroshima and Nagasaki tumor registries are of high quality but often lack information on specific tumor histology. Diagnostic accuracy of the mortality data derived from death certificates is known to vary among different diseases but is especially inaccurate for liver cancer[3]. To overcome these problems, we conducted a detailed pathology review of reported and suspected liver cancer cases in the LSS cohort. The analysis of incidence data obtained from this review confirmed a significant dose response for primary liver cancer in relation to radiation exposure. An excess relative risk of 0.81 per Sv was similar for males and females, but the excess absolute incidence was higher in males, reflecting the higher background liver cancer rates in males[2].

This paper focuses on the histological features of liver tumors and accompanying cirrhotic changes in the atomic-bomb survivors. We found the atomic-bomb liver cancer cases to be predominately of the hepatocellular carcinoma type. This is in remarkable contrast to the excess cholangiocarcinoma and hemangiosarcoma cases that characterize the Thorotrast-induced liver tumor series[4–8]. HCC is the most common type of liver cancer in Japan and has been attributed to a high prevalence of hepatitis B (HBV) and C virus (HCV) infections[9]. However, little is known about the relationship of CC with HBV and HCV. Therefore, we also wanted to characterize the relationship of HCC and CC to HBV and HCV infections in the present series.

MATERIALS AND METHODS

Subjects and Pathology Review

The Radiation Effects Research Foundation's LSS cohort consists of 120,321 Japanese citizens, of whom 93,741 were present in Hiroshima or Nagasaki at the time of the 1945 atomic bombings and were also residing there at the time of censuses conducted in 1950,

1951, and 1952[10]. Mortality follow-up of this cohort has been ongoing since 1950. Incident cases of cancer were ascertained by linkage to population-based cancer registries in Hiroshima and Nagasaki[11].

Methods used to ascertain LSS liver cancer incidence cases for this study are described elsewhere[2]. Briefly, all subjects reported to have any of the following diagnoses were identified and reviewed: cancers of the liver and biliary systems, chronic liver disease or other liver disorders, disorders of the gallbladder and biliary tract, and pancreatic cancer or other pancreatic disease. Deaths recorded as due only to unspecified cancer of the respiratory tract or digestive systems were also reviewed. A panel of three pathologists (TF, HI, and MY) reviewed a total of 3,902 cases meeting these criteria who were identified from the tumor and tissue registries, the RERF autopsy and surgical pathology programs, the RERF clinical follow-up program (the Adult Health Study), and death certificates. Tissue samples were available for 80% of cases from autopsies, 15% from surgical biopsies, and 5% from needle biopsies. The cases were first examined independently by the three pathologists who then met jointly to review their diagnoses and to reach a consensus diagnosis when they disagreed; cases were rejected unless agreement was unanimous. Tissue slides were first prepared by hematoxylin and eosin stains. When diagnoses were unclear, additional slides were prepared using the periodic acid-Schiff staining method for mucopolysaccharide observation within the tissue or cell or using a silver impregnation specific for structural elements, including elastic tissue. Histological classification followed that proposed by the World Health Organization[12], and the degree of cancer-cell atypia was evaluated by Edmondson's grading scheme[13]. Histological evidence of cirrhotic changes was obtained from non-neoplastic liver tissue, and the changes were characterized according to the four types proposed by Anthony *et al*[14].

The pathology panel accepted 830 cases as primary liver cancer occurring during the 1958–1987 study period, but many of these cases were death-certificate-only cases or for other reasons lacked the medical records and tissue samples needed for histologic verification. The pathology panel conducted histological review of 423 of these cases; they rejected 69 as having unclear histology and accepted the remaining 354 as histologically confirmed PLC. Initially the panel disagreed on the histological diagnoses of 41 (11.6%) of these cases that they later accepted unanimously. The panel also reviewed and accepted four cases originally thought to have been diagnosed during the study period that were later determined to have occurred slightly outside it. Because there was no bias in the selection of these four cases and because of the small number of cholangiocarcinoma cases, we added these cases to the study, making a total of 358.

HBV and HCV

To detect hepatitis-B surface antigen (HbsAg), the pathology panel reviewed orcein-stained tissue slides[15] and HBV-immunostained tissue slides (DAKO LSAB kit, Universal K681) prepared from neoplastic and non-neoplastic, formalin-fixed and paraffin-embedded liver tissues. To increase the accuracy of HBV testing, we also used PCR to test archival tissues for expression of genes encoding hepatitis B viral antigens as described elsewhere[16]. DNA was extracted from 5-mm-thick-sections of tissue, and three separate HBV loci were

amplified, the S, pre-C, and X regions, using the following specific primer sets for each locus: S region: MD03, 5'-CTTGGATCCTATGGGAGTGG-3' and MD06, 5'-CTCAAGCTTCATCATCCATATA-3'; pre-C region: P20, 5'-AGGCATAAATTGGTCTGCGC-3' and M1, 5'- ACGAGAGTAACTCCACAGTAGCTCC-3'; and X region: XP22, 5'-CCAGCAATGTCAACGACCG-3' and XM0, 5'-ATTTATGCCTACAGCCTCC-3'. We were able to perform HbsAg tests on frozen (−80°C) or freeze-dried serum samples from 51 subjects for whom tissue-based HBV staining or PCR results were available using radioimmunoassay procedures and commercially available reagents. All 45 of the persons whose serum was negative for HbsAg were negative by tissue staining and/or PCR; five (83%) of the six persons whose serum was HbsAg-positive were positive by one of these methods. Because tissue staining and PCR appeared to be more likely to falsely classify HBV-positive subjects as negative instead of falsely classifying negative subjects as positive, we classified anyone testing HBV-positive by staining or PCR as positive to maximize the sensitivity of our tests. We classified everyone else testing negative as negative since the specificity of our tests was high. Based on our serum results, we can assume that a high percentage of our subjects testing HBV-positive by staining or PCR were positive for HbsAg, indicating active HBV infection. In all, HBV test results were available for 254 HCC cases (82.7%) and 47 CC cases (88.7%); unknown HBV-status was due to lack of tissue samples or their excessive degradation over time.

To determine HCV status, RNA was extracted from one 5-mm-thick section of paraffin-embedded liver tissue. The method of detecting the HCV genome and ensuring the integrity of mRNA in each sample is described elsewhere[17]. Briefly, the 5'-untranslated region of the HCV genome was amplified using our specific primer sets. After RT-PCR amplification, HCV-positive samples were identified by hybridizing with a radiolabeled oligomer probe that recognizes a sequence between the two primers. We were able to test frozen serum samples (−80°C) for 43 subjects, for whom HCV results of tissue-based RT-PCR tests were available. These samples were tested, under code, for HCV antibodies by ELISA-2 tests using commercially available reagents and by qualitative RT-PCR tests. Fifteen (65%) of the 23 subjects testing HCV-negative by either serum test were confirmed; the others tested positive by tissue-based RT-PCR. Of the 20 subjects testing positive by either serum test, 14 (70%) also tested positive by tissue-based RT-PCR, the other six being negative. HCV test results were available for 200 HCC (65%) and 32 CC cases (60%); unknown HCV-status was due to lack of archival tissue samples or their excessive degradation over time.

Because serum samples were available for just a small fraction of subjects for whom tissue samples were available, analysis was based entirely on the tissue-based measures. Misclassification of HBV and HCV status is likely to have occurred randomly among the two sets of cases, thereby biasing odds ratio estimates towards a finding of no association. Both HBV and HCV test results were available for 198 HCC and 32 CC cases.

Radiation Exposure

The amount of a-bomb radiation to which each subject's liver was exposed was derived from the DS86 system[18]. This dosimetry system provides estimated organ doses from gamma rays and neutrons based on physical calculations of yield coupled with individual data about

location during the bombings and shielding by buildings, terrain, and body tissue. We assumed a relative biologic effectiveness (RBE) factor of 10, multiplying the neutron dose by this amount and adding it to the gamma dose to allow for the differential effectiveness of gamma rays and neutron particles, as described by Thompson *et al*[1].

Analytical Methods

Histologically verified cases represent less than half of the total liver cancer cases that the panel accepted as primary liver cancer incident cases and that were reported by Cologne *et al*[2]. Archival tissue samples and medical records permitting histologic verification were more likely to be available when subjects received autopsies. Since LSS subjects with higher radiation exposures were more likely to receive autopsies[19], the radiation exposures of groups of CC and HCC cases will depend partly on how many were autopsied. Thus, we restricted comparison of radiation exposures of HCC and CC cases to subjects who had autopsies. Because we were concerned that cirrhosis and HBV and HCV infections might be more frequently detected in subjects receiving autopsies due to the increased quantity of tissue and possible differences in tissue quality, comparison of these risk factors was also limited to autopsied subjects. Odds ratios (OR's) comparing HCC and CC cases were estimated as measures of the strength of association for the variables of interest: radiation dose, cirrhosis and HBV/HCV infection.

Unconditional logistic regression was used to calculate OR's of cirrhosis versus no cirrhosis for infection with HBV and/or HCV; the OR's were adjusted for attained age, year of diagnosis, and sex. In addition, analyses of the relationship of HCC versus CC to HBV and HCV were first performed with analysis restricted to non-cirrhotic cases in view of these viruses' involvement in the development of liver cirrhosis. OR's for HBV and HCV infections were calculated by unconditional logistic regression, adjusting for attained age, year of diagnosis, sex, and either HBV or HCV status. Later, this analysis was repeated including cases with and without cirrhosis. Three radiation dose (liver dose in Sv) categories were used: 0, >0 to 0.5, and 0.5 or greater. Under DS86, kerma doses up to 0.005 Gy were considered zero dose. Unconditional logistic regression was used to calculate OR's, comparing autopsied HCC and CC cases, with adjustment made for attained age, year of diagnosis, sex, and HBV and HCV status. All *p*-values calculated were two-tailed. These analyses were performed using the Statistical Analysis System (SAS) version 6.12 (SAS Institute Incorporated, Cary, North Carolina). Families of subjects approved autopsies, and the study was approved by the RERF Human Investigation Committee.

RESULTS

The predominant histological type of liver cancer was HCC, accounting for 84.4% of the 358 liver tumors, with CC being the other major tumor type (14.8%) (Table 1). There were two cases of combined HCC and CC and one case of hepatoblastoma. No cases of hemangiosarcoma were found. As shown in Table 2, among HCC cases, the trabecular carcinoma

Table 1. Histological features of primary liver tumors in the Life Span Study.

Histologic type	Number of cases	Percent
Hepatocellular carcinoma	302	84.4
Cholangiocarcinoma*	53	14.8
Combined hepatocellular carcinoma and cholangiocarcinoma	2	0.6
Hepatoblastoma	1	0.3
Total	358	100.1

* All were adenocarcinomas not of the cystadenocarcinoma subtype.

Table 2. Histologic subtype and grade of hepatocellular carcinoma tumors.

Characteristic	Number of cases	Percent
Histologic subtype		
Trabecular	250	82.8
Compact	39	12.9
Pseudoglandular	10	3.3
Scirrhous	3	1.0
Total	302	100.0
Grade		
I	8	2.6
II	198	65.6
III	91	30.1
IV	5	1.7
Total	302	100.0

subtype predominated (82.8%), followed in decreasing order by the compact (12.9%), pseudoglandular (3.3%), and scirrhous (1%) subtypes. HCC showing Grade I cellular atypia was encountered in seven (2%) cases of trabecular-type carcinoma. On the other hand, HCC with Grade IV cellular atypia was seen in all three cases of scirrhous-type carcinoma. HCC with Grade II or III cellular atypia was observed in trabecular, pseudoglandular, and compact-type carcinomas.

About three-quarters of HCC cases were male (n = 219) and just a quarter, female (n = 83), but for CC, there were slightly more female than male cases (Table 3). Comparing HCC to CC cases, male gender was a significant risk factor for HCC (OR = 2.7, 95% CI: 1.47–5.11). Among males, HCC cases were significantly younger than CC cases at diagnosis of cancer; the median age at diagnosis being 63 for male HCC cases and 70 for male CC cases ($p = 0.04$, Kolmogorov-Smirnov test) (Table 3). Female HCC cases were also younger at diagnosis than female CC cases, but this difference was not statistically significant. Both male and female HCC cases were significantly younger than CC cases at the time of exposure to the atomic bombings (males: $p = 0.03$; females: $p = 0.01$, Kolmogorov-Smirnov test). About two-thirds of the cases were from Hiroshima and one-third from Nagasaki, reflecting the dis-

Table 3. Summary characteristics of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) cases

Characteristic	HCC cases (n=302)		CC cases (n=53)		HCC-CC comparisons
	Male (n=219)	Female (n=83)	Male (n=25)	Female (n=28)	*p*-value
Age at diagnosis (median)	63	67	70	73	Females: 0.46* Males: 0.04*
Radiation dose to liver in Sv (mean)	0.149	0.094	0.192	0.045	0.60†
Age at exposure to bomb (median)	32	36	41	42.5	Females: 0.01* Males: 0.03*
Hiroshima residents (number, percent)	144 (65.8)	64 (77.1)	15 (60.0)	16 (57.1)	
Nagasaki residents (number, percent)	75 (31.2)	19 (22.9)	10 (40.0)	12 (42.9)	
Presence of cirrhosis (number, percent)	145 (72.9% of 199)	64 (78.1% of 82)	2 (8.3% of 24)	1 (3.7% of 27)	0.001‡
HBV-positive (number, percent)	53 (29.8% of 178)	21 (27.6% of 76)	2 (8.7% of 23)	2 (8.3% of 24)	0.004‡
HCV-positive (number, percent)	57 (41.9% of 136)	42 (65.6% of 64)	3 (23.1% of 13)	4 (21.0% of 19)	0.001‡

* Kolmogorov-Smirnov nonparametric test.

† T-test, two-tailed

‡ Summary Cochran-Mantel-Haenszel chi square test, two-tailed

tribution of the subjects in the LSS cohort.

Major differences between HCC and CC related to the frequencies of accompanying cirrhotic changes in the liver and HBV/HCV infection status. The largest difference found was in the frequency of accompanying cirrhotic changes, which were found in about three-quarters of HCC cases and just 5% of CC cases. A significant but less striking difference was found in HBV infections (about 30% of HCC cases versus 8% of CC were infected); the difference in HCV-positive results between HCC and CC cases (about 50% versus 20%) was also statistically significant. Among HCC cases, females were significantly more likely to be HCV-infected than males (65.6% versus 41.9%; $p = 0.002$, Fisher-exact test) (Table 3). Among the 209 hepatocellular carcinoma cases who also had cirrhosis, the mixed-type cirrhosis subtype predominated (72%), followed by micronodular cirrhosis (12%), macronodular cirrhosis (11%), and undetermined subtype (5%).

Analysis of cirrhosis among autopsied HCC cases was performed to explore its relation to HBV and HCV status. With only three CC cases with cirrhosis, there were too few to conduct this type of analysis. Altogether, 93 (70%) of 133 HCC cases with known HBV and HCV status had cirrhosis. The crude OR of HCC (relative to CC) for having cirrhosis was 46.6. As shown in Table 4, OR's of cirrhosis were not significantly different than unity for the

Table 4. Hepatitis B (HBV) and C (HCV) viral infections and findings of cirrhosis among autopsied hepatocellular carcinoma cases

	HBV– HCV–	HBV+ HCV–	HBV– HCV+	HBV+ HCV+	HBV or HCV unknown	Total	%
No cirrhosis	12	9	11	3	21	56	24%
Cirrhosis	40	27	50	16	43	176	76%
Odds ratio of cirrhosis	1.0	0.9	1.0	1.0	0.6		
(95% confidence interval)*		(0.3–2.6)	(0.4–2.8)	(0.3–7.0)	(0.2–1.4)		

* Odds ratios and confidence intervals were calculated using subjects negative for both HBV and HCV as the reference category and were adjusted for attained age, year of diagnosis, and sex.

Table 5. Comparison of HBV and HCV infections among non-cirrhotic, autopsied subjects with either hepatocellular carcinoma (HCC) or cholangiocarcinoma (CC)

Characteristic	CC Number (%)	HCC Number (%)	Crude Odds Ratio	Adjusted Odds Ratio	95% Confidence Interval
Hepatitis B*					
Unknown	4	4			
No	37 (92%)	38 (72%)	1.0	1.0	
Yes	3 (8%)	15 (28%)	4.9	10.9†	2.1–83.2
Hepatitis C					
Unknown	16	22			
No	22 (79%)	21 (60%)	1.0	1.0	
Yes	6 (21%)	14 (40%)	2.4	4.3†	1.1–20.5

***** HBV-positive by either orsein or immunohistological stain or by polymerase chain reaction (PCR) or negative by all three measures. Two HCC cases with cirrhosis were positive by immunohistological stain but negative by orcein stain; all other staining results agreed.

† Odds ratio estimates of relative risk were adjusted for attained age, year of diagnosis, sex, and either HBV or HCV infection status.

presence of either or both HBV and HCV, adjusting for attained age, year of diagnosis, and sex.

HBV and HCV infections are related to cirrhosis and HCC, but cirrhosis is not involved in the pathogenesis of CC. Therefore, the association of liver cancer with HBV and HCV was first examined in cases without cirrhosis (Table 5). Adjusting for attained age, year of diagnosis, sex, and HCV status, non-cirrhotic HCC cases were 10.9 times more likely to be infected with the hepatitis B virus than were non-cirrhotic CC cases (95% CI: 2.1–83.2). HCC cases were also substantially and significantly more likely to be infected with the hepatitis C virus than were CC cases. Forty percent of the HCC cases were HCV-positive compared to 21 percent of the CC cases, a 4.3-fold difference (95% CI: 1.1–20.5). When cases both with and without cirrhosis were included in the analysis, the OR of HCC versus CC for HBV decreased

Table 6. Comparison of A-bomb radiation exposure among cirrhotic and non-cirrhotic, autopsied subjects with either hepatocellular carcinoma (HCC) or cholangiocarcinoma (CC)

Liver dose, Sv	CC Number (%)	HCC Number (%)	Crude odds ratio	Adjusted odds ratio*	95% Confidence Interval
0[†]	24 (56%)	126 (56%)	1.0	1.0	
>0[†,‡]–	16 (36%)	82 (37%)	1.0	0.8	(0.32–2.25)
0.5[§] +	4 (9%)	16 (7%)	0.8	4.0	(0.31–132.18)

* Odds ratio estimates of relative risk were adjusted for attained age, year of diagnosis, sex, and HBV and HCV infection status.

[†] Technically, under DS86, kerma doses up to .005 Gy (<0.003 Sv liver dose) were recorded as zero.

[‡] Mean radiation dose for category: 0.11 Sv

[§] Mean radiation dose for category: 1.24 Sv

from 10.9 to 7.1 and that for HCV slightly increased from 4.3 to 6.3, with both 95% CI's still excluding the null value. HBV and HCV infections were both significantly associated with HCC when CC cases are used as the reference group. The association of CC with these viral infections in the development of CC cannot be determined because our comparison is limited to HCC and CC cases.

As shown in Table 6, the distribution of HCC cases by dose groups was comparable to that of CC cases, and there was no significant trend in the OR's associated with radiation doses. We found a significant positive association among subjects with HCC between liver radiation dose and tumor size ($p = 0.02$, Jonckheere-Terpstra test). We did not find a relationship between tumor stage at diagnosis and radiation dose for these subjects, but staging information was available for a very limited number of HCC cases. We found no relationship between tumor size and radiation dose among subjects with CC ($p = 0.90$).

DISCUSSION

In Japan and many parts of the world, HCC is the predominant type of liver cancer. The histological distribution of primary liver cancers in the atomic-bomb survivors follows this pattern of background liver cancer incidence. The predominance of HCC in the atomic-bomb series is in remarkable contrast to the distribution of histological types of liver cancer observed among patients who received Thorotrast. In Thorotrast-exposed patients in Germany[7], Denmark[8], Portugal[6] and Japan[4], HCC comprises from 6% to 38% of liver cancers, whereas the majority of tumors found are CC (from 32% to 46%) and hemangiosarcoma (from 28% to 61%) (Table 7).

The earlier analysis of liver cancer incidence data inclusive of all histological types showed a significant risk associated with radiation exposure in the atomic-bomb survivors[2]. The present data demonstrated no significant differences in the OR's of HCC versus CC for radiation exposure, so the magnitudes of the relative risks associated with radiation exposure

Table 7. Primary liver tumors by histologic type occurring among persons exposed to Thorotrast, adapted from Anderson *et al*.[8)]

Cohort Study (reference number)	Radiation exposure	Year of end of follow-up	Total patients with primary liver cancer*	Hepato-cellular carcinoma %	Cholangio-carcinoma %	Hemangio-sarcoma %
Germany[7)]	Thorotrast	1988	292	24	43	32
Denmark[8)]	Thorotrast	1992	119	38	34	28
Portugal[6)]	Thorotrast	1974	31	6	32	61
Japan[4)]	Thorotrast	1990	103	22	46	32

* Includes subjects with specific diagnoses of hepatocellular carcinoma, cholangiocarcinoma or hemangiosarcoma, excluding persons with multiple tumors, tumors with other histologies, and tumors not otherwise specified.

are comparable for HCC and CC. These results are in agreement with the cohort analysis comparison of HCC and CC cases conducted by Cologne *et al.,* although both our study and the earlier one are limited by the lack of CC cases[2)]. Since five-sixths of the primary liver cancer cases in the atomic-bomb series are HCC, one may deduce that the excess risk of liver cancer in the atomic-bomb survivors is in large part derived from HCC. If relative risks of a similar magnitude are applied to the high background risk of HCC and low background risk of CC, the predominance of HCC in the present series may be expected.

Radioactive thorium dioxide from injected Thorotrast migrates to the connective tissue and results in greater radiation exposures of nearby liver bile duct and vascular cells than hepatic cells. In contrast, the atomic-bomb survivors received mostly penetrating gamma rays, resulting in whole-body exposures involving not only bile-duct and vascular cells in the liver and hepatic cells but also bone marrow and hematopoietic cells. So, the comparable magnitude of relative risks for HCC and CC in the atomic-bomb series may be expected from the uniform exposure to radiation. While hepatic cells are the possible target, bone marrow cells may also be considered in light of a possible immunological impairment suspected in atomic-bomb survivors, as suggested by the increased prevalence of HBV carriers in heavily exposed survivors[20)]. In an earlier Japanese study, all 16 HCC cases diagnosed in patients exposed to Thorotrast had trabecular and/or pseudoglandular histologic patterns[21)], which is comparable to the 260 out of 302 HCC cases (86%) we found to have these patterns in the present study.

Excess liver cancer risk has been reported from epidemiological studies of a few other, but not all, irradiated populations. Excess liver cancer mortality has been found in participants in weapons testing by the United Kingdom[22)] and in uranium miners[23–24)], but no excess risk has been found in populations receiving radiation for medical reasons, e.g, ankylosing spondylitis[25)], peptic ulcer[26)], benign gynecological bleeding disorders[27)], and cervical cancer[28)], or in UK and North American populations occupationally exposed to low levels of radiation[29–30)]. A two-fold, but nonsignificantly increased risk of stomach and liver cancer was reported among persons living near the Semipalatinsk nuclear test site in Kazakhstan, where 37,200 residents received exposures of 350–990 mSv[31)]. If there is a sex effect (women having lower risks) as suggested from the LSS incidence data[2)] and by this study, it may explain

some of the differences, for example, the lack of excess liver cancer risk in patients who received radiotherapy for gynecological diseases and cervical cancer. However, males comprised 84% of the irradiated ankylosing spondyltics[25] and 85% of the UK and North American nuclear industry workers studied by Cardis *et al*[29]. We note that irradiated populations other than the atomic-bomb survivors reside in areas where the background risk of liver cancer is relatively low with low prevalences of HBV and HCV infections. It may be that a substantially high background liver cancer risk is necessary for radiation-associated excess to be detected or that there is interaction between radiation exposure and HCV or HBV infection. The present study, based on a comparison of two groups of cancer cases, could not address this question, but it is currently being investigated by a case-control study nested in the LSS cohort.

This study's finding that HCC cases were about 11 times more likely to be HBV-positive than were CC cases is consistent with results from case-control studies of HCC and CC conducted in Korea[32]. In the Korean study, the OR of HCC for HBV surface-antigen positivity was about 64 times higher than the OR of CC for HBV (OR's: 83.6, 1.3, respectively). Persistent exposure to the hepatitis B virus can cause chronic active hepatitis and ultimately cirrhosis, in which HCC can develop[33]. Although the pathogenesis of HCC is not yet fully understood, it is known that HBV-DNA is incorporated into the genome of hepatic cells infected with HBV[34]. We found that HCC cases were 4.3 times more likely to be infected with HCV than were CC cases. These results also agree with those from the Korean case-control study, which found that the OR of HCC for HCV infection was 6.3 times higher than that of CC for HCV (OR's: 24.7, 3.9, respectively)[32].

Chronic alcohol abuse and alcoholic cirrhosis have long been recognized as a cause of liver cancer[35]. Because we could not measure the alcohol consumption of our subjects, we could not determine if there was an interaction between alcohol use and viral infections in the etiology of HCC and CC or adjust our odds ratios and their confidence intervals for this factor. However, since there is no reason to suspect a strong correlation between alcohol use and either viral status or radiation exposure, our failure to adjust for alcohol use is unlikely to have had much effect on the odds ratios we report.

In the present study, HCV measures were completely based, and HBV measures were partly based, on analysis of formalin-fixed, paraffin-embedded liver tissue samples up to 30 years old. A previous study of these samples showed that 44% of our 1952 to 1960 liver samples and 80% of our 1986 to 1989 liver samples could be successfully amplified[17]. Although prevalences of HBV and HCV infections may be underestimated in this study, the effects of underestimation should equally apply for both HCC and CC cases. Such nondifferential misclassification of exposures would cause the OR's reported in this study to be underestimated but not overestimated. In a study of Japanese HCC patients treated at a Tokyo hospital from 1984 to 1994, 2.9% were negative for HCV-RNA and HBV-DNA using an analysis of formalin-fixed, paraffin-embedded liver tissue similar to that performed for this study[9]. In comparison, 30% of our 1958 to 1987 HCC cases were negative for both viruses. This difference between studies is likely to reflect differences in the ages of our tissue samples, changes over time in HBV and HCV prevalences in Japan, or differences in the age

structure of the two populations.

Liver cirrhosis is a major risk factor for HCC as evident from the very large OR. One puzzling finding in the present analysis is that HBV and/or HCV infection did not affect the OR for cirrhosis among HCC cases. These results may imply that factors other than HBV or HCV are involved in liver cirrhosis in the pathway to HCC, or that HBV and/or HCV infection can lead to HCC without involving liver cirrhosis. The case-control study in the LSS currently in progress may shed light on the nature of associations between cirrhosis, viral infections, and radiation exposure in the LSS.

ACKNOWLEDGEMENTS

The authors thank Drs. Jun Nagano and Kojiro Koyama for their assistance and helpful comments, Mr. Shoji Nishio for his help with the tumor and tissue registry, and Mrs. Rosalyn Vu for editorial assistance. We are grateful for the support and cooperation kindly extended to us in this study by the Tumor Registry Committees of the Hiroshima and Nagasaki City Medical Associations, the Tumor Tissue Registry Committees of the Hiroshima Prefectural Medical Association and Nagasaki City Medical Association, and the many medical institutions and people engaged in medical work in the two cities. RERF is a private nonprofit foundation funded equally by the Japanese Ministry of Health and Welfare and the U.S. Department of Energy through the National Academy of Sciences. Additional funding for this project was provided by contract number NCI-4893-8-001 from the U.S. National Institutes of Health.

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