Hepatitis Virus Infection and Chronic Liver Disease among Atomic-Bomb Survivors.

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INTRODUCTION:
Increased liver cancer and cirrhosis among atomic-bomb survivors have been reported based on mortality studies or tumor registries. In the Adult Health Study (AHS) cohort, a significant excess risk was detected for chronic hepatitis and liver cirrhosis. Hepatitis C and B virus (HCV, HBV) infection plays a crucial role in the etiology of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. In Japan, more than 60-75% of hepatocellular carcinoma cases are related to chronic HCV infection and 20-25% are positive for the hepatitis B surface antigen. Three previous studies on HBV in the AHS during 1969-70, 1973-75, and 1979-81 have consistently shown that prevalence of HBV surface antigen demonstrated increasing prevalence with radiation dose. The purpose of this study is to investigate whether radiation exposure altered the prevalence of hepatitis virus infection or accelerated the progress toward chronic hepatitis after hepatitis virus infection.

METHODS:
Subjects and Methods
1. Study subjects
The AHS was established in 1950 to observe the late health effects of radiation exposure among atomic-bomb survivors in Hiroshima and Nagasaki. The original AHS cohort consisted of about 20,000 atomic-bomb survivors selected from residents in Hiroshima and Nagasaki using the 1950 national census supplementary schedules and the Atomic-Bomb Survivors Survey. Since 1 July 1958, AHS subjects have been followed through biennial medical examinations. In 1977, the original AHS sample was enlarged by 2,436 survivors and 1021 people exposed in utero.

The subjects of the analysis for HCV were 6,121 AHS participants (2,112 men and 4,009 women; 3,252 in Hiroshima and 2,369 in Nagasaki with age and radiation dose distributions as shown in Table 1) who underwent medical examination in Hiroshima and Nagasaki during 1993 through 1995. The analysis for HBV was based on 5,577 subjects after excluding 544 persons who were positive for anti-HCV. This study was approved by the Human Investigation Committee at RERF.

Table 1. Study subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Group</th>
<th>Mean</th>
<th>N</th>
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<tbody>
<tr>
<td>&lt;50</td>
<td>48.4</td>
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<td>50-54</td>
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<td>55-59</td>
<td>56.9</td>
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<td>65-69</td>
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<tr>
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<table>
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<tr>
<th>Radiation Dose (whole-body kerma, Gray)</th>
<th>Group</th>
<th>Mean</th>
<th>N</th>
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<tr>
<td>0&lt;1d≤0.2</td>
<td>0.094</td>
<td>819</td>
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<tr>
<td>0.2&lt;d≤0.5</td>
<td>0.333</td>
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<tr>
<td>0.5&lt;d≤1</td>
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<tr>
<td>1&lt;d≤1.5</td>
<td>1.215</td>
<td>412</td>
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<tr>
<td>1.5&lt;d≤2</td>
<td>1.733</td>
<td>192</td>
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<tr>
<td>2&lt;d≤3</td>
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<tr>
<td>3&lt;d≤4</td>
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<tr>
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</table>

2. Dosimetry
Radiation doses were based on the DS86 dosimetry system,[12] which estimates whole-body and organ doses after correcting for shielding by buildings, terrain, and surrounding body tissue. Because there is no single organ system exclusively involved in hepatitis infection, we used whole-body kerma dose without weighting the neutron and gamma components by a relative biological efficiency (RBE) factor, i.e. using an RBE of 1.

3. Laboratory Methods

Among all AHS participants, levels of anti-HCV, HBs antigen (HBsAg) and anti-HBs antibody (anti-HBs), and anti-HBc antibody (anti-HBc) were measured using commercial measurement kits. Anti-HCV was measured using passive hemagglutination kits (PHA, Abbott HCV-PHA 2nd Generation, Dynabott, Tokyo). In screening tests, individuals were diagnosed as having anti-HCV antibody when agglutination was found in a serum diluted $2^5$. We defined a titer of $2^{12}$ and over as high. HBsAg and anti-HBs were measured using reverse passive hemagglutination (RPHA, Special Immunological Lab., Tokyo) and PHA (Special Immunological Lab., Tokyo), respectively. Levels of serum anti-HBc were determined using PHA (Red-Cross Hospital, Hiroshima). In screening tests, individuals were diagnosed as having antibody or antigen when agglutination was found in serum diluted $2^2$ (4-fold) for HBsAg, $2^3$ (8-fold) for anti-HBs, and $2^4$ (16-fold) for anti-HBc.

All participants underwent liver function tests using a serum autoanalyzer (Hitachi 7050, Tokyo, Japan).

Trained nurses interviewed participants about their own and family member’s histories of liver disorders, prior blood transfusions, acupuncture, and alcohol intake.

4. Clinical procedure

Chronic liver disease, primarily chronic hepatitis and cirrhosis, were diagnosed by the examining physician during clinical examinations consisting of medical history, general physical examination, and abnormal liver function persisting for more than six months, all conducted according to established diagnostic procedures and recorded using the four-digit International Classification of Diseases (ICD) nomenclature. Examining physicians were blind to subjects’ radiation-exposure status.

5. Statistical Methods

Logistic regression was used to examine effects of age or birth year, radiation exposure, and questionnaire factors. Prevalence of each measurement with any particular values of radiation exposure or questionnaire variables was equal to the corresponding relative-prevalence ratios times the baseline prevalence:

$$P_j\text{(city, sex, age or birth year, } e, d, t, u, h) = R_E(e) \times R_D(d) \times R_T(t) \times R_A(u) \times R_H(h) \times P_0\text{(city, sex, age or birth year)}$$

where $e, d, t, u,$ and $h$ represent group or continuous values of the variables, radiation exposure ($e$), drinking ($d$), transfusion ($t$), acupuncture ($u$), and history ($h$). Each factor had a comparison group (baseline value) with relative prevalence 1, e.g., $R_E(0) = 1$ for subjects with radiation doses equal to 0, $R_T$ (never transfused) = 1 for subjects who reported never having received a transfusion. For continuous variables, $R_F(f)$ was estimated as a continuous function of the factor value $f$; otherwise, $R_F$ was estimated for individual categories of the factor F.

Data were analyzed using the generalized logistic-regression procedure Generalized Models for Binary Observations’ (GMBO) in the Epicure software (Hirosoft International, Seattle, Wa) with an identity link.

RESULTS:

1. Anti-HCV antibody prevalence

Overall anti-HCV prevalence was 9.4% for men versus 8.6% for women and 10.0% in Hiroshima versus 7.2% in Nagasaki. Seropositivity of anti-HCV antibody was 2.5 times higher among those with history of blood transfusion and 1.2 times higher among those with family history of liver disease, whereas acupuncture showed no association with anti-HCV.

No relationship was found between anti-HCV prevalence and radiation dose, after adjusting for age, sex, city, history of blood transfusion, drinking, and family history, but prevalence of anti-HCV was significantly lower overall among the radiation-exposed people (relative prevalence 0.84, $p=0.022$) compared to people with estimated radiation dose 0 Gy. No significant interaction was found between any of the above mentioned risk factors and radiation dose.

Among the 6,121 AHS participants, five persons were diagnosed as having liver cancer or history of liver cancer and 394 persons (6.4%) as having chronic hepatitis or liver cirrhosis. All five
liver cancer cases were anti-HCV-positive, and 186 other chronic hepatitis or liver cirrhosis cases (47.2%) were anti-HCV-positive. After adjustment for radiation dose, the relative risk of chronic hepatitis or liver cirrhosis for those who were anti-HCV-positive was 13.24 (95% CI 9.26-17.22, p < 0.001).

Prevalence of chronic hepatitis or liver cirrhosis increased with radiation dose among both anti-HCV-positive (slope=3.04, 95% CI −1.05~9.02) and anti-HCV-negative (slope=0.16, 95% CI −0.05~0.46) individuals (Fig 1). Although the slope of the dose response was nearly 20-fold higher among anti-HCV-positive individuals, the difference was only marginally significant (p = 0.097).

Figure 1. Relative risk of liver disease by dose, depending on HCV status
Points display adjusted relative risk in summary dose groups. Solid lines are the fitted dose responses using individual data and adjusted doses to correct for bias due to random dosimetry error. Separate dose responses are shown for anti-HCV- and anti-HCV+ persons. Dashed lines are 95% confidence intervals on the fitted dose responses.

2. HBs antigen, anti-HBs antibody, and anti-HBc antibody
Overall prevalence was 1-2% for HBs antigen (HBsAg), around 37% for anti-HBs antibody (anti-HBs), and 18% for anti-HBc antibody (anti-HBc). There was no sex difference in HBsAg; anti-HBs and anti-HBc prevalence was higher in men than women. Prevalence of HBsAg decreased with age, but anti-HBs increased with age. There was no relationship between HBsAg prevalence and blood transfusion, acupuncture, or family history of liver disease.

Prevalence of HBsAg and anti-HBc increased with whole-body kerma (Figures 2 and 3). However, no trend with radiation dose was found in the anti-HBs prevalence.

Figure 2. HBs antigen prevalence by radiation dose (adjusted for birth year [1927] and city; no history of liver disease
Prevalence of chronic hepatitis or liver cirrhosis increased with radiation dose after adjusting background prevalence for age, sex, and HBsAg positivity (slope=0.17, 95% CI 0.04–0.48). In the background, prevalence of chronic hepatitis or liver cirrhosis in people with HBsAg-positive was approximately three times higher that that in those without HBsAg (RR for HBsAg positive=3.31, p=0.006). However, the slope of the dose response did not differ between HBsAg positive and HbsAg negative individuals (slope: HBV positive 0.91/Gy, HBV negative 0.11/Gy, difference p=0.66) (Figure 4).

![Figure 3. Hepatitis B c-Antibody Prevalence by Radiation Dose (adjusted for birth year [1927] and gender; no history [self or family] of liver disease)](image)

![Figure 4. Relative risk of liver disease by dose, depending on HBs antigen status](image)

Points display adjusted relative risk in summary dose groups. Solid lines are the fitted dose responses using individual data and adjusted doses to correct for bias due to random dosimetry error. Separate dose responses are shown for anti-HBs- and anti-HBs+ persons. Dashed lines are 95% confidence intervals on the fitted dose responses.

3. Attributable fraction and population attributable fraction of chronic hepatitis or liver cirrhosis according to radiation exposure and viral infection

Two types of attributable fraction were determined: 1) dose-hepatitis specific attributable fraction and 2) population attributable fraction. The dose-hepatitis specific attributable fractions show what proportion of cases of chronic hepatitis or liver cirrhosis with a particular dose or hepatitis status are attributable to the exposure or virus (or both). The population attributable fraction is what proportion of cases of chronic hepatitis or liver cirrhosis in the entire 6,121 AHS sample are due to radiation exposure, virus, or both.
The prevalences of hepatitis infection and the dose distributions in the sample of 6,121 persons are as follows:

- Prevalence of HBsAg+: 1.7%
- Prevalence of anti-HCV+: 8.9%
- Mean whole-body kerma:
  - among all persons with known dose (including 0): 0.38 Gray
  - among all persons with non-zero dose estimates: 0.77 Gray

Given exposure to these risk factors for chronic hepatitis or liver cirrhosis, the population attributable fractions are as follows:

- Radiation exposure only (HB,C negative):
  - among all 6,121 persons: 1.0%
  - among all persons with known dose (including 0): 5.2%
  - among persons with non-zero dose estimate: 9.8%
- HBV only: 74.2%
- HCV only: 85.7%
- Radiation/HBV: 79.5%
- Radiation/HCV: 86.6%

If a radiation-exposed (non-zero dose) person in this sample has liver disease and is negative for both B and C virus, there is about a 10% overall chance that the disease was due to radiation exposure; this risk can be higher or lower, depending on what the actual dose was (0% at zero dose, about 34% at 4 Gy). However, if a radiation-exposed person has liver disease and is positive for HBV (HCV−), there is only about a 5% (79.5 - 74.2) chance that their disease was due to radiation exposure. If a radiation-exposed person has liver disease and is positive for HCV (HBV−), there is only about a 1% (86.6 - 85.7) chance that their disease was due to radiation exposure. This is because the relative effect of hepatitis virus (particularly HCV) is large compared to that of radiation.

**DISCUSSION**

Hepatocellular carcinoma develops frequently in patients with advanced stages of chronic liver disease. In Japan, more than 60-75% of cases of hepatocellular carcinoma are related to chronic hepatitis C virus and 20-25% of cases are positive for the hepatitis B surface antigen. Testing positive for anti-HCV indicates either recovery from prior HCV infection or an HCV carrier. In Japan, 100% of those with a high anti-HCV (≥ 212) titer are reported to be HCV carriers, and 42% of men and 19% of women with low anti-HCV titer (from 21 to 211) are HCV carriers. Neither anti-HCV prevalence nor high anti-HCV titer was related to radiation dose in the present investigation. Prevalence of anti-HCV was significantly lower overall among the radiation-exposed people compared to nonexposed, even with adjustment for blood transfusion and family history. Anti-HCV prevalence is known to differ by geographic region. However, the difference of prevalence between the exposed and the unexposed group cannot be considered just a regional difference because the same tendency was observed in both Hiroshima and Nagasaki. The difference is conceivably due to some socioeconomic or other unknown factor in the region near the hypocenter that were not investigated in the present study.

After acute HCV exposure, approximately 80% of patients evidence chronic hepatitis. However, there are people found to have anti-HCV at the time of blood donation or health examination who are asymptomatic and whose liver function is normal. Among the AHS population, no dose response was found in the prevalence of anti-HCV-positive individuals even with stratification for low and high anti-HCV-positive titer. However, the increase in prevalence of chronic liver disease with radiation dose was quite high among anti-HCV-positive individuals, suggesting that radiation exposure might accelerate the progress of chronic liver diseases showing abnormal liver function due to HCV infection. Radiation dose may affect the course of HCV-associated chronic hepatitis regardless of whether HCV infection occurs before or after radiation exposure. The clinical presentation of HCV infection may vary depending on the host immune system and the source and duration of infection. In previous immunological studies of peripheral blood lymphocytes among atomic-bomb survivors, the proportion of CD4+ T cells decreased with radiation dose whereas the proportion of B-cell subsets significantly
increased with dose. Thus, A-bomb radiation may have altered the balance or interaction between T- and B-cell subsets. Subsequently, liver damage resulting from HCV infection may have advanced among the A-bomb survivors.

At ABCC-RERF, three studies have been conducted to examine the relationship between HBV infection and radiation dose. All studies showed higher prevalence of the HBsAg in the heavily exposed than in the controls, but no relationship was found between anti-HBs and radiation dose. The present study also indicated that prevalence of HBsAg increased with radiation dose, while no relationship was found between radiation dose and prevalence of ant-HBs, which are consistent with the previous studies. It is likely that increased HBV infection with radiation dose contributes to increased chronic liver disease and subsequent hepatocellular carcinoma.

In the background, prevalence of chronic hepatitis in people who were HBsAg positive was approximately three times higher that that in those without HBsAg. There was no difference in the slope between HBsAg-positive and HbsAg-negative individuals. There was no evidence that radiation accelerated the progress of chronic hepatitis from the HBV carriers. The results on attributable fraction of chronic liver disease by radiation and hepatitis virus infection showed that, although the joint effect of radiation and virus (primarily HCV) leads to a greater number of chronic hepatitis cases, the attributable risk for radiation among viral positive cases is smaller than among viral negative cases.

CONCLUSIONS:
In conclusion, no dose-response relationship was found between positivity of anti-HCV antibody and radiation dose, while prevalence of HBs antigen increased with radiation dose. Increased hepatitis B virus infection with radiation dose may make some contribution to increased chronic liver disease and subsequent hepatocellular carcinoma. It is unlikely that radiation exposure alters the prevalence of hepatitis C virus infection. However, a possible increase in the radiation dose-response of chronic liver disease among anti-HCV antibody positive individuals warrants further study, because it suggests that radiation exposure may accelerate the progress of chronic liver disease associated with hepatitis C virus infection.

REFERENCES