Serum interleukin-6 associated with hepatocellular carcinoma risk: A nested case–control study

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Inflammatory markers have been associated with increased risk of several cancers, including colon, lung, breast and liver, but the evidence is inconsistent. We conducted a nested case–control study in the longitudinal cohort of atomic-bomb survivors. The study included 224 hepatocellular carcinoma (HCC) cases and 644 controls individually matched to cases on gender, age, city and time and method of serum storage, and countermatched on radiation dose. We measured C-reactive protein (CRP) and interleukin (IL)-6 using stored sera obtained within 6 years before HCC diagnosis from 188 HCC cases and 605 controls with adequate volumes of donated blood. Analyses with adjustment for hepatitis virus infection, alcohol consumption, smoking habit, body mass index (BMI) and radiation dose showed that relative risk (RR) of HCC [95% confidence interval (CI)] in the highest tertile of CRP levels was 1.94 (0.72–5.51) compared to the lowest tertile (p = 0.20). RR of HCC (95% CI) in the highest tertile of IL-6 levels was 5.12 (1.54–20.1) compared to the lowest tertile (p = 0.007). Among subjects with BMI > 25.0 kg/m², a stronger association was found between a 1-standard deviation (SD) increase in log IL-6 and HCC risk compared to subjects in the middle quintile of BMI (21.3–22.9 kg/m²), resulting in adjusted RR (95% CI) of 3.09 (1.78–5.81; p = 0.015). The results indicate that higher serum levels of IL-6 are associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-related factors and radiation exposure. The association is especially pronounced among subjects with obesity.

Key words: C-reactive protein, interleukin-6, obesity, hepatocellular carcinoma, nested case–control study
Abbreviations: BMI: body mass index; CI: confidence interval; CRP: C-reactive protein; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IL-6: interleukin-6; RERF: Radiation Effects Research Foundation; RR: relative risk; SD: standard deviation
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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) are recognized as crucially important risk factors for HCC, whereas an increase of HCC without HBV and HCV infection (non-B, non-C HCC) has been noted recently in Japan.1,2 Although periodic follow-up with imaging, tumor markers such as alpha-fetoprotein (AFP) and fibrosis markers are recommended, these strategies have not been sufficient for early detection of HCC in chronic liver disease, especially in non-B, non-C liver disease. Therefore, it is necessary to identify biomarkers that may be useful to narrow down a high-risk subgroup for HCC. A large number of epidemiologic studies have shown that obesity and diabetes mellitus are associated with increased risks of such malignant tumors as colon, prostate and breast, as well as HCC.3–11 Our earlier study also demonstrated that obesity [body mass index (BMI) > 25.0 kg/m²] 10 years before HCC diagnosis was significantly associated with increased risk of HCC, independently of HBV and HCV infection, alcohol consumption, smoking habit and radiation exposure.12 It has been suggested that cell proliferation activity of insulin due to hyperinsulinemia or chronic inflammation may promote
carcinogenesis by DNA damage, enhancement of cellular proliferation and inhibition of apoptosis.\textsuperscript{4,13} In recent years, some studies have suggested that blood levels of inflammatory markers or cytokines also related to insulin resistance—such as C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor (TNF)—may reveal a biological mechanism by which risks of colon, lung and breast cancers increase,\textsuperscript{14–17} but other studies have not supported such associations.\textsuperscript{18,19}

Several studies have demonstrated that elevated serum levels of IL-6 are associated with increased risk of HCC in female chronic hepatitis C patients,\textsuperscript{20} and that the combination of serum levels of IL-6 and alpha-fetoprotein improves sensitivity in diagnosing HCC or predicting future HCC development in chronic hepatitis B patients.\textsuperscript{21} A few experimental studies using a mouse model have demonstrated that estrogen-mediated inhibition of IL-6 production by Kupffer cells reduces liver cancer risk in females,\textsuperscript{22} and that obesity-promoted HCC development was bound up with elevated production of the tumor-promoting cytokines, such as IL-6 and TNF, which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3.\textsuperscript{23} In several other cancers,\textsuperscript{24,25} it has been suggested that IL-6 and STAT3 may also contribute toward a general enhancement of cancer risk by high BMI.

With the aim of investigating whether serum levels of CRP and IL-6 are associated with risk of HCC and, if so, whether that risk is independent of HBV and HCV infection, alcohol consumption, smoking habit, BMI and radiation exposure, we conducted a nested case–control study using sera collected from a prospective cohort study of atomic-bomb survivors. We subsequently evaluated whether the association between serum IL-6 levels and HCC risk is modified by alcohol consumption, smoking habit, BMI or radiation dose to the liver using analyses based on subgroups of those factors.

**Material and Methods**

**Cohorts**

The Atomic Bomb Casualty Commission (ABCC) and its successor, the Radiation Effects Research Foundation (RERF), established the prospective Adult Health Study cohort in 1958, in which more than 20,000 gender-, age- and city-matched proximal and distal atomic-bomb survivors and persons not present in the cities at the time of bombings have been examined biennially in outpatient clinics in Hiroshima and Nagasaki.

**Cases and controls**

Incident cancer cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, confirmed and supplemented by additional cases detected via pathological review of related diseases.\textsuperscript{26} As described in our previous studies,\textsuperscript{3,27} 359 primary HCC cases were diagnosed among 18,660 Adult Health Study participants between 1970 and 2002, who visited our outpatient clinics before their diagnosis. Of these, 229 cases had serum samples obtained within 6 years before HCC diagnosis (average: stored sera obtained 1.2 years before diagnosis). After excluding five cases with inadequate stored serum, 224 cases remained for our previous studies. There were no important differences in characteristics such as alcohol consumption, smoking habit, BMI or radiation dose to the liver (among exposed persons) between HCC cases excluded because of nonavailability of stored serum and those included in our study.

As described in our previous studies,\textsuperscript{3,27} 644 controls were selected from the at-risk cohort members matched to the case on gender, age, city and time and method of serum storage, and countermatched on radiation dose in nested case–control fashion.\textsuperscript{28} Counter matching (to increase statistical efficiency for studying joint effects of radiation and other factors) was performed using four strata based on whole-body (skin) dose: zero dose (<0.0005 Gy), <0.05 Gy, <0.75 Gy and ≥ 0.75 Gy (nonzero categories correspond roughly to tertiles of skin dose among all eligible exposed cases). At the time of each case diagnosis, one control serum was selected at random from each of the three dose strata not occupied by the case in the cohort risk set.

**Laboratory tests**

Virological assays of HBV and HCV were performed on 211 cases and 640 controls with sufficient stored sera for these assays as previously described.\textsuperscript{29,30} HBV infection (HBV+) status was defined as positive for HBsAg or having a high titer of anti-HBc Ab (positive for anti-HBc Ab of samples diluted 200-fold). HCV infection (HCV+) status was defined as positive for HCV RNA. Non-B, non-C status was defined as negative for HBsAg and not having a high titer of anti-HBc Ab (HBV-) as well as negative for HCV RNA (HCV-).
Serum levels of CRP were measured using an autoanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan) and a high-sensitivity assay kit (Nissui Pharmaceutical, Tokyo, Japan) containing anti-CRP monoclonal antibodies. The detection limit of CRP was 0.08 mg/L. The intra-assay variability was determined by assaying two pooled serum samples (mean CRP level: 0.62 and 1.68 mg/L, respectively) 20 times in a single day, and the respective coefficients of variation (CVs) were 1.12 and 0.95%. The interassay variability was determined by assaying two quality control samples (mean CRP level: 2.14 and 4.71 mg/L, respectively) once a day 12 for days; the respective CVs were 4.1 and 1.2%. Serum levels of IL-6 were measured using the multiplex bead array assay on the Luminex Complete System 200 (Luminex Corp., Austin, TX), with MILLIPLEX MAP kits (Millipore, Billerica, MA) according to the manufacturer’s instructions. Human serum adipokine panel B (HADK2-61K-B) was used for IL-6. The intra-assay variability was determined by assaying two pooled serum samples with and without including a quality control sample (mean IL-6 level: 4.29 and 144.82 pg/mL, respectively) 15 times in a single day, and the respective CVs were 8.6 and 7.5%. The interassay variability was determined by assaying two quality control samples (mean IL-6 level: 31.02 and 171.62 pg/mL, respectively) once a day for 7 days; the respective CVs were 7.9 and 13.7%.

Radiation dose
Radiation dose to the liver was estimated for each subject according to Dosimetry System DS02. A weighted sum of the gamma dose in gray plus ten times the neutron dose in gray was used.

Alcohol consumption, smoking habit and BMI
Self-administrated questionnaires on lifestyle-related factors were given to Adult Health Study participants in 1965 during attendance at the outpatient clinic and in 1978 by mail survey. Information on alcohol consumption was obtained from the 1965 questionnaire when available, with missing data complemented using the 1978 mail survey. Mean ethanol amounts were calculated as grams per day, as previously described. Information on smoking habit was obtained from the 1965 questionnaire. Subjects were categorized as never, current or former smoker. BMI (kg/m²) was calculated from height and weight measured in the outpatient clinic of the Adult Health Study. Subjects were classified based on BMI quintiles with cut points of 19.5, 21.2, 22.9 and 25.0. Following the recommendations for Asian people by the WHO, the International Association for the Study of Obesity and the International Obesity Task Force, BMI 21.3–22.9 kg/m² was considered as normal, 23.0–25.0 kg/m² as overweight and >25.0 kg/m² as obese.

Statistical analyses
The nested case–control design is analyzed using a partial likelihood method analogous to that used for cohort follow-up studies, which is in practice the same as the conditional binary data likelihood for matched case–control studies except that the subjects (cases and controls) in the study are not completely independent owing to the possibility of repeated selection. Radiation risk was estimated using an excess relative risk (ERR) model (ERR = RR − 1) to conform to other analyses of the atomic-bomb survivor cohort. Bias in control doses due to selecting controls using countermatching was corrected using weights as described elsewhere. Risks for all other factors were assessed using a log-linear model. In analyses based on continuous values, CRP and IL-6 were transformed using the natural logarithm. Analyses using CRP or IL-6 groups used tertiles computed among controls. A two-degree-of-freedom heterogeneity test was performed by comparing the deviance of the model with tertiles to that without, using the lowest tertile as the comparison group. We fit log-linear regression models for the effect of a 1-standard deviation (SD) increase in IL-6 and tested for interaction with each of the other risk factors individually using the same heterogeneity test, with degrees of freedom depending on the number of categories of the other risk factor; we report the p value for the pairwise test comparing the interaction parameter in the highest to lowest level of each other risk factor. We also assessed various models for log relative risk of HCC with continuous level of IL-6—linear, linear-quadratic and linear spline—using the Akaike information criterion (AIC). Analyses were conducted using Epicure (HIROSOFT International Corp., Seattle, WA).

Results
Characteristics of cases and controls
Table 1 shows characteristics of HCC cases and matched controls. Because of matching, cases and controls were comparable with respect to gender, age, city and time and method of serum storage. Prevalence of HBV and/or HCV infection status in HCC cases is higher than in controls. Compared to the controls, higher proportions of HCC cases had a history of alcohol consumption exceeding 40 g of ethanol per day, were obese (BMI > 25.0 kg/m²) and were current smokers. Median serum levels of CRP were 0.72 mg/L among HCC cases and 0.59 mg/L among controls. Median serum levels of IL-6 were 4.88 pg/mL among HCC cases and 2.90 pg/mL among controls. HCC cases also received on average higher radiation doses to the liver compared to controls.

Correlations among CRP, IL-6, alcohol, BMI and radiation dose
Table 2 shows Spearman rank-correlation coefficients (r) between serum levels of CRP and IL-6, alcohol consumption,
BMI 10 years before HCC diagnosis and radiation dose to the liver. Serum levels of CRP were positively correlated with serum levels of IL-6 among both cases (r = 0.46) and controls (r = 0.29). Serum levels of CRP were modestly correlated with BMI among both cases (r = 0.15) and controls (r = 0.28), whereas correlations between serum levels of IL-6 and BMI were not significant among either cases (r = 0.11) or controls (r = 0.06). Neither alcohol consumption nor radiation dose showed any evidence of correlation with either marker.

### Risk of HCC according to serum levels of CRP and IL-6

Table 3 shows the association between CRP and HCC risk based on tertiles of serum CRP levels. Analyses with adjustment for HBV and HCV infection, alcohol consumption, smoking habit, BMI 10 years before HCC diagnosis and radiation dose showed that relative risks (RRs) of HCC [95% confidence interval (CI)] in the middle tertile (0.37–0.96 mg/L) and highest tertile (>0.96 mg/L) of CRP levels were 2.11 (0.73–6.54; p = 0.17) and 1.94 (0.72–5.51; p = 0.20), respectively, compared to...
Table 2. Spearman rank-correlation coefficients between CRP, IL-6, alcohol, BMI and radiation dose among HCC cases and controls

| Variables                          | CRP          |       | IL-6          |       |
|------------------------------------|--------------|-------|---------------|-------|
|                                    | Correlation  | p Value | Correlation    | p Value |
| HCC cases                          |              |        |               |        |
| CRP                                | –            | –      | –             | –      |
| IL-6                               | 0.46         | <0.001 | –             | –      |
| Alcohol consumption (g ethanol/day)| 0.01         | 0.9    | –0.02         | 0.83   |
| BMI 10 years before diagnosis      | 0.15         | 0.049  | 0.11          | 0.14   |
| Radiation dose to the liver        | –0.09        | 0.26   | –0.08         | 0.30   |
| Controls                           |              |        |               |        |
| CRP                                | –            | –      | –             | –      |
| IL-6                               | 0.29         | <0.001 | –             | –      |
| Alcohol consumption (g ethanol/day)| –0.003       | 0.94   | 0.05          | 0.28   |
| BMI 10 years before diagnosis      | 0.28         | <0.001 | 0.06          | 0.13   |
| Radiation dose to the liver        | –0.02        | 0.64   | –0.06         | 0.13   |

Table 3. Relative risks of HCC by tertile of serum levels of CRP

| Tertile of CRP                      | No. of cases/controls1 | Crude RR (95% CI) | p Value for heterogeneity |
|-------------------------------------|------------------------|-------------------|--------------------------|
| Low < 0.37 mg/L                     | 49/120                 | 1.00 (0.36–1.15)  | 0.10                     |
| Middle 0.37–0.96 mg/L               | 29/98                  | 1.54 (0.62–3.92)  | 0.28                     |
| High > 0.96 mg/L                    | 59/109                 | 1.90 (0.87–4.36)  | 0.11                     |
|                                    |                        |                   |                          |
| Adjusted RR (95% CI)2               | 1.00                   | 0.36              | 0.11                     |
| p Value                             | –                      |                   |                          |
| Adjusted RR (95% CI)3               | 1.00                   | 2.11 (0.73–6.54)  | 0.32                     |
| p Value                             | –                      | 0.17              | 0.20                     |

1Number of subjects for whom information available for all factors included in a log-linear model: 137 HCC cases and 327 controls.
2Adjusted for HBV/HCV infection, excluding three HBV+/HCV+ individuals.
3Adjusted for HBV/HCV infection, alcohol consumption, smoking habit, BMI 10 years before diagnosis and radiation dose to the liver.

Table 4. Relative risks of HCC by tertile of serum levels of IL-6

| Tertile of IL-6                     | No. of cases/controls1 | Crude RR (95% CI) | p Value for heterogeneity |
|-------------------------------------|------------------------|-------------------|--------------------------|
| Low < 2.01 pg/mL                    | 13/103                 | 1.00              | <0.001                   |
| Middle 2.01–4.46 pg/mL              | 48/107                 | 2.87 (1.02–8.91)  | 0.025                    |
| High > 4.46 pg/mL                   | 71/103                 | 4.09 (1.46–12.9)  | 0.007                    |
|                                    |                        |                   |                          |
| Adjusted RR (95% CI)2               | 1.00                   | 0.045             | 0.007                    |
| p Value                             | –                      |                   |                          |
| Adjusted RR (95% CI)3               | 1.00                   | 3.85 (1.16–14.7)  | 0.023                    |
| p Value                             | –                      | 0.027             | 0.007                    |

1Number of subjects for whom information available for all factors included in a log-linear model: 132 HCC cases and 313 controls.
2Adjusted for HBV/HCV infection, excluding three HBV+/HCV+ individuals.
3Adjusted for HBV/HCV infection, alcohol consumption, smoking habit, BMI 10 years before diagnosis and radiation dose to the liver.

Those in the lowest tertile (<0.37 mg/L; heterogeneity p = 0.32).

Table 4 shows the association between IL-6 and HCC risk based on tertiles of IL-6. Analyses with adjustment for HBV and HCV infection, alcohol consumption, smoking habit, BMI 10 years before HCC diagnosis and radiation dose showed that RRs of HCC (95% CI) in the middle tertile (2.01–4.46 pg/mL) and highest tertile (>4.46 mg/L) of IL-6
levels were 3.85 (1.16–14.7; \( p = 0.027 \)) and 5.12 (1.54–20.1; 0.007), respectively, compared to those in the lowest tertile (<2.01 pg/mL; heterogeneity \( p = 0.023 \)).

Additional analyses were conducted to examine the association between CRP or IL-6 and non-B, non-C HCC risk, although there were relatively few cases with non-B, non-C status (31 cases). Analyses with adjustment for alcohol consumption, smoking habit, BMI 10 years before HCC diagnosis and radiation dose showed that RRs of non-B, non-C HCC (95% CI) in the middle and highest tertiles of IL-6 were 56.3 (4.27–2,000) and 98.0 (6.74–4,500), respectively, compared to those in the lowest tertile (heterogeneity \( p < 0.001 \)) after the same adjustment. The wide confidence bounds are presumably due to the small numbers of non-B, non-C HCC cases.

We also examined the possibility of a nonlinear relation between serum levels of CRP or IL-6 and HCC risk. There was no evidence of any systematic relationship between CRP and HCC risk (Fig. 1a). The log RR of HCC increased linearly with logarithm of serum IL-6 level after adjustment for alcohol consumption, smoking habit, BMI and radiation dose (\( p = 0.015, \text{AIC} = 132.63; \) Fig.1b). Although HCC risk appears to level off or decline at high values of IL-6 (Fig.1b), neither a negative quadratic term (\( p = 0.17, \text{AIC} = 132.73 \)) nor a linear spline (\( p = 0.10, \text{AIC} = 133.95 \), with best fit obtained using a join point at log IL-6 = 1.6 or IL-6 = 4.95) revealed any statistically significant departure from the log-linear model. Although the appearance of a downturn at high values of IL-6 may be spurious, lack of statistical significance could also be due to the large uncertainty in estimated risk for IL-6 (high upper bound on confidence intervals for IL-6 groups).

### Interaction between IL-6 level and gender, lifestyle-related factors or radiation for risks of HCC

Table 5 shows the association between IL-6 and HCC risk by selected subgroups. Stronger association was found between a 1-SD increase in log IL-6 and HCC risk among subjects with BMI of >25.0 kg/m\(^2\) (obese) 10 years before diagnosis than among subjects with BMI of 21.3–22.9 kg/m\(^2\) (normal), resulting in adjusted RR (95% CI) of 3.09 (1.78–5.81; \( p \) for interaction = 0.015). However, there was no significant difference in association between IL-6 and HCC risk among females compared to males, among subjects with alcohol consumption of 40 g of ethanol per day compared to never drinkers, among current smokers compared to never smokers or among subjects exposed to \( \geq 1.0 \) Gy radiation compared to subjects exposed to <0.001 Gy radiation.

Additional analyses were conducted to examine the association between IL-6 and non-B, non-C HCC risk by selected subgroups. Similarly, a stronger association was found between a 1-SD increase in log IL-6 and non-B, non-C HCC risk among subjects with BMI of >25.0 kg/m\(^2\) than among subjects with BMI of 21.3–22.9 kg/m\(^2\), resulting in adjusted RR (95% CI) of 5.01 (1.51–34.0; \( p \) for interaction = 0.025). The results suggest that elevated serum levels of IL-6 among obese subjects are more strongly associated with increased risks of non-B, non-C HCC as well as overall HCC compared to subjects with normal weight.

### Discussion

Our study demonstrated that elevated serum levels of IL-6 are associated with increased risk of HCC, independently of hepatitis virus infection, lifestyle-related factors—such as alcohol consumption, smoking habit and BMI—and radiation.
exposure. Significant association was observed between ele-

vated serum levels of IL-6 and increased risk of non-B, non-

C HCC, whereas the association with elevated serum levels of

CRP was only marginally significant. Among subjects with

obesity, an even stronger association was observed between

elevated serum levels of IL-6 and increased risk of HCC

(non-B, non-C HCC as well as all HCC).

Several studies have demonstrated that elevated serum

level of CRP is associated with poor prognosis in HCC

patients, whereas few cohort studies have shown a significant

association between CRP level and HCC risk. In our study,

the association between serum level of CRP and HCC risk

was not significant, after adjusting for HBV and HCV infec-
tion, lifestyle-related factors and radiation dose. However, it

has been reported that positive association between CRP level

and degree of hepatic steatosis occurs among obese patients

with nonalcoholic fatty liver disease, and CRP level is useful

not only for distinguishing nonalcoholic steatohepatitis

(NASH) from simple nonprogressive fatty liver but also for

predicting the severity of liver fibrosis in steatohepatitis.

Table 5. Relative risks of HCC associated with a 1-SD increase in log IL-6 level

|                          | RR   | 95% CI   | p Value for interaction$^1$ |
|--------------------------|------|----------|-----------------------------|
| All HCC                  | 1.84 | 1.50, 2.28 |
| Gender                   |      |          |                             |
| Males                    | 1.78 | 1.36, 2.38 |
| Females                  | 1.91 | 1.41, 2.68 |
| Alcohol consumption (g ethanol per day) |    |          |                             |
| None                     | 1.91 | 1.40, 2.69 |
| ≥40                      | 1.88 | 1.69, 3.53 |
| Smoking habit            |      |          |                             |
| Never                    | 2.09 | 1.48, 3.07 |
| Current smoker           | 1.61 | 1.19, 2.23 |
| BMI (kg/m$^2$) 10 years before diagnosis |      |          |                             |
| 21.3–22.9                | 1.26 | 0.80, 1.99 |
| >25.0                    | 3.09 | 1.78, 5.81 |
| Radiation dose to the liver (Gy) |      |          |                             |
| 0≤ <0.001                | 2.01 | 1.43, 2.89 |
| ≥1.0                     | 2.50 | 1.38, 5.10 |
| Non-B, non-C HCC         | 1.62 | 1.14, 2.39 |
| Gender                   |      |          |                             |
| Males                    | 1.09 | 0.60, 1.96 |
| Females                  | 2.13 | 1.32, 3.84 |
| Alcohol consumption (g ethanol per day) |    |          |                             |
| None                     | 1.86 | 1.09, 3.73 |
| ≥40                      | 2.09 | 0.57, 11.0 |
| Smoking habit            |      |          |                             |
| Never                    | 2.04 | 1.13, 4.16 |
| Current smoker           | 1.35 | 0.78, 2.39 |
| BMI (kg/m$^2$) 10 years before diagnosis |      |          |                             |
| 21.3–22.9                | 0.84 | 0.31, 2.02 |
| >25.0                    | 5.01 | 1.51, 16.0 |
| Radiation dose to the liver (Gy) |      |          |                             |
| 0≤ <0.001                | 1.71 | 0.89, 3.44 |
| ≥1.0                     | 2.66 | 1.06, 10.1 |

$^1$p Value for interaction is from the likelihood ratio test for a difference in IL-6 risk between high-risk and re-

ference categories of the other factor, while adjustment was made for main effects and interactions of all cat-

ergories of the other factor.
cases. In our study, analyses with adjustment for lifestyle-related factors and radiation dose in non-B, non-C subjects showed that the risk of non-B, non-C HCC is significantly higher in the middle or highest tertile of serum CRP levels than in the lowest tertile, and that the risk increases with elevated serum levels of CRP (though only with marginal statistical significance). This result is consistent with published findings that background liver disease of non-B, non-C HCC may be partially caused by NASH or steatohepatitis.

Several studies have reported that higher serum IL-6 level precedes the development of HCC in female chronic hepatitis C patients or chronic hepatitis B patients. Estrogen-mediated inhibition of IL-6 production by Kupffer cells may explain such gender disparity in HCC development.

An animal study also showed gender-based differences in IL-6 production associated with liver cancers. Previous studies have also demonstrated that serum IL-6 level increases in patients with established HCC. IL-6 is a multifunctional cytokine that plays a prominent role in immune response, cell survival, apoptosis and proliferation.

In our study, the association between serum levels of IL-6 and HCC risk was significant after adjusting for HBV and HCV infection, lifestyle-related factors and radiation dose. Elevated serum levels of IL-6 were associated with increased risk of HCC irrespective of gender. Additionally, analyses with adjustment for lifestyle-related factors and radiation dose in HCC cases and controls of non-B, non-C type showed that non-B, non-C HCC risk is significantly higher in the middle or highest tertile of serum IL-6 levels than in the lowest tertile, and that the risk significantly increases with elevated serum levels of IL-6. These results are consistent with published findings that elevated IL-6 level is associated with the development of type 2 diabetes or insulin resistance, which are considered to be factors contributing to progression in non-B, non-C HCC as well as HCC.

Obesity and diabetes mellitus have recently earned recognition as risk factors for HCC. Our previous study also demonstrated that obesity 10 years before HCC diagnosis was an independent risk factor for HCC, and that there was a significant multiplicative interaction in HCC risk between obesity and HCV infection. Obesity contributes to a high rate of visceral fat storage. Increases in production of cytokines such as TNF-α, IL-6, monocyte chemoattractant protein-1 and leptin secreted from adipose tissue and/or macrophages accumulated in such tissues cause hepatic steatosis and oxidative stress through insulin resistance, resulting in the development of HCC. A recent experimental study using a mouse model indicated that obesity promotes HCC development by enhancing production of the tumor-promoting cytokines such as IL-6 and TNF, which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3.

In our study, elevated serum levels of IL-6 were significantly associated with increased risk of HCC, especially among subjects with obesity, after adjusting for all other categories of the other risk factor. That trend changed little when the association between IL-6 levels and non-B, non-C HCC risk was examined. Other factors related to HCC risk among obese subjects such as genotype may affect the interaction between IL-6 and obesity, when taking into account the fact that correlations between serum levels of IL-6 and BMI were not significant among HCC cases and controls. Nevertheless, monitoring of IL-6 levels may be crucial to early detection of HCC irrespective of HBV and/or HCV infection, especially for individuals with chronic liver disease or fatty liver disease with obesity.

The strengths of our study include its prospective cohort base with high follow-up rate and nested case–control design, which minimize selection bias. It is difficult and expensive to perform full cohort analyses of serum biomarkers such as IL-6 and CRP, whereas the nested case–control design used here can provide substantial reductions in cost and effort with little loss of statistical efficiency.

We also incorporated, in a strict and in-depth manner, hepatitis virus infection status of HCC cases measured before diagnosis (measured at comparable ages among matched controls). Furthermore, we included such potential HCC risk factors as alcohol consumption, smoking habit and BMI in the multivariate analyses, because several studies have demonstrated that inflammatory markers including CRP and IL-6 levels are associated with such lifestyle-related factors. However, we cannot completely exclude the possibility of residual confounding.

A limitation of our study is that use of hormones, aspirin and nonsteroidal anti-inflammatory drugs, which are related to CRP levels, could not be adjusted as confounders, because participants have only been asked detailed information on such kinds of medication since 1991. Another is that we used stored sera obtained within 6 years before HCC diagnosis. The reason is that to render primary diagnosis of HBV and/or HCV infection status of cases and controls of serum samples obtained from study participants between 1970 and 2002, de novo HCV infection in particular could not be denied outright regarding those obtained between 1970 and 1989. Therefore, the findings of elevated IL-6 levels associated with HCC risk (also measured within 6 years of diagnosis) may include a mixture of precancerous change and defense against tumor formation or growth. It suggests that elevated IL-6 levels may represent not cause but effect for increased risk of HCC, although causality cannot be inferred from our study. However, for early identification and management of HCC, measurement and monitoring of IL-6 levels for individuals with chronic liver disease or fatty liver disease may be meaningful, irrespective of HBV and/or HCV infection.
In conclusion, elevated serum levels of IL-6 were associated with increased risk of HCC, even after adjusting for HBV or HCV infection, alcohol consumption, smoking habit, BMI and radiation dose. Elevated IL-6 levels associated with non-B, non-C HCC risk were also observed, although it was estimated among a relatively small number of non-B, non-C HCC cases. Moreover, elevated serum levels of IL-6 were significantly associated with increased risk of HCC, especially among subjects with obesity. Elevated serum levels of CRP were only marginally associated with increased risk of non-B, non-C HCC, whereas monitoring of CRP and IL-6 levels in combination with tumor markers may be more robust in predicting subsequent HCC among individuals with non-B, non-C liver disease. An in-depth understanding of the mechanisms by which IL-6 levels are associated with increased risk of HCC, independently of hepatitis virus infection, lifestyle-related factors and radiation exposure, should lead to better prevention and therapeutic strategies.

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