Serum Testosterone:Estradiol Ratio and the Development of Hepatocellular Carcinoma among Male Cirrhotic Patients

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Abstract

The reason for the large male predominance in the occurrence of hepatocellular carcinoma (HCC) remains unknown, and sex hormones may contribute to this phenomenon. We examined possible associations of serum levels of testosterone, free testosterone, estradiol, sex hormone binding globulin, and testosterone:estradiol ratio (T:E2 ratio) with HCC development in a follow-up study of 46 Japanese male patients with liver cirrhosis predominantly of hepatitis C virus origin (76%). Serum samples were collected between December 1985 and December 1987, and the patients were completely followed until the end of 1995 for an average of 5.1 years. During the follow-up period, 20 patients (43%) developed HCC. Univariate analysis demonstrated that serum T:E2 ratio and testosterone were significant predictors of HCC; the hazard ratios (and 95% confidence intervals) in the middle and upper tertiles relative to the lower tertile were 2.0 (0.5–7.6) and 4.0 (1.1–14.6; P trend = 0.03) for T:E2 ratio and 0.8 (0.2–3.1) and 2.9 (1.0–8.5; P trend = 0.05) for testosterone. Adjustment for age, serum albumin, hepatitis virus markers, and other clinicobiological measurements that are possibly associated with estradiol and sex hormone binding globulin were not evident. These results indicate that elevated serum testosterone, together with decreased serum estradiol, may promote the development of HCC in cirrhosis.

Introduction

HCC develops much more frequently in men than in women. At least 2–3-fold male predominance in the incidence of primary liver cancer, largely HCC, is universally observed (1). The reason for this large sex difference remains to be elucidated. As for the situation in Japan, the prevalence of hepatitis C virus infection, the most important etiological factor of HCC in this country (2–4), has been shown to be similar or only slightly different between both sexes according to blood donor data (3, 5, 6). Other potential risk factors more common in Japanese men than women comprise alcohol consumption and cigarette smoking. However, heavy drinking was estimated to account for only 13% of male HCC occurrences in our previous case-control study in a high risk area of Japan (7), and the causal link between smoking and HCC still remains uncertain (7). These findings necessarily raise the possibility that sex hormones may be involved in the etiology of HCC.

Numerous animal experiments have underscored a promoting role of testosterone in murine hepatocarcinogenesis (8–13), although the role of estrogens remains in dispute (13–18). In contrast, analytic epidemiological data on sex hormones and HCC have been sparse and controversial, except for the link between oral contraceptive use and HCC (19). Yu and Chen (20) first described a positive association between serum testosterone level and risk of HCC in a nested case-control study in Taiwan, where hepatitis B virus represents a major causative agent. This positive association was replicated in a subsequent nested case-control study in Shanghai (21), but the association was confounded by chronic hepatitis B virus infection, which was positively related to serum testosterone level. In a French follow-up study of male patients with LC mostly attributable to alcohol abuse (22), neither testosterone nor estradiol level in serum was predictive of HCC occurrence. Because of the clear need for further studies on this issue, we performed a follow-up study of male cirrhotic patients mainly of hepatitis C virus origin, taking into account the effects of other clinicobiological measurements that are possibly associated with both serum hormone levels and HCC risk.

Materials and Methods

Subjects. The details of the subjects and the methods have been described elsewhere (23). In brief, a total of 100 Japanese patients with LC who were attending or were admitted to the Third Department of Internal Medicine and the Second Department of Surgery at Kyushu University Hospital between December 1985 and December 1987 were recruited, as one of patient groups in case-control studies of HCC and LC, according to the following selection criteria: (a) without evidence of HCC based on ultrasonography, computed tomography, and/or serum α-fetoprotein level; (b) 40–69 years of age; (c) residents in Fukuoka or Saga prefecture (adjacent to Fukuoka prefecture); and (d) of Japanese nationality. In addition, patients with special forms of LC (primary or secondary biliary cirrhosis and cirrhosis attributable to autoimmune hepatitis, parasitosis, congestive heart failure, or metabolic disorders) were excluded. Patients were regarded as having been enrolled when an interview survey on their life styles, including a past history of alcohol drinking, was successfully conducted by a trained interviewer. Among the 100 patients, sera from 75 patients (48 men and 27 women) were collected on the date of enrollment for outpatients or during admission for inpatients. Of the 75 patients, 48 men were followed in this study. In data analysis, we further eliminated 2 men whose follow-up period was <6 months as described below, leaving 46 patients (27 patients from the medicine department and 19 patients from the surgery department; 35 outpatients and 11 inpatients; 44 residents in Fukuoka prefecture and 2 residents in Saga prefecture). The median age was 56 years. The diagnosis of LC was based on histology for 17 patients (37%), laparoscopy for 8 patients (17%), and evident clinical signs (e.g., ascites and esophageal varices) and laboratory/imaging findings for 21 patients (46%). The causes of LC were considered to be hepatitis C virus infection (n = 26), both hepatitis C virus infection and alcohol (ethanol use ≥80 ml/day for ≥10 years; n = 8), hepatitis B virus infection (n = 4), both viral infections (n = 1), alcohol (n = 4), and cryptogenic (n = 3). According to the Child-Turcotte stage, 23 patients (50%) were classified as stage A at enrollment, 18 patients (39%) as stage B, and 5 patients (11%) as stage C. None of the 46 patients had received IFN therapy during follow-up.

Follow-Up. The end point in this study was defined as the development of HCC as a final diagnosis >6 months after enrollment. The follow-up began on the date of enrollment and finished upon the diagnosis of the end point, death from other causes, loss to follow-up, or December 31, 1995, whichever came first. For 33 patients who had been attending the cooperating departments or relevant hospitals until the last date of follow-up, serum α-fetoprotein level had been measured every month, and ultrasound and/or computed tomography examination of the liver had been performed every 3–6 months, followed by...
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RESULTS

Average concentrations (±SD) of serum testosterone, free testosterone, estradiol, and SHBG were 5.4 ± 2.1 ng/ml, 10.5 ± 4.4 pg/ml, 28.0 ± 12.7 pg/ml, and 83.5 ± 29.4 nm/l, respectively. Serum T:E2 ratio ranged from 31 to 722 with an average (±SD) of 226 ± 127.

Table 1 shows the cumulative 5-year HCC incidence and the crude HRs according to the tertile classification of each hormonal measurement. Raised levels of testosterone and T:E2 ratio were associated with increased risk (P = 0.03 and 0.06, respectively, by log-rank tests), and the dose-response relation was clearest for T:E2 ratio (Table 1 and Fig. 1); increased risk was observed both for patients with less than the median length (4.4 years) of follow-up [HR (and 95% CI) in the upper tertile relative to the middle and lower tertiles: 2.2 (0.7–6.8) for testosterone and 2.0 (0.7–6.0) for T:E2 ratio] and for patients with ≥4.4 years of follow-up [corresponding HRs: 4.1 (0.8–20.5) for testosterone and 3.5 (0.7–17.3) for T:E2 ratio]. Elevated risk was also noted for SHBG, yet the association did not reach statistical significance. Unlike testosterone, free testosterone was associated with insignificantly decreased risk. The estradiol level was not predictive of HCC risk in univariate analysis.

Age adjustment had a little influence on the HRs shown in Table 1 (data not shown). However, some of those estimates were materially changed by additional adjustment for serum albumin level (model 1 in Table 2), which was strongly inversely associated with HCC risk as reported previously (23), and was also correlated positively with testosterone [Spearman rank correlation coefficient (r) = 0.16], free testosterone (r = 0.50), and T:E2 ratio (r = 0.20) and inversely with estradiol (r = −0.20) and SHBG (r = −0.25); the HRs for testosterone and T:E2 ratio substantially increased, and the inverse association with free testosterone observed in crude analysis disappeared.

When the effects of other clinicobiological variables were further controlled for (model 2 in Table 2), the positive associations with testosterone and T:E2 ratio became even stronger, but with statistical instability as indicated by wide CIs, and an upward gradient in HCC risk with increasing free testosterone emerged. Finally, controlling additionally for hormonal measurements (model 3 in Table 2) did not essentially alter the positive associations with testosterone, free testosterone, and T:E2 ratio. In this final model, the upper tertile for either estradiol or SHBG appeared to present lower risk than did the corresponding lower tertile, although not statistically significant.

A subgroup analysis of patients who tested seropositive for antibody to hepatitis C virus but seronegative for hepatitis B surface antigen (n = 34) gave similar HRs for serum testosterone and T:E2 ratio as those in Tables 1 and 2. For example, the age- and albumin-adjusted HRs (and 95% CI) in the middle and upper tertiles as

**Table 1 HCC incidence and associated HRs according to serum levels of hormonal measurements among 46 male cirrhotic patients**

<table>
<thead>
<tr>
<th>Testosterone (ng/ml)</th>
<th>HCC/Total no.</th>
<th>5-year HCC incidence</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4.4</td>
<td>5/15</td>
<td>29%</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>4.4–6.1</td>
<td>4/15</td>
<td>18%</td>
<td>0.8 (0.2–3.1)</td>
</tr>
<tr>
<td>≥6.2</td>
<td>11/16</td>
<td>63%</td>
<td>2.9 (1.0–8.5)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>0.03b</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Free testosterone (pg/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>HCC/Total no.</th>
<th>5-year HCC incidence</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8.2</td>
<td>&lt;8.2</td>
<td>7/15</td>
<td>42%</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>8.2–11.5</td>
<td>8.2–11.5</td>
<td>7/15</td>
<td>39%</td>
<td>0.6 (0.2–1.8)</td>
</tr>
<tr>
<td>≥11.6</td>
<td>≥11.6</td>
<td>6/16</td>
<td>31%</td>
<td>0.5 (0.2–1.5)</td>
</tr>
<tr>
<td>p</td>
<td>p</td>
<td>0.45b</td>
<td>0.26</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Estradiol (pg/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>HCC/Total no.</th>
<th>5-year HCC incidence</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20.8</td>
<td>&lt;20.8</td>
<td>7/15</td>
<td>27%</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>20.8–30.3</td>
<td>20.8–30.3</td>
<td>7/16</td>
<td>43%</td>
<td>1.3 (0.5–3.7)</td>
</tr>
<tr>
<td>≥30.4</td>
<td>≥30.4</td>
<td>6/15</td>
<td>44%</td>
<td>1.1 (0.4–3.3)</td>
</tr>
<tr>
<td>p</td>
<td>p</td>
<td>0.89b</td>
<td>0.89</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SHBG (nm/l)</th>
<th>Testosterone (ng/ml)</th>
<th>HCC/Total no.</th>
<th>5-year HCC incidence</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;66.1</td>
<td>&lt;66.1</td>
<td>4/15</td>
<td>22%</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>66.1–102.2</td>
<td>66.1–102.2</td>
<td>8/15</td>
<td>47%</td>
<td>2.5 (0.7–8.2)</td>
</tr>
<tr>
<td>≥102.3</td>
<td>≥102.3</td>
<td>8/15</td>
<td>41%</td>
<td>2.4 (0.7–8.1)</td>
</tr>
<tr>
<td>p</td>
<td>p</td>
<td>0.25b</td>
<td>0.15</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>T:E2 ratio</th>
<th>Testosterone (ng/ml)</th>
<th>HCC/Total no.</th>
<th>5-year HCC incidence</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;162</td>
<td>&lt;162</td>
<td>3/15</td>
<td>16%</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>162–245</td>
<td>162–245</td>
<td>7/15</td>
<td>42%</td>
<td>2.0 (0.5–7.6)</td>
</tr>
<tr>
<td>≥246</td>
<td>≥246</td>
<td>10/15</td>
<td>47%</td>
<td>4.0 (1.1–14.6)</td>
</tr>
<tr>
<td>p</td>
<td>p</td>
<td>0.06b</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

*Based on the Kaplan-Meier method.

*P* for the difference between HCC incidence curves by a log-rank test.

*P* for linear trend based on the Cox proportional hazards model, in which a score-dependent variable denoting the median value for each hormonal measurement category was included.
compared with the lower tertile were 1.3 (0.2–6.6) and 4.2 (0.9–19.5) for testosterone (P for trend = 0.04) and 3.1 (0.8–12.6) and 5.5 (1.3–23.0) for T:E2 ratio (P for trend = 0.02). For other etiological categories, the sample size (5 patients seropositive for hepatitis B surface antigen and 7 patients seronegative for both viral markers) was too small to perform separate analyses.

**DISCUSSION**

Our results indicate that elevated levels of serum T:E2 ratio and testosterone are predictive of HCC risk among male cirrhotic patients. Of particular note was that the positive associations were substantially strengthened after controlling for other clinicobiological parameters including serum albumin level, one of the liver function measurements that reflect the severity of cirrhosis. Previous clinical investigations found that serum estradiol (or estrone:testosterone ratio, the reciprocal of our variable, was higher in patients with HCC and LC than in patients with LC alone or control subjects, suggesting that hormonal imbalance as manifested by elevated estradiol and/or decreased testosterone levels might be involved in hepatocarcinogenesis (26–29). However, such a finding can be attributed to the advanced severity of LC among HCC patients, which is well recognized to reflect the severity of cirrhosis. Initial clinical observations of benign and even malignant hepatocellular tumors in women taking oral contraceptives (44–46) prompted researchers to perform relevant animal experiments. Early data demonstrated promoting effects of estrogens, such as estradiol-17-phenylpropionate and estradiol benzoate (15), mestranol (16), and ethinyl estradiol (17), on the development of hepatocellular liver tumors in female rats. Yager et al. (18) showed that chronic treatment of ethinyl estradiol to rats caused an inhibition of chemical hepatocarcinogenesis in male rats (41). Finally, androgen receptors are, more frequently and at a higher concentration, detected in human HCC than in the surrounding cirrhotic parenchyma (42, 43).

Conversely, the role of estrogens in the pathogenesis of HCC remains controversial. Initial clinical observations of benign and even malignant hepatocellular tumors in women taking oral contraceptives (44–46) prompted researchers to perform relevant animal experiments. Early data demonstrated promoting effects of estrogens, such as estradiol-17-phenylpropionate and estradiol benzoate (15), mestranol (16), and ethinyl estradiol (17), on the development of hepatocellular liver tumors in female rats. Yager et al. (18) showed that chronic treatment of ethinyl estradiol to rats caused an inhibition of chemical hepatocarcinogenesis in male rats (41). Finally, androgen receptors are, more frequently and at a higher concentration, detected in human HCC than in the surrounding cirrhotic parenchyma (42, 43).
regenerative liver growth, after an initial transient increase in liver growth. Shimizu et al. (13) also demonstrated a suppressive effect of estradiol valerate on glutathione S-transferase-positive foci in rats. The discrepancy across studies may result partly from the differences in sex steroid compounds used and the administration dose of each compound. Estrogen receptors have been detected in both human HCC and the surrounding liver tissue but usually at a lower concentration in the former (43, 47–49). In addition, estrogen receptors mutated in the hormone-binding domain have been implicated in a carcinogenic process among male cirrhotic patients through the escape from hormonal control (50).

Although a significant independent association with serum estradiol was not evident in this study, the fully adjusted analysis revealed a risk reduction (HR, 0.4) in the upper tertile of the estradiol level, and the T:E2 ratio was found to be the best predictor of HCC among hormonal measurements. This suggests that elevated serum estradiol may confer a somewhat decreased risk of HCC, although the role of serum testosterone appeared predominant. The causal link between oral contraceptive use and HCC has been established in areas with a low prevalence of hepatitis virus infection (19), yet such evidence has not been evident in this study, the fully adjusted analysis revealed a lack of association. This suggests that elevated serum estradiol is not the primary effect of oral contraceptive use and HCC, but other factors may play a role.

To our knowledge, no other studies have examined serum T:E2 ratio as a predictor of HCC development in cirrhosis. This study was small and had less precision to estimate the associated HRs; these studies are completely needed to consolidate our findings.

ACKNOWLEDGMENTS

We are grateful to T. Hayashi for interviewing study subjects and to the staff members of the Third Department of Internal Medicine and the Second Department of Surgery, Faculty of Medicine, Kyushu University, for their kind cooperation.

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