Increased Serum Concentrations of Tumor Necrosis Factor-α Are Associated with Disease Progression and Malnutrition in Hepatocellular Carcinoma

Background. Increased serum tumor necrosis factor-α (TNF-α) concentrations are associated with disease progression in some cancers. Also, TNF-α is known as an important mediator of cancer cachexia. This study was conducted to investigate the relationship between serum TNF-α levels and disease and nutritional status in patients with hepatocellular carcinoma.

Methods. Thirty-one male cirrhotic patients with hepatocellular carcinoma (mean age: 65 ± 2 years), 26 male cirrhotic patients without hepatocellular carcinoma (mean age: 59 ± 3 years), and 25 male control subjects (mean age: 67 ± 2 years) were included. Body fat mass was examined by bioelectrical impedance analysis. Serum TNF-α levels were measured by immunoassay. Hepatocellular carcinoma progression was staged by Okuda’s classification.

Results. Serum TNF-α values in 31 patients with hepatocellular carcinoma and 26 patients with cirrhosis were significantly above those of controls (12.3 ± 0.7 pg/mL vs. 11.3 ± 1.2 pg/mL vs. 5.8 ± 0.7 pg/mL; p < 0.01), but showed no differences between hepatocellular carcinoma and cirrhotic patients. When hepatocellular carcinoma patients were grouped according to Okuda’s classification, the serum TNF-α levels significantly increased with disease progression (p < 0.05). Only in patients at stage III (n = 5), but not at stages I (n = 13) and II (n = 13), was the serum TNF-α levels greater than those in cirrhotic patients (p < 0.05). The serum albumin values and body fat mass in patients with hepatocellular carcinoma were both lower than in controls [(34 ± 1 g/L vs. 42 ± 1 g/L; p < 0.01); (15.9 ± 1.2 kg vs. 18.9 ± 0.8 kg; p < 0.05), respectively]. Further, both decreased significantly with disease progression by Okuda staging [(37 ± 1 g/L vs. 32 ± 2 g/L vs. 30 ± 1 g/L; p < 0.01); (19.4 ± 1.6 kg vs. 13.9 ± 1.8 kg vs. 11.7 ± 2.0 kg; p < 0.05); respectively]. Finally, a negative correlation was found between serum TNF-α and both fat mass (r = -0.40; p < 0.05) and serum albumin (r = -0.45; p < 0.05).

Conclusions. Our study demonstrated that serum TNF-α levels were increased in patients with hepatocellular carcinoma, and associated with disease severity and nutrition status. However, serum TNF-α should not be used as a marker to early diagnose hepatocellular carcinoma in cirrhotic patients.

Hepatocellular carcinoma (HCC), the most common malignancy in Taiwan, generally carries an unfavorable prognosis. In patients in advanced stages, the tumor growth and spread are rapid, and often associated with progressive depletion of fat and muscle mass, a condition termed cachexia. It has been reported that about 50% of patients with advanced HCC eventually die of cachexia and/or related complications.

Tumor necrosis factor-α (TNF-α), a polypeptide secreted from activated macrophages or monocytes, is a
pleiotrophic cytokine with immunoregulatory and metabolic functions.\textsuperscript{6}

It can mediate biological responses in several immune disorders, such as fever, septic shock, and arthritis. Also, TNF-\textsubscript{c} can exert a stimulatory effect on natural killer cells, and play a potential role in antitumor cytolytic responses. In experimental animals, administration of TNF-\textsubscript{c} has been shown to suppress activity of lipoprotein lipase and to stimulate the proteolytic system, both of which may increase the catabolism of body mass, resulting in weight loss.\textsuperscript{7-9} Nonetheless, this cachetic effect of TNF-\textsubscript{c} can be mitigated by specific anti-TNF-\textsubscript{c} antibodies. Elevated serum concentrations of TNF-\textsubscript{c} have been described in patients with HCC.\textsuperscript{10,11} However, the relationship of elevated serum TNF-\textsubscript{c} levels to tumor progression and malnutrition in HCC has been little studied. In view of the potential tumor cytoidal and cachetic effects of TNF-\textsubscript{c} in \textit{in vitro} or \textit{in vivo} experiments, we conducted the present study to investigate the relationship between circulation TNF-\textsubscript{c}, nutritional features and disease staging in patients with HCC.

METHODS

Patients

Thirty-one male cirrhosis-associated HCC patients with a mean age of 65 ± 2 y/o, and 26 male cirrhotic patients with a mean age of 59 ± 3 y/o were consecutively enrolled from the outpatient clinic of the Kaohsiung Veterans General Hospital, Taiwan. The diagnosis of cirrhosis was based on the typical findings of hepatic cirrhotic appearance, splenomegaly, esophageal varices and ascites (by ultrasonography and upper gastrointestinal endoscopy examinations), with supporting biochemical data.\textsuperscript{12} The diagnosis of HCC was confirmed by either biopsy or elevated alpha-fetoprotein together with image studies. Exclusion criteria for entry into the study included (i) concomitant acute complications, such as gastrointestinal hemorrhage, hepatic encephalopathy or clinical signs of infection; (ii) presence of ascites or peripheral edema; (iii) renal insufficiency; and (iv) diabetes mellitus or other endocrine diseases. Of 31 HCC patients, 12 were associated with hepatitis B virus infection, 11 with hepatitis C, 4 with both hepatitis B and C, and 4 were cryptogenic. Of 26 cirrhotic patients, 13 were hepatitis B-related, 9 hepatitis C-related, and 4 cryptogenic. Hepatic functions were classified according to the Pugh-Child’s scoring system,\textsuperscript{13} and staging of HCC by the Okuda staging system.\textsuperscript{14} In addition, 25 male control subjects (mean age: 65 ± 2 y/o) were enrolled from physical check-up units in our hospital and considered normal on the basis of history, physical examination, and biochemical screening. The Hospital Ethics Committee approved the study and all patients and control subjects gave their informed consent.

Laboratory investigation and immunoassays

All blood samples were drawn preoperatively after overnight fasting, and none of all the patients had received any drug therapy or blood transfusion before blood collection. Serum was separated from blood cells immediately at 1000 \textsuperscript{g} and then stored at -70 °C until subsequent analysis. The serum albumin (reference range: 37-53g/L), total bilirubin (3.4-27.2 \textsuperscript{mole/L}), and alanine aminotransferase (0-40 u/L) were measured at a central laboratory in our hospital. Serum TNF-\textsubscript{c} levels were determined in duplicate with enzyme-linked immunosorbent assay kits (Qantikine human TNF-\textsubscript{c}, R D Systems, Minneapolis, MN, USA). The intra-assay and inter-assay coefficients of variation were 3.5% and 5.8%, respectively. The lowest sensitivity limit of TNF-\textsubscript{c} assay was 0.18 pg/mL.

Anthropometric measures and body composition measures

Body weight and height of all the subjects were measured by an auto-anthropometer, Nakamura KN-5000A (Nakamura, Tokyo, Japan). Body weight was measured to the nearest 0.1 kg with subjects barefoot and wearing light indoor clothing. Body height was recorded to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Body compositions were measured by bioelectrical impedance analyzer (Model BIA-101, RJ Systems, Detroit, MI Company, USA) with a radiofrequency current of 800-microampere alternating current at 50 kHz. Current injector electrodes were placed on hands.
and feet with the patient lying in supine position. All placements of skin electrodes and readings were performed by the same skilled person. Bioimpedance analysis was performed on the same morning that blood samples were obtained after overnight fast, and the procedure was repeated twice. The coefficient of variation between measurements was less than 2%. Lean body mass, total body water and fat mass (FM) were calculated according to the formula provided by the software manufacturer.

Statistical analysis

All data are expressed as mean ± SEM. The difference was determined by Mann-Whitney U non-parametric test for comparison between two groups and by Kruskal-Wallis One-way analysis of variance for comparison among more than two groups. The relationship between two mutually dependent variables was analyzed by Spearman’s correlation and expressed as a correlation coefficient (r). Results were considered statistically significant at p < 0.05.

RESULTS

The baseline demographic and hepatic biochemical data of the 31 patients with HCC and 26 patients with cirrhosis are listed in Table 1. The serum TNF-α levels in 31 patients with HCC were significantly higher than those of the control groups (12.3 pg/mL vs. 5.8 pg/mL; p < 0.01), and showed a positive association with the Pugh-Child’s staging (10.7 pg/mL vs. 13.8 pg/mL; p < 0.05). When the HCC patients were grouped according to Okuda’s classification, the serum TNF-α concentrations significantly increased with disease severity (10.5 pg/mL vs. 12.3 pg/mL vs. 16.7 pg/mL; p < 0.05) (Fig. 1). At a value of 12.9 pg/mL (2 standard deviations above the mean concentrations of stage I and II patients), the sensitivity and specificity for identifying patients at stage III were 100% and 60%, respectively. As compared with serum TNF-α in cirrhotic patients without HCC, only patients at stage III, but not those in stages I and II, had significantly higher values (p < 0.05).

The serum creatinine, albumin and body FM were all significantly lower in HCC patients than in control subjects [(88.4 1 g/L vs. 106.1 1 g/L; p < 0.01); (34.1 g/L vs. 42.1 g/L; p < 0.01); (15.9 1.2 kg vs. 18.9 0.8 kg; p < 0.05)], indicating malnutrition status in patients with HCC. Further, serum albumin and body FM decreased significantly with disease progression by Okuda staging [(37.1 g/L vs. 32.2 g/L vs. 30.1 g/L; p < 0.01); (19.4 1.6 kg vs. 13.9 1.8 kg vs. 11.7 2.0 kg; p < 0.05); respectively]. A negative correlation was observed between serum TNF-α and albumin (r = -0.45; p < 0.05), and body FM (r = -0.40; p < 0.05), respectively (Fig. 2), but there was no relationship between serum TNF-α and serum creatinine (r = -0.12, p = 0.53).

Table 1. Baseline demographic, body composition, biochemical, and hormonal data in cirrhotic patients with HCC, without HCC, and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Cirrhosis/c HCC (n = 31)</th>
<th>Cirrhosis/s HCC (n = 26)</th>
<th>Control subjects (n = 25)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y/o)</td>
<td>65 ± 2</td>
<td>59 ± 3</td>
<td>65 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 0.8</td>
<td>23.7 ± 0.4</td>
<td>24.4 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>15.9 ± 1.2</td>
<td>16.4 ± 0.8</td>
<td>18.9 ± 0.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>44.5 ± 1.2</td>
<td>47.1 ± 1.2</td>
<td>48.5 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>ALT (u/L)</td>
<td>145.1 ± 64.1</td>
<td>65.1 ± 9.9</td>
<td>24.4 ± 1.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Bilirubin (mol/L)</td>
<td>32.3 ± 6.8</td>
<td>28.9 ± 1.7</td>
<td>17.1 ± 1.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>34.1 ± 1.0</td>
<td>36.1 ± 1.0</td>
<td>42.1 ± 1.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Creatinine (mol/L)</td>
<td>88.4 ± 8.8</td>
<td>88.4 ± 8.8</td>
<td>106.1 ± 8.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>12.3 ± 0.7</td>
<td>11.3 ± 1.2</td>
<td>5.8 ± 0.7</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

ALT = alanine transferase; BMI = body mass index; NS = not significant; /c = with; /s = without; p values were obtained with the Kruskal-Wallis One-way analysis of variance.
Body FM showed no correlation with various hepatic functional status by Pugh-Child’s classification in all patients with HCC.

**DISCUSSION**

In this study, we found that increased serum TNF-α levels were associated with disease progression in patients with HCC, and correlated negatively with body FM and blood albumin. Although we did not identify the source of TNF-α in patients with HCC, it is possible that raised serum TNF-α levels in HCC may be caused by decreased clearance or increased production. The liver is the major site of clearance and metabolism of biologically active TNF-α. The association between serum TNF-α levels and worse Pugh-Child’s grades in patients with HCC indicated a reduced clearance of circulating TNF-α due to impaired liver function. In addition, it has been shown that liver regeneration in rat hepatic tumor model may increase TNF-α production by Kupffer cells and pit cells (hepatic natural killer cells), and thus contribute to elevated serum TNF-α in HCC. Finally, it has been shown in *in vitro* studies that both insulin and growth hormone stimulate the production of TNF-α from macrophages. As hyperinsulinemia and elevated growth hormone are frequently observed in liver cirrhosis, we speculated that insulin and growth hormone might also play a role for the increased circulating TNF-α levels in cirrhotic patients with HCC.

Elevated serum TNF-α concentrations have been described in several human malignancies, and shown to correlate with the disease extent. In our study, it was also found that serum TNF-α was associated with tumor progression in HCC. Using a cutoff value of serum TNF-α at 12.9 pg/mL could identify HCC patients at an advanced stage with a very high sensitivity, though the specificity is moderate. We surmised that the association between serum TNF-α and cancer staging might reflect a compensatory response of TNF-α to counteract tumor progression, just as TNF-α was shown to exert an antitumor effect in *in vitro* studies. As determination of TNF-α levels in blood was convenient, serum TNF-α would seem to be a potential marker to predict disease...
severity of HCC preoperatively. However, the serum TNF-α levels between patients with cirrhosis and early-staged HCC showed no differences. A similar observation has also been reported in patients with hepatitis C-related liver cirrhosis and HCC. It was presumed that in early-staged HCC the elevated serum TNF-α might mainly result from hepatic dysfunction as noted in liver cirrhosis. With progression of disease stages, the HCC itself would be another factor to activate TNF-α system, as found in other malignancies. Taken together, our study showed that serum TNF-α values could be a potential indicator of disease severity, but should not be used to early diagnose HCC in cirrhotic patients.

In experimental cancer models, TNF-α is postulated to be involved in the development of cachexia. However, the relationship between TNF-α and malnutrition in human cancer cachexia remains unclear. A negative correlation between serum TNF-α levels and serum albumin, a parameter of visceral protein pool, has been reported in patients with prostate and pancreatic cancers. In our study, a similar observation was made, implying that TNF-α could be also involved in hypalbuminemia in HCC. It was possible that the relationship between TNF-α and albumin might only reflect an epiphenomenon due to impaired hepatic function in patients with HCC. However, it has been shown in mice that administration of TNF-α inhibits hepatic synthesis of albumin and reduces serum albumin concentration. This suggested that the activated TNF-α system would further downregulate serum albumin values in cirrhotic patients with HCC.

Lower body FM was another feature of malnutrition in our patients with HCC. As disease progression, the body FM was decreased significantly with correlation to serum TNF-α levels. As body FM showed no significant relationship with various Pugh-Child’s status, the probability that the association between TNF-α and FM was mediated by hepatic dysfunction seemed less likely. It has been shown in animal studies that injection of TNF-α decreases food intake with loss of body fat by inhibiting lipoprotein lipase activity in adipose tissue. Also, in adipose tissue of cancer patients, low synthetic rate of lipoprotein lipase has been found. It was supposed that TNF-α might play a role in depletion of fat reserve in patients with HCC, though we did not measure lipoprotein lipase activity in this study.

Low serum creatinine levels have been proposed to represent decreased skeletal muscle mass in liver cirrhosis, though other causes, such as decreased hepatic creatine synthesis and increased tubular creatinine secretion, may also contribute to low cirrhotic serum creatinine. It has been shown in patients with chronic renal failure that TNF-α correlates with serum creatinine, and is suggested as a potential mediator of protein wasting. However, we could not demonstrate a relationship between serum TNF-α and creatinine level. This implied that TNF-α might not directly be involved in catabolism of muscle protein in HCC, as shown in some animal experiments.

In summary, serum TNF-α levels were increased in patients with HCC and associated with tumor staging and nutrition parameters. Although we cannot demonstrate a direct causal relationship for this association, it suggests that TNF-α may be one of the factors involved in disease progression and malnutrition in patients with HCC.

REFERENCES


