Evolution of Proinflammatory Cytokines in Hepatocellular Carcinoma Patients Undergoing Orthotopic Liver Transplantation

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OBJECTIVE To analyze the expression and levels of serum proinflammatory cytokines including tumor necrosis factor alpha (TNF-α), and interleukin (IL)-6 in patients with hepatocellular carcinoma (HCC), who received orthotopic liver transplantation (OLT).

METHODS The blood samples of 20 consecutive HCC patients who underwent liver transplantation were detected and analyzed for the clinical serum biochemical parameters, TNF-α and IL-6. Blood samples were drawn from the radial artery at planned time points: preoperatively, intraoperatively, and postoperatively. Levels of serum TNF-α and IL-6 were detected with enzyme-linked immunosorbent assay (ELISA).

RESULTS The levels of serum TNF-α and IL-6 increased significantly at reperfusion phase compared with those detected preoperatively (P < 0.01), and the level of serum IL-6 remained significantly higher until the third day after the liver transplantation. There was a significant correlation between TNF-α and IL-6 (P < 0.001).

CONCLUSION This research into the effects of the proinflammatory cytokines on liver transplantation has provided new insights into the mechanisms of ischemia and reperfusion injury to OLT.

KEY WORDS: liver transplantation, carcinoma, hepatocellular, reperfusion, cytokines.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common tumors in the world, with a global incidence of 500,000 new cases per year and 82% of the cases (and deaths) occurring in developing countries (with 55% in China)1,2. HCC is up to 4 times more common in men than in women with 60%-90% of these tumors developing from liver cirrhosis3. In China, approximately 200,000 people die from HCC every year. Liver transplantation, which allows both resection of the entire, potentially tumor-bearing, liver and elimination of the cirrhosis, holds great theoretical appeal in treating this disease. However, ischemia, reperfusion injury, and antigen-independent events, are unavoidable consequences of liver transplantation, and these events are also implicated in a variety of non-transplant conditions, including liver resection and hypovolemia in the liver, which may partially
determine the outcome of transplant and non-transplant clinical settings. The ischemia and reperfusion injury cause up to 10% of primary graft nonfunctioning, and can lead to a higher incidence of both acute and chronic rejection, and are also main risks for developing complications and mortality in liver transplantation patients[4-7].

Whilst the mechanisms of injury are complex, it has been reported that primary graft nonfunctioning is strongly associated with the involvement of the impaired sinusoidal endothelial and activated Kupffer cells[8,9]. The potential mediators involved in ischemia and reperfusion injury are numerous. They include proinflammatory cytokines such as tumor necrosis factor alpha (TNFa); interleukin (IL) 1β; and IL-6. These mediators are produced in large amounts by macrophages. The macrophages that produce them are Kupffer cells and endothelial cells. They are released rapidly by various stimuli. Recent studies have highlighted a relationship between the perioperative serum levels of proinflammatory cytokines and the clinical outcomes after liver transplantation[10,11]. These levels directly affect endothelial cells (ECs) and hepatocyte damage and hepatic sequestration of polymorphological neutrophils (PMNs), forming the crucial step in full-scaled ischemia and reperfusion injury[12,13]. In this study, researchers examined the evolutions of serum proinflammatory cytokines (TNFa and IL-6) in patients with hepatocellular carcinoma (HCC) receiving orthotopic liver transplantation (OLT).

Patients and Methods

There were 20 consecutive patients with HCC receiving OLT in the Organ Transplantation Center in Tianjin Medical University Cancer Hospital and Institute in this study. These patients were aged 20-60 years, with 15 being male and 5 female. The study was approved by the institutional ethics committee in the hospital. Informed consent was obtained from each participant. All donor livers were from cadaveric donors. The procedures applied in taking the donor livers met all appropriate institutional guidelines of the Tianjin First Central Hospital, Tianjin Medical University, China, and Chinese Governmental regulations concerning the ethical use of donated organs. University of Wisconsin solution was used for graft preservation. In general anesthesia, midazolam (0.05-0.1 mg/kg), fentanyl (2-5 μg/kg), propofol (1-2 mg/kg), pipercuronium bromide (0.08-0.2 mg/kg) were used. Throughout the whole procedure, each recipient’s arterial blood pressure, central venous pressure, mean pulmonary artery pressure (MPAP), pulmonary artery wedge pressure (PAWP), electrocardiogram, oxygen saturation (SpO2) and end-tidal CO2 (PETCO2) were closely monitored. Postoperatively, the patients received standard post-liver transplantation care in the intensive care unit, and received identical immunosuppressive therapy with tacrolimus (0.05-0.15 mg/kg/day), daclizumab (1 mg/kg), mycophenolate mofetil (750 mg twice a day), and an initial high-dose methylprednisolone (10 mg/kg), which was gradually reduced to 4-8 mg per day within 3 months after transplantation.

Sample collection

Blood samples were taken from each patient’s radial artery at 6 time points: T1, preoperatively; T2, 15 min after entering the anhepatic phase; T3, 30 min after anhepatic phase; T4, 15 minutes after starting the reperfusion of the graft; T5, 60 min after reperfusion of the graft; D3, on the third day after surgery. Blood samples were collected into vacutainers for measurement. No longer than 60 min after collection, the samples were centrifuged (4500 rpm) at 4°C for 10 min, and then the supernatant serum was removed carefully.

Methods

Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of serum TNF-α and IL-6, using kits purchased from R&D Systems (Abingdon, UK). Determinations of serum TNF-α and IL-6 were done in a 96-well microtiter plate, and a microplate reader (Tecan Spectra III, Vienna, Austria) at 492 nm was employed to read the samples on the plate. The levels of TNF-α and IL-6 in the experimental samples were calculated based on a standard curve.

Statistical analysis

For each parameter, values measured at each sampling time (T2 to D3) were compared to those measured at T1. Statistical analysis was performed using the two-tailed Student’s t-test. The Pearson correlation coefficient was used to analyze the relationship between the levels of serum TNF-α and IL-6. P < 0.05 was regarded as statistically significant throughout the study.

Results

The participants’ clinical features are summarized in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
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<tbody>
<tr>
<td>Gender (male/female)</td>
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<tr>
<td>Mean age, years (range)</td>
<td>48 (20-60)</td>
</tr>
<tr>
<td>Cause of disease</td>
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</tr>
<tr>
<td>Hepatitis B virus</td>
<td>16</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>4</td>
</tr>
<tr>
<td>Child-Pugh score</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>9</td>
</tr>
<tr>
<td>B + C</td>
<td>11</td>
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<tr>
<td>TNM stage</td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>7</td>
</tr>
<tr>
<td>III-IVa</td>
<td>13</td>
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</tbody>
</table>
The levels of serum TNF-α and IL-6 at different time points are shown in Fig. 1. The levels of TNF-α and IL-6 increased significantly at 15 and 60 min after reperfusion compared with those before the surgery ($P < 0.01$), and the levels of serum IL-6 remained significantly higher until the third day after the surgery ($P < 0.01$). However, the levels of serum TNF-α decreased to preoperative levels on the third day ($P > 0.05$). There was a significant correlation between the levels of serum TNF-α and IL-6 ($r = 0.499$, $P < 0.001$).

**Discussion**

Liver transplantation remains the definitive treatment for patients with HCC complicated by cirrhosis. Since the publication of the “Milan criteria”, OLT has progressed into becoming the universally accepted treatment for HCC. The results of OLT for HCC continue to improve with time\[14,15\]. However, hepatic ischemia/reperfusion injury is a multifactorial process that greatly affects graft functioning right after the liver transplantation and late changes, and it is also a main cause of complications and mortality after the liver transplantation\[16,17\]. Different injury mechanisms contribute to the overall pathophysiology of hepatic ischemia/reperfusion injury\[18-20\]. It has been reported that primary graft nonfunctioning is strongly associated with the involvement of impaired sinusoidal endothelial and activated Kupffer cells\[21,22\].

This study shows that the levels of serum TNFα and IL-6 increase significantly at reperfusion phase and there is a correlation between the levels of serum TNFα and IL-6. The increase of TNFα and IL-6 might be play an important role in manifestations associated with ischemia/reperfusion injury. TNFα is now involved as a mediator in various physiological and pathophysiological events, including inflammation as well as differentiation and cell apoptosis. In previous studies by this research group, activation of apoptotic mediators in the ischemic liver, mainly after reperfusion has been well documented\[19\], indicating that the apoptosis process of sinusoidal endothelial cells and hepatocytes exists in ischemia/reperfusion injury\[23,24\]. TNFα might be the most potent cytokine inducing apoptosis that is rapidly released upon reperfusion of the ischemic liver\[23,24\]. IL-6 is an important pleiotropic acute reactant cytokine involved in inflammatory responses with elevated serum levels documented in numerous conditions\[25,26\]. A recent study showed that IL-6 plays a critical role in resistance to liver ischemia–reperfusion injury after liver transplantation. The results obtained in the present study further imply that the expression and increased level of IL-6 might correlate to immune graft injury and consequent reparation for the injury.

An understanding of the inflammatory process surrounding graft reperfusion may, therefore, provide the rationale for developing new therapeutic strategies that will reduce or even eliminate the risks of patients developing ischemia and reperfusion injury after liver transplantation; this warrants further research.

**Conflict of interest statement**

No potential conflicts of interest were disclosed.

**References**


