

Effects of radical cholecystectomy on nutritional and immune status in patients with gallbladder carcinoma

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INTRODUCTION

Carcinoma of the gallbladder is the most common neoplasm in biliary tract, and its incidence has been rising in recent years. The rate of correct diagnosis in early gallbladder carcinoma has been raised after the wide use of CT, ultrasound scans and frozen section examination. Now radical cholecystectomy is advocated as the best management for patients with early gallbladder carcinoma. In the present study, the diagnosis of 27 patients with gallbladder carcinoma was confirmed correct, and the patients underwent radical cholecystectomy, and their nutritional and immune assessments were performed pre- and post-operatively.

PATIENTS AND METHODS

Patients

From September 1993 to December 1997, 27 patients with gallbladder carcinoma who had undergone radical cholecystectomy were selected, and they were admitted to the First and Second Affiliated Hospitals of Xi'an Medical University. The patients included eight males, aged 46-65 years, with a mean of 57 years; nineteen females, aged 50-67 years, with the mean of 59 years. Four patients were diagnosed as having polyposis preoperatively, and diagnosed as carcinoma during the operation by the frozen section technique. In five patients with preoperative diagnosis of carcinoma, metastasis had not been found during the operation. No severe systemic disease was found, e.g. recent myocardial infarction, cerebral vascular accidents, uncontrollable diabetes mellitus or hypertension. Antibiotics were administered

postoperatively to prevent infection, intravenous infusion was routinely administered immediately after surgery and maintained at least for one week. The tumors were graded as stage I, II and III (Nevin stage) by histological examination. All of the resected specimens were sent for histological examination. No chemotherapy or radiotherapy was administered preoperatively.

Methods

The nutritional and immune status of the patients were assessed preoperatively (1 wk before surgery), and on d 3, d 7, d 14 and d 21 postoperatively.

Nutritional assessment Biochemical parameters evaluating the patient's nutritional status consisted of serum levels of albumin, cholesterol, iron, magnesium, zinc, and transferrin determined by an automated calorimetric technique (SMAC), and total iron binding capacity (TIBC) determined by radioimmunoassay.

Immune studies The immune status includes T lymphocyte subpopulation-CD₄, CD₈; immunoglobulins (IgG, IgA and IgM); complements (C₃ and C₄); and serum interleukin-2 and soluble interleukin-2 receptor were evaluated. T lymphocyte subpopulation assessment: blood samples were taken with venopuncture and anticoagulated by heparin. Mononuclear cells were isolated after the sample had been washed twice with Hank's balanced salt solution, and the concentration of the cells was regulated at $2 \times 10^2/\text{mL}$ with RPMI-1840 (Cow serum). Cell suspensions (0.2 mL) were placed in 24-well plates, and added cow serum 0.1 mL, PHA 0.05 mL, LPS 0.05 mL and 10% RPMI-1640 0.6 mL respectively. The plates were incubated at 37°C with 5% CO₂ for 72 h. Cultured supernatants were harvested and kept at -30°C. The cell suspension was assigned equally into three parts, which was washed once, added with CD₄ and CD₈ monoclonal antibody (monoclonal antibody was provided by Luo Yang Hua Mei Co.), stand for 2 h at 4°C; then each was washed twice again; a sheep-anti-mouse IgG fluorescent antibody 50 µL was added into each tube, stand for 2 h at 4°C, and washed twice again. After harvesting, cell suspension was studied under the fluorescence microscopy and the scintillation cells were quantitated as percentage of 100 or 200 lymphocytes.

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Assay of IgA, IgG, IgM and complements Serum IgG, IgA, IgM, C₃ and C₄ were quantitated by nephelometric immunoassay.

Assay of serum IL-2, sIL-2R Serum IL-2 and sIL-2 levels were measured by a "Sandwich" enzyme-linked immunosorbent assay with two antibodies, the diagnostic reagents were supplied by Norman Bethune Medical University.

Statistical analysis

For each variable, multiple analysis of variance for repeated measurements was used to compare the values measured before operation with those measured at four subsequent time points. The results were presented as mean and standard error ($\bar{x} \pm s$) based on the mixed model of repeated measurement analysis. Statistical analysis was performed with SAS software. *P* values <0.05 were considered statistically significant.

RESULTS

The results of the nutritional and immunological assessment at various time points are demonstrated in Table 1.

Preoperation

As shown in Table 1, in patients with gallbladder carcinoma, the preoperative nutritional and immune status was all within the assigned normal ranges.

Third postoperative day

All the nutritional parameters decreased greatly after surgery, especially the serum iron, transferrin, cholesterol, TIBC, magnesium, and zinc ($P<0.01$, respectively).

The serum levels of immunoglobulins (IgA, IgG, IgM) and C₃, C₄ complements decreased significantly ($P<0.01$), serum IL-2 levels, CD₄ and CD₈ levels also reduced greatly ($P<0.01$), and CD₈ and sIL-2R levels increased significantly ($P<0.01$).

First postoperative week

Compared with the third postoperative day, all the nutritional parameters increased slightly, particularly the serum albumin, magnesium. Serum cholesterol, TIBC, transferrin, iron and zinc were still lower than their preoperative levels ($P<0.01$). CD₄, CD₈, CD₄/CD₈, IL-2, sIL-2 values differed significantly from those of the preoperative stage ($P<0.01$). The serum levels of immunoglobulins (IgG, IgA, IgM) and complements (C₃ and C₄) were significantly higher than those of the third postoperative day, but they were still lower than those of the preoperative day.

Second postoperative week

Compared with the third postoperative day and the first postoperative week, albumin, magnesium and zinc recovered to the preoperative levels ($P>0.05$), however, the levels of TIBC, transferrin and iron were still significantly lower than those of the preoperative ones ($P<0.01$). Compared with the third postoperative day and the first postoperative week, the serum levels of immunoglobulins (IgG, IgA, IgM) and complements (C₃ and C₄) gradually recovered, and IL-2, CD₄, CD₈, sIL-2R levels and CD₄/CD₈ ratio were not statistically different from the preoperative levels.

Third postoperative week

The nutritional evaluation showed continuous improvement in the third postoperative week, most of the nutritional parameters returned to the preoperative levels, except for the serum levels of iron, transferrin and TIBC. The immune parameters IL-2, sIL-2R, CD₄, CD₈, CD₄/CD₈ ratio, C₃, C₄ immunoglobulin levels (IgG, IgA, IgM) also returned to the preoperative levels, with no statistical difference ($P>0.05$).

Table 1 Nutritional and immunity status in patients with gallbladder carcinoma ($\bar{x} \pm s$)

Parameters	Preoperative	Postoperative			
		3rd day	1st week	2nd week	3rd week
Nutrition					
Albumin(g/mL)	3.6 \pm 0.07	3.01 \pm 0.14 ^b	3.49 \pm 0.13 ^a	3.50 \pm 0.12	3.58 \pm 0.15
Cholesterol(mg/dL)	215 \pm 7.81	78.16 \pm 7.22 ^b	85.23 \pm 7.41 ^b	131.45 \pm 6.85 ^b	148.62 \pm 8.41 ^b
TIBC(μ g/dL)	251.15 \pm 12.95	129.18 \pm 8.15 ^b	162.67 \pm 9.83 ^b	194.15 \pm 10.15 ^b	199.34 \pm 9.68 ^b
Transferrin(mg/dL)	221.07 \pm 12.11	99.77 \pm 8.98 ^b	131.29 \pm 9.01 ^b	175.25 \pm 9.31 ^b	186.21 \pm 10.18 ^b
Iron(mg/dL)	65.12 \pm 6.15	23.39 \pm 4.93 ^b	23.41 \pm 5.01 ^b	32.47 \pm 5.42 ^b	39.87 \pm 5.87 ^b
Magnesium(mg/dL)	2.13 \pm 0.06	1.57 \pm 0.05 ^b	1.99 \pm 0.05 ^b	2.04 \pm 0.06	2.06 \pm 0.06
Zinc(mg/dL)	106.1 \pm 3.56	59.85 \pm 5.13 ^b	88.66 \pm 4.43 ^b	105.8 \pm 3.71	109.9 \pm 3.81
Immunity					
C ₃ (mg/dL)	148.1 \pm 5.15	70.21 \pm 5.6 ^b	96.23 \pm 4.68 ^b	118.56 \pm 5.11 ^a	123.73 \pm 6.22
C ₄ (mg/dL)	25.15 \pm 1.15	14.38 \pm 1.41 ^b	18.79 \pm 1.45 ^a	24.82 \pm 1.21	24.98 \pm 1.16
T lymphocyte subpopulation					
CD ₄ (%)	44.15 \pm 2.01	33.22 \pm 1.88 ^b	39.96 \pm 2.02 ^b	42.37 \pm 2.00 ^a	43.99 \pm 1.97
CD ₈ (%)	34.12 \pm 1.69	29.47 \pm 3.77 ^b	26.21 \pm 3.13 ^b	39.69 \pm 3.00 ^b	35.81 \pm 1.20
CD ₄ /CD ₈ (ratio)	1.3 \pm 0.18	1.1 \pm 0.08 ^b	1.5 \pm 0.19 ^b	1.07 \pm 0.14 ^b	1.20 \pm 0.16 ^b
IL-2(IU/mL)	9.95 \pm 2.95	4.81 \pm 1.81 ^b	5.99 \pm 2.30 ^b	8.41 \pm 2.93 ^a	9.89 \pm 3.01
sIL-2R(U/mL)	754.2 \pm 141.5	478.3 \pm 87.57 ^b	597.4 \pm 96.81 ^b	692.1 \pm 112.6	748.9 \pm 138.8

For each variable, multiple repeated measurements analysis of variance and *t* test were used to compare the values before operation with those measured after operation, ^a $P<0.05$, ^b $P<0.01$, vs before operation.

DISCUSSION

Carcinoma of the gallbladder is one of the most common neoplasms in biliary tract, and 40%-100% cases are complicated with gallstones^[1,2], but correct diagnosis of gallbladder carcinoma in its early stage accounted for only 19.1%, and 53.3% cases are always diagnosed as cholecystitis and gallstone^[3-5]. More and more clinical experiences indicate that radical cholecystectomy for early carcinoma is the most effective treatment^[6-8]. In the present study, though all patients with gallbladder carcinoma were well prepared to receive the radical cholecystectomy, their nutritional and immune status still deteriorated remarkably immediately after the extensive surgical resection. The reasons might be that: ① Large volume of body fluid lost during and after the surgery; ② the radical cholecystectomy is a complex operation needing long time and wide scope of resection. Sumiyoshi^[9] and Wang *et al.*^[10] studied the effect of surgery as an injury factor on nutritional and immune status in patients with carcinoma, it is coincident with our findings in this report. Our investigation showed that all of the nutritional parameters but the serum levels of iron, TIBC and transferrin recovered within 3 wk after operation. Hickey *et al.*^[11] advocated that supplemental vitamins and minerals, e.g. iron should be given postoperatively when deficiencies are suspected. Our conclusion is that adequate iron should be supplemented after the radical cholecystectomy for gallbladder carcinoma in the third postoperative week, and the serum levels of minerals should be monitored routinely after surgery.

The immune study showed remarkable decrease of serum IgA, IgM, IgG and C₃, C₄ complement, IL-2, CD₄, CD₄/CD₈ ratio, and the remarkable increase of serum sIL-2R and CD₈ ($P < 0.01$) on d3 after operation. IL-2 is a T-cell derived soluble lymphokine whose main bioactivity is to stimulate the activated T cell (Th, Ts, Tc) to reproduce continually, proliferate and is the key mediator in cell and humoral immunity and immune regulation. The balance between IL-2 and its receptor regulates the immune status. T cell serves as the center in controlling cellular immune status which can affect directly the occurrence, development and progression of tumor^[12]. T cell's regulating function is mainly performed by CD₄ and CD₈ T cells. CD₄⁺ T cells can help B cell produce antibody and CD₈⁺ T cells can suppress B cell to produce antibody. The stable balance between them keeps normal immune response of the organism. Surgery, as an injurious factor, broke the balance between CD₄ and CD₈, however T cell's immune regulating function is demanded finally by the organism. In gallbladder carcinoma in an early stage, the serum IL-2, CD₄, CD₈, CD₄/CD₈ ratio, sIL-2R recovered remarkably in the first postoperative week. In early

postoperative stage, the serum levels of immunoglobulins and complement reduced remarkably. This evidence suggests the results are possibly influenced by surgical stress and the diluting effect of the postoperative massive fluid therapy. The immune parameters returned to the preoperative levels within 2 wk-3 wk after surgery, suggesting that T cell plays a more important role in the immune regulating system.

The present study suggests that radical cholecystectomy for early gallbladder carcinoma might have a mild and transient adverse effect on the cell-mediated immune response during the early postoperative period. Because of tumor's own direct products, tumor cell's metabolites and immuno-complex in body circulation, which depress the anti-tumor action of the immune cells^[13,14], patients' immune status deteriorated remarkably in the middle and late stage. For gallbladder carcinoma, radical cholecystectomy in its early stage with complete resection of the tumor and removal of lymphnodes should be performed, thus the immune inhibitors in the tumor mass can be excluded. These factors played important roles in the recovery of immune function.

REFERENCES

- Shi JS, Zhou LS, Wang ZR, Luo J, Wang L, Hao XY, Ma QJ, Li FZ, Wang T, Ren B, Lu Y, Liu SG. Retrospective analysis of 830 extra-hepatic biliary carcinoma. *Zhonghua Waike Zazhi*, 1997; 35:645-647
- Ekbohm A, Hsieh CC, Yuen J, Tricopoulos D, McLaughlin JK, Lan SJ, Adami HO. Gallstones and bile duct cancer. *Lancet*, 1993;342: 1262-1265
- Chijiwa K, Sumiyoshi K, Nakayama F. Impact of recent advances in hepatobiliary imaging techniques on the preoperative diagnosis of carcinoma of the gallbladder. *World J Surg*, 1991;15:322-327
- Henson DE, Albores Saavedra J, Corle D. Carcinoma of the gallbladder: histologic types, stage of disease, grade, and survival rates. *Cancer*, 1992;70:1493-1496
- Sumiyoshi K, Nagai E, Chijiwa K, Nakayama F. Pathology of carcinoma of the gallbladder. *World J Surg*, 1991;15:315-321
- Busse PM, Cady B, Bothe A Jr., Jenkins R, McDermott WV, Steele G Jr., Stone MD. Intraoperative radiation therapy for carcinoma of the gallbladder. *World J Surg*, 1991;15:352-356
- Gagner M, Rossi RL. Radical operations for carcinoma of the gallbladder: present status in North America. *World J Surg*, 1991; 15:344-347
- Gall FP, Kockerling F, Scheele J, Schneider C, Hohenberger W. Radical operations for carcinoma of the gallbladder: present status in Germany. *World J Surg*, 1991;15:328-336
- Mulvihill SJ, Pellegrini CA. Postoperative care. In: Current surgical diagnosis. 10th ed. VS: Lange Medical Publication, 1994:15-23
- Wang LS, Lin HY, Chang CJ, Fahn HJ, Huang MH, Jeff Lin CF. Effects of en bloc esophagectomy on nutritional and immune status in patients with esophageal carcinoma. *J Surg Oncol*, 1998; 67:90-98
- Hickey MS, Arbeit JM, Way LW. Surgical metabolism and nutrition. In: Current surgical diagnosis and treatment. 10th ed. VS: Lange Medical Publication, 1994:143-194
- Lotze MT, Finn OJ. Recent advance in cellular immunology: implications for immunity to cancer. *Immunol Today*, 1991;11:190-194
- Jiao XY, Shi JS, Gao JS, Zhou LS. Determination of levels of cellular immunity and humoral immunity in patients with gallbladder carcinoma. *Zhongguo Puwai Jichu Yu Lingchuang Zazhi*, 1999;6:227-229
- Jiao XY, Shi JS, Gao JS, Zhou LS, Han WS, Liu G, Lu Y. Study on the serum IL-2, sIL-2R and CEA levels in patients with gallbladder carcinoma. *Zhonghua Gandan Waike Zazhi*, 1999;5:342