Hepatocellular Carcinoma Patients May Benefit From Postoperative Huaier Aqueous Extract After Liver Transplantation

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ABSTRACT

Background. Liver transplantation has been the first choice for most early- or intermediate-stage hepatocellular carcinoma (HCC) cases. However, postoperative anti-HCC therapies remain controversial. In this study, we aimed to evaluate the safety and efficacy of Huaier aqueous extract (Jinke), when used as an adjuvant postoperative anti-HCC therapy.

Methods. We retrospectively collected the clinical and follow-up data of HCC patients who underwent liver transplantation at our center. We divided them into 2 groups: a control liver transplantation group and a Huaier treatment group. The baseline characteristics, tumor characteristics, intraoperative data, postoperative recovery, long-term overall survival rate, and tumor-free survival rate were compared between the 2 groups.

Results. Fifty-three patients were included in our study, including 28 patients who underwent postoperative Huaier therapy and 25 patients who underwent liver transplantation without postoperative Huaier therapy. The baseline and tumor characteristics were similar between the 2 groups. None of the patients in the Huaier group experienced any severe adverse events. The long-term predictive overall survival was similar between the 2 groups ($P = .202$). However, the Huaier group had a higher predictive tumor-free survival rate than the control group ($P = .029$). And the 10- and 30-month predictive tumor recurrence rates were 17.9% and 35.7% in the Huaier group, which were significantly lower than those in the control group (60% and 64%; $P < .05$).

Conclusions. HCC patients may benefit from Huaier therapy after liver transplantation, but a longer follow-up time and larger cohort study may be necessary to be sure.

HEPATOCELLULAR CARCINOMA (HCC) is the 5th leading cancer type worldwide and the 2nd leading cause of cancer-related deaths. Most of the disease burden in Asia and Africa is due to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection; there are >750,000 cases diagnosed and 1 million deaths annually, and China accounts for >50% of HCC cases worldwide [1]. The radical therapies for HCC include liver transplantation (LT), hepatic resection, and radiofrequency ablation (RFA) [2]. The efficacy of RFA in HCC has been demonstrated in small cases (diameter ≤3 cm) but not in larger cases [3], even though most HCC cases were diagnosed at the intermediate or advanced stage. LT could be viewed as the optimal treatment for HCC because LT treats the tumor and the underlying liver disease [4–6]. However, even with radical LT, HCC recurrence ranges from 18.9% to 78.3% and is the leading reason for HCC morbidity [7]. Therefore, postoperative adjuvant therapies, such as TACE, systematic chemotherapy, and sorafenib, may be automatic choices for these cases. However, there is still controversy regarding these adjuvant therapies [8–10].

Some medicines, such as Huaier, have been clinically used to treat various cancers, such as hepatocellular carcinoma [11], breast cancer [12], lung cancer [13], colorectal cancers [14], and other system cancers [15]. However, the safety and efficacy of Huaier in preventing recurrence of HCC after LT are still unclear, even though some studies

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Table 1. Baseline Demographic and Tumor Characteristics of the Two Groups of Patients

Characteristic	Huaier Group (n = 28)	Control Group (n = 25)	*P* Value
Age (y)	49.9 ± 8.4	50.3 ± 10.1	.890
Sex (M/F)	27/1	25/0	.345
Weight (kg)	66.6 ± 9.8	69.5 ± 8.6	.272
Height (cm)	166.6 ± 5.6	170.4 ± 4.3	.014
BMI (kg/m^2)	23.9 ± 3.0	24.8 ± 4.2	.402
BMI <26/≥26	22/6	21/4	.617
Cirrhosis etiology (HBV/non-HBV)	28/0	23/2	.131
HBV-DNA <1,000/≥1,000	20/8	13/12	.149
Child score (A/B/C)	11/8/9	10/9/6	.680
Total bilirubin (μmol/L)	45.0 ± 26.8	46.3 ± 17.7	.299
Albumin (g/L)	31.5 ± 10.9	30.2 ± 14.9	.181
Hemoglobin (g/L)	105.2 ± 33.4	112.3 ± 29.9	.351
Creatinine (μmol/L)	77.4 ± 12.5	81.4 ± 10.2	.217
Platelet count (10^3/μL)	101.5 ± 53.1	103.2 ± 62.9	.862

Abbreviations: BMI, body mass index; HBV, hepatitis B virus.

have demonstrated the efficacy of Huaier when used as an adjuvant therapy in combination with other therapies, such as TACE [16], RFA [11], and liver resection [11]. Therefore, in the present study, we aimed to evaluate the safety and efficacy of Huaier in treating HCC as a postoperative adjuvant therapy after LT.

PATIENTS AND METHODS

We collected data on HCC patients who underwent LT from January 2009 to August 2014 at our center. The following were the inclusion criteria: age 18–70 years, HCC diagnosed by means of pathology, Child A or B liver function, Eastern Cooperative Oncology Group score of 0–1, undergoing LT, no preoperative or postoperative adjuvant therapy except Huaier, and available for follow-up after the operation. The following were the exclusion criteria: patients with impaired heart or lung function who could not tolerate LT, HCC with multiple targets that could not be treated by LT, HCC with main hepatic vein or portal vein thrombosis or extrahepatic metastasis, HCC with a postoperative diagnosis of biliary carcinoma or other mixed cell liver cancer, patients who underwent other radical therapies, such as RFA or liver resection, and patients who received other postoperative therapies, such as sorafenib. Based on the inclusion and exclusion criteria, 53 HCC patients were included in the present study. We divided them into 2 groups according to postoperative adjuvant therapy, namely, the Huaier group (28 cases) and the control group (25 cases). Then, comparisons were made between the 2 groups regarding baseline characteristics, tumor characteristics, intraoperative data, postoperative recovery, and, particularly, long-term follow-up results, including the patients' overall survival, tumor-free survival, and tumor recurrence rates.

The diagnosis of HCC in the TACE group was based on a serum hepatitis virus test, 2 imaging scans (contrast-enhanced ultrasound, double-phase helical computerized tomographic [CT] scan, or magnetic resonance imaging), and the serum alpha-fetoprotein (AFP) level. For the LT patients, HCC was retrospectively demonstrated by means of histopathologic examination. The chief physician or deputy chief physician, with >20 years of LT experience, performed the surgeries at our center. All surgical procedures were performed with the use of general anesthesia. The details of the living- or deceased-donor LT were described in our previous study [5].

All patients underwent bimonthly follow-up at the outpatient clinic for the first six months after discharge from the hospital as

Table 2. Comparison of Tumor Characteristics Between the Two Groups of Patients

Characteristic	Huaier Group (n = 28)	Control Group (n = 25)	*P* Value
AFP level (ng/mL)	984.6 ± 4,325.6	4,901.3 ± 20,416.4	.335
0–800	24	15	.036
>800	4	10	
Tumor number	1.5 ± 0.9	1.7 ± 1.2	.465
1	20	16	.546
2–3	6	6	
Multiple	2	3	
Overall tumor diameter (cm)	6.0 ± 3.3	6.7 ± 2.7	.196
Largest tumor diameter (cm)	4.9 ± 3.1	6.1 ± 2.8	.167
BCLC stage (A/B)	19/9	12/13	.147
NLR	2.7 ± 2.5	3.2 ± 3.8	.567
0–4	23	21	.859
>4	5	4	
Tumor differentiation (good/moderate/poor)	10/11/7	8/10/7	.754
Microvascular invasion (yes/no)	10/18	8/17	.778

Abbreviations: AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer.

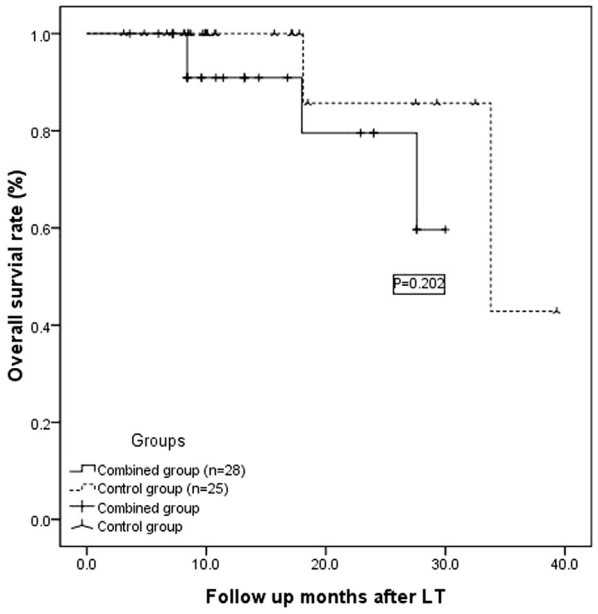

Fig 1. Predictive long-term overall survival comparison between the 2 groups. The Huaier and control groups had similar predictive long-term overall survival rates ($P = .202$). Abbreviation: LT, liver transplantation.

follows. During the follow-up visits, the AFP level and liver function were assessed, and ultrasound sonography or a CT scan was performed. The frequency of outpatient clinic visits thereafter varied according to the patients' condition, tumor recurrence, and liver function. Chest radiography and bone scintigraphy were performed when extrahepatic HCC recurrences were suspected. The therapies for HCC recurrence mainly depended on the patients' liver function and tumor location and included RFA, and transcatheter arterial chemoembolization (TACE).

Continuous data are expressed as mean ± SD. Survival curves were estimated by means of the Kaplan-Meier method and compared with the use of the log-rank test. The demographic data of the 2 groups were compared with the use of the Fisher exact test for categoric variables and the Mann-Whitney *U* test for continuous variables. Differences between categoric data were analyzed with the use of the chi-square and 2-tailed Fisher exact test. The overall survival and tumor-free survival rates were obtained with the use of Kaplan-Meier analysis, and the differences in the survival curves between the 2 groups were statistically compared by means of the log-rank test. Two-tailed $P < .05$ was considered to be statistically significant. The inclusion of variables in the final model was based on biologic and statistical considerations. All statistical analyses were performed with the use of the SPSS 17.0 statistical package (SPSS, Chicago, Illinois).

RESULTS

Based on the inclusion and exclusion criteria in our study, 53 patients were included in our study; 28 patients underwent persistent Huaier therapy after LT, and 25 patients underwent LT without postoperative Huaier therapy. Table 1 summarizes the baseline characteristics of the 2 groups of patients. The mean height in the Huaier group was higher than that in the control group ($P = .014$); however, no significant differences were observed between the 2 groups regarding the patients' weight, body mass index (BMI), age, or sex. Only 1 female patient was included in our study. Most of the HCC cases were caused by HBV infection, including 27 patients (96.4%) in the Huaier group and 23 patients (92%) in the control group ($P = .131$). There was no significant difference in the preoperative liver function, total bilirubin level, albumin level, hemoglobin level, creatinine level, or platelet count (all $P > .05$).

The preoperative tumor characteristics were compared and are presented in Table 2. The mean numbers of tumors were 1.5 in the Huaier group and 1.7 in the control group, which were not significantly different ($P = .465$). In addition, the overall tumor diameter and largest tumor diameter in these 2 groups were not significantly different. When we staged HCC according to the Barcelona Clinic Liver Cancer staging system, most patients were stage A, including 19 (67.9%) in the Huaier group and 12 (48%) in the control group, only a few were stage B, and none were stage C. The differences were not significant ($P = .147$). The Huaier group's AFP level was much lower than the control group's. However, this difference was not significant (984 ng/mL vs 4,901 ng/mL; $P = .335$). The Huaier group had fewer cases with the AFP level >800 ng/mL than the control group ($P = .036$). There were 5 cases (17.9%) with NLR >4 in the Huaier group and 4 (16%) in the control group ($P = .859$). Finally, tumor differentiation and HCC with microvascular invasion were similar between the 2 groups ($P > .05$).

During the follow-up, 6 patients died (4 in the Huaier group and 2 in the control group), all of them from tumor

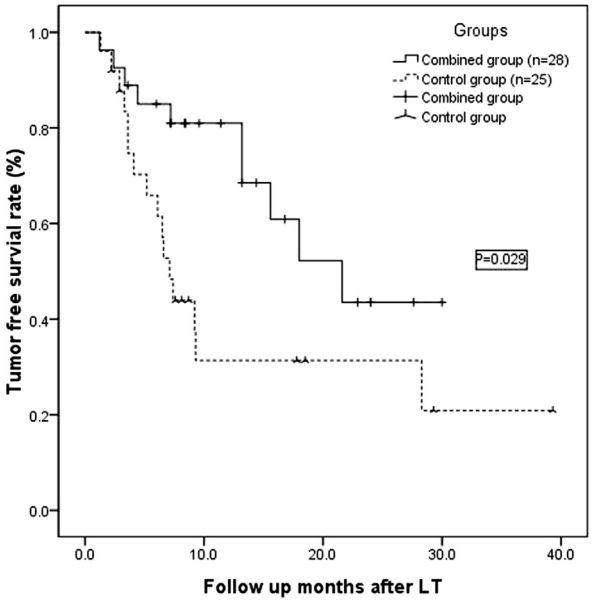

Fig 2. Predictive long-term tumor-free survival comparison between the 2 groups. The Huaier group patients may benefit from postoperative Huaier in terms of predictive long-term tumor-free survival compared with the control group ($P = .029$). Abbreviation: LT, liver transplantation.

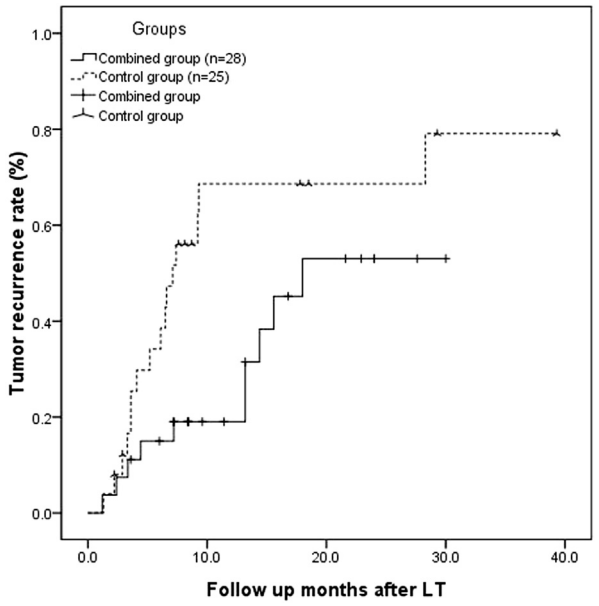

Fig 3. Tumor recurrence rate comparison between the 2 groups. The tumor recurrence rate was significantly higher in the control group than in the Huaier group ($P < .05$). Abbreviation: LT, liver transplantation.

recurrence. The overall survival was not significantly different between the 2 groups ($P = .202$; Fig 1). Twenty-six patients were diagnosed with tumor recurrence or metastasis (10 in the Huaier group and 16 in the control group), and the predictive tumor recurrence–free survival rate in the Huaier group was significantly higher than that in the control group ($P = .029$; Fig 2). When we compared the predictive tumor recurrence rate between the 2 groups, the 10- and 30-month predictive tumor recurrence rates were 17.9% and 35.7%, respectively, in the Huaier group, which were significantly lower than those in the control group (60% and 64%, respectively; $P < .05$; Fig 3). The most common sites of tumor recurrence or metastasis included liver recurrence, liver recurrence and lung metastasis, liver recurrence and bone metastasis, liver recurrence and abdominal lymph node metastasis, and lung metastasis. The therapy protocol for treating recurrence or metastasis included tTACE (20 cases, 76.9%), resection (2 cases, 7.7%), TACE combined with RFA (2 cases, 7.7%), TACE combined with systemic chemotherapy (1 case, 3.8%), and TACE combined with liver resection (1 case, 3.8%). Five patients underwent TACE to prevent recurrence. Univariable and multivariable analyses indicated that post-LT persistent Huaier treatment may contribute to better tumor-free survival rates (hazard ratio, 1.128; 95% confidence interval, 1.082–1.313; $P = .046$).

DISCUSSION

There are multiple HCC treatment strategies, including hepatic resection, RFA, chemotherapy, radiotherapy, transplantation, and complementary therapies. However, until now, only resection, RFA, and LT have been considered as radical therapy for early-stage HCC. Owing to the limitations of RFA and liver resection in small HCCs, RFA and resection should be considered in only a few HCC patients [17]. However, the post-LT HCC recurrence ranged from 14.2% to 68.7% in a large-cohort study [2]. Sorafenib has been shown to be effective in treating advanced-stage HCC, prolonging the survival time by 3 months in these patients, but the usefulness of sorafenib as an adjuvant therapy after curative therapy was not demonstrated in a worldwide, phase III, randomized, double-blind, placebo-controlled clinical trial of sorafenib as an adjuvant therapy after radical resection or LT [2]. Therefore, patients do not benefit from adjuvant sorafenib after radical resection or LT [18].

Huaier has a long history, spanning 3 millennia, and includes a variety of complex compounds, with natural origins, that have been widely used in clinical treatment, including cancer treatment, in China. Compared with other treatments, Huaier emphasizes the adjustment of human body functions and enhancement of body immunity, followed by the activation of anticancer activities, improvement of quality of life, and prevention of tumor metastasis and recurrence after surgery, radiotherapy, and chemotherapy [19]. Previous experimental studies have indicated that Huaier has effective anticancer activities, including apoptotic induction, antiangiogenesis, antimetastasis, drug resistance reversal, and systemic immune activation.

The effective ingredients of Huaier extract are proteoglycans, which consist of 41.53% polysaccharides, 12.93% amino acids, and 8.72% water. Proteoglycans are the most effective anticancer element among the isolated ingredients of Huaier extract, which has been confirmed in liver cancer H22 [11,20]. Some studies have reported that Huaier extract can inhibit angiogenesis both in vitro and in vivo. In addition, it has been posited that Huaier inhibits angiogenic properties (proliferation and differentiation) [21]; reduces proliferative ability, as well as inhibits motility, adherence, and the formation of blood vessels [22]; inhibits the mobility and invasion of cancer cells [23]; and regulates the mRNA expression of some angiogenic-related genes [22]. Meanwhile, in vivo studies have demonstrated that the Huaier extract significantly decreases the microvessel density and vascular endothelial growth factor expression in HCC tumor tissues [16]. All of these studies have suggested that Huaier can suppress tumor growth and stimulate tumor necrosis. Meanwhile, Huaier can improve and activate systemic immunity. Tumor immunity includes cell-mediated immunity and humoral immunity, and the former has more important roles than the latter. Some experimental studies have reported that Huaier can enhance the activity of natural killer cells (one of the main components of the anticancer immune response) in gastric cancer patients and breast cancer patients. Meanwhile, Huaier may increase the number of CD4+/CD8+ T cells, which may also contribute to its enhancement of immunity in cancer patients [24,25].

Until now, few studies have focused on the clinical use of Huaier for HCC. In the present study, we first evaluated the efficacy and safety of Huaier in HCC after LT. The baseline and tumor characteristics were similar between the 2 groups, and even with a similar long-term overall survival, the patients who received Huaier postoperatively seemed to have a decreased tumor recurrence rate. In our center, some patients have benefited from persistent use of Huaier over a period of 8 years and have not experienced severe adverse events, but they did not have improved long-term tumor-free survival rates after hepatic resection or LT. The main reason for this finding may be the limited follow-up time; the longest follow-up time was only 39.3 months, and longer follow-up may yield more objective results.

The main limitations of the present study were limited sample size, retrospective nature, and single-center analysis. To overcome these limitations, and based on the results of the present study, we are performing a prospective multicenter study with a large sample size. Meanwhile, the median follow-up time in this study was insufficient, and we plan to prolong the follow-up time to $\geq$5 years for each case to observe the long-term outcomes.

In conclusion, HCC patients may benefit from the administration of postoperative Huaier with decreased tumor recurrence rates, but more evidence is needed to validate this finding.

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