Int J Clin Exp Med 2017;10(10):14699-14709
www.ijcem.com /ISSN:1940-5901/IJCEM0055043

Original Article

Traditional Chinese medicine of Xiao-Chaihu-Decoction and radical surgery predicts improved prognostic outcomes in primary liver cancer patients

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Received April 10, 2017; Accepted September 11, 2017; Epub October 15, 2017; Published October 30, 2017

Abstract: To analyze the efficacy of traditional Chinese medicine (TCM) treatment of Xiao-Chaihu-Decoction combined with naturopathic medicine therapy on survival outcomes in primary liver cancer (PLC) patients received surgical treatment. From January, 2010 to May, 2012, 251 outpatients underwent previous treatment of surgical resection were incorporated and randomly divided into three groups: 95 cases in group A treated with Xiao-Chaihu-Decoction and naturopathic medicine therapy, 86 patients in group B treated with the Xiao-Chaihu-Decoction only, and another 70 cases in group C received naturopathic medicine therapy. Immune function of all patients were checked for assessing curative effect, in combination with the measurement of AFP and CA199 levels in the serum. The survival time was counted monthly from the starting day of the treatment to patients' death or to the date of the end of the follow-up. Recurrence rate was also recorded and compared among groups. No obvious statistical difference was found in general characteristics among groups before treatment, such as age, gender, and karnofsky performance score (KPS), etc. Both natural killer (NK) cells activity improved and CD^+_4/CD^+_8 values increased after treatment significantly (both $P<0.05$). The activity of NK cell and CD^+_4/CD^+_8 value in group A increased obviously than those in group B and group C after treatment (all $P<0.05$). Through follow-up, there were 191 cases of deaths and 60 cases survived at the end of follow-up, the 24 and 36 month-survival rate of group A was 31.58% and 26.32%, respectively; while the 24 month-survival rate of group B and group C was 27.91% and 25.71%, and the 36 month-survival rate was 22.09% and 22.86%. Kaplan-Meier curve showed that the survival rate of patients in group A was higher than that in group B and group C significantly, statistical difference was confirmed by Log-rank test. AFP and CA199 levels were both reduced after treatment in all groups (all $P<0.05$). There is a strong synergistic effect of Xiao-Chaihu-Decoction and naturopathy in improving prognostic survival outcomes as well as lowering recurrence rate in PLC patients. This study highlights that combining conventional approaches and traditional Chinese medicine regimens may be a possible therapeutic pathway for the treatment of malignancies in future.

Keywords: Traditional Chinese medicine, primary liver cancer, synergistic effect, natural killer cells

Introduction

Liver cancer is a third leading cause of cancer-related mortality and accounts for the death of approximately 700,000 annually worldwide, with a grim growth in incidence and mortality over the past 20 years in the world [1]. Liver cancer is divided into two major categories of primary and secondary, and primary liver cancer (PLC) has a rapid development of condition and severe clinical manifestations than secondary liver cancer [2]. Hepatocellular carcinoma and intrahepatic cholangiocarcinoma are the two most common histologic types of PLC, both of which show dismal clinical outcomes; and as a rare form of PLC, combined hepatocellular-cholangiocarcinoma shares morphologic characteristics with the other two major types of PLC [3]. The distribution of PLC shows variations among geographic regions and by sex, where an over eighty percentages of cases/deaths are found in developing countries, mostly in China alone [4]; and the age standardized rates for men are extremely higher than that for

women [5]. Leading risk factor for PLC is hepatitis B virus (HBV) infection, accounting for more than 50% cases worldwide, followed by the hepatitis C virus (HCV) infection [6]. Apart from this, other known factors, such as aflatoxins, alcohol, drinking water pollution, cirrhosis, sex hormones and a host of genetic factors involvement, do contribute a lot to the origin and progression of PLC [7-10].

In recent years, surgical and chemical treatment, HBV vaccination, antiviral treatment and surveillance are aggressively employed as strategies for early management and further prevention of PLC [11, 12]. Despite efforts, the incidence of PLC is increasingly high at an alarming rate, and the survival rate is still kept in an extremely low rate at 5% after diagnosis or intervention. Therefore, effective treatment methods are in urgent need to address the issue of public health crisis [13]. Furthermore, previous studies have also supported that combined treatment is evidently superior to monotherapy treatment for PLC patients in terms of long-term survival improvement and focal lesions control [14, 15].

Complementary and Alternative Medicine (CAM) has gained worldwide popularity in recent years, and traditional Chinese medicine (TCM) is one of the most popular CAM forms worldwide [16]. Over thousands of year's development, TCM has accumulated abundant clinical experience and has formed a comprehensive and unique medical system with the use of herbs or herbal formulae [17]. During the past half century, the effect of TCM has become widely accepted on infectious diseases, hypertension and cardiovascular diseases [18-20]. TCM is frequently used to treat cancer, either alone or in combination with Western medicines [21]. For a long time, Chinese herbal medicine has been widely recognized as an antineoplastic medicine, which has been popularly used clinically in the treatment of malignancies, such as non-small cell lung cancer, pancreatic cancer and gastric cancer [22-24]. Meanwhile, naturopathic medicine is an alternative therapy employing natural modalities and non-invasive treatment [18], including herbalism, acupuncture, massage, nature cures (diet/nutrition, exposure to nature elements, fasting, etc.) and physical medicine (sports medicine, exercise, etc.) [19].

In present period, the combination of TCM therapies on the basis of radical surgery has been promoted for the treatment of PLC, yet there were few studies on the clinical experiment of this topic, and most of them concentrated on one single TCM therapy or did not combined with the evaluation of prognostic efficacy indexes [25, 26]. We hence conduct a prospective study to analyze the effect of TCM treatment combined with radical surgery on survival outcomes and recurrence in patients diagnosed with PLC.

Materials and methods

Ethics statement

The present prospective study was conducted conforming to the guidelines of the ethics committee at The Second Hospital of Jilin University. All subjects have signed the informed consent prior to the performance of predefined treatment strategy. The study was carried out in accordance to the declaration of Helsinki [27]. Incorporated patients all agreed to accept treatment approaches according to and in full compliance with the recommendation of attending physicians in our hospital.

Patient eligibility

A total of 251 patients confirmed with PLC were enrolled in the present research, who all used to undergo surgical treatment from January, 2010 to May, 2012 in The Second Hospital of Jilin University. They were consisting of 220 males and 31 females with different ages from 23-75 years (mean age of 51.23±8.77 years). All patients incorporated in this study conformed to the follow criteria: (1) patients diagnosed with PLC by imaging and pathological examination; (2) patients in stage II and III, assessed according to the International Union Against Cancer (UICC) staging systems [28]; (3) Child-Pugh Score of the liver function [29] was A, and with no absolute contraindications for surgery; (4) expected survival time over 3 months; (5) no medical history of Chinese herbal medicine treatment during the past 3 months before treatment. Patients who met any of the following criterion were excluded: (1) age was under 20 or over 75 years old; (2) secondary hepatocarcinoma or primary hepatocarcinoma, diagnosed by exploratory operation; (3) underwent anticancer therapy (chemotherapy, radio-

therapy, surgical treatment and intervention therapy) during the latest 3 months and got effects; (4) pregnant or lactating woman and psychotics; (5) long-term smoking (smoking history of 10 years or above) or heavy drinking (50 g per day or more-alcohol quantity in grams per typical day); (6) accompanied with primary diseases, such as severe cardio-cerebral vascular diseases or hemopoietin system diseases; (7) accompanied with lumbar spinal stenosis; (8) the Child-Pugh Score was B or C; (9) patients with incomplete clinical information or unwilling to accept the research project. Baseline characteristics of each subject were carefully recorded; furthermore, the examination of alpha-fetoprotein (AFP) and CA199 levels was performed by radioimmunoassay as described before [30].

Grouping and treatment

Using the grouping method on the basis of the patients' data on admission to achieve random grouping of patients, 95 cases of patients were enrolled (83 males and 12 females, 24-72 years old, mean age 52.61±8.60 years) in group A who were accepted to be treated with Xiao-Chaihu-Decoction and naturopathic medicine therapy (group A). And 86 patients (79 males and 7 females, 23-75 years old, mean age of 50.12±10.13 years) were willing to be treated with the supplementation of Xiao-Chaihu-Decoction only (group B). Another 70 cases (58 males and 12 females, 25-68 years old, mean age 51.33±9.41 years) were categorized in the group C and accepted to receive naturopathic medicine therapy. Patients in group B received Xiao-Chaihu-Decoction regimen based on the addition and subtraction theory of TCM, detailed therapies were conducted in accordance with the symptom and willing of involved patients (Appendix I). Furthermore, patients in group C underwent naturopathic treatment, including acupoint application, nature cures (diet/nutrition), combined therapeutic apparatus and meridian therapy. Acupoint application was performed based on patients' conditions, balanced with diet and nutrition, combined therapeutic apparatus for discomfort improvement such as hypochondriac pain, stomach pain, diarrhea, indigestion, and chills, shortness of breath, ascites and other symptoms, also covering meridian therapy to dredge the meridian. With respect to the above therapies, all involved patients accepted treatment and all the treatment processes were properly implemented, including the accurate dose and the preset duration of medication, referral, and compliance with the dietary restrictions of individual drugs.

Observation of the curative effect

To assess the curative effect, immune function of all the included patients were checked via blood test one week before surgery and four weeks later after the surgery, respectively. Therapeutic effects were evaluated based on the World Health Organization (WHO) criteria, and the curative effect was then classified as complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), according to the Response Evaluation Criteria in Solid Tumors (RECIST) [31]. CR means the disappearance of all known targeted initial spots, and the effect was maintained for at least 4 weeks which was supported by normal laboratory and imaging parameters. PR was defined as a decrease of ≥30% (but not 100%) of the sum of the longest diameters of predetermined target lesions. PD was regarded as an increase of ≥20% (but not 100%) of the sum of the longest diameters of predetermined target lesions, or with new or enlarging lesions (target or nontarget). And the SD was considered as a possible decreased of the sum of the longest diameters of predetermined target lesions, but between the scope of PR and PD. The objective response rate (ORR) = (CR+PR)/n*100%; the stable rate = (CR+PR+SD)/n*100%; and the failure rate = PD/n*100%.

The clinical follow-up was done before December, 2015 by consulting the medical records or making telephone calls. The survival time was counted monthly from the starting day of the treatment to patients' death or to the date of the last follow-up. The period, from diagnosis to death or to the date of the end of the follow-up, was defied as overall survival (OS) time. The recurrence of tumor within 3 years was also measured after the therapy. The 12, 24 and 36 months-survival rate were calculated subsequently.

Statistical analysis

All data were analyzed with the SPSS18.0 software (IBM, Armonk, NY, USA) and PraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, USA). Continuous data was expressed as mean

Table 1. Comparisons in general data among groups after applying Xiao-Chaihu-Decoction and naturopathy on the basis of radical therapy

	Group A (n = 95)	Group B (n = 86)	Group C (n = 70)	t/χ^2	*P*
Gender (Male/Female, n)	83/12	79/7	58/12	2.901	0.235
Age (years)	52.61±8.60	50.12±10.13	51.33±9.41	1.596	0.205
KPS	65.21±10.14	69.33±11.47	87.67±12.62		
Body weight	58.05±9.43	60.16±8.98	59.73±9.11	1.327	0.267
Positive AFP (n, %)	76 (80.00)	78 (90.70)	61 (87.14)		
Negative AFP (n, %)	19 (20.00)	8 (9.30)	9 (12.86)		
Positive HBsAg (n, %)	86 (90.53)	78 (90.70)	62 (88.57)		
Combined hepatitis (n, %)	19 (20.00)	15 (17.44)	12 (17.14)		
UICC staging (n, %)					
Stage II	51 (53.68)	43 (50.00)	36 (51.43)		
Stage III	45 (47.37)	43 (50.00)	34 (48.57)	0.178	0.915
Tumor size (n, %)					
>5 cm	38 (40.00)	41 (47.67)	30 (42.86)		
≤5 cm	57 (60.00)	46 (53.49)	40 (57.14)	1.428	0.839
≤3 cm	7 (7.37)	9 (10.47)	7 (10.00)		
Integrated tumorcapsule (n, %)	67 (70.53)	58 (67.44)	46 (65.71)		
Single nodular tumor (n, %)	57 (60.00)	58 (67.44)	43 (61.43)		
Invasion (n, %)					
Vessel invasion	19 (20.00)	21 (24.42)	15 (21.43)		
Bile duct invasion	0 (0)	0 (0)	0 (0)	1.914	0.384
Other	76 (80.00)	65 (75.58)	55 (78.57)		
Pathological type (n, %)					
Hepatocellular carcinoma	83 (87.37)	78 (90.70)	60 (85.71)		
Cholangiocarcinoma	10 (10.53)	6 (6.98)	6 (8.57)	1.200	0.878
Mixed carcinoma of liver	4 (3.16)	3 (3.49)	4 (5.71)		

Note: KPS, karnofsky performance score; AFP, alpha fetal protein; HBsAg, hepatitis B surface antigen; UICC, Union for International Cancer Control.

± standard deviation and tested with t test, and categorical variables were expressed as percentage and rates, which was compared using χ^2 test. For data that were not normal distribution, rank-sum non-parametric test were conducted. Kaplan-Meier was involved for mapping survival curve, Log-rank test was used to analyze the relationship between different therapies and prognosis, and multivariate regression analyses were employed to identify possible factors affecting survival outcomes based on the Cox's proportion hazards model. All tests were two-tailed and $P<0.05$ indicates a statistically significance.

Results

Comparison of baseline characteristics

In group A, 76 patients were detected with positive AFP; for serum HBsAg, 86 patients were detected to be positive; 19 patients were accompanied with liver cirrhosis; 51 patients were rated as stage II and 45 patients were stage III (UICC staging system); the tumor size of 38 patients were larger than 5 cm; integrated tumor capsule were observed in 67 patients; single nodular tumor were seen in 57 patients; vessel invasion was found in 19 patients and no bile duct invasion was observed; there were 83 cases with hepatocellular carcinoma, 10 cases with cholangiocarcinoma and 4 cases with the mixed carcinoma of liver. In group B, 78 patients were detected with positive AFP; 78 patients were detected with positive serum HBsAg; 15 patients were accompanied with liver cirrhosis; patients of stage II and stage III were both 43, respectively; tumor size in 41 patients were larger than 5 cm; integrated tumor capsule were observed in 58 patients; single nodular tumor were seen in 58 patients;

Table 2. Comparison of cellular immunity function among groups after applying Xiao-Chaihu-Decoction and naturopathy on the basis of radical therapy

	Group A		Group B		Group C	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Activity of NK	25.37±11.0	34.15±11.33*,▲	24.88±12.14	20.97±14.84#	25.60±10.24	21.88±12.45#
CD^+_3	49.34±9.92	48.96±12.01	46.83±12.32	45.28±12.04	47.23±10.20	45.62±11.86
CD^+_4	31.14±9.31	32.67±10.13	31.58±9.24	30.03±8.17	32.00±8.92	31.34±9.12
CD^+_8	23.63±8.52	22.50±8.91	22.83±8.16	23.19±8.23	23.44±7.99	22.67±8.48
CD^+_4/CD^+_8	1.41±0.29	1.64±0.38*,▲	1.66±0.41	1.25±0.33#	1.57±0.44	1.19±0.33#

Note: *, compared with in group A before treatment, $P<0.05$; #, compared with in group B and group C before treatment, $P<0.05$; ▲, compared with group B and group C after treatment, $P<0.05$.

Table 3. Tumor responses evaluation and comparison among groups after applying Xiao-Chaihu-Decoction and naturopathy on the basis of radical therapy

	Group A	Group B	Group C
Cases (n)	95	86	70
CR (n)	2	0	0
PR (n)	32	21	19
SD (n)	35	31	26
PD (n)	26	34	9
ORR = CR+PR (n, %)	34 (35.79)*	21 (24.42)	19 (27.14)
Stable rate = CR+PR+SD (n, %)	69 (72.63)*	52 (60.47)	45 (64.29)

Note: CR, complete remission; PR, partial remission; SD, stable duration; PD, progression disease; ORR, objective response rate. χ^2 test to compare the proportion or rates. *, compared with in group B and group C before treatment, $P<0.05$.

Table 4. The 12, 24 and 36 month-survival rates comparison among groups after applying Xiao-Chaihu-Decoction and naturopathy on the basis of radical therapy

	Group A	Group B	Group C
Survival rate			
12 month (n, %)	34 (35.79)	30 (34.88)	35 (34.29)
24 month (n, %)	30 (31.58)*	24 (27.91)	18 (25.71)
36 month (n, %)	25 (26.32)*	19 (22.09)	16 (22.86)
Recurrence rate			
12 month (n, %)	15 (15.79)*	23 (26.74)	19 (27.14)
24 month (n, %)	33 (34.74)*	44 (51.16)	33 (50.47)
36 month (n, %)	45 (47.37)*	65 (75.58)	54 (77.14)

Note: *, compared with group B and group C, $P<0.05$.

vessel invasion was seen in 21 patients; there were 78 cases with hepatocellular carcinoma, 6 cases with cholangiocarcinoma and 3 cases with the mixed carcinoma of liver. Furthermore, in group C, 61 patients were detected with positive AFP, 62 patients with positive serum HBsAg, 12 patients with liver cirrhosis, 36 patients with stage II and 34 patients with stage III; the tumor size of 30 patients were larger than 5 cm; integrated tumor capsule in 46 patients; single nodular tumor in 43 patients; vessel invasion in 15 patients and without bile duct invasion; 60 cases with hepatocellular carcinoma. Portal vein tumor thrombus and bile duct cancer thrombus were seen in neither groups. Comparison of the clinical data among groups showed no statistic difference ($P>0.05$), detailed information was presented in **Table 1**.

Detection of the cellular immunity function before/after the surgery

In group A, the activity of NK cell and CD^+_4/CD^+_8 value increased significantly after treatment ($P<0.05$); while CD^+_3, CD^+_4 and CD^+_8 values showed no significant difference before and after the treatment (all $P>0.05$). Moreover, compared with group B and group C, the activity of NK cell and CD^+_4/CD^+_8 value in group A increased obviously after the treatment (all $P<0.05$), indicating that the perioperative integrated therapies were likely to improve patients' cellular immunity function, suggesting a synergistic effect of Xiao-Chaihu-Decoction and naturopathy in the treatment of PLC. Comparison of cellular immunity function among groups were shown in **Table 2**.

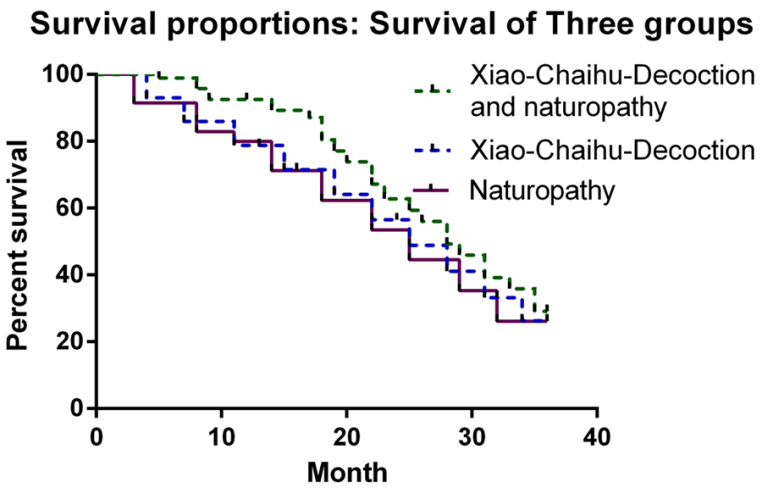

Figure 1. Overall survival among groups evaluated using the Kaplan Meier survival analysis method. The overall survival rate of patients received Xiao-Chaihu-Decoction and naturopathic medicine therapy was higher than that of patients accepted Xiao-Chaihu-Decoction or naturopathic medicine therapy.

Table 5. Association of different therapies and clinical characteristics with the prognosis of primary liver cancer patients

Variables	χ^2	*P*
Gender	7.696	0.021
Age	10.521	0.047
AFP	7.774	0.036
HBsAg	9.829	0.015
Combined hepatitis	4.006	0.033
UICC staging	3.255	0.043
Tumor size	7.869	0.020
Invasion	1.469	0.194
Pathological type	0.798	0.296
Integrated Xiao-Chaihu-Decoction and naturopathy	9.738	0.002
Xiao-Chaihu-Decoction therapy	4.628	0.031
Naturopathy therapy	0.142	0.566

Note: AFP, alpha fetal protein; HBsAg, hepatitis B surface antigen; UICC, Union for International Cancer Control.

Assessment of the therapeutic effect on the basis of tumor response

There were 2 case of CR, 32 case of PR, 35 cases of SD and 26 cases of PD in group A, of which the ORR, stable rate and failure rate was 35.79%, 72.63% and 27.37%, respectively **Table 3**. While in group B, there was 0 case of CR, 21 cases of PR, 31 cases of SD and 34 cases of PD, corresponding ORR, stable rate and failure rate was 24.42%, 60.47% and 39.53%, respectively. In group C, there was 0 case of CR, 19 cases of PR, 26 cases of SD and 25 cases of PD, and the ORR, stable rate and failure rate was 27.14%, 64.29% and 35.71%, respectively. χ^2 test result showed that the therapeutic effect of group A was better than group B and group C (both $P<0.05$), but the difference was not significant when compared between group B and group C ($P>0.05$).

Survival rate and recurrence rate comparison

All subjects were followed-up for 3 years until December, 2015. In group A, 6 patients were lost to follow-up, and the follow-up rate was 93.68%; 8 patients were lost to follow-up in group B, with a follow-up rate of 90.70%; and the follow-up rate in group C was 92.86% (66/70). The survival analysis showed that the 24 and 36 month-survival rate of group A was 31.58% (30/95) and 26.32% (25/95), respectively; while the 24 month-survival rate of group B and group C was 27.91% (24/86) and 25.71% (18/70), and the 36 month-survival rate was 22.09% (19/86) and 22.86% (16/70). These data showed that the 24 month-survival rate and 36 month-survival rate of group A were higher than those of group B and group C (both $P<0.05$). But the 12 month-survival rate showed no significant difference among groups (35.79% (34/95) vs. 34.88% (30/86) vs. 34.29% (35/70)) (all $P>0.05$) (**Table 4**). As shown in **Figure 1**, overall survival among groups was evaluated using the Kaplan Meier survival analysis method, it could be found that the overall survival rate of group A was higher than that of group B and group C, Log-rank test indicated that there was statistical difference ($P<0.05$).

In assessing the recurrence rate, in group A, the 12 month-recurrence rate was 15.79%, 24 month-recurrence rate was 34.74% and the 36 month-recurrence rate was 47.37%. The 12, 24

Table 6. Multivariate analysis of influencing factors for primary liver cancer patient survival after interventional therapy

Variables	Overall survival					
	β	SE	Wald	*P*	OR	95% CI
Integrated Xiao-Chaihu-Decoction and naturopathy	-2.01	0.28	12.36	0.012	0.51	0.26-0.63
Combined hepatitis	1.91	0.33	10.1	0.028	1.73	1.54-5.67
Tumor size	2.18	0.54	11.45	0.027	1.96	1.31-5.83

Note: β: regression coefficient; SE: standard error; Wald: test statistics; P: *P* value; OR: odds ratio; 95% CI: 95% confidence interval.

Table 7. The comparison of KPS score, AFP, and CA199 among groups after applying Xiao-Chaihu-Decoction and naturopathy on the basis of radical therapy

	Group A		Group B		Group C	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
KPS score	65.21±10.14	91.08±12.81$^{*,\#}$	69.33±11.47	78.11±11.09*	87.67±12.62	81.79±11.93*
AFP (ng/mL)	267.36±28.92	34.56±5.40$^{*,\#}$	274.25±22.58	55.77±8.54*	265.47±27.24	49.50±6.81*
CA199	241.46±34.24	24.56±8.30$^{*,\#}$	233.67±32.19	65.29±11.24*	248.34±30.11	57.40±9.30*

Note: KPS, karnofsky performance score; AFP, alpha fetal protein. *, compared with the value before treatment, *P*<0.05; #, compared with group B and group C, *P*<0.05.

and 36 month-recurrence of group B was 26.74%, 51.16% and 75.58%, respectively. Meanwhile, in group C, the 12, 24 and 36 month-recurrence was 27.14%, 50.47% and 77.14%, respectively. Conspicuously, the recurrence rate of group B and group C was significantly higher than group A (all *P*<0.05). Besides, a comparison between group B and group C indicated that there was no apparent statistical differences between them (*P*>0.05).

Cox proportion hazards regression analysis

To identify factors which contribute to survival, univariate analysis was performed. By including age, gender, AFP, HBsAg, combined hepatitis, tumor size, etc., it was found that gender, age, AFP, HBsAg, combined hepatitis, UICC staging, tumor size, and integrated Xiao-Chaihu-Decoction and naturopathy might be correlated with the survival of patients, which was illustrated in **Table 5**. Furthermore, factors entering the equation of univariate analysis were further enrolled for multivariate regression analysis using Cox's proportional-hazards model, variables that put into the regression equation were integrated Xiao-Chaihu-Decoction and naturopathy, combined hepatitis and tumor size (**Table 6**), suggesting that the above factors were the independent factors for the survival outcomes of patients. To be specific, integrated Xiao-Chaihu-Decoction and naturopathy might be a protective prognostic factor, whereas combined hepatitis and tumor size were risk factors in the overall survival outcome of PLC patients (OR = 0.51, 1.73 and 1.96, respectively, all *P*<0.05).

KPS scores AFP and CA199 levels changes after treatment

As shown in **Table 7**, karnofsky performance score (KPS) scores were significantly improved after treatment in all groups, and evidently in group A. Furthermore, before and after treatment comparison of AFP and CA199 levels suggested that both indexes were reduced after treatment in all groups; besides, the decreasing amplitude in group A was remarkably obvious than that in group B and group C, showing statistical differences (all *P*<0.05).

Discussion

In this study, it supported the consistent role of the efficacy of TCM treatment combined with radical surgery on survival and recurrence in patients diagnosed with PLC, as we hypothesized. Our study also highlighted the synergistic effect of Xiao-Chaihu-Decoction and naturopathy in PLC patients who received surgical treatment. Firstly, the activity of NK cell and CD^{+}_{4}/CD^{+}_{8} values in patients received integrated Xiao-Chaihu-Decoction and naturopathy

increased obviously after the treatment when compared to patients treated with single treatment only, suggesting that the perioperative treatment with Xiao-Chaihu-Decoction and naturopathy were more likely to improve patients' cellular immunity function after surgical treatment. Generally speaking, acting as crucial effector cells of the innate immune system, NK cells appear to be the first-line of defense against invading pathogens and malignant transformation through their natural induction of cytotoxicity and specific cytokine profiles production [32]. At the same time, NK cells are widely distributed in human body throughout lymphoid and non-lymphoid tissues, with highest frequency in the lung and followed by the liver, there is no doubt that NK cells may exhibit a role in antiviral, antifibrotic, and antitumor responses in the liver [33-35]. Therefore, it was not difficult to understand the significant reduction of NK cells activity in PLC patients than in those patients received treatment, possible reason might be that tumor-surveillance functions of NK cells was suppressed in cancerous tumor in the liver [36]. And there was an evident elevation of NK cells activity in patients treated with compound Chinese medicine after surgical treatment, which highlights the impactful role of integrated Chinese-western therapy in PLC patient, which might be attributed to the augmented cytolytic activity of NK cells in the liver [37]. Further, T cells mediated cellular immunity is especially important in antitumor immunity, CD^+_4 and CD^+_8 acting as helper T cells and cytotoxic T lymphocyte, respectively, both trigger efficient antitumor immune response [38]. Our study further discovered that CD^+_4/CD^+_8 values were suppressed before treatment, revealing a poor immune function and antitumor effect. And both values were significantly higher in integrated Chinese-western therapy treatment patients than those treated with single treatment, which strengthened our opinion that there might be a synergistic effect of integrated Chinese-western therapy in PLC patients.

And whether integrated Xiao-Chaihu-Decoction and naturopathy therapy correlated with the improved prognosis in these patients was further investigated in the study. Survival exploration results indicated that survival rates in all therapeutic regimens were improved as time went on in the follow-up period, suggesting the curative effects of Xiao-Chaihu-Decoction and naturopathy treatment in these patents. And further comparison among groups found that there was significantly increasing in survival rate in the combined group, demonstrating that the effect of integration treatment was much better than that of single treatment. Currently, surgery and other therapeutic regimes, such as chemotherapy, radiotherapy and immunotherapy, are the main stream anti-cancer therapies being applied for controlling liver cancer and prolonging survival time [39, 40]. Yet current curative effect is not optimistic, for example, radical operation may result in various complications such as upper gastrointestinal bleeding, liver function failure and disorders of the immune system [41]. And patients prone to intrahepatic recurrence during postoperative follow-up, which is closely correlated with the multicenter growth characteristics of liver cancer and possible associated with hepatitis [42, 43]. Recent pre-clinical and clinical studies proposed that TCM combined with conventional western medicine can significantly improve the treatment outcomes early in the course of the disease. It was speculated that some novel TCM delivery forms can enhance the bio-availability and pharmacological effects of TCM, so as to rectify the problems of conventional dosage forms or related complications [44]. With respect to the outcome of recurrence, on the basis of surgical therapy, Xiao-Chaihu-Decoction and naturopathy treatment showed an apparently lower recurrence rate, which to some extent also proved that a combination of Xiao-Chaihu-Decoction, naturopathy and surgery did help greatly to the promotion of immune reconstitution [45].

Xiao-Chaihu-Decoction has been applied in clinical and demonstrated to be effective in blocking the development of hepatitis to liver fibrosis, so as to delay tumor progression [46]. Xiao-Chaihu-Decoction can inhibit the replication of hepatitis virus, protecting liver cells, preventing liver damage, and suppressing liver fibrosis, as well as possessing the role of immune regulation, and the effect of anti-HBV and anti-tumor, etc. [47, 48]. Besides, Xiao-Chaihu-Decoction may be responsible for the inhibition the cell cycle G0/G1 stage of cancer cell line, suppression of cell proliferation and apoptosis, thereby inhibiting the transformation of liver cirrhosis to cancer [47, 49]. Also, on the belief in human body's ability to heal itself, naturopathic practice is suggested to have few

adverse effect comparing conventional medicine [50]. Meanwhile, levels of AFP and CA199 were also measured to further verify the treatment efficacy of Xiao-Chaihu-Decoction and naturopathy. Clinically, AFP has been recognized as the only widely used serum marker for liver cancer which is crucial for the determination of postoperative survival and prognostic outcomes after treatment [51]; meanwhile, CA199 is a broad spectrum tumor marker that is detected to be increased in malignancies [52]. The present study indicated that both indexes were reduced after treatment in all groups, especially in patients underwent Xiao-Chaihu-Decoction and naturopathy, which proved the hypothesis of the synergistic effect of the above therapy.

However and importantly, there were some limitation of this study that should be improved in our future research. Firstly, patients within group received different kind of herbal medicines and naturopathy medicines, a better solution would be the interaction of different medicines and sub-grouping of different therapies, which required a larger sample size and more complex statistical analysis. In addition, to further confirm the role of TCM in the treatment of liver cancer and a clear comparison of different therapies, larger sample size and longer follow-up period should be considered in our future research on the basis of a complete random experimental design. In conclusion, we find a strong synergistic effect of TCM using Xiao-Chaihu-Decoction and naturopathy in improving survival rate as well as lowering recurrence rate in the PLC patients. This study highlights that combining conventional approaches and traditional Chinese medicine regimens may be an effective therapeutic strategy to promote the future practice in the treatment of human malignancies. Also worth-noting is that, more studies address the issue of the mechanism of TCM in liver cancer will be a key to uncover its full potential in future.

Acknowledgements

This research was partially supported by Key Project of Tianjin Science and Technology Support Program (15ZCZDSY00890), National Natural Science Foundation of China (81471761, 81501568) and National Science and Technology Support Program of China (2012BAI15B-06).

Disclosure of conflict of interest

None.

Authors' contribution

Hai-Peng Yu and Yan Zhao writing of the manuscript. Hai-Peng Yu and Yan Zhao: analysis and interpretation of the results. Lan-Lan Yang: Design of the study. Hai-Peng Yu and Yan Zhao: carried out the experiments. All authors reviewed the manuscript.

Ethics statement

Compliance with Ethical Standards.

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