ORIGINAL ARTICLE

Efficacy of Early Treatment on 52 Patients with Preneoplastic Hepatitis B Virus-Associated Hepatocellular Carcinoma by Compound *Phyllanthus Urinaria* L.*

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ABSTRACT **Objective**: To observe the change in the number of antibodies of preneoplastic hepatocellular carcinoma (HCC) using early treatment by Compound *Phyllanthus Urinaria* L. (CPUL) on patients with preneoplastic hepatitis B virus (HBV)-associated HCC. **Methods**: A total of 102 cirrhosis patients with regenerative or dysplastic nodules whose sera were tested positive for at least one of these six proteins (five up-regulated genes URG4, URG7, URG11, URG12 and URG19, and one down-regulated gene DRG2) were assigned randomly to two groups using continual random codes by SPSS software. Fifty-two patients were in the treatment group and 50 patients were in the control group. CPUL was used in the treatment group for 3 years, while the control group did not receive any treatment. The changes in HBV-DNA level, number of antibodies, and hepatocarcinogenesis occurred were observed. Patients who did not develop HCC were followed up for another 2 years. **Results**: HBV-DNA levels decreased ⩾2log in 22.2% (10/45) of patients in the treatment group in contrast to only 5.0% (2/40) of patients in the control group (P=0.0228). The number of antibodies that were tested positive in the treatment group (1.08±1.01) was significantly lower compared with the control group (2.11±1.12) after 24 months of drug treatment (P<0.01). Both the positive rates of anti-URG11 (33/52) and anti-URG19 (31/52) were over 60% at baseline in the two groups, and were decreased to 48.1% (25/52) and 46.2% (24/52) respectively at 36 months of drug treatment, while the rates increased to 68.0% (34/50) and 66.0% (33/50) respectively (P=0.0417, P=0.0436) in the control group. The positive rate of anti-DRG2 was increased to 55.8% (29/52) at 36 months of drug treatment, while in the control group was decreased to 36.0% (18/50, P=0.0452). Among the 102 patients who developed HCC, 2 were in the treatment group and 9 were in the control group, meaning that a significant difference between the two groups (P=0.0212). In 11 patients who developed HCC, anti-URG11 and anti-URG19 were always positive, while anti-DRG2 was negative. Patients newly developing HCC were 6 (20.0%) in the control group, and only one (2.5%) in the treatment group (P=0.0441) during 2-year follow-up after the end of the treatment. **Conclusions**: Anti-URG11, anti-URG19 and anti-DRG2 could be used as early markers in the prediction of the therapeutic efficacy of CPUL in treating preneoplastic HCC. CPUL is useful in preventing or delaying the development of HBV-associated cirrhosis to HCC.

KEYWORDS carcinoma, hepatic cell, serologic preneoplastic markers of hepatocellular carcinoma, compound *phyllanthus urinaria* L.

In China, the mortality of primary hepatocellular carcinoma (PHC) has reached 20.40 per 100,000 people, and accounts for 45% of PHC mortality around the world.[1] Although primary (pathogenic) prevention of hepatocellular carcinoma (HCC), including hepatitis B vaccination, has been undertaken in China, the chronic hepatitis B virus (HBV) infection rate is as high as 7.18%.[2] Therefore, secondary prevention, namely the early detection, diagnosis and treatment of HCC, is very important.

Preneoplastia of HCC is a histopathological abnormity of liver that is easy to cancerate.[3] Most researchers believe that dysplastic nodules, or atypical adenomatous hyperplasia (AAH) with

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cirrhosis, are the precancerous lesions of HCC. The diameters of AAH are usually more than 0.8 cm, which are significantly larger than the regenerative nodules of surrounding hepatic tissues. In 1986, the first case of hapatocarcinogenesis derived from AAH in hepatitis B associated cirrhosis was reported.[4] Terada, et al[5] has shown that among 38 patients with AAH, 19 patients have obvious lesions of HCC. Nakashima, et al[6] also reported that among 53 patients with AHH, 11 patients had HCC. These findings confirmed that AHH might be preneoplastia of HCC.

Although cirrhosic nodule is preneoplastia of HCC, and can be detected by pathological and imaging approaches,[7] more than 30% of the HCC patients have a lack of dysplastic nodules.[8,9] Furthermore, preneoplastia of HCC can occur anytime in chronic hepatitis B (CHB) patients. Therefore, it is beneficial to develop an easy serological detection method for preneoplastia of HCC. In this study, we provide a simple method for the diagnosis of preneoplastic HCC, use a novel compound *phyllanthus urinaria* L. (CPUL) to treat preneoplastic of HCC, and report efficacy assessment markers for the treatment of preneoplastic HCC.

METHODS

Diagnostic Criteria

The diagnoses of regenerative or dysplastic nodule with HBV-associated cirrhosis were based on the separate criteria of cirrhosis nodule set by the International Association for the Study of Liver Diseases (IASLD) in 1995.[10,11] The criteria include: (1) HBsAg positive; (2) changes of hepatic cirrhosis detected by ultrasound B (thickening of liver capsule, obviously uneven, serration or wave-like surface, blunting liver edge, and asymmetrical echo of liver parenchyma); and (3) regenerative nodule larger than 0.8 cm detected by computerized tomography (CT) or magnetic resonance imaging (MRI).

Inclusion Criteria

All of the eligible patients included the following criteria: (1) had cirrhosis; (2) were tested positive for more than one of the six antibodies (5 up-regulated genes URG 4, URG 7, URG 11, URG 12 and URG 19 and 1 down-regulated gene DRG 2) of preneoplastic HCC; (3) had regenerative or dysplastic nodule detectable by CT or MRI; and (4) had filled in the patient informed consent before the study voluntarily.

Exclusion Criteria

Patients who would be excluded from the study possessed one of the following criteria: (1) had tested negative for all of the six antibodies of preneoplastia of HCC; (2) had psychotic, kidney, cardiovascular, hematological system and other life-threatening acute or chronic diseases; or, (3) were on nucleoside analogue antiviral therapy.

The study protocol was approved by the Ethics Committee of Shenzhen Hospital Affiliated to Guangzhou University of Chinese Medicine, China. Informed consent was obtained from each patient before the study. All 362 cirrhosis patients were enrolled from July 2002 to July 2009. A total of 102 patients were selected from 362 patients with eligible inclusive criteria. These patients with confirmed diagnoses were then randomly divided into treatment group (52 cases, among them, 19 patients were decompensated cirrhosis) and control group (50 cases, among them, 20 patients were decompensated cirrhosis), using continual random codes by SPSS software 13.0. These patients included 81 males and 21 females, and ranged from 35 to 65 years old. All of the diagnoses were based on serum biochemistry, CT and MRI.

Preneoplastic Antibody Test of HCC

HepG2 cells were stably transfected with HBx or the bacterial chloramphenicol acetyltransferase (CAT) gene. Up-regulated genes URG4, URG7, URG11, URG 12 and URG19, as well as a down-regulated gene DRG2 were identified in the transfected cell line. Sera of patients with HBV chronic infections were tested for the presence of these six proteins by enzyme-linked immunosorbent assay. All the eligible patients were tested for these six antibodies associated with preneoplastic HCC.[12,13]

Therapeutic Methods

The treatment group was given CPUL (add on anti-inflammatory and hepatoprotection therapy when serum ALT was elevated) for 3 years; while the control group was not given treatment (add on anti-inflammatory and hepatoprotection therapy when serum ALT was elevated). Patients who had developed HCC from cirrhosis with regenerative or dysplastic nodule in both groups were subjected to interventional therapy, including transcatheter hepatic arterial chemoembolization (TACE), radiofrequency tumor ablation (RITA) and percutaneous ethanol

injection (PCEI).

The ingredients of CPUL included *phyllanthus urinaria* L. 30 g, *Scutellaria barbata* 30 g, *Curcumae* 15 g, *Astragalus* L. 30 g and *Edible tulip* 10 g. All of the drug granules of herbs were made in Guangdong Yifang Pharmaceutical Co., Ltd., China (Batch number: 1012006) with a National Good Manufacture Practice certification.

The concentrated Chinese medicine granules indicated above were melted in 200 mL of water. One hundred milliliters of the medicine were taken by the patients in the morning and evening, respectively, for no less than 20 days per month. One course of the therapy was 12 months and 3 courses were used. Patients continued therapy for a follow-up period of 2 years.

Observational Methods

HBV Markers

HBV markers were tested by enzyme-linked immunosorbant assay (ELISA) with kits purchased from Shanghai Kehua Bio-engineering Co., Ltd., China. HBV was quantified by real time quantitative polymerase chain reaction (PCR) using kits from Shenzhen-based Bio-engineering Co., Ltd., China, on an ABI PRISM 7000 sequence detection system (Applied Biosystems Inc., CA, USA).

Alpha Fetoprotein

Alpha fetoprotein (AFP) was detected by enzyme immunoassay (normal value 20 μg/L) using kits obtained from Shanghai Haiyin Biotechnology Center (China).

CT and MRI Examination

Under CT (Picker UltraZ super, Germany), hepatic cirrhosis with regenerative nodule was high in density and small low-density when enhanced. HCC had low density, which was enhanced in the arterial phase. MRI (Dutch Philips Philips Intera 1.5T high-field superconducting MRI, Phillip, Holland) showed that regenerative nodule had high or equal signal in the summary of T1-weighted imaging, and equal or low signal in T2-weighted imaging. However, HCC was displayed as high- or low-signal in the T1, but always as high-signal in the T2, which was helpful for identification. The diagnoses were made by more than two radiologists.

Statistical Analysis

All analyses were performed using SPSS13.0 (Statistical Product and Service Solutions SPSS, Inc., Chicago, IL, USA). Data were expressed as means ± standard deviations. The antibody markers and HBV DNA levels were compared between the two groups using χ^2 test. Fisher's exact probability test was applied to the incidences of adverse reactions. Differences with *P* value lower than 0.05 in two-tailed tests were considered significant.

RESULTS

Baseline of HBV Patients with Cirrhosis

The 102 patients were chosen among the 362 cirrhosis patients after long-term observations. Clinical baseline characteristics are shown in Table 1. The selected patients had no difference in the signs of baseline statistics between the treatment group and the control group (*P*>0.05).

Table 1. Characteristics of 102 HBV Cirrhosis Patients with Regenerative or Dysplastic Nodules (Case)

Group	Case	Age (Year)	Gender (M/F)	HBeAg (+)	Anti-HBe (+)
Treatment	52	51.2 (35–65)	41/11	27	25
Control	50	49.7 (36–65)	40/10	27	24

Changes of Virus Markers and HBV-DNA Levels

None of the patients was detected with HBsAg loss after 36 months of drug treatment. There were no significant difference in HBeAg seroconversion between the two groups (*P*=0.8928). However, HBV-DNA levels were decreased ≥2log in patients in the treatment group compared with the control group (*P*=0.0228, Table 2).

Table 2. Changes of HBV-DNA Levels and HBeAg Status at Baseline and Post-treatment (Case)

Group	Case	HBeAg			HBV-DNA*		
		Pre-treatment HBeAg(+)	Post-treatment HBeAg seroconversion	Rate of seroconversion (%)	Pre-treatment HBV-DNA (+)	Post-treatment HBV-DNA decreased ≥2log	Effective rate (%)
Treatment	52	27	8	29.6	45	10	22.2
Control	50	27	6	22.2	40	2	5.0

Notes: *HBV-DNA quantification. Normal value< 5.0×10^2 copies/mL

Serum Antibody of Preneoplastic HCC

The testing results of the six antibodies in the two groups at baseline and after treatment are shown in Table 3. The difference in hepatocarcinogenesis rate was statistically significant between the two groups (*P*=0.0212). The average number of antibodies of preneoplastic HCC in patients that tested positive was not statistically different between the two groups after 12 months of treatment, but was statistically different after 24 months of treatment (*P*<0.01). All 102 HBV cirrhosis patients (100%) were tested positive for preneoplastic HCC antibodies. Among them only 28 (27.5%) had AFP levels higher than 20 μg/L.

Changes of Anti-URG11, Anti-URG19 and Anti-DRG2

More than 60.0% of the patients in both groups were tested positive for anti-URG11 and/or anti-URG19 antibodies at baseline, and were higher than the positive rates of other antibodies tested (Table 3). Both the positive rates of anti-URG11 and anti-URG19 were decreased to 48.1% and 46.2% respectively at 36 months of drug treatment, while the rates increased to 68.0% and 66.0% respectively in the control group (*P*=0.0417, *P*=0.0436). Positive rates of anti-DRG2 were increased to 56.0% at 36 months of drug treatment, while the control group was decreased to 36% (*P*=0.0452, Table 3).

Changes of Anti-URG11, Anti-URG19 and Anti-DRG2 in Patients Developed into HCC

Among the 102 patients, 2 in the treatment group and 9 in the control group developed HBV-associated HCC. All 11 HCC patients were tested positive for more than three of the six preneoplastic HCC antibodies, and the antibody test results were variable during the course of the disease. For example, anti-URG11 and anti-URG19, but not anti-DRG2, were always detected in hepatocarcinogenesis patients. But after drug treatment, anti-DRG2 was detected but anti-URG11 and anti-URG19 were not detected in the patients. In control group, 4 patients who developed HCC within 12-month or 24-month were treated by TACE, RFTA or PEI therapies and tested subsequently. The results indicated that the anti-URG11 and anti-URG19 were negative and anti-DRG2 was positve (Table 3).

Follow-up

Among those patients who did not develop HBV-associated HCC in the treatment endpoint, 80.0% (40/50) of patients in the treatment group and 73.2% (30/41) of patients in the control group were followed up for 2 years. The patients in the treatment group continued to take CPUL. Among them, 6 (20.0%) new patients developed HBV-associated HCC in the control group. However, only 1 patient (2.5%) developed HCC in the treatment group. There was significant difference between the two groups (χ^2=4.051, *P*=0.0441). A total of 3 (5.8%) patients developed HCC in the treatment group and 15 (30.0%) patients developed HCC in the control group during the 5 years. Statistically significant difference was found between the two groups (χ^2=10.298, *P*=0.0013, Figure 1).

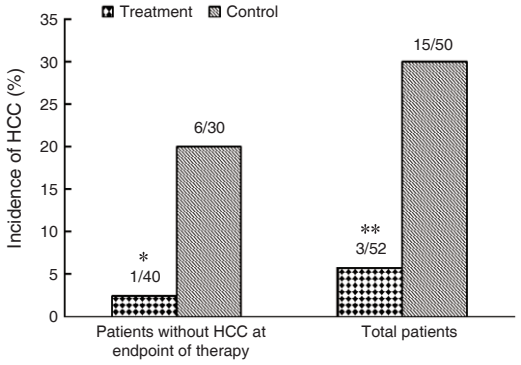

Figure 1. Incidence of HCC during A Follow-up of Five Years

Notes: $^{*}P$<0.05, $^{**}P$<0.01, compared with the control group

DISCUSSION

According to statistics, 86.5% of HCC are diagnosed from among cirrhosis patients in China.[14] Cirrhosis is the major lesion phase that causes hepatocarcinogenesis and can be diagnosed by imaging and pathologic examinations. Hepatocarcinogenesis develops from regenerative nodule (RN), low-grade dysplasia nodule (LGDN), high degree of dysplasia nodule (HGDN), HCC in center of dysplasia, a small area HCC, and finally to a larger area of HCC.[15] Recently, cirrhosis with RN and DN is considered preneoplastic, so we choose 102 patients with regenerative or dysplastic nodules as study subjects.

It is reported that the risks of LGDN, HGDN and DN with micro-focal HCC are 19.2%–21.5%, 5.7%–9.6% and 8%, respectively.[16] In cirrhosis patient, the presence of RN with micro-focal HCC is strong evidence of preneoplastia.[17] However, the prognosis of RN is not clear. Follow-up examinations of 33 patients

Table 3. Distribution of Antibodies at Baseline and After Treatment [Case (%)]

Item	Time (Month)	Treatment group (52 cases)	Control group (50 cases)	*P* value
Mean of number of antibodies ($\bar{x} \pm s$)	0	1.69 ± 1.01	1.83 ± 0.61	0.4011
	12	1.67 ± 0.78	1.72 ± 0.73	0.7391
	24	1.08 ± 1.01	2.11 ± 1.12	0.0000
	36	0.68 ± 1.17	2.72 ± 1.06	0.0000
URG4	0	26 (50.0)	27 (54.0)	0.6861
	12	27 (51.9)	25 (50.0)	0.8460
	24	26 (50.0)	28 (56.0)	0.5439
	36	25 (48.1)	30 (60.0)	0.2272
URG7	0	27 (51.9)	26 (52.0)	0.9938
	12	30 (57.7)	27 (54.0)	0.7073
	24	29 (55.8)	26 (52.0)	0.7026
	36	27 (51.9)	28 (56.0)	0.6796
URG11	0	33 (63.5)	30 (60.0)	0.7191
	12	30 (57.7)	31 (62.0)	0.6573
	24	29 (55.8)	33 (66.0)	0.2901
	36	25 (48.1)	34 (68.0)	0.0417
URG12	0	27 (51.9)	27 (54.0)	0.8336
	12	28 (53.8)	26 (52.0)	0.8519
	24	30 (57.7)	28 (56.0)	0.8630
	36	26 (50.0)	28 (56.0)	0.5439
URG19	0	31 (59.6)	30 (60.0)	0.9684
	12	30 (57.7)	28 (56.0)	0.8630
	24	27 (51.9)	31 (62.0)	0.3043
	36	24 (46.2)	33 (66.0)	0.0436
DRG2	0	24 (46.2)	22 (44.0)	0.8270
	12	25 (48.1)	22 (44.0)	0.6796
	24	27 (51.9)	20 (40.0)	0.2272
	36	29 (55.8)	18 (36.0)	0.0452
AFP (>20 ng/L)	0	2	1	
	12	1	4	
	24	3	6	
	36	3	8	
Total		9 (17.3)	19 (38.0)	0.0192
HCC was newly found by CT or MRI	0	0	0	
	12	0	1	
	24	1	3	
	36	1	5	
Total		2 (3.8)	9 (18.0)	0.0212

Notes: data are expressed as case (%) except mean of number of antibodies as $\bar{x} \pm s$

with dysplastic nodules showed that 46% of the nodules disappeared, 42% were static, and only 12% developed to HCC.[16] The regenerative nodules were similar in morphology while the prognoses were different.

HBxAg affects gene expressions and promotes the growth, survival and canceration of liver cells.[18,19] HBxAg also affects the expressions of URG4, URG7, URG11, URG12, URG19, and DRG2 in cirrhotic and premalignant lesions. HBxAg is released into the blood stream when the liver cells are damaged, and leads to the production of corresponding antibody.[11,12]

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Table 4. Presence, Fequency and Distribution of Antibodies in 11 Patients with HBV-associated HCC

Patient	1	2	3	4	5	6	7	8	9	10	11
Group	CG	CG	CG	CG	CG	CG	CG	CG	CG	TG	TG
Gender	M	F	M	M	M	M	M	M	M	M	F
Age (Year)	47	45	48	65	48	45	38	53	47	48	51
Month	0 12 24 36*	0 12 24 36*	0 12 24* 36	0 12 24* 36	0 12 24* 36	0 12 24* 36	0 12 24 36*	0 12 24 36*	0 12 24 36*	0 12 24 36*	0 12 24 36*
Treatment	TACE PCEI		TACE	TACE	TACE RITA						
URG4	− − − +	− − − +	+ + + −	− − − +	+ − − −	+ + − +	+ − + +	− − + +	− − + +	− − + +	+ + + −
URG7	+ − + −	− − − −	− − + −	− + + −	+ + − +	+ + + −	− − − −	+ + + −	− + + +	− − − −	+ − − +
URG11	+ + + −	− + + +	+ + + −	+ + + −	+ + + −	+ − + +	+ + + +	+ − − +	+ + − +	+ + + +	+ + − +
URG12	− + − +	+ + + −	+ + + −	− − + −	+ + + −	− + − −	+ + − −	− + − +	+ + − −	+ + + +	+ + − −
URG19	+ + − +	− − + +	+ + + −	+ + + −	− + + −	− + + +	− + − +	− + + +	− + + +	+ − + +	− − + +
DRG2	− − + −	− − − −	− − − +	− − − +	− − − +	− − − −	− − − −	+ + − −	− − − −	− − − −	+ − − −

Notes: antibody profiles of individual HBV patients who developed HCC during the period of observation. *The point in time when the diagnosis of HCC was made. CG: control group; TG: treatment group

These reflect changes in the expressions of HBxAg target genes, which stimulate tumor growth, and are consistent with previous results that these antibodies are associated with multistep carcinogenesis.[20]

URG11 (bata-catenin) is up-regulated by HBxAg and promotes cell growth and accumulation in agar. URG11 also accelerates tumor growth in nude mice. The presence of URG11 in serum indicates early canceration.[21,22] URG19 (vascular endothelial growth factor receptor3, VEGFR3) is another essential cancer protein that stimulates vein growth.[23-25] URG7 may inhibit Fas-mediated apoptosis.[26] Overall, the high levels of expressions of these cancer proteins activate canceration through different mechanisms including mitogen-activated protein kinase, insuline-like growth factor, nuclear factor-B, activating protein 1, signal transducer and activator of transcription[27] and phosphatidylinositol 3-kinases pathways.[28] Therefore, these antigens are up-regulated proteins that stimulate HCC. Sui1 is discovered recently as a cancer inhibitory protein.[29] DRG2 (Suil) is an elongation initiation factor in protein translation initiation that facilitates the recognition of ribosome to adenine, uracil, and guanosine (AUG).[30] In this study, DRG2 could also be found in patients with cirrhosis of liver regeneration or dysplastic nodule related by chromic HBV infection. It was probably one of the reasons that some of these cirrhosis patients did not develop HCC.

Our early immunological studies indicated that URG11 and URG19 are commonly detected in patients with HBV-associated HCC, while DRG2 and URG7 are detected in patients with dysplastic and/or regenerative nodules.[12] Therefore, the differences in the expression patterns of the six antigens in chronic HBV-infected patients suggest the differences in prognoses. In this context, it is proposed that DRG2 is a tumor suppressor in hepatocarcinogenesis. When combined with URG7, DRG2 may suppress the expression of URG7, and thereby suppress the growth of tumor.[11,12]

Chinese medicines are known to improve immune functions and suppress the development of HCC. In long-term clinical and scientific research, we have triturated CPUL. Thyagarajau, et al[31] suggested that *phyllanthus urinaria* L. decreased the risk of hepatocarcinogenesis from 85.0% to 20.0%. *Scutellaria barbata* can inhibit HepG2 cell proliferation, induce cell cycle block, and increase cell apoptosis, which may relate to the activation of flavonoids and phenolic compounds.[32] *Edible tulip* (*Cremastra appendiculata*) containing polysaccharide and phenols (hemicellulose) inhibits human colon cancer (HCT-8) and hepatoma (Bel7402) cell growth,[33] and acts as a potent inhibitor of basic fibroblast growth factor (bFGF)-induced *in vitro* and *in vivo* angiogenesis of the chorioallantoic membrane in chick embryo.[34] *Astragalus* L. contains polysaccharide, saponins and flavonoids, and improves the immune system.[35] It also displays antifibrotic effects by inhibiting collagen synthesis and proliferation in hapatic stellate cells.[36] β-elemene curcumenol and curzerenone from oil of *Curcumae volatile* can inhibit the proliferation of liver cancer cells.[37] Therefore, CPUL may suppress the development of HCC, mainly by improving the immune system, regressing liver fibrosis, inducing hepatocarcinoma cell cycle block and inhibiting angiogenesis.

Our results indicated that when long-term Chinese medicine was used as a maintenance therapy, the risk of hepatocarcinogenesis declined. In addition, our immunological studies suggested that URG11, URG19 and DRG2, rather than AFP, were useful markers in diagnosing patients who developed HCC.[12,38] Therefore, these antibodies may serve as new indicators in the treatment of preneoplastic HCC.

We also showed that CPUL suppresses HBV replication to some extent. Clinical studies showed that several patients treated with CPUL have achieved undetectable HBV-DNA levels, but relapse occurred when treatment ceased.[39-41] This result indicates that Chinese medicine slightly prevents HBV-DNA replications.[42] The antiviral treatment may be important for patients with chronic HBV infection in preventing the development of HCC, but not important for patients with cirrhosis.[43,44] George, et al[45] reported no impact of persistent virologic response with cirrhosis on HCC risk in therapy. We also demonstrate that CPUL. suppresses liver fibrosis, because the levels of liver fibrosis serum markers are much lower after drug treatment. Drug treatment may reverse the development of HCC from cirrhosis.

It is worth noticing that about 15% of hepatitis B carriers or chronic hepatitis B patients without cirrhosis develop into HBV-associated HCC in China.[46,47] In our prior observations, several patients with chronic hepatitis B directly developed HBV-associated HCC. More than three indicator antigens were detected in these patients. This suggests that HCC may develop not only from cirrhosis, but also from any phase in chronic HBV infection. Hence, it is important to test for the indicator antigens in chronic HBV infected patients, which is one of the objectives of this research.

In short, patients with early HCC are often asymptomatic. Therefore, most diagnoses are made at a later stage in patients with advanced HCC. AFP has been used as a tumor marker for HCC, although its low specificity has made its continued use controversial. This study suggests that URG11, URG19, and DRG2 could be used as the markers for the prediction of preneoplastic HCC. Chinese medicine may block or delay the development of HBV cirrhosis to HCC. Long-term clinical studies with larger sample size are needed to further validate the efficacy of CPUL in treating preneoplastic HCC.

Competing Interests

All authors declare that there are no financial competing interests.

Authors' Contributions

TONG Guang-dong and ZHOU Da-qiao designed the research. TONG Guang-dong, ZHOU Da-qiao, and HE Jing-song performed the research. TONG Guang-dong, ZHOU Da-qiao and ZHANG Xi analyzed the data. TONG Guang-dong, ZHOU Da-qiao, and WEI Chun-shan drafted the manuscript. ZHANG Xi carried out the immunoassays. HE Jing-song performed the statistical analyses. WEI Chun-shan conceived and coordinated the study. All authors read and approved the final manuscript.

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