An-Luo-Hua-Xian Pill Can Improve the Regression of Liver Fibrosis in Chronic Hepatitis B Patients Treated With Entecavir

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Research Article

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Abstract

Background & Aims: Chronic hepatitis B (CHB) can cause liver fibrosis and lead to cirrhosis even cancer, antiviral therapy can reverse liver fibrosis with limited effect, thus we aimed to evaluate the effect of An-Luo-Hua-Xian pill (ALHX) on fibrosis regression in CHB patients treated with ETV.

Methods: Treatment naïve patients with CHB from Oct 1st, 2013 to Dec 31st, 2020 were randomly treated with ETV alone or combined with ALHX (ETV+ALHX). Patients' demographic, laboratory and liver histology data before and after 78 weeks of treatment were collected. Ishak fibrosis score (F) was used and fibrosis regression means F decreased ≥ 1 after treatment.

Results: In total, 394 patients with a second liver biopsy after treatment were included in per-protocol population: 132 patients in ETV group and 262 patients in ETV+ALHX group. After 78 weeks of treatment, the fibrosis regression rate in the ETV+ALHX group were significantly higher than ETV group in baseline F ≥ 3 patients: 124/211 (58.8%) verse 45/98 (45.9%), $p=0.035$. The degradation rate of liver stiffness measurement (LSM) is also consistent with it: 154/262 (73.0%) in ETV+ALHX group and 60/132 (61.2%) in ETV group, $p=0.037$. Logistic regression analysis showed that combined with ALHX was related to fibrosis regression (OR=1.94, $p=0.018$), and family history of hepatocellular carcinoma was on the contrary (OR=0.41, $p=0.031$).

Conclusions: ETV combined with ALHX can significantly increase liver fibrosis regression in CHB patients.

1. Introduction

Chronic hepatitis B virus (HBV) infection is one of the serious public health problems endangering people's health especially in China¹. Liver fibrosis caused by chronic hepatitis B (CHB) is the key in occurrence of liver cirrhosis and liver cancer. If CHB patients are not diagnosed and treated in time, most of them will die of decompensated liver cirrhosis or HCC. CHB accounts for 30% of all liver cirrhosis death and 40% of HCC death². A large number of research results showed that effective antiviral therapy can help to improve HBV related liver fibrosis and the improvement rate of hepatic fibrosis in newly treated patients with CHB patients treated with entecavir (ETV) for 48 weeks was 32% - 39%³. Although liver fibrosis has not been improved in more than half of patients, there are still no clearly recommended anti-fibrosis drugs.

In recent decades, through continuous research and experiments, Traditional Chinese medicine (TCM) has made remarkable progress in the treatment of chronic liver disease, and many drugs for the treatment of liver fibrosis have been approved by the State Drug Administration of China. Studies have shown that TCM alone or combined with anti HBV drugs has a good effect in delaying or reversing liver fibrosis/cirrhosis^{4,5}. An-Luo-Hua-Xian pill (ALHX) is a Chinese patent drug containing more than ten kinds of TCMs, such as Rehmannia glutinosa (Di-Huang), Radices pseudo-ginseng (San-Qi) and Leech (Shui-

Zhi). “Luo” in Chinese means collateral branches of the pathway system that runs the “Qi” and blood of the whole body, “Xian” means fibrosis, “An” means to calm or pacify and “Hua” means to soften or alleviate. ALHX can regulate immunity, improve liver microcirculation, promote hepatocyte injury and repair collagen synthesis and promote collagen degradation⁶. Our previous study already found that in significant fibrosis (fibrosis grade ≥ 3) CHB patients, ETV combined with ALHX can significantly improve the regression rate of liver fibrosis/cirrhosis than ETV alone⁷. But further expand the sample size to confirm the curative effect, form a standardized treatment scheme for reversing liver fibrosis and promote its application is needed.

Thus, based on the previous research, we continued to carry out a prospective randomized controlled trial in order to further clarify the anti-fibrosis effect of ALHX in CHB patients.

2. Methods

2.1 Study design and patients

This is a multicenter, open-label prospective randomized controlled trial in 33 hospitals in mainland China. Eligible patients consented to participate in the study between Oct 1st, 2013 to Dec 31st, 2020. The inclusion criteria were: 1) 18-70 years old; 2) positive of hepatitis B surface antigen (HBsAg+) ≥ 6 months or pathologically judged as chronic HBV infection; 3) HBV DNA positive; 4) regular follow-up. Exclusion criteria were: 1) other types of viral hepatitis: hepatitis C virus infection (HCV), hepatitis D virus (HDV) or human immunodeficiency virus (HIV) co-infection and other chronic liver diseases (autoimmune hepatitis, drug-induced liver damage, genetic, nonalcoholic fatty liver); 2) decompensated manifestations of liver cirrhosis, including ascites, hepatic encephalopathy, gastrointestinal bleeding, hepatorenal syndrome, spontaneous bacterial peritonitis and other complications of liver cirrhosis or primary liver cancer; 3) unstable diabetes, hypertension, thyroid diseases and autoimmune diseases; 4) patients with serious diseases of heart, lung, kidney, brain, blood and other important organs with dysfunction; 5) patients with severe neurological and mental diseases (such as epilepsy, depression, mania, schizophrenia, etc.); 6) pregnant and lactating women.

Patients were randomly assigned to either the ETV+ALHX group or the ETV group, in the ratio of 2:1, by simple randomization with no stratification. Randomized treatment was open-label. Patients were assigned to a serial number by the study coordinator which was linked to a computer-generated randomization list assigning the treatment regimens.

All patients signed informed consent before enrollment. Procedures followed were in accordance with the Helsinki Declaration and was approved by The Ethical Committees of Peking University First Hospital. The complete protocol for the clinical trial has been registered at ClinicalTrials.gov (NCT01962155 and NCT03568578).

2.2 Liver biopsy and scoring system

Liver histological specimens (paraffin embedded) by patients who underwent a percutaneous ultrasound-guided liver biopsy will be transported to the central laboratory for unified interpretation, specimens were considered adequate for scoring with a length more than 2.0 cm and containing at least 11 portal tracts and assessed by two professional liver pathologists from Capital Medical University affiliated Beijing You-An Hospital under double-blind conditions. If the results of the two pathologists are different or the interpretation results have large difference with the local pathology, the results will be determined under the joint discussion of the third pathologist.

The evaluation of liver fibrosis (F) was divided into 0-6 stages and necroinflammatory scores was using modified histological activity index (HAI) grading by Ishak scoring and grading system, $F \geq 3$ was considered significant fibrosis⁸. Fibrosis changes after treatment: regression means F decreased ≥ 1 after treatment and progress means F increased at least one stage after treatment. Histological improvement means HAI decreased at least two grade and no progression (increased more than and equal to one stage) in fibrosis after treatment.

2.3 Endpoints

The primary endpoint includes liver fibrosis regression and liver stiffness measurement (LSM) degradation. LSM was divided into four levels^{9,10}: $LSM < 7.4$, $7.4 \leq LSM < 9.0$, $9.0 \leq LSM < 12.0$ and ≥ 12.0 , "LSM degradation" means grade after treatment decreased at least one than before. "LSM degradation" means that the level after treatment is at least one level lower than that before treatment.

Secondary endpoints included histological improvement and non-invasive fibrosis indexes degradation, biochemical, virological and serological responses. Non-invasive fibrosis indexes: fibrosis-4-score (FIB-4) and aspartate aminotransferase to platelet ratio index (APRI) were calculated by biochemical datas. FIB-4 was divided into three levels^{11,12}: $FIB-4 < 1.45$, $FIB-4 \geq 3.25$ and between them, "FIB-4 degradation" means the same as LSM. APRI was divided into three levels¹³: $APRI < 1.00$, $APRI \geq 2.00$ and between them, "APRI degradation" means the same as LSM too.

Qualitative detection of HBsAg was performed using available enzyme immunoassays (Roche Diagnostics Co., Penzberg, Germany), HBV DNA was tested by Roche COBAS AmpliPrep/COBAS TaqMan (Roche Co., Penzberg, Germany) with a minimum detection value of 20 IU/mL and quantification of serum hepatitis B core antibody (qAnti-HBc) was measured by a newly chemiluminescent particle immunoassay (Wantai Co., Xiamen, China), which were all tested in the central laboratory in Peking University First Hospital. Biochemical data and transient elastography results (liver stiffness measurement, LSM) were collected in local center.

2.4. Statistical analysis

Quantitative variables were expressed as the median (lower and upper quartiles) or mean $\pm$ standard deviation. Categorical variables were demonstrated with number and percentage. The t-test or Kruskal-Wallis analysis were used to compare continuous variables, and Chi-square or Fisher's exact tests were used to categorical variables. Logistic regression was used to analyze the related factors of fibrosis

regression. The $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS 24.0 (SPSS, Chicago, IL).

All authors had access to the study data and reviewed and approved the final manuscript.

3. Results

3.1 Study population

From Oct 1st, 2013 to Dec 31st, 2020, a total of 1328 HBsAg (+) patients were assessed, 780 CHB patients with liver biopsy were randomized. A total of 394 patients with a second liver biopsy after treatment were finally enrolled in the per-protocol population: 132 patients in the ETV group and 262 patients in the ETV+ALHX group. The enrollment protocol of patients was showed in Figure 1. Both groups were mainly male patients and the average age was about 40 years old, body mass index (BMI) is about 23-24 kg/m². There was no significant difference between two groups in the baseline demographic and clinical characteristics except HAI grade. The baseline characteristics of the patients were presented in Table 1. Baseline characteristics of patients with significant fibrosis were presented in Supplement Table 1.

Table 1
Baseline demographic and clinical characteristics.

	ETV	ETV+ALHX	<i>p</i> -value
N	132	262	
Age, year	40.68±11.58	40.92±10.07	0.831
<40, n(%)	63(47.7)	125(47.7)	
≥40, n(%)	69(52.3)	137(52.3)	
Male sex, n(%)	104(78.8)	190(72.5)	0.177
BMI(kg/m ²)	23.52±3.03	24.00±3.27	0.163
Family history of HBV infection, n(%)	57(43.2)	131(50.4)	0.177
Family history of HCC, n(%)	17(12.9)	31(11.9)	0.775
History of drinking, n(%)	4(3.0)	5(1.9)	0.491
History of smoking, n(%)	26(19.7)	65(24.9)	0.248
HBsAg positive duration, year	10(3, 20)	11(4, 20)	0.270
WBC(×10 ⁹ /L)	5.49±1.5, ,5.36	5.33±1.4, ,5.11	0.295
LY%	34.99±8.6, ,35.50	35.13±8.8, ,35.10	0.879
HGB(g/L)	146.77±16.9, ,150	144.74±16., ,147	0.259
PLT(×10 ⁹ /L)	161.08±50.1, ,158.50	156.87±52.6, ,153.00	0.447
ALT(U/L)	57.30(37.71, 106.00)	56.00(34.00, 105.71)	0.173
AST(U/L)	39.38(28.00, 67.95)	41.00(29.00, 67.95)	0.053
ALP(U/L)	86.00(73.80, 112.00)	82.46(67.00, 108.64)	0.516
GGT(U/L)	37.65(25.10, 73.80)	41.33(25.00, 78.15)	0.872
ALB(g/L)	43.37±4.5, ,43.05	42.60±4.8, ,43.00	0.650
TBiL(μmol/L)	15.40(10.80, 19.60)	14.60(11.70, 20.00)	0.251
DBiL(μmol/L)	4.95(3.35, 6.90)	4.60(3.40, 7.50)	0.123

All values shown are based on available data, and numeric data are represented as (mean±SD, median) or median(range); Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; BMI, body mass index; WBC, white blood cell; LY%, percentage of lymphocyte; HGB, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyl transferase; ALB, albumin; TBiL, total bilirubin; DBiL, direct bilirubin; Cr, creatinine; TCHO, total cholesterol; TG, triglyceride; AFP, alpha fetoprotein; INR, international normalized ratio; LSM, liver stiffness measurement; qAnti-HBc, quantitative anti-hepatitis B core antigen; HAI, liver histologic activity scores of inflammatory; F, liver fibrosis score.

	ETV	ETV+ALHX	<i>p</i> -value
Cr(μmol/L)	66.77±13.0, ,66.00	67.84±14.5, ,66.90	0.960
TCHO(mmol/L)	4.26±0.8, ,4.09	4.40±0.8, ,4.42	0.122
TG (mmol/L)	1.10±0.5, ,0.95	1.15±0.5, ,1.02	0.411
AFP(ng/mL)	4.59(2.82, 7.59)	4.60(2.75, 15.32)	0.815
INR	1.09±0.1, ,1.09	1.09±0.1, ,1.08	0.975
HBV DNA(log ₁₀ IU/ml)	5.78±1.7, ,6.01	5.63±1.9, ,5.75	0.470
HBsAg(log ₁₀ IU/ml)	3.32±0.7, ,3.41	3.36±0.7, ,3.39	0.589
HBeAg(+), n(%)	84(63.6)	138(52.7)	0.053
qAnti-HBc(log ₁₀ IU/ml)	3.73±0.7, ,3.75	3.85±0.7, ,3.91	0.149
LSM (kPa)	10.45(7.80, 16.30)	11.90(8.30, 18.00)	0.117
HAI			0.025
≤ 4, n(%)	41(31.1)	68(26.0)	
5-6, n(%)	50(37.9)	72(27.5)	
7-9, n(%)	30(22.7)	83(31.7)	
≥10, n(%)	11(8.3)	39(14.9)	
F			0.306
< 3, n(%)	34(25.8)	51(19.5)	
3-4, n(%)	68(51.5)	153(58.4)	
5-6, n(%)	30(22.7)	58(22.1)	
All values shown are based on available data, and numeric data are represented as (mean±SD, median) or median(range); Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; BMI, body mass index; WBC, white blood cell; LY%, percentage of lymphocyte; HGB, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyl transferase; ALB, albumin; TBil, total bilirubin; DBil, direct bilirubin; Cr, creatinine; TCHO, total cholesterol; TG, triglyceride; AFP, alpha fetoprotein; INR, international normalized ratio; LSM, liver stiffness measurement; qAnti-HBc, quantitative anti-hepatitis B core antigen; HAI, liver histologic activity scores of inflammatory; F, liver fibrosis score.			

3.2 Endpoints

3.2.1 Primary endpoint

Patients with baseline $F \geq 3$ in the ETV+ALHX group has a significantly higher rate (124/211, 58.8%) of fibrosis regression than in the ETV group (45/98, 45.9%) after 78w treatment, $p=0.035$. (Table 2). All patients in ETV+ALHX group also has a higher fibrosis regression rate than ETV group but without significant difference: 130/262 (49.6%) and 53/132 (40.2%), $p=0.075$. (Table 2.). The distribution of F stage at baseline and after 78w treatment for both groups were shown in Figure 2. Different baseline in fibrosis changes after treatment were showed in supplement Table 2.

Table 2
Treatment efficacy of the ETV group and ETV+ALHX group.

	ETV	ETV+ALHX	p-value
All patients, n	132	262	
ALT normalization, n(%)	83/90(92.2)	159/178(88.8)	0.449
HBV DNA decreased $\geq 2 \times \lg$, n(%)	118(89.4)	238(90.8)	0.646
HBV DNA ≤ 20 IU/ml, n(%)	100(75.8)	203(77.5)	0.702
HBeAg clearance, n(%)	26(19.7)	44(16.9)	0.498
HBeAg seroconversion, n(%)	9(6.8)	11(13.3)	0.271
Histological improvement ¹ , n(%)	61(46.2)	145(55.3)	0.087
HAI decreased ≥ 2 , n(%)	74(56.1)	173(66.0)	0.053
Fibrosis changes ² :			
Regression, n(%)	53(40.2)	130(49.6)	0.075
Progression, n(%)	30(25.1)	45(17.2)	0.185
LSM degradation ¹ , n(%)	85(64.4)	179(68.3)	0.434
FIB-4 degradation ² , n(%)	47(35.6)	96(36.6)	0.840
APRI degradation ³ , n(%)	47(35.6)	107(40.8)	0.315
F ≥ 3 patients, n	98	211	
ALT normalization, n(%)	65/70(92.9)	126/144(87.5)	0.235
HBV DNA decreased $\geq 2 \log_{10}$, n(%)	90(91.8)	191(90.5)	0.708
HBV DNA ≤ 20 IU/ml	75(76.5)	166(78.7)	0.672
HBeAg clearance, n(%)	14(14.3)	33(15.8)	0.733
HBeAg seroconversion, n(%)	1(1.0)	8(3.8)	0.174
Histological improvement ¹ , n(%)	48(49.0)	123(58.3)	0.125
HAI decreased ≥ 2 , n(%)	52(53.1)	142(67.3)	0.016
Fibrosis changes ² :			
Regression, n(%)	45(45.9)	124(58.8)	0.035
Progression, n(%)	13(13.3)	29(13.7)	0.909

	ETV	ETV+ALHX	p-value
LSM degradation ¹ , n(%)	60(61.2)	154(73.0)	0.037
FIB-4 degradation ² , n(%)	33(33.7)	77(36.5)	0.630
APRI degradation ³ , n(%)	37(37.8)	88(41.7)	0.510

We also measured fibrosis regression in LSM, APRI and FIB-4. Patients with baseline $F \geq 3$, LSM degradation rate in ETV+ALHX group (73.0%) was much higher than ETV group (61.2%), $p=0.037$. FIB-4 and APRI in the two groups before and after treatment were also showed in Table 2. The distribution of LSM, APRI and FIB-4 at baseline and after treatment and changes of them between the two groups before and after treatment were all showed in Figure 3.

3.2.2 Secondary endpoint

The normalization rate of ALT, the rate of HBV DNA decreased $\geq$ twice the log value, the rate of HBV DNA ≤ 20 IU/ml, HBeAg clearance rate and HBeAg seroconversion rate in ETV+ALHX group and ETV group were all without significant difference. (Table 2)

But the rate of HAI decreased ≥ 2 grade (in baseline $F \geq 3$ patients), ETV+ALHX group was significantly higher than ETV group (67.3% vs 53.1%, $p=0.016$). Histological improvement rate in ETV+ALHX group also has a higher trend both in all patient (55.3% vs 46.2%) and $F \geq 3$ patients (58.3% vs 49.0%). (Table 2).

3.3 Factors related to fibrosis regression

We divided significant fibrosis patients (baseline $F \geq 3$) into regression group and not regression group (the stage of baseline F is equal to or increased after treatment) according to the treatment outcome. And we found that ETV combined with ALHX had a higher rate in fibrosis regression (73.4% vs 62.1%, $p=0.035$) and family history of HCC made patients more difficult to reach fibrosis regression (7.7% vs 17.1%, $p=0.026$). Variables with $p < 0.2$ in univariate analysis were selected into logistic regression for multivariate analysis, we found that combined with ALHX, no family history of HCC, high PLT and low INR made a significant difference related to fibrosis regression. Detailed results were showed in Table 3.

Table 3
Analysis of related factors of fibrosis regression (in F $\geq$ 3 patients).

	Regression	Not regression	<i>uni</i>	<i>p</i> ¹	<i>multi</i>	<i>p</i> ²
N	169	140				
ETV+ALHX group	124(73.4)	87(62.1)	4.46	0.035	1.94(1.12, 3.37)	0.018
Age(years)	41.12 $\pm$ 10.51	42.34 $\pm$ 10.72	-1.00	0.316		
<40, n(%)	79(46.7)	58(41.4)	0.88	0.349		
Male sex, n(%)	121(71.6)	102(72.9)	0.06	0.81		
Family history of HBV infection, n(%)	81/167(48.5)	72(51.4)	0.26	0.610		
Family history of HCC, n(%)	13/168(7.7)	24(17.1)	6.39	0.011	0.41(0.18, 0.92)	0.031
History of drinking, n(%)	5(3.0)	4(2.9)	0.00	0.958		
HBsAg positive duration, year	11(4, 20)	10(2, 20)	-0.26	0.794		
BMI(kg/m ²)	23.98 $\pm$ 3.4, 23.62	24.22 $\pm$ 3.0, 24.22	-0.64	0.524		
WBC($\times 10^9$ /L)	5.47 $\pm$ 1.5, 5.30	5.32 $\pm$ 1.5, 5.18	0.77	0.443		
LY%	34.93 $\pm$ 7.8, 34.6	34.83 $\pm$ 9.6, 35.9	0.11	0.918		
HGB(g/L)	143.91 $\pm$ 17.3, 147	146.22 $\pm$ 16.7, 148	-1.19	0.237		
PLT($\times 10^9$ /L)	157.57 $\pm$ 52.2, 155.00	144.86 $\pm$ 46.0, 143.00	2.25	0.026	1.00(1.00, 1.01)	0.012
ALT(U/L)	56.00(38.00, 101.28)	56.00(35.00, 101.28)	1.16	0.265		
AST(U/L)	41.00(28.00, 70.00)	41.00(30.09, 69.26)	1.11	0.239		
ALP(U/L)	87.15(71.05, 109.22)	89.32(71.28, 114.91)	-0.11	0.914		
GGT(U/L)	46.00(28.50, 80.00)	42.78(24.91, 83.81)	-0.16	0.870		
ALB(g/L)	42.46 $\pm$ 4.9, 43.00	41.68 $\pm$ 4.8, 42.00	1.41	0.161		0.943
TBil(μ mol/L)	14.70(11.40, 20.80)	15.90(11.45, 20.30)	1.27	0.177		0.217

	Regression	Not regression	<i>uni</i>	<i>p</i> ¹	<i>multi</i>	<i>p</i> ²
DBil(μmol/L)	4.70(3.21, 7.50)	4.70(3.40, 7.65)	1.71	0.065		0.077
Cr(μmol/L)	66.97±14.1, 66.00	65.81±12.9, 65.40	0.75	0.457		
TCHO(mmol/L)	4.42±0.9, 4.31	4.23±0.7, 4.20	1.85	0.060		0.688
TG(mmol/L)	1.19±0.6, 1.01	1.08±0.5, 1.02	1.59	0.113		0.798
AFP(ng/mL)	4.53(2.94, 12.98)	5.00(3.10, 16.45)	-0.08	0.940		
INR	1.10±0.1, 1.08	1.11±0.1, 1.10	-1.43	0.154	0.10(0.01, 0.87)	0.037
HBV DNA(log ₁₀ IU/ml)	5.62±1.9, 5.68	5.62±1.7, 5.83	-0.26	0.797		
HBsAg(log ₁₀ IU/ml)	3.32±0.6, 3.38	3.22±0.6, 3.31	1.32	0.189		0.092
HBeAg(+), n(%)	93/167(55.7)	80/136(58.8)	0.30	0.583		
qAnti-HBc(log ₁₀ IU/ml)	3.88±0.6, 3.90	3.81±0.8, 3.82	0.75	0.448		
LSM (kPa)	11.90(8.50, 18.55)	13.45(8.90, 19.90)	-0.64	0.520		
HAI			2.42	0.490		
≤4, n(%)	38(22.5)	42(30.0)				
5-6, n(%)	53(31.4)	39(27.9)				
7-9, n(%)	49(29.0)	39(27.9)				
≥10, n(%)	29(17.2)	20(14.3)				
F			30.67	0.000	2.49(1.81, 3.42)	0.000
3-4	99(58.6)	122(87.1)				
5-6	70(41.4)	18(12.9)				

3.4 Safety

There were two patients in ETV+ALHX group had mild diarrhea, but after evaluated by the attending physician, symptomatic treatment was given and they all relieved soon, so the dosage was not reduced. No noticeable severe adverse effects related to the drug use were observed during the study for both groups.

In addition, two patients in ETV+ALHX group withdrew from the study due to liver cancer, but they were not related to the experimental drug after evaluation: one is 57-year-old with a family history of HCC and one is 33-year-old with a known HBsAg positive time of 29 years.

4. Discussion

In this study, we found that combined with ALHX has a significant efficacy on the regression of liver fibrosis in CHB patients using ETV, especially in baseline $F \geq 3$ patients ($p=0.035$), using transient elastography to estimate liver fibrosis, the LSM degradation rate in ETV+ALHX group was significantly higher than the ETV group ($p=0.037$) in baseline $F \geq 3$ patients, non-invasive fibrosis indexes such as FIB-4 and APRI, the degradation rates in ETV+ALHX group were also higher, although without statistical difference.

Moreover, we calculated that in significant fibrosis patients (baseline $F=3-4$), ETV+ALHX group had a higher rate of decreased to less than 3 (<3) after treatment than ETV group (30.1% vs 19.1%, $p=0.090$), and in cirrhosis patients (baseline $F=5-6$), the rate were 17.2% and 3.3% ($p=0.089$). And the rate of cirrhosis regression to less than 5 (<5) after treatment in the two groups were 75.9% and 63.3% ($p=0.217$). This tendency is consistent with other studies such as Kong et al.¹⁴ found that ETV combined with ALHX can significantly increase the survival rate of decompensated hepatitis B patients after 5-year (67.85% vs 26.19%, $p<0.01$). Animal model studies have proved that ALHX does play a positive role in anti-fibrosis: Lu. et al.¹⁵ showed it can reverse carbon tetrachloride (CCl₄) induced liver fibrosis in rats, and its mechanism may be through affecting transforming growth factor β -1 (TGF β -1), which inhibits the activation of hepatic stellate cells (HSC) and plays an anti-fibrosis role. Wang. et al.¹⁶ found that the anti-fibrosis effect of ALHX may occur through the upregulation of peroxisome proliferator-activated receptor-gamma (PPAR γ) expression and down regulation of nuclear factor-kappa B (NF- κ B) / Inhibitor α of NF- κ B (I κ B α) signaling pathway. PPAR γ plays an important role in adipocyte differentiation and lipid metabolism. Its antifibrotic effect may play a role by regulating the adipogenic phenotype of HSC.¹⁷ NF- κ B acts as a key regulator of inflammation, NF- κ B transcriptional activity increased and maintained at a high level when HSC is activated¹⁷. However, more clinical studies based on a larger population and more in-depth mechanism studies are still needed to clarify its anti-fibrosis effect.

Other Chinese patent drugs such as Biejia-Ruangan (BR) and Fu-Zheng-Hua-Yu (FZHY) also have an anti-fibrosis effect in clinical therapy. Rong et al.¹⁸ enrolled 1000 CHB patients (705 with second biopsy), the rate of fibrosis regression after 72week treatment was significantly higher in ETV+BR group than ETV group (40% versus 31.8%, $p=0.0069$). Huang. et al.¹⁹ found that BR inhibited hepatic collagen deposition and improved liver injury in CCl₄ induced hepatic fibrosis rats, which might also be associated with down regulation of TGF- β -Smad pathway. As early as 2005, a multicenter clinical trial confirmed the anti fibrosis effect and safety of FZHY in CHB patients²⁰. Gui. et al²¹ carried out a single center clinical study (46 CHB patients with second liver biopsy) showed that ETV+ FZHY had significantly higher rate of fibrosis regression (82% vs 54%, $p<0.05$). In mice with fibrosis induced by carbon (CCl₄) and

dimethylnitrosamine (DMN), it was observed that FZHY could not only have the effect of anti-fibrosis, but also improve CCl₄ and DMN induced sinus capillary formation, angiogenesis and angiogenesis related factors' expression²². Hepatic sinusoidal capillarization and angiogenesis in the fibrous septum connecting the portal vein and the central hepatic vein are two key events leading to liver cirrhosis.²³. Further controlled studies are needed to compare the efficacy of these TCMs.

Loomba²⁴ et al. found that family history of HCC multiplies the risk of HCC at HBV infection: the cumulative risk was 15.8% vs 7.5% ($p < 0.001$) with or without family history of HCC. And our study suggests that it affects the regression of liver fibrosis, so it may lead to the progression of fibrosis and eventually develop into liver cirrhosis and HCC. Therefore, in guidelines for the diagnosis and treatment of CHB, it is suggested that patients with a family history of HCC should start antiviral treatment.^{3,25}. Univariate and multivariate analysis in our study also showed that in significant fibrosis patients, there was a negative correlation between histological fibrosis regression and a family history of HCC. As we know HBV has at least nine different genotypes and genotypes B and C are the most prevalent in China and genotype C is associated with earlier progression to HCC²⁶. Our research further supports the recommendations of the guidelines and the anti-fibrosis combined with antiviral treatment of these CHB patients should start it as soon as possible. However, relationship between family history of HCC and fibrosis not to regress or the incidence of HCC still need further mechanism research to clarify.

We have several limitations. Firstly, only nearly half of patients with first liver biopsy did a second liver biopsy after treatment, although we tried our best to communicate with patients, but partly because of the COVID-19, some patients just couldn't return to hospital intime for follow-up. Secondly, ALHX is a patent Chinese drug containing more than ten kinds of TCM, we have not studied the anti- fibrosis effect of single component in depth. Our research showed that ALHX combined with antiviral therapy can significantly reverse liver fibrosis, but its specific anti-fibrosis components need to be studied. Thirdly, our study only observed the results of 78w of treatment, we still need a longer follow-up to observe and we are working on it.

5. Conclusion

In CHB patients using ETV, combined with ALHX can increase liver fibrosis regression rate after 78w treatment in baseline $F \geq 3$ patients, whether from the perspective of invasive liver biopsy or non-invasive such as LSM, etc. Logistic regression analysis also proved that ALHX is associated with the regression outcome while family history of HCC is opposite. So, in significant fibrosis patients, we suggest that antiviral therapy should be combined with ALHX as soon as possible.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with Ethical Requirements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. Informed consent was obtained from all patients for being included in the study.

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Figures

Figure 1

Flowchart of enrollment.

Figure 2

Distribution of fibrosis score in the two groups before and after 78w treatment.

Figure 3

Distribution of LSM, APRI and FIB-4 in the two groups before and after treatment (A, B and C) and changes of them between the two groups before and after treatment (D, E and F).

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