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Syndrome of liver depression and spleen deficiency is a primary TCM syndrome of response to entecavir + FuZheng HuaYu in patients with HBV-related liver fibrosis

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ABSTRACT

Objective: Although clinical studies have found that Chinese patent medicine FuZheng HuaYu tablet/capsule can promote the reversal of HBV-related liver fibrosis, not all sufferers have histopathological responses. This study aims to explore the correlation between traditional Chinese medicine (TCM) syndromes and response to entecavir + FuZheng HuaYu (ETV + FZHY) in patients with HBV-related liver fibrosis.

Methods: This a multi-center cross-sectional study. According to the different treatment strategies that sufferers have ever received, a total of 437 cases were included and divided into ETV + FZHY group and ETV + placebo group. And based on the relevant efficacy determination criteria, the two groups were subdivided into efficacy responders and non-responders. Then, TCM clinical questionnaire information of these patients were collected for subsequent analysis to acquire relevant syndrome elements and TCM syndromes.

Results: No matter what group was, the first three frequency of TCM pathological position in efficacy responders were as follows: Liver > Spleen > Stomach (TCM concepts). As for the ETV + FZHY group, the first three frequency of pathological nature was ranked as Qi deficiency > Dampness > Heat. Compared with the non-responders, the frequency of Spleen, Stomach, Qi deficiency, Heat, and Qi movement stagnation was significantly increased in the efficacy responders ($P < 0.05$). In terms of TCM syndromes, the frequency increase of Syndrome of liver depression and spleen deficiency (LDSD), in the efficacy responders, changed more obviously than the non-responders ($Chi^2 = 6.32$, $P = 0.0006$).

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Conclusions: TCM syndrome elements of Spleen, Stomach, Qi deficiency, Heat, and Qi movement stagnation were closely associated with efficacy responders with HBV-related liver fibrosis in the ETV + FZHY group. Moreover, LDSD was a primary TCM syndrome in these responders.

1. Introduction

Hepatitis B virus (HBV), a hepatotropic virus, can cause acute and chronic liver diseases with high morbidity and mortality [1,2]. Its infection is still a major public health problem around the world. As is well-known, HBV is also one of the most important etiologies for liver fibrosis. And liver fibrosis, with the characteristics of the activation of hepatic stellate cell (HSC) and progressive deposition of collagen extracellular matrix (ECM), is the common pathological basis of most chronic liver diseases and a final destination of chronic hepatocellular damage [3]. If it is not controlled in time, liver cirrhosis or decompensated cirrhosis even hepatocellular carcinoma (HCC) will occurred as time passes, seriously affecting sufferers' health and reducing their lifetimes [4]. Therefore, regressing liver fibrosis is as important as inhibiting HBV replication.

Fuzheng Huayu (FZHY), as a therapy in chronic liver diseases because of its dual anti-inflammatory and antifibrotic properties, has successfully completed phase II clinical trials [5]. Meanwhile, Entecavir (ETV) + FZHY has also proved a higher rate of necroinflammatory improvement and fibrosis regression than ETV alone in HBV and hepatitis C virus patients [5,6]. Currently, FZHY is widely used in clinical treatment of liver fibrosis and cirrhosis [7]. And the previous multi-center study of our research team also confirmed that ETV + FZHY, this therapeutic schedule of anti-virus + anti-fibrosis, can significantly improve the histological reversal rate of HBV-related liver fibrosis. However, about one third of these sufferers were still lack significant histological responses [8].

Traditional Chinese medicine (TCM) syndrome (namely "Zheng" in Chinese pinyin) is the essential reflection of the internal and external pathogenic factors of the body at a certain stage in the process of the occurrence and development of the disease [9]. And it is manifested by the corresponding symptom, tongue, pulse, physical appearance, complexion and expression, which can reveal the pathogenesis, location, nature, and progression of disease [10]. Furthermore, the pathological constitution associated with the TCM syndrome can determine the internal tendency of the disease to a certain extent [11,12]. Although TCM has certain advantages in treating HBV-related liver fibrosis, not all patients have a histological response to the therapeutic schedule. Considering these current situations above, our study aims to observe the epidemiology characteristics of TCM syndrome about HBV-related liver fibrosis, and then explore the TCM syndrome characteristics of patients in response to therapeutic effect, thereby providing more precise protocol for the clinical guidance of the therapeutic strategy and then improving clinical efficacy.

2. Materials and methods

This research protocol was approved by Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (Ethical approval number: 2014-331-27-01). Details can be found in Fig. S1 (Supplementary material). Furthermore, all included subjects volunteered to sign informed consent. The experiments conformed to the principles set out in the Declaration of Helsinki and the NIH Belmont Report.

2.1. Data sources

This is a cross-sectional study. The cases included in this study were 437 patients with HBV-related liver fibrosis attending the inpatient department and outpatient department of 20 hospitals around the China from September 9, 2014 to October 25, 2018.

2.2. Diagnostic criteria

Chronic hepatitis B (CHB) is diagnosed according to the *Diagnostic criteria for chronic hepatitis B* [13]. Liver fibrosis is determined according to the *Guidelines for the diagnosis and treatment of liver fibrosis in integrative medicine practice* [14].

The diagnosis of TCM syndrome is based on a physician's assessment of the patient through the Four Examinations of TCM: inspection, auscultation and olfaction, inquiry, and palpation. These examinations are used to gather relevant information about the TCM pathological position and nature characteristics, distinguishing their primary and secondary relationships. This process allows for the comprehensive organization and summarization of the main TCM syndrome elements, ultimately leading to the diagnosis of each patient's TCM syndrome.

Specifically, "inspection" involves observing changes in a patient's spirit, color, form, and demeanor. "Spirit" refers to their mental and spiritual state, "color" relates to the external appearance of the organs' qi and blood in terms of complexion, "form" pertains to signs of bodily fullness or weakness, and "demeanor" encompasses dynamic expressions of agility or sluggishness. This involves observing the patient's face, mouth, nose, teeth, tongue, coated tongue, limbs, and skin to understand their "spirit".

"Auscultation and olfaction" entail listening to the patient's voice, breathing, coughing, vomiting, belching, and other sounds they make. It also involves using the sense of smell to detect the patient's body odor, bad breath, phlegm, urine, and feces odors.

"Inquiry" involves asking the patient about the onset and progression of their condition, as well as inquiring about symptoms such as chills, fever, sweating, head and body sensations, urination, defecation, diet, chest and abdominal sensations, ears, mouth, and various other conditions.

"Palpation" includes pulse diagnosis and touch examination. Pulse diagnosis involves feeling the pulse and interpreting its characteristics, while touch examination involves using the hands to touch and assess various parts of the patient's body to determine factors like temperature, hardness, resistance, or tenderness, aiding in the diagnostic process.

This is how TCM physicians assess patients to identify and diagnose their specific TCM syndromes. In this study, syndrome differentiation of TCM is diagnosed according to *the Science of syndrome differentiation on syndrome element* [15].

2.3. Inclusion criteria

(i) Previous history of hepatitis B or HBV-DNA positive ≧ 6 months. (ii) Age 18–65 years old. (iii) Two liver biopsies involving in the histologic evaluation before and after treatment. (iv) The assessment of liver fibrosis before treatment: Ishak score ≧ F2 and Child-Pugh score grade A. (v) Completed 48 weeks of treatment course. Histological characteristics of normal liver and fibrotic liver tissues including Ishak staging can be found in Fig. S2 (Supplementary material).

2.4. Exclusion criteria

(i) Decompensated cirrhosis or even liver cancer. (ii) Entecavir resistance. (iii) Severe diseases related to heart, spleen, lung, renal, encephalopathy, or psychosis. (iv) Uncontrollable diabetes. (v) Immunodeficiency.

2.5. Clinical grouping and efficacy criteria

According to the different intervention measures, all the included patients were divided into control group (ETV + Placebo) and treatment group (ETV + FZHY). The placebo is identical in appearance and packaging to the experimental drug (FZHY). Each capsule contains 0.4 g of starch, and the recommended dosage is four capsules per administration, which equals 1.6 g per dose. The regimen calls for three administrations per day, totaling 4.8 g daily. It is essential to take the medication after meals. Besides, the efficacy

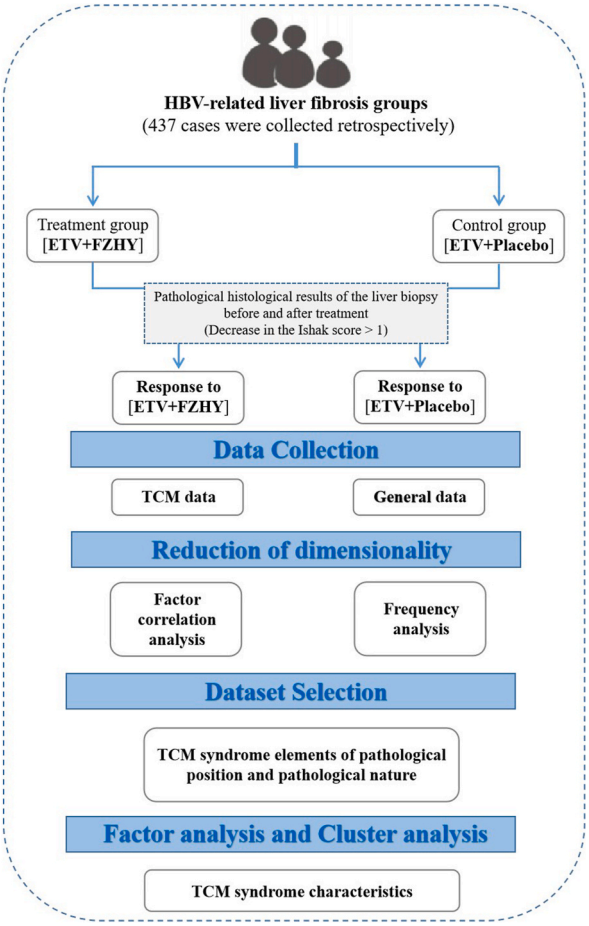

Fig. 1. Flow chart of this study.

determination criteria were based on the pathological histological results of the liver biopsy before and after treatment, a 1-point decrease at least in the Ishak score was regarded as effective, or ineffective [16].

2.6. Methods

TCM symptoms and signs of the included patients before treatment were collected retrospectively. Based on the clinical TCM data and previous document researches, a total of 88 items of TCM four diagnostic methods (inspection, auscultation and olfaction, inquiry, and palpation) were established as TCM questionnaire of HBV-related liver fibrosis. And then, all collected data were transferred to the computer for subsequent analysis. The researchers involved in the information collection are TCM practitioners engaged in clinical work in liver department or institute of liver diseases. And they are trained in a unified manner. During the period, the relevant data of each center will be checked regularly by telephone.

2.7. Statistics

SPSS version 26.0 software was used for statistical analysis. Measurement indicators directly record the original data while qualitative indicators are quantified and then input. If the data fit a normal distribution and homogeneity of variance, One-way ANOVA was performed. If statistical differences existed between groups, the Tamhane test was further used for pairwise comparison. If the data did not conform to the normal distribution or had heterogeneity of variance, either the Kruskal-Wallis test or the Games-Howell test was analyzed. Frequency number analysis was used to study the distribution of the patient's basic data obtained from the questionnaire, and then used the KMO test and the Bartlett's spherical test as the adaptability test to evaluate the feasibility of the factor analysis. Finally, the methods of factor analysis and cluster analysis were used to analyze the distribution of the syndrome characteristics in patients with HBV-related liver fibrosis. Count data were expressed as n (%), which were analyzed for statistical analysis using chi-square (χ^2) test. Pairwise comparisons between the frequency distributions were analyzed by meta-quantification. In order to identify the relevant covariates, multivariate logistics regression analysis was used to correct the effects of sex, age, and BMI factors on the outcomes of our study. Test level was $\alpha = 0.05$; Statistical differences determined as $P < 0.05$.

3. Results

The flow chart of this cross-sectional study was illustrated in Fig. 1.

3.1. General information

A total of 437 sufferers (including 335 males and 102 females) with HBV-related liver fibrosis were involved in this study. There were effective crowds and ineffective crowds respectively between the two groups. Among them, the average age was 49.91 ± 9.05 and the average body mass index (BMI) was 23.55 ± 3.06. No matter what gender, age or BMI was, the differences were not statistically significant through statistical analysis ($P > 0.05$), which indicated that they were comparable. Detailed information can be found in Table 1 and Fig. 2.

3.2. Extraction of syndrome elements

3.2.1. Factor correlation analysis

Because of $KMO = 0.912 > 0.05$, this data was considered acceptable for factor analysis. Additionally, the *Bartlett*'s spherical test approximated χ^2 value was 12547.007 ($P = 0.000$), indicating a good correlation among the variables.

3.2.2. Frequency analysis

Frequency statistics were performed on 88 symptoms and signs of TCM questionnaires. Among these, 64 pieces of symptomatic information with a frequency >10 % were selected. Besides, 22 pieces of information with a frequency >5 % were selected from tongue, pulse, and pain types. Finally, a total of 86 pieces of information about TCM four diagnostic methods were included.

Table 1
General data of patients with HBV-related liver fibrosis.

Groups	Gender		Age	BMI
	Male	Female		
F–R	104	35	49.10 ± 9.00	23.07 ± 3.23
F-NR	71	12	50.19 ± 9.50	23.66 ± 2.65
E-R	89	20	49.36 ± 8.78	23.68 ± 2.66
E-NR	71	25	51.31 ± 8.96	23.96 ± 3.45
P value	0.215		0.247	0.134

Annotations: F–R=Response to FZHY + ETV. **F-NR**= No-response to FZHY + ETV. **E-R**=Response to ETV + Placebo. **E-NR**=No-response to ETV + Placebo.

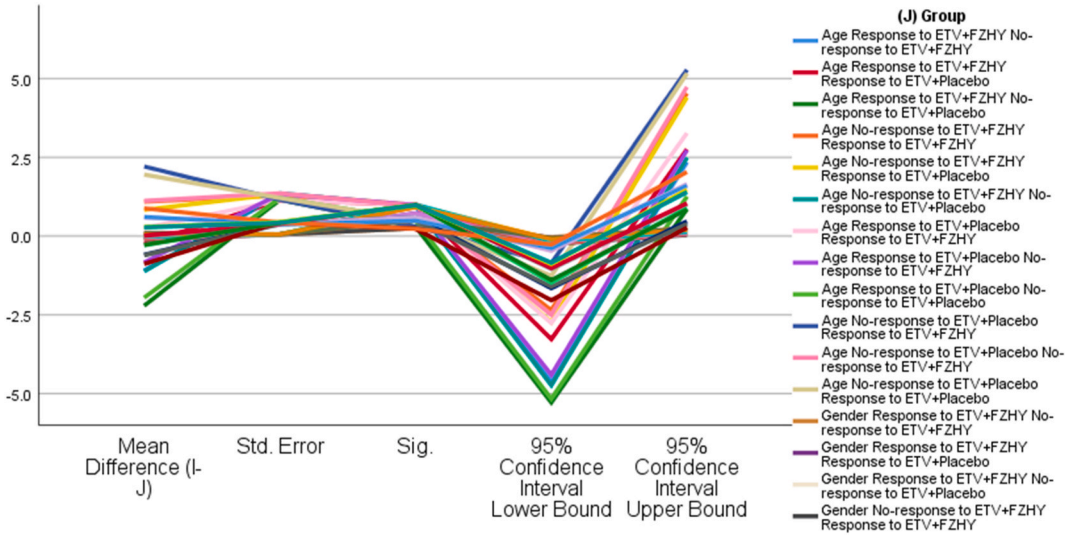

Fig. 2. Results of pairwise comparisons between groups.

3.3. Factor analysis

3.3.1. Common factor extraction on TCM four-diagnosis information

According to the factor analysis rules and the TCM practice status of HBV-related liver fibrosis, 26 common factors with a characteristic value > 1 were extracted. Using principal component analysis (PCA), the factor load matrix was obtained with 36 iterations through the rotation by Caesar-normalized maximum variance method. The 26 common factors in the symptom group of these patients were screened out according to the factor load coefficient with the absolute load value > 0.5. Ultimately, the corresponding syndrome elements of pathological position and pathological nature of each common factor were obtained. Relevant Details were shown in Table 2.

3.3.2. Frequency distribution of TCM syndrome elements in efficacy responders and non-responders

Based on the above common factors distribution, the frequency distribution of seven syndrome elements of pathological position and 10 syndrome elements of pathological nature were calculated (Fig. 3A and B). Then this frequency distribution between the efficacy responders and non-responders in the two groups were counted and compared (Table 3). No matter what group was, the frequency of pathological position in efficacy responders was ranked from high to low as follows: Liver > Spleen > Stomach > Gallbladder > Heart > Lung and Kidney (TCM concepts). As for the ETV + FZHY group, the frequency of pathological nature in efficacy responders was ranked as Qi deficiency > Dampness > Heat > Stasis > Qi movement stagnation > Blood deficiency > Yang hyperactivity > Qi stagnation > Yin deficiency and Yang deficiency. As for the ETV + Placebo group, the frequency of pathological nature in efficacy responders was listed as Dampness and Qi deficiency > Heat and Stasis > Blood deficiency > Qi movement stagnation > Yang deficiency > Yin deficiency and Yang hyperactivity > Qi stagnation. Besides, compared with the non-responders in ETV + FZHY group, the frequency of Spleen, Stomach, Heat, Qi movement stagnation, and Qi deficiency was significantly increased in the efficacy responders ($P < 0.05$).

3.4. Cluster analysis

3.4.1. Cluster analysis for TCM syndrome

As shown in Table 4, using R-type clustering, the Euclidean square distance was selected to cluster the 26 common factors into seven categories based on the dendrogram and clinical practice.

3.4.2. Frequency distribution of TCM syndromes in efficacy responders and non-responders

As for the ETV + FZHY group, the frequency of TCM syndromes in efficacy responders was ranked from high to low as follows: Syndrome of liver depression and spleen deficiency (LDSD) > Syndrome of liver-gallbladder dampness-heat (LGDH) > Syndromes of liver-kidney yin deficiency and spleen-kidney yang deficiency (LKYD/SKYD) > Syndrome of liver fire flaming upward (LFFU) > Syndrome of heart-kidney noninteraction (HKnI) > Syndrome of dampness-heat in the spleen and stomach (DHSS) > Syndrome of lung qi deficiency (LQD). The ranking list of non-responders in this group was LDSD > LGDH > LKYD/SKYD > DHSS > LFFU > LQD > HKnI. As for the ETV + Placebo group, the TCM syndromes in efficacy responders ranked from high to low were LDSD > LGDH > LKYD/SKYD > DHSS > LFFU > HKnI > LQD. The ranking list of non-responders in the control group was LDSD > LGDH > LKYD/SKYD > LFFU > DHSS > LQD > HKnI. Details of these above frequency distribution and rankings were shown in Table 5 and Fig. 4 (A and B).

Table 2
Common factors on TCM four-diagnosis information.

Common factors	TCM four diagnostic information	Syndrome elements of pathological position	Syndrome elements of pathological nature	Frenquency (%)
F1	vexation (0.469), long face (0.514), depression (0.532), irritableness (0.375)	Liver	Qi movement stagnation; Heat	25 (5.72 %)
F2	heavy-headedness (0.627), giddy head (0.641), unwilling to use your mind (0.563)	/	Dampness	6 (1.37 %)
F3	dryness in the eyes (0.557), blurred vision (0.542), tired eyes (0.659), swollen and painful eyes (0.505)	Liver	Blood deficiency; Qi deficiency	20 (4.58 %)
F4	down energy (0.776), reduced strength (0.672), lassitude in legs (0.632), heavy body (0.669), weak (0.648), sleepy (0.653), mind uncomfortable (0.597), lassitude in loin (0.501)	Spleen	Qi deficiency; Dampness	64 (14.65 %)
F5	chest distress (0.511), breathe hard (0.486), flustered (0.432), dizziness (0.443)	Lung、Liver	Qi deficiency	2 (0.46 %)
F6	undersleep (0.548), difficulty falling asleep (0.690), sleep lightly (0.689), dreaminess (0.443)	Heart	Yang hyperactivity	4 (0.92 %)
F7	nocutria (0.623), dark urine (0.465)	Kidney	Yin deficiency	1 (0.23 %)
F8	easy cold (−0.383), easy hunger (0.448)	Stomach	Qi deficiency; Yin deficiency	1 (0.23 %)
F9	skin itch (0.668), dental ulcer (0.464)	/	Blood deficiency; Yin deficiency	1 (0.23 %)
F10	feverish sensation of five centers (0.643), sweat more (0.673), night sweat (0.638), the whole body heat (0.430)	/	Yin deficiency	1 (0.23 %)
F11	obstructed stool (0.669), dry stool (0.470), bitter taste (0.660), ozostomia (0.589), xerostomia (0.449), sticky mouth (0.423), tinnitus (0.474)	Liver、Spleen、Stomach	Dampness; Heat	75 (17.16 %)
F12	headache (0.660), slight pain on head (0.821), swelling pain on head (0.800)	/	Qi deficiency; Stasis	5 (1.14 %)
F13	the whole body pain (0.599), slight pain on the whole body (0.739), leg cramp (0.369), sensation of chill (0.403)	Liver	Blood deficiency; Yang deficiency	8 (1.83 %)
F14	hiccup (0.349)	Stomach	Heat	0 (0 %)
F15	poor appetite (0.694), anorexia greasy food (0.549), nausea (0.250)	Spleen、Stomach、Gallbladder	Dampness	16 (3.66 %)
F16	abdominal pain (0.814), abdominal slight pain (0.811), hypochondriac pain (0.733), hypochondriac slight pain (0.738)	Liver、Spleen、Stomach	Qi deficiency; Stasis	102 (23.34 %)
F17	distension and depression (0.354), loose stool (0.447), abdominal distension (0.376)	Spleen、Stomach	Qi deficiency	13 (2.97 %)
F18	edema of lower limbs (0.723)	Kidney	Yang deficiency	0 (0 %)
F19	bleeding gums (0.380), red tongue (0.826), the whole body soreness (0.847)	/	Heat; Dampness	4 (0.92 %)
F20	abdominal distension and pain (0.701), hypochondriac distension and pain (0.773)	Liver、Spleen、Stomach	Qi stagnation	6 (1.37 %)
F21	yellowish coating (0.888), yellow coating (0.844)	/	Dampness; Heat	15 (3.43 %)
F22	liver palms (0.641), darkish complexion (0.729), varicose sublingual veins (0.381)	Liver	Stasis	25 (5.72 %)
F23	icteric sclera (0.726), color yellow staining (0.743), tender tongue (0.438), spider angioma (0.432)	Liver、Gallbladder	Dampness; Heat; Stasis	21 (4.81 %)
F24	tongue with tooth prints (0.797), cyanosed sublingual veins (0.369), yellowish complexion (−0.483)	Spleen	Qi deficiency; Stasis	5 (1.14 %)
F25	string pulse (0.726), red complexion (0.757)	Liver	Heat	15 (3.43 %)
F26	pale complexion (0.510), fat tongue (0.670), weak tongue (−0.834), white coating (−0.784)	/	Qi deficiency	2 (0.46 %)

Additionally, to compare the differences between the efficacy responders and non-responders, a quantitative meta-analysis using a random effects model was conducted to analyze the distribution frequency of these TCM syndromes between the two groups (Fig. 5). In terms of ETV + FZHY group, the frequency increase of LDSD ($P = 0.0432$), LGDH ($P = 0.0121$), LKYD/SKYD ($P = 0.0095$), and LFFU ($P = 0.0077$) in the efficacy responders, especially LDSD, changed more obviously than the non-responders ($\chi^2 = 6.32$, $P = 0.0006$) (Table 5 and Fig. 5A), indicating that these three TCM syndromes could be favorable ones for the efficacy response. In terms of ETV + Placebo group, the frequency increase between responders and non-responders was not obvious among TCM syndromes. And there was no statistically significant difference between the two groups ($\chi^2 = 2.16$, $P = 0.86$) (Table 5 and Fig. 5B).

3.5. Multivariate logistics regression analysis

In order to adjust potential confounding factors (including gender, age and BMI) to ensure the validity of our conclusions, multivariate logistics regression analysis was performed. As shown in Table 6, when LGDH was used as a reference group, after adjusting BMI and gender or age, results showed that both age and gender failed to influence the outcomes of our study. However, after adjusting gender and age, patients with high BMI had potential risk of the onset of LGDH compared to LDSD (RR = 0.381) and LKYD/

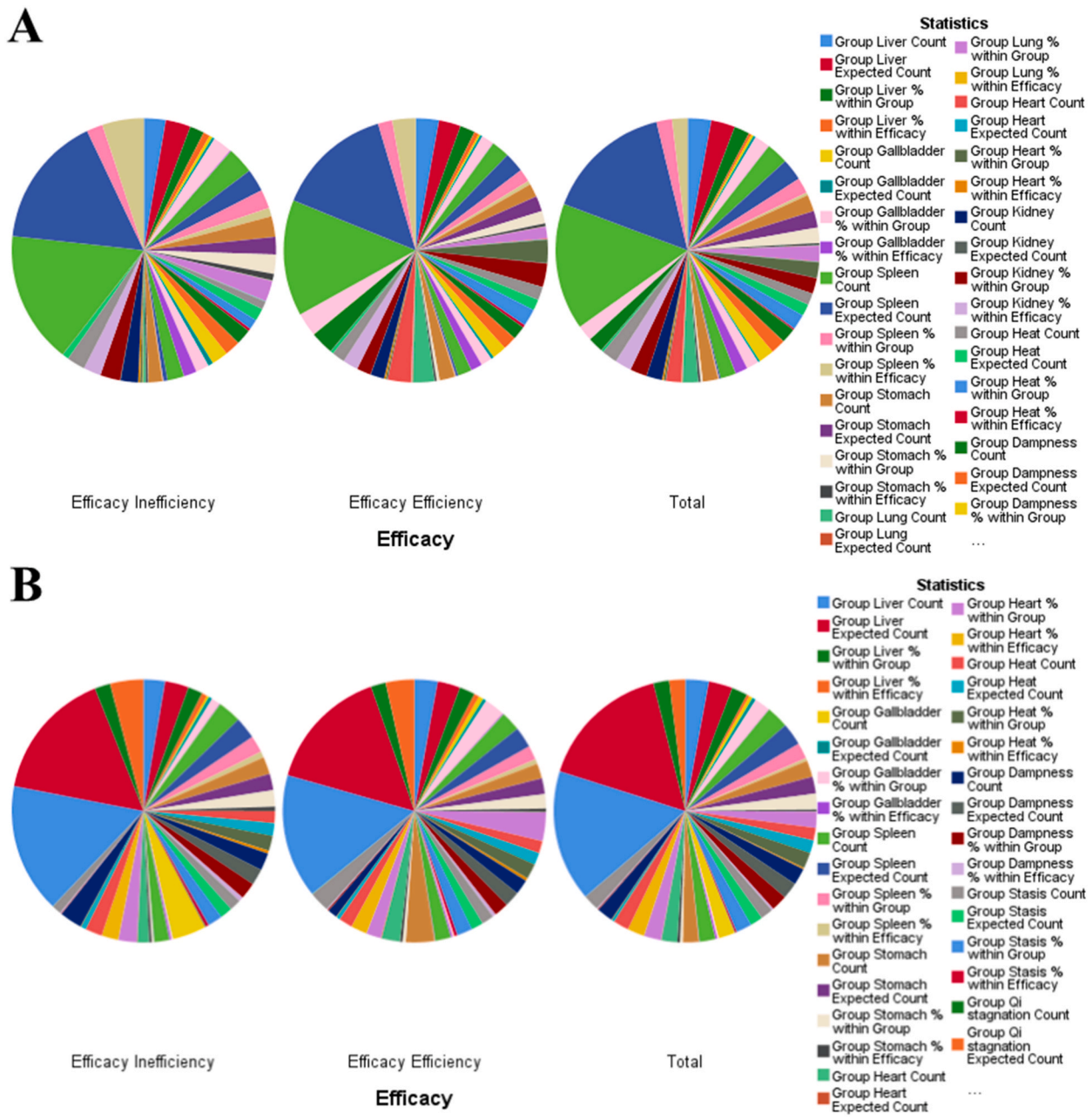

Fig. 3. Frequency distribution of syndrome elements between the two groups (A. ETV + FZHY group; B. ETV + Placebo group).

SKYD (RR = 0.277) among TCM groups.

4. Discussion

Over recent decades, liver fibrosis has increased markedly around the world especially China, which could be associated with viral hepatitis, alcohol liver, fatty liver, and autoimmune liver diseases [17]. As one of the most important etiologies for liver fibrosis, HBV infection is still a serious public health problem. Therefore, early diagnosis and treatment for HBV-related liver fibrosis are very important. Removal of the etiology by antiviral therapy is beneficial for blocking and reversing liver tissue fibrosis. In China, the clinical practice of anti-virus + anti-fibrosis indicates that the combination therapy significantly improves the clinical efficacy [5–8]. FZHY is one of the Chinese patent medicines against liver fibrosis recommended by the guidelines. Although the therapeutic schedule of anti-virus + anti-fibrosis (ETV + FZHY) could achieve certain satisfactory effects, some same patients with HBV-related liver fibrosis did not see improvements in health [8]. This actuality could be related to abnormalities of internal biological indexes and different

Table 3
Comparison of frequencies in efficacy responders and non-responders on syndrome elements.

Syndrome elements	F–R (n = 139)	F-NR (n = 83)	Chi-squared value	P value	E-R (n = 109)	E-NR (n = 106)	Chi-squared value	P value
Liver	99 (71.22 %)	53 (63.86 %)	1.6853	0.1944	82 (75.23 %)	66 (62.26 %)	1.7316	0.1882
Gallbladder	8 (5.76 %)	7 (8.43 %)	0.3580	0.5496	16 (14.68 %)	6 (5.66 %)	1.1035	0.2947
Spleen	77 (55.4 %)	64 (77.11 %)*	6.7396	0.0095	69 (63.30 %)	71 (66.98 %)	0.2585	0.6113
Stomach	58 (41.73 %)	50 (60.24 %)*	6.3911	0.0115	53 (48.62 %)	52 (49.06 %)	0.0166	0.8974
Lung	1 (0.72 %)	1 (1.2 %)	0.0039	0.9493	0 (0 %)	0 (0 %)	–	–
Heart	3 (2.16 %)	0 (0 %)	–	–	1 (0.92 %)	0 (0 %)	–	–
Kidney	1 (0.72 %)	0 (0 %)	–	–	0 (0 %)	0 (0 %)	–	–
Heat	57 (41.01 %)*	19 (22.89 %)	9.7287	0.0018	43 (39.45 %)	36 (33.96 %)	1.1357	0.2877
Dampness	58 (41.73 %)	38 (45.78 %)	0.2974	0.5852	52 (47.71 %)	52 (49.06 %)	0.0572	0.8110
Stasis	48 (34.53 %)	32 (38.55 %)	0.5563	0.4556	43 (39.45 %)	35 (33.02 %)	0.0269	0.8694
Qi stagnation	2 (1.44 %)	1 (1.2 %)	0.0518	0.8199	0 (0 %)	3 (2.83 %)	–	–
Qi movement stagnation	13 (9.35 %)*	1 (1.2 %)	5.0860	0.0242	6 (5.5 %)	5 (4.72 %)	1.1035	0.2947
Yang hyperactivity	3 (2.16 %)	0 (0 %)	–	–	1 (0.92 %)	0 (0 %)	–	–
Blood deficiency	7 (5.04 %)	5 (6.02 %)	0.1392	0.7091	11 (10.09 %)	6 (5.66 %)	1.5561	0.2128
Qi deficiency	60 (43.17 %)	48 (57.83 %)[a]	5.3140	0.0211	52 (47.71 %)	55 (51.89 %)	0.1929	0.6604
Yin deficiency	1 (0.72 %)	0 (0 %)	–	–	1 (0.92 %)	2 (1.89 %)	0.0805	0.7767
Yang deficiency	1 (0.72 %)	0 (0 %)	–	–	5 (4.59 %)	2 (1.89 %)	0.2929	0.5886

Annotations: F–R=Response to FZHY + ETV. **F-NR**= No-response to FZHY + ETV. **E-R**=Response to ETV + Placebo. **E-NR**=No-response to ETV + Placebo. With theoretical frequencies <1, Fisher's Exact Test was conducted. *$P < 0.05$ *vs* F-NR.

Table 4
Cluster of TCM syndromes in patients with HBV-related liver fibrosis.

Categories	Common factors	TCM syndrome	Frequency (%)
Category 1	F1, F12, F16, F17, F20, F22, F24, F26	LDSD	183 (41.88 %)
Category 2	F2, F11, F21, F23	LGDH	117 (26.77 %)
Category 3	F3, F4, F9, F13, F18	LKYD/SKYD	93 (21.28 %)
Category 4	F5, F8	LQD	3 (0.69 %)
Category 5	F6, F7, F10	HKnI	6 (1.37 %)
Category 6	F14, F15	DHSS	16 (3.66 %)
Category 7	F19, F25	LFFU	19 (4.35 %)

Annotations: LDSD=Syndrome of liver depression and spleen deficiency. **LGDH**=Syndrome of liver-gallbladder dampness-heat. **LKYD/SKYD**=Syndromes of liver-kidney yin deficiency and spleen-kidney yang deficiency. **LQD** = Syndrome of lung qi deficiency. **HKnI**=Syndrome of heart-kidney noninteraction. **DHSS**=Syndrome of dampness-heat in the spleen and stomach. **LFFU**=Syndrome of liver fire flaming upward.

Table 5
Comparison of frequencies in efficacy responders and non-responders on TCM syndromes.

TCM syndromes	F–R (n = 139)	F-NR (n = 83)	Chi-squared value	P value	E-R (n = 109)	E-NR (n = 106)	Chi-squared value	P value
LDSD	60 (13.73 %)*	39 (8.92 %)	4.0793	0.0432	40 (9.15 %)	44 (10.07 %)	0.1575	0.6919
LGDH	36 (8.24 %)*	19 (4.35 %)	6.2984	0.0121	33 (7.55 %)	29 (6.64 %)	0.2934	0.5881
LKYD/SKYD	26 (5.95 %)*	16 (3.66 %)	6.7305	0.0095	26 (5.95 %)	25 (5.72 %)	0.0267	0.8707
4LQD	1 (0.23 %)	1 (0.23 %)	–	–	0 (0 %)	1 (0.23 %)	–	–
5HKnI	4 (0.92 %)	0 (0 %)	–	–	2 (0.46 %)	0 (0 %)	–	–
6DHSS	3 (0.69 %)	6 (1.37 %)	3.3625	0.0667	4 (0.92 %)	3 (0.69 %)	0.0065	0.9365
7LFFU	9 (2.06 %)*	2 (0.46 %)	7.1039	0.0077	4 (0.92 %)	4 (0.92 %)	–	–

Annotations: Fisher's Exact Test. *$P < 0.05$ *vs* F-NR. **LDSD=**Syndrome of liver depression and spleen deficiency. **LGDH**=Syndrome of liver-gallbladder dampness-heat. **LKYD/SKYD**=Syndromes of liver-kidney yin deficiency and spleen-kidney yang deficiency. **LQD** = Syndrome of lung qi deficiency. **HKnI**=Syndrome of heart-kidney noninteraction. **DHSS**=Syndrome of dampness-heat in the spleen and stomach. **LFFU**=Syndrome of liver fire flaming upward.

external symptoms. Considering these, in the case of patients with comparable baseline, our study, based on data mining technologies, conducted the researches on TCM syndromes and syndrome elements, thereby finding the TCM clinical characteristics of efficacy responders and then providing more precise therapeutic strategy and improving clinical efficacy.

Syndrome differentiation is the basic principle of diagnosis and treatment of diseases in TCM. The understanding of the "Zheng" (TCM syndrome) of the disease determines the principles and methods of treatment. In terms of study on TCM syndromes, on one hand, both factor analysis and cluster analysis are currently common multivariate statistical methods. Through these statistical methods, dimension reduction is analyzed for the four-diagnosis information, which makes the high-dimensional complex structure flattened. And it presents the TCM syndrome characteristics of real-world diseases [18]. Syndrome elements can reflect the disease

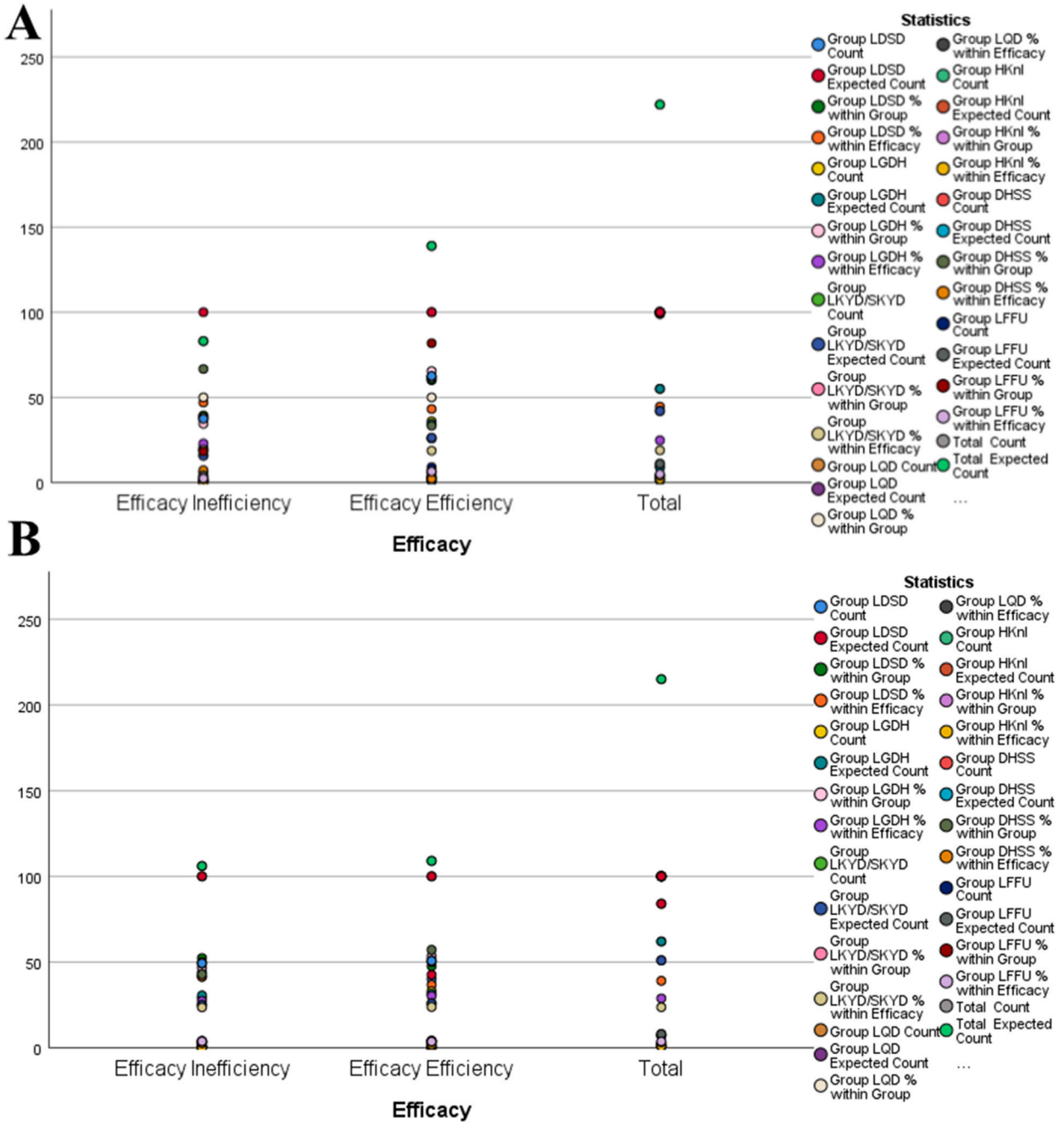

Fig. 4. Frequency distribution in efficacy responders and non-responders on TCM syndromes (A. ETV + FZHY group; B. ETV + Placebo group).

characteristics of pathological position and pathological nature. Meanwhile, TCM syndromes can further reflect the combination law of these position and nature. The integration of syndrome elements and TCM syndromes can enable us to have a more three-dimensional understanding for the disease [19]. On the other hand, The purpose of this study is to investigate the baseline TCM syndromes characteristics of the HBV related liver fibrosis patients with response to ETV + FZHY treatment, and to explore the individual differences of treating the same disease with different methods and TCM syndromes realm convergence of these responders. At enrollment, symptoms and signs were considered as independent variables. Be included in this study as long as the disease diagnostic criteria and criteria for efficacy of Ishak in liver biopsy before and after treatment were met. Moreover, from the perspective of the basic theory of TCM, since each patient is an independent individual, although suffering from the same disease, differences in symptoms and signs will inevitably occur due to different dietary structure and living habits. And the differences between the distribution of symptoms and signs can exactly reflect the thought of TCM syndrome differentiation so as to explore the TCM syndrome

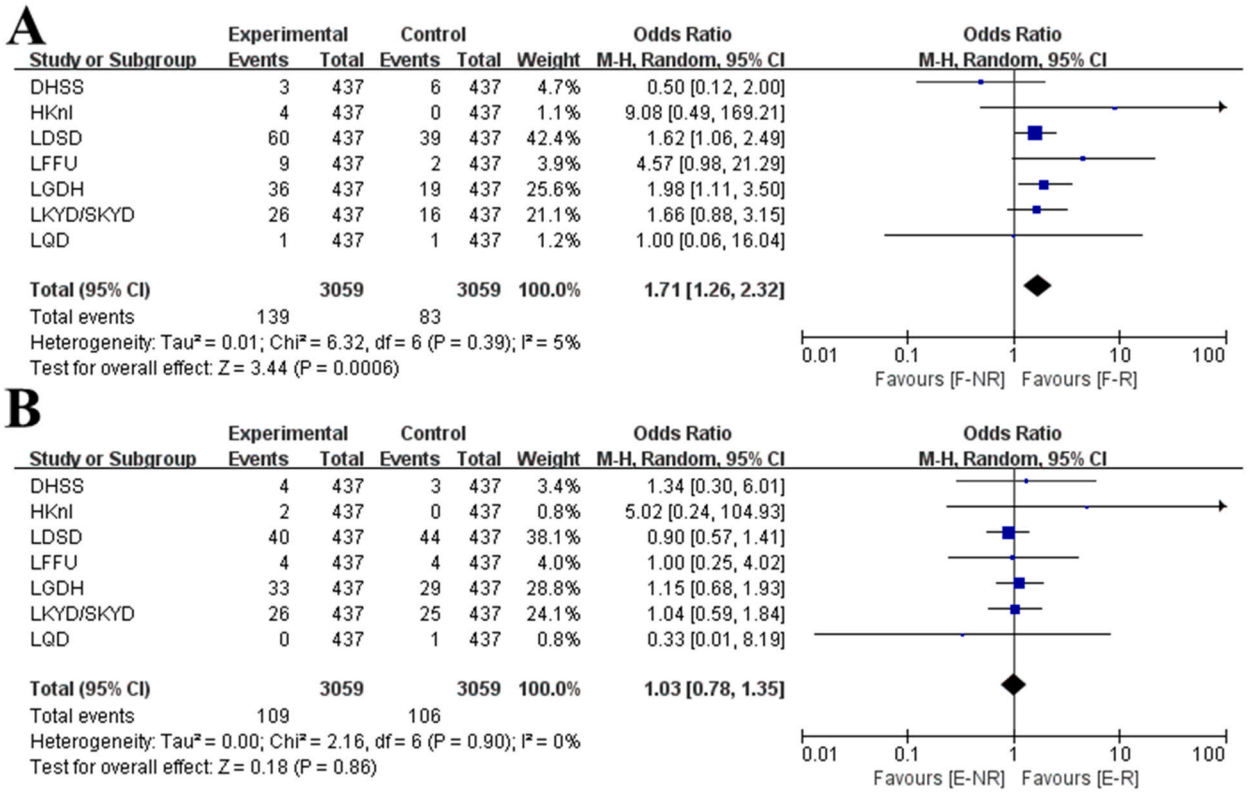

Fig. 5. Meta-analysis of the distribution frequency of the TCM syndromes (A. ETV + FZHY group; B. ETV + Placebo group).

characteristics of these responders. Therefore, it can be concluded that the difference in efficacy is caused by the correlation between the treatment plan and the differences in symptoms and signs.

Our findings, in terms of the treatment of HBV-related liver fibrosis, can reveal the differences of syndrome elements and TCM syndromes in efficacy responders and non-responders. Meanwhile, multivariate logistics regression analysis indicated that both age and gender failed to influence the outcomes of our study. On one hand, as for the frequency distribution of syndrome elements, no matter what treatment strategy was, the first three frequencies of pathological position in efficacy responders were Liver, Spleen, and Stomach (TCM concepts). Moreover, liver, a syndrome element as the highest frequency of pathological position, was a key role in this disease. Besides, Qi deficiency, Dampness, and Heat were the first three frequencies of syndrome elements of pathological natures in efficacy responders. In the ETV + FZHY group, compared with the non-responders, Heat and Qi movement stagnation had significantly increased frequency in the responders. These results of syndrome elements mentioned above suggested they could be closely involved in the formation of TCM syndrome characteristics of the efficacy responders with HBV-related liver fibrosis. On the other hand, as for the frequency distribution of TCM syndromes, whatever therapeutic strategy was, LDSD ranked first, followed by LGDH and LKYD/SKYD. Furthermore, in the ETV + FZHY group, these three TCM syndromes especially LDSD in the efficacy responders had higher frequency increase than those in non-responders, suggesting the three syndromes could be favorable ones for the efficacy response. When combined with the above results of syndrome elements and TCM syndromes for analysis, we found that the findings were consistent with our expectations. Meanwhile, these selected syndrome elements were closely related to these "Zheng" (including liver depression and spleen deficiency, liver-gallbladder dampness-heat, dampness-heat in the spleen and stomach). From the perspective of TCM theory, HBV infection belongs to dampness-heat evil, which can be susceptible to personal physical and surroundings, thereby causing individual differences and clinical susceptibilities for patients with HBV-related liver fibrosis. This also explained why the same treatment regimens in the same population had different clinical efficacies. Based on the findings about TCM clinical syndrome characteristics of patients in response to therapeutic effect, some certain symptoms and signs of TCM also had significance in stratifying these sufferers in response or non-response to ETV + FZHY. And it also reflected the theory of TCM syndrome differentiation and treatment. Besides, our findings also provided an acceptable explanation that the disparity between Western medicine's disease concepts and TCM syndromes in terms of appraising treatment outcomes in integrative medicine [18].

However, result of this study showed that dampness and heat were the first three frequency of pathological nature for efficacy responders in the ETV + FZHY group, which may be associated with the ability of HBV to establish persistence [20]. It was reported that the infection of the primates including human beings with a low-dose HBV inoculum of 1 or 10 genome-equivalent would lead to the infection of 100 % hepatocytes and viral persistence with sever immunopathology [21]. From the perspective of TCM theory, HBV, as an exogenous evil, got into the body and then colonized the liver, which could contribute to the weak of human body health (namely

Table 6
Multivariate logistics regression analysis in TCM groups.

TCM groups	Independent variables	RR	Std. Err.	z	P	95 % Confidence Interval for Exp(B)	
LGDH	(base outcome)					Lower Bound	Upper Bound
LDSD	BMI (BMI: normal as a reference)						
	On the low side	0.68	0.574	−0.385	0.502	0.221	2.093
	On the high side	0.68	0.275	−0.386	0.161	0.397	1.165
	High*	0.381	0.408	−0.966	0.018	0.171	0.846
	Gender (Female as a reference)	0.82	0.287	−0.199	0.488	0.467	1.437
	Age (<45 as a reference)						
	45–59	1.08	0.309	0.077	0.802	0.589	1.981
	≧60	1.745	0.416	0.557	0.181	0.772	3.943
LKYD/SKYD	BMI						
	On the low side	0.566	0.742	−0.569	0.444	0.132	2.425
	On the high side	0.79	0.315	−0.236	0.453	0.426	1.463
	High*	0.277	0.552	−1.284	0.02	0.094	0.816
	Gender (Female as a reference)	0.657	0.335	−0.42	0.21	0.34	1.268
	Age (<45 as a reference)						
	<60	1.717	0.381	0.54	0.156	0.814	3.619
	≧60	2.062	0.501	0.723	0.149	0.772	5.506
LQD	BMI						
	On the low side	3.457E-08	0	−17.18	.	3.457E-08	3.457E-08
	On the high side	1.174	1.444	0.16	0.912	0.069	19.875
	High	2.388E-08	9490.898	−17.55	0.999	0	.
	Gender (Female as a reference)	0.428	1.632	−0.849	0.603	0.017	10.478
	Age (<45 as a reference)						
	<60	0.352	1.495	−1.045	0.484	0.019	6.583
	≧60	3.249E-08	7371.97	−17.242	0.998	0	.
HKnI	BMI						
	On the low side	2.375E-08	9487.833	−17.556	0.999	0	.
	On the high side	0.266	1.14	−1.323	0.246	0.029	2.491
	High	1.004E-08	7304.131	−18.416	0.998	0	.
	Gender (Female as a reference)	0.441	1.106	−0.819	0.459	0.05	3.856
	Age (<45 as a reference)						
	<60	1.989	1.182	0.688	0.561	0.196	20.173
	≧60	8.353E-08	5089.46	−16.298	0.997	0	.
DHSS	BMI						
	On the low side	1.423	1.182	0.353	0.765	0.14	14.427
	On the high side	1.93	0.585	0.657	0.261	0.613	6.074
	High	2.41E-08	4732.058	−17.541	0.997	0	.
	Gender (Female as a reference)	1.204	0.68	0.185	0.785	0.317	4.567
	Age (<45 as a reference)						
	<60	0.914	0.706	−0.09	0.899	0.229	3.643
	≧60	1.73	0.869	0.548	0.528	0.315	9.495
LFFU	BMI						
	On the low side	2.161E-08	5437.556	−17.65	0.997	0	.
	On the high side	0.437	0.581	−0.827	0.154	0.14	1.365
	High	1.187E-08	4217.251	−18.249	0.997	0	.
	Gender (Female as a reference)	1.019	0.717	0.019	0.979	0.25	4.158
	Age (<45 as a reference)						
	<60	1.097	0.731	0.092	0.899	0.262	4.593
	≧60	4.728E-08	2936.775	−16.867	0.995	0	.

Annotations: LDSD=Syndrome of liver depression and spleen deficiency. **LGDH**=Syndrome of liver-gallbladder dampness-heat. **LKYD/SKYD**=Syndromes of liver-kidney yin deficiency and spleen-kidney yang deficiency. **LQD** = Syndrome of lung qi deficiency. **HKnI**=Syndrome of heart-kidney noninteraction. **DHSS**=Syndrome of dampness-heat in the spleen and stomach. **LFFU**=Syndrome of liver fire flaming upward. *$P < 0.05$; RR: relative risk.

Qi deficiency). Together with fatty and greasy diets and unhealthy living habits, patients with HBV infection were more susceptible to these surroundings and led to the production of dampness-heat evil (namely endogenous evil). As time passes, the interaction of exogenous and endogenous evils certainly would generate or aggravate stasis (TCM theory of stasis caused by long illness) while the stasis would also influence and enhance these evils' virulence and persistence in turn. Considering ETV used for anti HBV virus and FZHY used for removing blood stasis, these explanations, to some extent, could elaborate the reason why both dampness and heat were the pathological nature for efficacy responders in the ETV + FZHY group. This was also consistence with the result of multivariate logistics regression analysis that patients with high BMI had potential risk of the onset of LGDH compared to LDSD (RR = 0.381) and LKYD/SKYD (RR = 0.277) among TCM groups.

There were several limitations which need to be noticed in our study. First, although this analysis was based on data from multi-centers in Eastern, Southern, Central of China, it is necessary to validate the results from other regions of China or even other countries. Second, a prospective cohort validation study should be conducted to further confirm the reliability of our findings. Third, although

baseline characteristics were comparable between the two groups, differences in blood pressure, alanine aminotransferase, aspartate aminotransferase, etc. may fluctuate the reliability of our findings. Therefore, these physiochemical indexes should be taken into consideration and used for stratification analysis in the future.

5. Conclusion

Evidence from this study showed that in the ETV + FZHY group, pathological positions of Spleen, and Stomach (TCM concepts) and pathological natures of Qi deficiency, Heat, and Qi movement stagnation in syndrome elements were closely associated with efficacy responders with HBV-related liver fibrosis. Furthermore, LDSD was a primary TCM syndrome in these responders.

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Data availability statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the first or corresponding author.

CRediT authorship contribution statement

Yun-kai Dai: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition. **Hai-na Fan:** Writing – review & editing, Software, Resources, Methodology, Formal analysis, Data curation. **Zhi-min Zhao:** Visualization, Supervision, Project administration, Investigation. **Li Shen:** Supervision, Resources, Formal analysis, Data curation, Conceptualization. **Cheng-hai Liu:** Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22216.

Abbreviations

TCM: traditional Chinese medicine;
ETV: entecavir;
FZHY: FuZheng HuaYu;
HBV: hepatitis B virus;
HSC: hepatic stellate cell;
ECM: extracellular matrix;
HCC: hepatocellular carcinoma;
FDA: Food and Drug Administration;
CHB: chronic hepatitis B;
BMI: body mass index;
PCA: principal component analysis;
F–R: response to FZHY + ETV;
F-NR: no-response to FZHY + ETV;
E-R: response to ETV + placebo;
E-NR: no-response to ETV + placebo;
LDSD: syndrome of liver depression and spleen deficiency;
LGDH: syndrome of liver-gallbladder dampness-heat;
LKYD/SKYD: syndromes of liver-kidney yin deficiency and spleen-kidney yang deficiency;
LQD: syndrome of lung qi deficiency;
HKnI: syndrome of heart-kidney noninteraction;
DHSS: syndrome of dampness-heat in the spleen and stomach;

LFFU: syndrome of liver fire flaming upward.

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