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Effect of Qianggan Pills combined with antiviral treatment on the fibrosis indexes, immune and inflammatory response in patients with compensated hepatitis b cirrhosis

Hong−Gang Huang✉

Department of Liver Diseases, Xinzhou District Hospital of TCM in Wuhan City Hubei Province, Wuhan City, Hubei Province, 430000

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ABSTRACT

Objective: To study the effect of Qianggan Pills combined with antiviral treatment on the fibrosis indexes, immune and inflammatory response in patients with compensated hepatitis b cirrhosis. **Methods:** A total of 88 patients with compensated hepatitis b cirrhosis treated in our hospital between April 2013 and March 2016 were collected and divided into observation group and control group according to single blind randomized control. Observation group of patients accepted Qianggan Pills combined with antiviral treatment and control group of patients received antiviral treatment alone. After 6 months of treatment, chemiluminescence method was used to detect serum fibrosis indexes, flow cytometer was used to detect peripheral blood T lymphocyte subset levels, and enzyme-linked immunosorbent assay (ELISA) was used to detect serum levels of inflammatory factors. **Results:** Before treatment, differences in fibrosis indexes, immune and inflammatory response indexes were not statistically significant between two groups of patients; after 6 months of treatment, serum LN, HA and Ⅳ-C levels of observation group were lower than those of control group, peripheral blood $CD3^+$ and $CD4^+$ T lymphocyte levels as well as $CD4^+/CD8^+$ ratio were higher than those of control group, and $CD8^+$ T lymphocyte level was lower than that of control group; serum PCT and CRP levels were lower than those of control group while IL-10 and IL-13 levels were higher than those of control group. **Conclusion:** Qianggan Pills combined with antiviral treatment can inhibit the fibrosis process, strengthen the body's immune function and also relieve systemic inflammatory response in patients with compensated hepatitis b cirrhosis.

1. Introduction

Hepatitis b cirrhosis is the liver cirrhosis caused by chronic hepatitis b progression, and it is the main type of liver cirrhosis at present in our country. There is no obvious liver dysfunction in patients with compensated hepatitis b cirrhosis, but the fibrosis persists, and it is the pathological foundation of later sustained liver damage and also the focus of the clinical treatment[1,2]. Antiviral therapy is the main treatment of patients with hepatitis b cirrhosis, it contains the activity of hepatitis b virus to prevent disease progression, but many studies believe that the effect of antiviral therapy alone is limited, and other drugs are needed to expand the curative effect. Qianggan Pills is composed of astragalus, salvia miltiorrhiza and many other traditional Chinese medicines, and it has been successfully applied in chronic hepatitis b[3,4]. In the following study, the effect of Qianggan Pills combined with antiviral treatment on the fibrosis indexes, immune and inflammatory response in patients with compensated hepatitis b cirrhosis was analyzed.

2. Information and methods

2.1 General information

A total of 88 patients with compensated hepatitis b cirrhosis treated

✉Corresponding author: Hong-Gang Huang, Department of Liver Diseases, Xinzhou District Hospital of TCM in Wuhan City Hubei Province, Wuhan City, Hubei Province, 430000.
Tel: 13100699060
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in our hospital between April 2013 and March 2016 were selected and patients themselves signed informed consent. According to the single blind randomized control method, the included patients were divided into observation group and control group (n=44). Control group included 24 male cases and 20 female patients, they were 43-78 years old, the course of hepatitis b was 5-18 years and 11 years in average, and the course of, cirrhosis was 1-6 years and 2 years in average; observation group included 25 male cases and 19 female patients, they were 42-76 years old, the course of hepatitis b was 4-16 years and 11 years in average, and the course of, cirrhosis was 1-7 years and 2 years in average. Two groups of patients were not statistically different in gender, age, course of hepatitis b and course of liver cirrhosis (P>0.05), and the research was approved by the hospital ethics committee.

2.2 Treatment methods

Both groups accepted albumin, liver protection and other symptomatic and supportive treatment. Control group received conventional treatment + antiviral treatment, which was as follows: oral administration of lamivudine tablets (Shandong Weifang Pharmaceutical Co., LTD., approved by H20123047), 100 mg/time, 6 months as a course of treatment. On the basis of conventional treatment, observation group accepted Qianggan Pills combined with antiviral therapy, which was as follows: oral administration of Qianggan Pills (astragalus, salvia miltiorrhiza, red paeony root, angelica, dried tangerine or orange peel, peach kernel, etc, were grinded into pills, 9 g/pill), 1 pill/time, 3 times/d, 6 months as a course of treatment. The usage and dosage of lamivudine tablets were the same as those of control group.

2.3 Observation indexes

2.3.1 Serum indexes

Before treatment and after 6 months of treatment, 2 mL of cubital venous blood was extracted from two groups of patients at the same point in time and centrifuged to get supernatant and determine the following indicators: (1) the fibrosis indicators: chemiluminescence method was used to detect serum fibrosis indexes, including laminin (LN), hyaluronic acid (HA) and collagen type Ⅳ (Ⅳ-C); (2) inflammatory response: ELISA kit (Sigma Company in the United States, the article number RA104, RA174, HC832 and YN912 respectively) instructions were followed to detect serum inflammatory factors procalcitonin (PCT), C-reactive protein (CRP), interleukin-10 (IL-10) and interleukin-13 (IL-13) levels.

2.3.2 Immune function

Before treatment and after 6 months of treatment, 2 mL of cubital venous blood was extracted from two groups of patients at the same point in time, and the flow cytometer (Guangzhou Yunxing Scientific Instruments Co., LTD., model FlowSight) was used to detect peripheral blood T lymphocyte subset levels, which was as follows: $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes as well as $CD4^+/CD8^+$ ratio.

2.4 Statistical methods

The data obtained in the study was input in software SPSS 21.0, measurement data was in terms of (Mean ± SD), comparison within same group before and after treatment was by paired t test, comparison between groups before and after treatment was by routine t test and P<0.05 was the standard of statistical significance in differences.

3. Results

3.1 Fibrosis indexes

Comparison of serum fibrosis indexes LN, HA and Ⅳ-C levels between two groups of patients was as follows: before treatment, differences in serum fibrosis indexes LN, HA and Ⅳ-C levels were not statistically significant between two groups of patients (P>0.05); after 6 months of treatment, serum liver fibrosis indexes LN, HA and Ⅳ-C levels of both groups were lower than those before treatment, and differences within same group were statistically significant before and after treatment (P<0.05); after 6 months of treatment, serum LN, HA and Ⅳ-C levels of observation group were lower than those of control group, and differences between groups were statistically significant (P<0.05), shown in Table 1.

3.2 T lymphocyte subsets

Comparison of peripheral blood T lymphocyte subset levels between two groups of patients was as follows: before treatment, differences in peripheral blood $CD3^+$, $CD4^+$ and $CD8^+$T lymphocyte levels as well as $CD4^+/CD8^+$ ratio were not statistically significant between two groups of patients (P>0.05); after 6 months of treatment, peripheral blood $CD3^+$ and $CD4^+$ T lymphocyte levels as well as $CD4^+/CD8^+$ ratio of both groups were higher than those before treatment while $CD8^+$ T lymphocyte levels were lower than

Table 1.

Comparison of serum fibrosis index levels (ng/mL).

Groups	n	LN		HA		Ⅳ-C	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation	44	174.38±20.19	91.27±10.09$^{*\#}$	193.26±24.37	102.46±13.25$^{*\#}$	143.25±18.19	81.25±9.17$^{*\#}$
Control	44	173.26±19.63	132.74±15.83*	194.75±23.54	159.37±18.62*	147.17±17.84	103.78±13.24*
t value		0.192	8.293	0.172	6.493	0.152	6.293
P value		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

Note: compared with same group before treatment, $^{*}P$<0.05; compared with control group after treatment, $^{\#}P$<0.05.

Table 2.

Comparison of peripheral blood T lymphocyte subset levels.

Groups	*n*	$CD3^+$		$CD4^+$		$CD8^+$		$CD4^+/CD8^+$	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation	44	50.13±5.28	59.63±6.57$^{*\#}$	29.37±3.51	39.59±4.35$^{*\#}$	31.27±3.65	23.73±2.81$^{*\#}$	1.13±0.12	1.74±0.25$^{*\#}$
Control	44	50.09±5.72	52.42±6.79*	29.46±3.48	32.57±3.87*	31.38±3.72	29.64±3.71*	1.12±0.14	1.28±0.19*
t value		0.182	6.492	0.152	7.192	0.192	6.983	0.115	6.273
P value		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

Note: compared with same group before treatment, $^{*}P<0.05$; compared with control group after treatment, $^{\#}P<0.05$.

Table 3.

Comparison of serum inflammatory factor levels.

Groups	*n*	PCT		CRP		IL-10		IL-13	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation	44	3.82±0.49	0.63±0.08$^{*\#}$	25.48±3.11	5.78±0.65$^{*\#}$	53.27±6.09	143.75±18.76$^{*\#}$	30.26±3.81	81.17±9.58$^{*\#}$
Control	44	3.78±0.42	2.73±0.34*	26.79±3.54	18.93±2.76*	52.19±6.34	84.73±9.15*	31.54±3.54	54.54±6.73*
t value		0.172	6.482	0.219	9.283	0.217	9.283	0.173	8.793
P value		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

Note: compared with same group before treatment, $^{*}P<0.05$; compared with control group after treatment, $^{\#}P<0.05$.

those before treatment, and differences within same group were statistically significant before and after treatment ($P<0.05$); after 6 months of treatment, peripheral blood $CD3^+$ and $CD4^+$ T lymphocyte levels as well as $CD4^+/CD8^+$ ratio of observation group were higher than those of control group while $CD8^+$ T lymphocyte level was lower than that of control group, and differences between groups were statistically significant ($P<0.05$), shown in Table 2.

3.3 Inflammatory factors

Comparison of serum inflammatory factors PCT (ng/mL), CRP (mg/L), IL-10 (μg/L) and IL-13 (pg/mL) levels between two groups of patients was as follows: before treatment, differences in serum PCT, CRP, IL-10 and IL-13 levels were not statistically significant between two groups of patients ($P>0.05$); after 6 months of treatment, serum PCT and CRP levels of both groups were significantly lower than those before treatment while IL-10 and IL-13 levels were significantly higher than those before treatment, and the differences within same group were statistically significant before and after treatment ($P<0.05$); after 6 months of treatment, serum PCT and CRP levels of observation group were significantly lower than those of control group while IL-10 and IL-13 levels were significantly higher than those of control group ($P<0.05$), and differences between groups were statistically significant ($P<0.05$), shown in Table 3.

4. Discussion

Liver fibrosis is the main pathological manifestation of patients with compensated hepatitis b cirrhosis, persistent hepatitis b virus replication leads to abnormal hepatic extracellular matrix proliferation and deposition, and the stellate cells are activated and then further combined with a variety of cytokines, which accelerate collagen deposition, secrete type O collagenase, destroy the basement membrane, cause Disse space capillarization, gradually damage liver detoxification function and form a vicious circle[5,6]. There is no obvious liver function decline in patients with compensated hepatitis b cirrhosis, patients in this period should receive positive anti-fibrosis treatment in order to protect the liver function and avoid the occurrence of decompensation state. Western medicine antiviral therapy is the basis to curb hepatitis b virus activity, but its effectiveness is limited, and its inhibiting effect on liver fibrosis is not significant[7]. Looking for efficient and reasonable means of adjuvant therapy is currently the research focus and hotspot in the treatment of compensated hepatitis b cirrhosis. TCM holds that the pathogenesis of liver fibrosis is the damp-heat toxin stagnation and immune barrier damage, the traditional Chinese medicine compositions of Qianggan Pills can regulate immune function and inhibit fibroplasia, and therefore, it is recommended by many scholars for the treatment of patients with liver cirrhosis.

In the study, Qianggan Pills was added in the treatment of observation group. The astragalus in the prescription can nourish qi to invigorate spleen and relieve immune complex deposition in liver; salvia miltiorrhiza promotes blood circulation to remove blood stasis, nourishes and cools blood, and can promote the liver cell regeneration and protect the liver cell mitochondria function. The degree of liver fibrosis is the most intuitive index to reflect clinical therapeutic effect, LN, HA and Ⅳ-C are the most commonly applied clinical indexes that are closely related to liver fibrosis, and with the increase of liver fibrosis, the serum levels of above factors increase[8,9]. After continuous 6 months of treatment, it was found that compared with control group, observation group were with lower serum fibrosis indexes LN, HA and Ⅳ-C levels, showing that adjuvant Qianggan Pills therapy can effectively curb the degree of fibrosis in patients with cirrhosis, which is mainly related to the anti-fibrosis activity of its effective components.

Autoimmune function in patients with liver cirrhosis can directly influence the outcome of disease, and many studies have confirmed the hepatitis b virus (HBV) does not destroy liver cells directly, but indirectly causes liver cell damage by immune function[10,11]. Cellular immunity is the main protective barrier for the host to clear

HBV, and when viral replication is active, the cellular immune function is mostly severely damaged[12]. T lymphocyte is the main effector cell of cellular immunity, $CD3^+$ T lymphocyte level represents the overall state of cellular immune function, $CD4^+$ T lymphocyte is also called helper T cell, $CD8^+$ T lymphocyte is also called cytotoxic T cell and $CD4^+/CD8^+$ ratio can directly reflect the body′s immune state [13]. The $CD4^+/CD8^+$ ratio declines when the body is in immunosuppressive state. In the study, the immune status of two groups of patients was evaluated, and it was found that compared with the control group, observation group of patients were with higher peripheral blood $CD3^+$ and $CD4^+$ T lymphocyte levels as well as $CD4^+/CD8^+$ ratio, and lower $CD8^+$ T lymphocyte level, showing that adjuvant Qianggan Pills therapy can effectively enhance the body′s cellular immune function, which is also one of the important mechanisms of suppressed fibrosis in observation group

Local hepatic and systemic inflammation plays an important role in the progression of hepatitis b cirrhosis, and the pro-inflammatory/ anti-inflammatory imbalance is the core link that leads to expanded inflammation and aggravated liver cell damage[14]. PCT and CRP are the commonly studied clinical pro-inflammatory factors, they can be detected in serum in the early inflammatory response, and their serum levels are positively correlated with the degree of inflammation[15,16]. IL-10 and IL-13 belong to anti-inflammatory factors, and IL-10 is mainly produced by the Th2 cells and the activated mononuclear macrophages, and can reduce inflammation and antagonize inflammatory mediators; IL-13 is produced by activated mast cells, etc., and it shares signal transduction system with IL-4 and exerts anti-inflammatory effect[17,18]. In the study, the levels of above inflammatory cytokines were detected, and it was found that compared with the control group, observation group were with lower serum PCT and CRP levels, and higher IL-10 and IL-13 levels after treatment, showing that Qianggan Pills can effectively balance the patients′ inflammatory state, enhance the body′s anti-inflammatory ability, inhibit pro-inflammatory factor activity, eventually optimize the environment in liver cells and suppress liver fibrosis progression.

To sum up, it is concluded as follows: Qianggan Pills combined with antiviral treatment can inhibit the fibrosis process, strengthen the body's immune function and also relieve systemic inflammatory response in patients with compensated hepatitis b cirrhosis, and it is worth popularization and application in clinical practice in the future.

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