




# Effect of the reduced glutathione antioxidation combined with conventional antiviral drugs on hepatic fibrosis in patient with hepatitis b cirrhosis

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## ABSTRACT

**Objective:** To study the effect of the reduced glutathione antioxidation combined with conventional antiviral drugs on hepatic fibrosis in patient with liver cirrhosis. **Methods:** A total of 300 patients with hepatitis b cirrhosis who were treated in Shangluo Central Hospital between August 2012 and August 2016 were collected and divided into the control group ( $n=159$ ) who received conventional antiviral therapy and the observation group ( $n=141$ ) who received reduced glutathione antioxidation combined with conventional antiviral drug therapy. The differences in serum levels of fibrosis indicators, inflammatory factors and oxidative stress indexes were compared between the two groups of patients before and after treatment. **Results:** Before treatment, differences in serum levels of fibrosis indexes, inflammatory factors and oxidative stress indexes were not statistically significant between two groups of patients. After treatment, serum HA, IV-C, LN, PCIII, PCT, IL-6, IL-22, IL-31, TNF- $\alpha$  and MDA levels of both groups of patients were lower than those before treatment while GSH-Px and T-SOD levels were higher than those before treatment, and serum HA, IV-C, LN, PCIII, PCT, IL-6, IL-22, IL-31, TNF- $\alpha$  and MDA levels of observation group after treatment were lower than those of control group while GSH-Px and T-SOD levels were higher than those of control group. **Conclusion:** Reduced glutathione antioxidation combined with conventional antiviral drugs can effectively inhibit the fibrosis process in patients with hepatitis b cirrhosis, which is because that it reduces the degree of inflammation and oxidative stress reaction.

## 1. Introduction

Liver cirrhosis caused by chronic hepatitis b is the most common type of cirrhosis in China, patients may be with different degrees of liver dysfunction, and severe cases may have upper gastrointestinal hemorrhage, hepatic encephalopathy, carcinogenesis, etc. There are extensive liver cell necrosis, connective tissue hyperplasia and fibrotic septum formation in the pathology of liver cirrhosis, which result in damage to the structure of the hepatic lobules and the formation of pseudolobules[1,2]. Liver fibrosis is the core factor leading to the occurrence and development of hepatitis b cirrhosis, the process of fibrosis also largely determines the ultimate outcome of patients[3,4], the role of clinical conventional antiviral treatment has limitations in inhibiting the process of fibrosis, and many scholars recommend to join other drugs to

expand the curative effect. Reduced glutathione can participate in the process of oxidation reduction in vivo, also has the functions such as protecting liver function, promoting protein synthesis and bilirubin metabolism, and can be used for the adjuvant treatment of liver diseases[5,6]. In the study, reduced glutathione combined with conventional antiviral drugs was used for the treatment of patients with hepatitis b cirrhosis, and the roles were discussed from liver fibrosis indexes, inflammatory factors, oxidative stress and other aspects.

## 2. Information and methods

### 2.1 General information

A total of 300 patients with hepatitis b cirrhosis who were treated in Shangluo Central Hospital between August 2012 and August 2016 were collected, and the patients themselves signed the informed consent form. After the therapies were reviewed, the patients were divided into the control group ( $n=159$ ) who received

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conventional antiviral therapy and the observation group ( $n=141$ ) who received reduced glutathione antioxidation combined with conventional antiviral drug therapy. Control group included 84 men and 75 women that were 48-72 years old; observation group included 73 men and 68 women that were 45-76 years old. The differences between the two groups were not significant in the gender and age distribution, and the hospital ethics committee approved the study.

## 2.2 Diagnostic criteria for hepatitis b cirrhosis

(1) Liver dysfunction: mild dysfunction in compensation period and significant dysfunction in decompensation period; (2) HBV-M positive; (3) elevated fibrosis indicator levels; (4) B ultrasound showed thickened liver capsule, unsmooth surface and enhanced liver parenchymal echo; (5) diagnosed by liver biopsy.

## 2.3 Inclusion and exclusion criteria

Inclusion criteria: (1) clearly diagnosed with hepatitis b cirrhosis; (2) not receiving systematic treatment before admission; (3) cooperating with treatment and inspection all the time and with complete data that could be compared. Exclusion criteria: (1) with previous history of hepatitis a and hepatitis c; (2) allergic to reduced glutathione; (3) combined with systemic infectious diseases; (4) combined with severe heart and kidney insufficiency.

## 2.4 Therapy

Both groups received routine treatment for patients with hepatitis b cirrhosis, including plasma, vitamin C, hemostasis, and anti-infection, etc. Patients in the control group received routine treatment and antiviral treatment, including adefovir dipivoxil (Youcare Pharmaceutical Group Co., Ltd., approved by H20110088) 10 mg, taken orally, 1 time/d, for continuous 3 months of treatment. Observation group of patients received conventional treatment + conventional antiviral drugs + reduced glutathione anti-oxidation treatment, specifically as follows: reduced glutathione (Chongqing YaoPharma Co., Ltd., approved by H20051599) 1 200 mg, added in 100 mL of normal saline, by intravenous drip, 1 time/d, for continuous treatment for 3 months.

## 2.5 Observation indexes

5.0 mL of fasting cubital venous blood was extracted from two groups of patients before and after treatment, anti-coagulated and then centrifuged at low speed to take upper serum, which was frozen in  $-70^{\circ}\text{C}$  environment for test. Enzyme-linked immunosorbent assay (ELISA) was used to detect serum levels of fibrosis indexes

hyaluronic acid (HA), collagen type IV (IV-C), laminin (LN) and type III procollagen (PCIII). ELISA was used to determine serum levels of inflammatory cytokines procalcitonin (PCT), interleukin-6 (IL-6), interleukin-22 (IL-22), interleukin-31 (IL-31) and tumor necrosis factor alpha (TNF- $\alpha$ ). The serum contents of oxidative stress indexes glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD) and malondialdehyde (MDA) were detected by ELISA.

## 2.6 Statistical methods

Statistical software was SPSS 20.0. Liver fibrosis indexes, inflammatory factors, oxidative stress indexes and other measurement data were in terms of mean  $\pm$  standard deviation, and comparison was by test.  $P < 0.05$  was the standard of statistical significance in differences.

## 3. Results

### 3.1 Liver fibrosis indexes HA, IV-C, LN and PCIII

Before treatment and 3 months after treatment, comparison of serum HA (mg/L), IV-C ( $\mu\text{g/L}$ ), LN ( $\mu\text{g/mL}$ ) and PCIII ( $\mu\text{g/L}$ ) levels between two groups of patients was as follows: before treatment, serum HA, IV-C, LN and PCIII levels were not statistically different between two groups of patients ( $P > 0.05$ ). Serum HA, IV-C, LN and PCIII levels of both groups of patients after treatment were significantly lower than those before treatment, and serum HA, IV-C, LN and PCIII levels of observation group after treatment were significantly lower than those of control group ( $P < 0.05$ ), shown in Table 1.

### 3.2 Inflammatory factors PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$

Before treatment and 3 months after treatment, comparison of serum inflammatory factors PCT (ng/mL), IL-6 (pg/mL), IL-22 (pg/mL), IL-31 (pg/mL) and TNF- $\alpha$  (pg/mL) levels between two groups of patients was as follows: before treatment, serum PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  levels were not statistically different between two groups of patients ( $P > 0.05$ ). Serum PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  levels of both groups of patients after treatment were significantly lower than those before treatment, and serum PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  levels of observation group after treatment were significantly lower than those of control group ( $P < 0.05$ ).

Table 1.

Changes in serum HA, IV-C, LN and PCIII levels before and after treatment.

Groups	n	Time	HA	IV-C	LN	PCIII
Control group	159	Before treatment	241.28 $\pm$ 30.64	182.16 $\pm$ 20.64	199.26 $\pm$ 21.28	153.48 $\pm$ 18.62
		After treatment	139.26 $\pm$ 15.88 <sup>a</sup>	124.37 $\pm$ 15.88 <sup>a</sup>	130.57 $\pm$ 15.64 <sup>a</sup>	91.66 $\pm$ 10.57 <sup>a</sup>
Observation group	141	Before treatment	240.61 $\pm$ 28.73	184.25 $\pm$ 19.57	201.43 $\pm$ 23.41	152.71 $\pm$ 17.95
		After treatment	74.28 $\pm$ 8.12 <sup>ab</sup>	73.28 $\pm$ 9.15 <sup>ab</sup>	74.29 $\pm$ 8.21 <sup>ab</sup>	50.27 $\pm$ 6.21 <sup>ab</sup>

Note: before treatment vs. after treatment within group, differences in a indexes were significant; observation group vs. control group, differences in b indexes were significant.

Table 2.

Changes in serum PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  levels before and after treatment.

Groups	n	Time	PCT	IL-6	IL-22	IL-31	TNF- $\alpha$
Control group	159	Before treatment	0.63 $\pm$ 0.09	341.28 $\pm$ 40.76	89.37 $\pm$ 9.65	21.27 $\pm$ 2.85	42.18 $\pm$ 5.39
		After treatment	0.42 $\pm$ 0.05 <sup>a</sup>	203.17 $\pm$ 24.63 <sup>a</sup>	59.23 $\pm$ 7.21 <sup>a</sup>	14.83 $\pm$ 1.79 <sup>a</sup>	26.31 $\pm$ 3.05 <sup>a</sup>
Observation group	141	Before treatment	0.61 $\pm$ 0.08	337.27 $\pm$ 39.62	90.16 $\pm$ 9.57	21.35 $\pm$ 2.92	43.56 $\pm$ 5.38
		After treatment	0.25 $\pm$ 0.04 <sup>ab</sup>	112.54 $\pm$ 13.29 <sup>ab</sup>	29.66 $\pm$ 4.52 <sup>ab</sup>	8.52 $\pm$ 0.91 <sup>ab</sup>	15.27 $\pm$ 2.09 <sup>ab</sup>

Note: before treatment vs. after treatment within group, differences in a indexes were significant; observation group vs. control group, differences in b indexes were significant.

Table 3.

Changes in serum GSH-Px, T-SOD and MDA levels before and after treatment.

Groups	n	Time	GSH-Px	T-SOD	MDA
Control group	159	Before treatment	2.16 $\pm$ 0.24	44.28 $\pm$ 5.09	5.72 $\pm$ 0.71
		After treatment	2.48 $\pm$ 0.31 <sup>a</sup>	53.57 $\pm$ 6.63 <sup>a</sup>	4.16 $\pm$ 0.53 <sup>a</sup>
Observation group	141	Before treatment	2.15 $\pm$ 0.23	43.76 $\pm$ 5.14	5.68 $\pm$ 0.69
		After treatment	2.86 $\pm$ 0.42 <sup>ab</sup>	69.28 $\pm$ 8.39 <sup>ab</sup>	3.05 $\pm$ 0.37 <sup>ab</sup>

Note: before treatment vs. after treatment within group, differences in a indexes were significant; observation group vs. control group, differences in b indexes were significant.

### 3.3 Oxidative stress indexes GSH-Px, T-SOD and MDA

Before treatment and 3 months after treatment, comparison of serum oxidative stress indexes GSH-Px (mg/L), T-SOD (U/L) and MDA (mmol/L) levels between two groups of patients was as follows: before treatment, serum GSH-Px, T-SOD and MDA levels were not statistically different between two groups of patients ( $P>0.05$ ). Serum GSH-Px and T-SOD levels of both groups of patients after treatment were significantly higher than those before treatment while MDA levels were significantly lower than those before treatment, and serum GSH-Px and T-SOD levels of observation group after treatment were significantly higher than those of control group while MDA level was significantly lower than that of control group ( $P<0.05$ ).

## 4. Discussion

Adefovir dipivoxil antiviral treatment is a routine means for hepatitis b cirrhosis of the liver, but antiviral treatment alone cannot reverse the dysfunction and related complications in patients with cirrhosis, and other mechanisms of drugs are needed to reverse the condition. Reduced glutathione is an important metabolism regulator in cells that can protect the protein containing sulphur in cell membrane from being damaged, it widely exists in the tissue cells in physiological conditions, and it has been confirmed that endogenous reduced glutathione levels decrease in patients with hepatitis b cirrhosis[7,8]. Exogenous reduced glutathione is the common means for clinical treatment of various kinds of liver diseases, and in the study, reduced glutathione and conventional antiviral drugs were used together for the treatment of patients with hepatitis b cirrhosis in order to clarify the optimizing effect of combination therapy on patients' conditions.

When chronic hepatitis b occurs, the body's self-defense reaction will lead to the apoptosis of hepatocytes, excessive apoptosis can result in the change of pathologic tissue fibrosis, and excessive fibrosis can result in cirrhosis of the liver. The hepatic fibrosis process includes the activation of stellate cells and the increase of

extracellular matrix synthesis, and their changes directly determine the final outcome of the disease[9,10]. HA is produced by connective tissue, the HA degradation decreases and serum HA content rises when hepatocytes are damaged. IV-C belongs to the vascular basement membrane components and is the earliest hyperplastic fibre, its degradation rate decreases and serum content increases when liver function declines[11,12]. LN is the glycoprotein which is deposited in the hepatic sinusoidal endothelium, its liver increases in the case of cirrhosis and its sensitivity to the diagnosis of liver cirrhosis is more than 90%. PCIII is directly correlated with the liver collagen synthesis, and the rising PCIII indicates the existence or aggravation of liver cirrhosis[13,14]. In this study, serum levels of above fibrosis indexes were compared between two groups of patients, and it was found that compared with those before treatment, serum HA, IV-C, LN and PCIII contents of both groups of patients were lower after treatment, indicating that both treatments can reduce the fibrosis degree in patients with hepatitis b cirrhosis; further compared with control group, the observation group were lower serum HA, IV-C, LN and PCIII contents after treatment, confirming that adding exogenous reduced glutathione can further inhibit liver fibrosis process, and the specific mechanism remains to be further defined.

The local hepatic and systemic inflammatory response caused by hepatitis b virus is an important cause of liver fibrosis, and the massively produced pro-inflammatory factors may activate hepatic stellate cells and prompt them to secrete extracellular matrix and massively gather within the liver[15]. PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  are the commonly studied inflammatory factors that are involved in hepatitis b cirrhosis at present, PCT is a new inflammatory factor, and it has good directivity to severe infectious diseases; IL-6 is the most typical pro-inflammatory factor that can promote the mononuclear macrophages to secrete IL-22, IL-31, TNF- $\alpha$  and other inflammatory mediators, which form local inflammation, can also increase pro-proliferation gene expression, and plays an important role in the occurrence of hepatitis b and fibrosis[16]. In this study, the differences in serum PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  levels were compared between two groups of patients before and after treatment, and it was found that compared with those before treatment, serum PCT, IL-6, IL-22, IL-31 and

TNF- $\alpha$  levels of both groups of patients were lower after treatment; further compared with the control group, the observation group were with serum PCT, IL-6, IL-22, IL-31 and TNF- $\alpha$  levels after treatment, confirming that adjuvant reduced glutathione therapy on the basis of antiviral treatment can effectively restrain the systemic inflammatory response, and this is one of the important mechanisms for it to inhibit fibrosis process.

Cytochrome P450 in hepatocytes, xanthine oxidoreductase, respiratory chain on mitochondrial membrane, etc., can all produce reactive oxygen species intermediate in the process of material metabolism, and under physiological state, the hepatic antioxidant system can control the production of oxygen free radicals and effectively decompose them. Liver function is in disorder and anti-oxidative defense ability decreases in the state of liver cirrhosis, and oxygen free radical generation/clearance is unbalanced, resulting in oxidative damage to tissue and cells[17,18]. The reduced glutathione can be combined with peroxide and free radicals to fight oxidative damage to the sulfhydryl group and inhibit the damage from oxygen free radicals to tissue organs. GSH-Px and T-SOD have antioxidant effects, MDA is the most typical oxidation product, and the balance state of the expression of the two can directly reflect the oxidative stress level of the body. In this study, the differences in serum levels of these oxidative stress indexes were compared between two groups of patients before and after treatment, and it was found that compared with those before treatment, serum GSH-Px and T-SOD contents of both groups were higher while MDA contents were lower after treatment; further compared with control group, the observation group were with higher serum GSH-Px and T-SOD contents, and lower MDA content after treatment, confirming that the antioxidant treatment by reduced glutathione can effectively suppress the oxidative stress reaction, and this is the fundamental mechanism for it to realize its anti-fibrosis effect.

Reduced glutathione anti-oxidation combined with conventional antiviral drug treatment of hepatitis b cirrhosis can effectively inhibit the liver fibrosis process, this is directly related to its effect on inhibiting inflammation and oxidative stress, and it is worthy of popularization and application in clinical practice in the future.

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