Original Article

Serum levels of IL-6 and TNF-α in chronic hepatitis B-induced Child-Pugh B cirrhosis patients after additional treatment of vitamins A and C and their value in evaluation of prognosis

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Abstract: Objective: The aim of this study was to explore expression of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in chronic hepatitis B (CHB)-induced Child-Pugh B cirrhosis patients after additional treatment of vitamins A and C, examining and their value in evaluating the prognosis of patients. Methods: A total of 60 cases of CHB-induced Child-Pugh B cirrhosis patients were selected and randomized into four groups, including the control group for basic treatment, Vit A group for basic treatment plus vitamin A, Vit C group for basic treatment plus vitamin C, and Vit A&C group for basic treatment plus both vitamin A and C. Measures involving liver function, degree of liver fibrosis, coagulation function, and levels of IL-6 and TNF-α in the four groups, before and after treatment, were recorded. The relationship between prognosis of CHB-induced cirrhosis and levels of IL-6 and TNF-α in patients was analyzed. Results: After treatment, levels of ALT (alanine transaminase), AST (aspartate transaminase), γ-GGT (γ-Glutamyl transpeptidase), TBiL (total bilirubin), and CHE (Cholinesterase) were significantly reduced, while levels of Alb (albumin) were significantly elevated (all P<0.05). Compared with other groups, the Vit A&C group was significantly lower in levels of ALT and CHE and significantly higher in levels of Alb (all P<0.05). Expression levels of IV-C (Collagen IV), PIIIP (procollagen-III peptide), HA (hyaluronic acid), LN (laminin), IL-6, and TNF-α, after treatment, in all groups were significantly lower than those before treatment (all P<0.05). Moreover, levels of IV-C, HA, and LN in the Vit A&C group were significantly lower than those in other groups after treatment (all P<0.05). PT-INR (prothrombin Time-international normalized ratio), TT (thrombin time), APTT (activated partial thromboplastin time), and PT (prothrombin time), after treatment, in all groups were significantly reduced. PTA (prothrombin time activity) and levels of FIB (fibrinogen) of the four groups were significantly elevated (all P<0.05). Compared with other groups, the Vit A&C group was significantly lower in INR, TT, APTT, and PT and significantly higher in PTA and levels of FIB (all P<0.05). Moreover, levels of IL-6 and TNF-α showed a significant correlation with changes in biochemical markers of liver cirrhosis. Conclusion: Additional treatment of vitamins A and C for CHB-induced Child-Pugh B cirrhosis patients can effectively improve liver function, prevent liver fibrosis, promote the recovery of coagulation function, and reduce expression levels of IL-6 and TNF-α. Furthermore, changes in levels of IL-6 and TNF-α can be used for evaluation of the prognosis of patients with CHB.

Keywords: Vitamin, chronic hepatitis B, inflammatory factor, prognosis

Introduction

Chronic hepatitis B (CHB) refers to an infectious disease caused by hepatitis B virus (HBV), with a duration of more than 6 months. CHB-induced chronic inflammatory diseases of the liver have become a worldwide public health issue [1, 2]. Most CHB patients have such symptoms, such as abdominal distension, nausea, and fatigue,

posing a severe threat to human health. The disease, in most patients, evolves into a chronic progressive phase, which is closely related to the occurrence of cirrhosis and liver cancer [3]. China tops most countries regarding hepatitis patients, with as many as 120 million HBV carriers. More than 200 million people, worldwide, estimated by the WHO, have chronic hepatitis B virus infections. Millions of the infections have

progressed to chronic liver disease, cancer, and even death [4]. In the treatment of CHB, long-term antiviral treatment can effectively inhibit HBV replication, thus alleviating liver damage, delaying the progression of liver disease, and reducing incidence of liver cancer [5]. Entecavir is currently the most effective nucleoside antiviral drug for treatment of CHB. It can selectively inhibit the replication of HBV and it is characterized by rapid onset of action, less adverse reactions, and low drug resistance [6]. However, patients cannot stop medication at any prescribed time. Otherwise, dizziness and headaches may occur with an increase in levels of ALT. This may lead to lactic acidosis, severe fatty liver complicated with hepatomegaly, and even death. Although viral replication in most patients with hepatitis B-induced cirrhosis is effectively controlled using nucleotide analogs, treatments for cirrhosis, such as inhibiting the activation of hepatic stellate cells, suppressing collagen proliferation, and facilitating collagen degradation, have received less attention [6-8].

The efficacy of vitamin D in the antiviral treatment of CHB has been widely recognized by the medical community [8]. Vitamin A, a natural pigment with high stability in nature, can improve oxidative stress induced by liver damage by increasing the effectiveness of oxygen free radical scavengers, such as superoxide dismutase. A previous study, including 140 patients with chronic hepatitis, showed vitamin A deficiency and gradual decline in serum retinol levels among patients with cirrhosis and hepatocellular carcinoma (HCC) [9]. Vitamin C can improve both liver detoxification and its metabolism. Insufficient intake of vitamin C may exacerbate liver damage and fibrosis in Gulo-/- (gulonolactone oxidase) mice [10]. The crucial roles that cytokines like IL-6 and TNF-α play in the recovery of liver failure have been demonstrated in previous studies [11], but the specific mechanisms of cytokines like IL-6 and TNF-α in occurrence and progression of liver disease have not been scientifically explained. Therefore, the present study aimed to explore expression of IL-6 and TNF-α in CHB-induced Child-Pugh B cirrhosis patients after additional treatment of vitamins A and C, examining their value in evaluating the prognosis of patients with CHB, aiming to provide a scientific basis for clinical treatment and improving patient quality of life.

Materials and methods

Baseline characteristics

A total of 60 cases of CHB-induced Child-Pugh B cirrhosis patients, from August 2017 to June 2018, in Ganzhou People's Hospital, were enrolled and randomized into four groups, including the control group for basic treatment, Vit A group for basic treatment plus vitamin A, Vit C group for basic treatment plus vitamin C, and Vit A&C group for basic treatment plus both vitamin A and C. Each group contained 15 cases. This study was approved by the Medical Ethics Committee of Ganzhou People's Hospital and informed consent was obtained from all patients and families.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients aged from 30.0 to 70.0 years old; (2) Patients diagnosed according to the diagnostic criteria for viral hepatitis established by the Chinese Medical Association in 2000 [12]; (3) Patients diagnosed as CHB-induced Child-Pugh B cirrhosis; (4) Patients that tested positive for HBsAg and HBeAg for more than 6 months; (5) Patients with degrees in education above middle school, showing good adherence during treatment; and (6) Patients without treatment of antiviral drugs, such as PEG-IFNα-2α, ordinary IFNα, and ETV, within half a year.

Exclusion criteria: (1) Patients with coagulopathy, endocrine diseases, and abnormal immune systems; (2) Patients with mental disorders or unconsciousness; (3) Patients with liver cancer, simple alcoholic liver disease, or non-alcoholic fatty liver; (4) Patients complicated with other hepatitis virus infections; (5) Patients complicated with severe dysfunction in heart, liver, kidney, blood, digestion, and nervous system; (6) Patients that are pregnant or lactating women; (7) Patients allergic to drugs used in this study; (8) Patients with abnormal thyroid function; and (9) Patients with a history of hepatotoxic drug use.

Methods

The control group was orally administrated liver-protecting tablets (Heilongjiang Sunflower Pharmaceutical Co., Ltd., China) for liver protection, 4 tablets a time, 3 times a day. They also received entecavir (Sino-US Shanghai Squibb

Pharmaceutical Co., Ltd., China) on an empty stomach, 0.5 mg a day. The Vit A group was given vitamin A (Sinopharm Holding Xingsha Pharmaceutical Co., Ltd., China), in addition to the treatment of control group, with 50,000 units a day. The Vit C group was given vitamin C (Guangdong Hengjian Pharmaceutical Co., Ltd., China), in addition to the treatment of control group, 200 mg a time, 3 times a day. Moreover, the Vit A&C group was given vitamin A and C with the same dosages as those in Vit A and Vit C groups, in addition to the treatment of control group. All patients were reexamined after 3 months of treatment.

Outcome measures

Primary indicators: Fasting venous blood samples of all patients were obtained in the early morning before treatment. The same operation was performed 3 months after treatment. After sample collection, serum was separated by centrifugation. This was followed by determination using automatic biochemical analyzer (Roche, Germany) concerning levels of alanine transaminase (ALT; Wuhan AmyJet Scientific Co., Ltd., China), aspartate transaminase (AST; Wuhan AmyJet Scientific Co., Ltd., China), total bilirubin (TBiL; Shanghai Jianglai Biotechnology Co., Ltd., China), and glutamyl transpeptidase (γ-GGT; Beijing Baiao Laibo Technology Co., Ltd., China).

Levels of laminin (LN; BioLegend, Cygnus, IBL, TSZ, USA), collagen IV (IV-C; Shanghai Qiyi Biotechnology Co., Ltd., China), procollagen-III peptide (PIIIP; Shanghai Huzhen Industrial Co., Ltd., China), and hyaluronic acid (HA; Shanghai Jingkang Biological Engineering Co., Ltd., China) were measured by enzyme-linked immunosorbent assay using a fully automated immunoanalyzer (Thermo, USA). Detailed steps referred to manufacturer instructions.

Determination of levels of interleukin-6 (IL-6; Shanghai Kanglang Biotechnology Co., Ltd., China) and tumor necrosis factor-α (TNF-α; Shanghai Chaoyan Biological Technology Co., Ltd., China) was performed using an automated enzymatic analyzer (Shanghai Xianjian instrument Co., Ltd., China).

Secondary indicators: Fasting venous blood samples, obtained before treatment and 3 months after treatment, were also used for determination of coagulation indexes and evaluation of

the activity of the plasma coagulation system. The blood sample was pipetted into a vacuum anticoagulative tube containing 0.2 mL of sodium citrate, followed by reverse mix and centrifugation. Next, prothrombin time-international normalized ratios (PT-INR; Shanghai Haling Biotechnology Co., Ltd., China), thrombin time (TT; Beijing Leagene Biotechnology Co., Ltd., China), activated partial thromboplastin time (APTT; Beijing Leagene Biotechnology Co., Ltd., China), and levels of fibrinogen (FIB; Beijing Solarbio Science & Technology Co., Ltd., China) in the sample blood were determined by the turbidimetric method using a fully automatic analyzer (Sysmex, Japan) and determination reagents (Dade Behring, USA).

Statistical analysis

Data obtained in this study were analyzed using SPSS software version 20.0. Measurement data are expressed as mean ± standard deviation ($\overline{x}$ ± sd). Measurement data with normal distribution between the two groups were compared using t-test. One-way analysis of variance (ANOVA) was used for multiple independent samples. Subsequent multiple comparisons were performed using the SNK method. Enumeration data are expressed as number/percentage (n/%), calculated using the χ^2 test and Fisher's Exact Probability test. Correlation between two sets of indicators was analyzed using Pearson's Chi-squared test. For all analyses, $P<0.05$ indicates statistical significance.

Results

Baseline characteristics

General characteristics, including gender, age, BMI, family history of hepatitis B, and duration of disease, were not significantly different among the four groups (all $P>0.05$). All patients in the four groups had no history of hepatotoxic drug use (**Table 1**).

Changes in liver biochemical indicators

There were no differences in levels of ALT, AST, γ-GGT, TBiL, Alb, and CHE among groups before treatment (all $P>0.05$). After treatment, levels of ALT, AST, γ-GGT, TBiL, and CHE in the four groups were significantly lower than those before treatment, while levels of Alb were higher than those before treatment, showing statistical significances (all $P<0.05$). Compared with the control group, Vit A group, and Vit C group,

Table 1. Baseline characteristics of the four groups ($\bar{x} \pm sd$)

	n (case)	Male/Female	Age (year)	BMI (kg/m^2)	Family history of hepatitis B (n, %)	Duration of disease
Control group	15	7/8	56.2±12.4	23.84±5.84	4 (26.67)	3.93±1.75
Vit A group	15	8/7	59.3±11.1	24.56±4.43	3 (20.00)	3.87±1.25
Vit C group	15	6/9	53.9±12.8	22.91±5.21	5 (33.33)	3.80±1.15
Vit A&C group	15	8/7	54.6±11.7	21.45±6.49	3 (20.00)	3.60±1.30
F/χ^2		0.536	0.595	0.659	0.978	0.163
P		0.911	0.621	0.581	0.807	0.921

Note: BMI, body mass index.
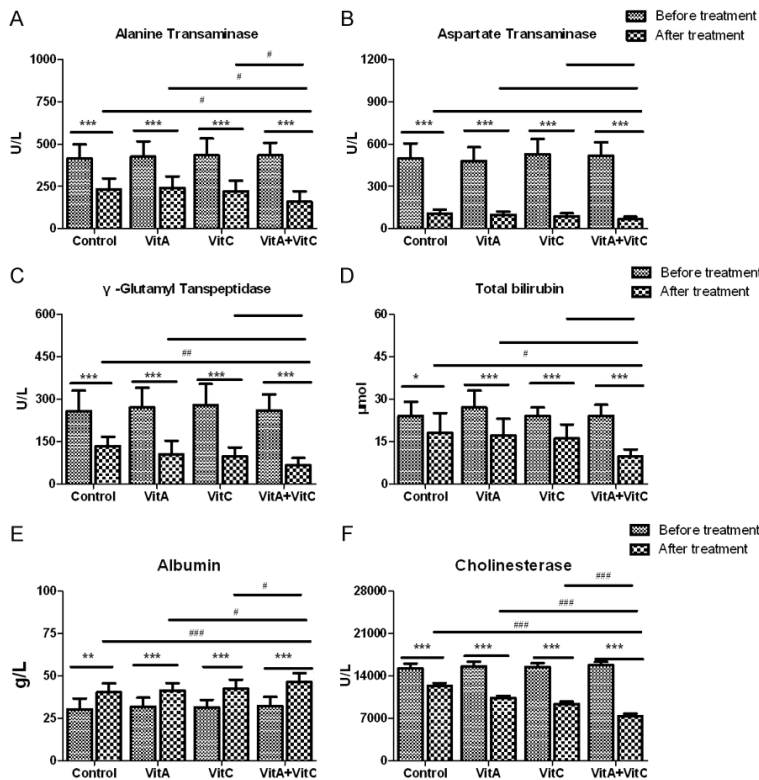
Figure 1. Intra-group and inter-group comparisons in liver biochemical indicators ($\bar{x} \pm sd$). A. Expression levels of alanine transaminase (ALT). B. Expression levels of aspartate transaminase (AST). C. Expression levels of γ-Glutamyl transpeptidase (γ-GGT). D. Expression levels of total bilirubin (TBiL). E. Expression levels of albumin (Alb). F. Expression levels of cholinesterase (CHE). The higher the value, the severer the liver damage. Intra-group comparison: *P<0.05, **P<0.01, ***P<0.001. Inter-group comparison: #P<0.05; ##P<0.01, ###P<0.001.

Changes in indicators of hepatic fibrosis

Before treatment, there were no differences in levels of IV-C, PIIIP, HA, and LN among groups (all P>0.05). After treatment, levels of IV-C, PIIIP, HA, and LN were significantly lowered in the four groups, showing statistical significance (all P<0.05). In addition, levels of IV-C, HA, and LN in the Vit A&C group were significantly lower than those in the control group, Vit A group, and Vit C group. Differences were statistically significant (all P<0.05). See **Figure 2**.

Changes in coagulation indicators

No differences were shown in PT-INR, TT, APTT, PT, PTA, and levels of FIB among the groups before treatment (all P>0.05). After treatment, INR, TT, APTT, and PT in the four groups were significantly elevated, while PTA and levels of FIB were significantly lowered, compared to those before treatment. Differences were statistically significant (all P<0.05). Compared with the control group, Vit A group, and Vit C group, the Vit A&C group was significantly lower in PT-INR, TT, APTT, and PT and significantly higher in PTA and levels of FIB. Differences were statistically significant (all P<0.05). See **Figure 3**.

expression levels of ALT and CHE were significantly lower and levels of Alb were significantly higher in the Vit A&C group (all P<0.05). Levels of γ-GGT and TBiL in the control group were significantly higher than those in the Vit A&C group. Differences were statistically significant (both P<0.05). See **Figure 1**.
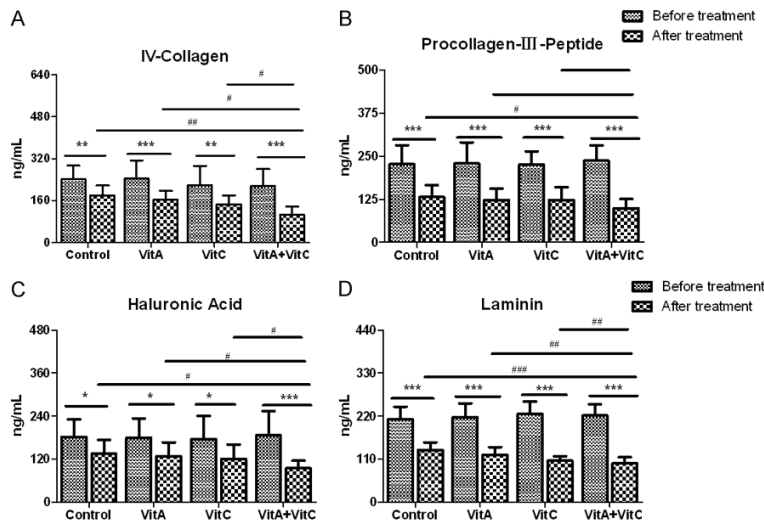
Figure 2. Intra-group and inter-group comparisons in indicators of hepatic fibrosis ($\bar{x}$ ± sd). A. Expression levels of collagen IV (IV-C). B. Expression levels of procollagen-III peptide (PIIIP). C. Expression levels of hyaluronic acid (HA). D. The expression levels of laminin (LN). The higher the value, the more likely liver fibrosis will occur. Intra-group comparison: *P<0.05, **P<0.01, ***P<0.001. Inter-group comparison: #P<0.05, ##P<0.01, ###P<0.001.

Changes in inflammatory markers

Figure 4 shows changes in levels of IL-6 and TNF-α in the four groups. Before treatment, there were no differences in expression levels of IL-6 and TNF-α among groups, showing no statistical significance (all P>0.05). After treatment, levels of IL-6 and TNF-α in the four groups were significantly lowered. Moreover, levels of IL-6 and TNF in the Vit A&C group were significantly lower than those in other groups, indicating statistical significance (all P<0.05).

Correlation between inflammatory factors and prognosis

Pearson's correlation and linear regression analysis showed that inflammatory factors, IL-6 and TNF-α, were significantly correlated with biochemical markers (ALT, AST, and TBiL) of liver cirrhosis. Differences were statistically significant (P<0.05). See **Figure 5**.

Discussion

Vitamins are essential micronutrients for proper functioning of human body in daily life. Studies have shown that the effects of fat-soluble vitamin E on liver fibrosis have been widely confirmed [13, 14]. Vitamin A is also a kind of fat-soluble vitamin, which is mainly distributed in the liver, blood, and eyeballs of animals [15].

Vitamin A is required for important physiological processes, but its relationship with restoration of liver damage has not been substantiated. B vitamins, like B2, B6, B12, and niacin, can prevent hepatic steatosis. Vitamin B12 is beneficial for removal of fat in the liver. Vitamin C, known as ascorbic acid, is an important water-soluble vitamin widely involved in biological redox reactions. However, it cannot be synthesized and stored in human body and must be taken in from the environment. Insufficient intake, malabsorption, or increased demand of vitamin C will aggravate liver damage in patients with liver disease. This is not conducive to disease recovery [16]. Therefore, the efficacy of additional treatment of vitamins for CHB-induced cirrhosis patients requires verification with more clinical data.

This study showed that basic routine treatment, with additional intervention of vitamin A and C, in cirrhosis patients caused a significant reduction in levels of liver function indicators, including ALT, AST, γ-GGT, TBiL, and CHE, as well as an increase in levels of Alb. Regarding effectiveness, vitamin A combined with vitamin C for was significantly better than a single vitamin or a non-vitamin treatment. Moreover, progression of liver fibrosis in patients receiving additional treatment of vitamin A plus vitamin C was significantly improved. Expression levels of IV-C, PIIIP, HA, and LN were significantly reduced. The mechanisms of those effects are that the imbalance of oxidation and antioxidants in patients with liver disease can lead to the accumulation of oxygen free radicals that cause lipid peroxidation and aggravate, to some degree, damage to the liver [17]. Moreover, vitamin A can regulate the proliferation of hepatic stellate cells and the synthesis of collagen, reduce the accumulation of scar tissue in liver, and facilitate the recovery of liver function [18]. Furthermore, vitamin C can effectively reduce lipid peroxidation, increase liver antioxidant capacity, inhibit Kupffer cell activi-
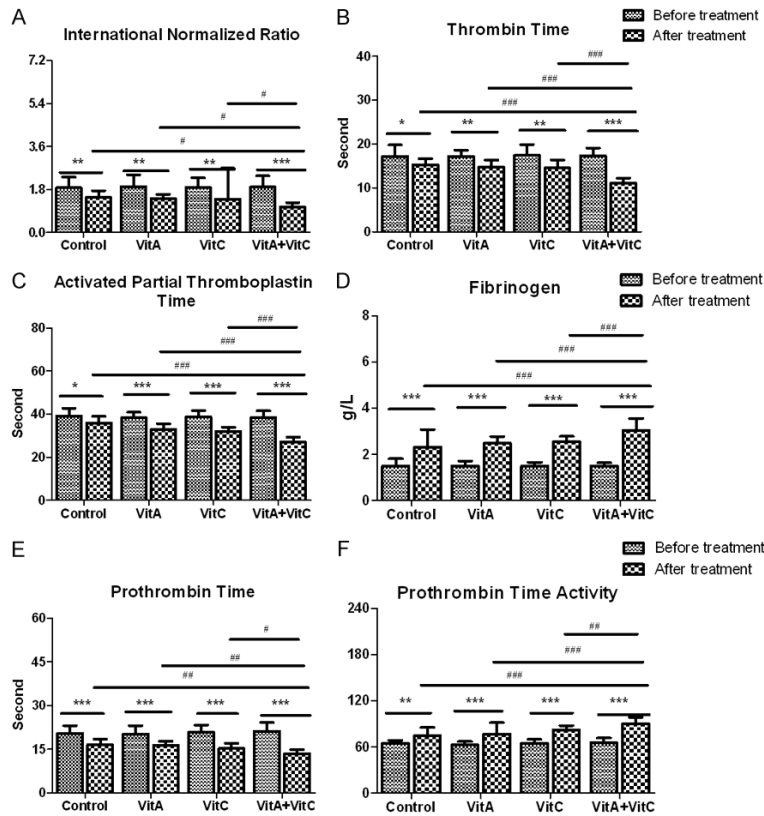
Figure 3. Intra-group and inter-group comparisons in coagulation indicators ($\bar{x} \pm sd$). A. Intra-group and inter-group comparisons of prothrombin time-international normalized ratio (PT-INR). B. Intra-group and inter-group comparisons of thrombin time (TT). C. Intra-group and inter-group comparisons of activated partial thromboplastin time (APTT). D. Intra-group and inter-group comparisons of the levels of fibrinogen (FIB). E. Intra-group and inter-group comparisons of prothrombin Time (PT). F. Intra-group and inter-group comparisons of prothrombin time activity (PTA). The higher the value, the more likely coagulation disorders appear. Intra-group comparison: *P<0.05, **P<0.01, ***P<0.001. Inter-group comparison: #P<0.05, ##P<0.01, ##P<0.001.
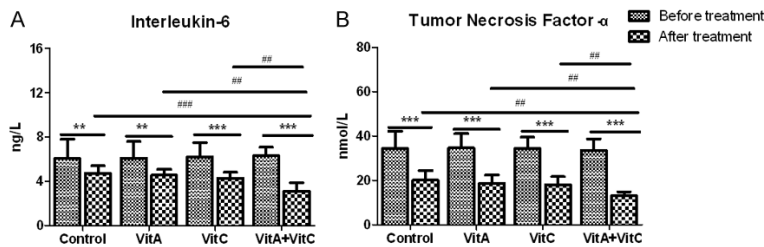

Figure 4. Intra-group and inter-group comparisons in inflammatory markers ($\bar{x} \pm sd$). A. Expression levels of interleukin-6 (IL-6). B. Expression levels of tumor necrosis factor-α (TNF-α). The higher the value, the severer the inflammation. Intra-group comparison: **P<0.01, ***P<0.001. Inter-group comparison: ##P<0.01, ###P<0.001.

The liver is the primary site of synthesis of most clotting factors. Damaged hepatocytes favor synthesis disorders of clotting factors, reduction in synthesis of anti-plasmin, and insufficient production of heparinase, leading to an increase in plasma heparin. This, in turn, causes abnormalities in the coagulation system [20]. Studies have shown that more severe liver damage leads to greater changes in levels of coagulation indicators, with both sides positively correlated [21]. This study showed that cirrhosis patients receiving additional treatment of vitamin A and C were far superior to those receiving a single vitamin or a non-vitamin treatment, regarding levels of liver function indicators. From this perspective, results confirm that combined treatment of vitamin A and vitamin C brings more benefits the recovery of patients. IL-6, also known as B-cell differentiation factor, is an important proinflammatory cytokine in the human body. It can regulate the synthesis of many reactive proteins in the acute phase. It is also a factor related to immune regulation in patients with chronic liver disease, presenting a high expression at early stages in acute and chronic liver injuries [22]. TNF-α is a key cytokine in the development of viral hepatitis. It increases platelet activation, with a result of abnormal blood coagulation, followed by aggravation in inflammation and fibrosis [23, 24]. Serum IL-6 levels are associated with the degree of hepatocellular necrosis and can, to some extent, reflect the severity of acute liver failure [25]. However, whether TNF-α and IL-6 can be used as new indicators of disease severity and clini-

ty, and lower levels of serum free fatty acids [19]. Combination of the two vitamins presents more benefits facilitating the recovery of liver function and improving the progression of liver fibrosis.
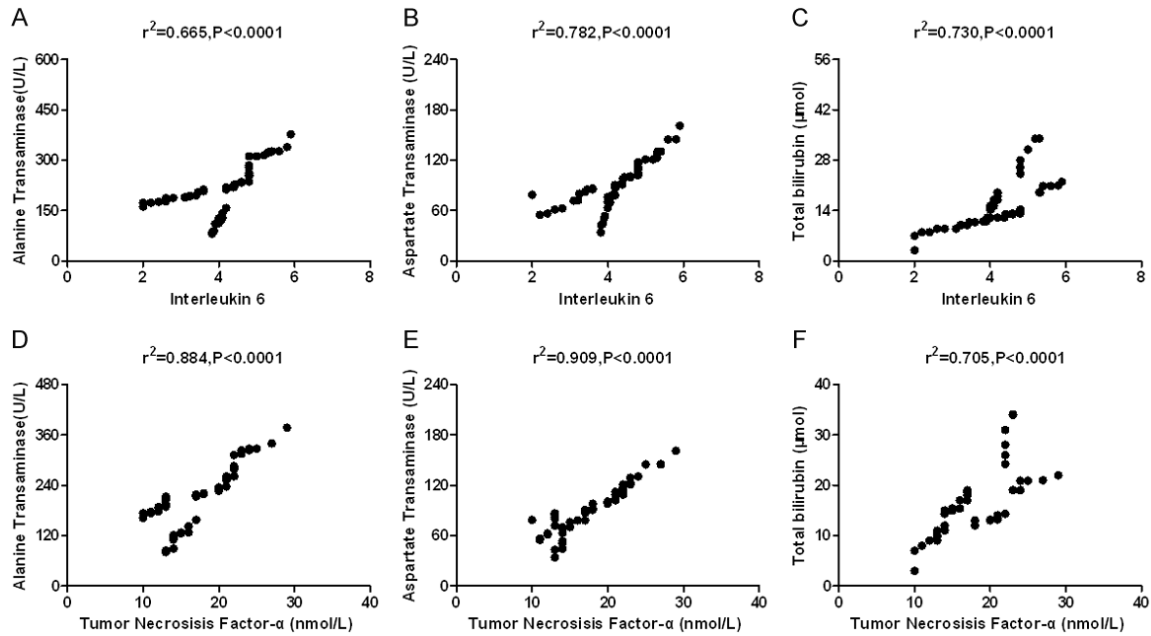
Figure 5. Correlation between inflammatory factors and biochemical markers of liver cirrhosis. A. Correlation between alanine transaminase (ALT) and interleukin-6 (IL-6). B. Correlation between aspartate transaminase (AST) and IL-6. C. Correlation between total bilirubin (TBiL) and IL-6. D. Correlation between ALT and tumor necrosis factor-α (TNF-α). E. Correlation between AST and TNF-α. F. Correlation between TBiL and TNF-α.

cal prognosis requires more clinical data support. Previous studies have reported that vitamins A and C can reduce expression of cytokines, like IL-6 and TNF-α, and inhibit expression of inflammatory factors in the body [26]. Levels of IL-6 and TNF-α in patients receiving additional treatment of vitamins A and C were significantly lower than those in the other three groups, again substantiating the results in those studies. In this study, inflammatory factors, IL-6 and TNF-α, were significantly correlated with biochemical markers, ALT, AST, and TBiL. Differences were statistically significant, further confirming that IL-6 and TNF-α serve important roles in evaluation of immune damage in liver diseases. However, the sample size was relatively small in this study and most patients received oral treatment at home. This factor may have led to low adherence and controllability. Therefore, further studies are necessary for verification.

In conclusion, additional treatment of vitamins A and C for CHB-induced Child-Pugh B cirrhosis patients can effectively improve liver function, prevent liver fibrosis, and promote the recovery of coagulation function. In addition, additional treatment can reduce expression levels of IL-6 and TNF-α. Changes in levels of IL-6 and TNF-α can be used for evaluation of the prognosis of patients with chronic hepatitis B. However, the molecular mechanisms require further scientific interpretation.

Disclosure of conflict of interest

None.

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