Brief Report

The Potential Role of Autophagy in Progression of Liver Fibrosis in Chronic Hepatitis B Patients Receiving Antiviral Treatment: A Brief Report

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Abstract

Despite antiviral treatment, some patients with chronic hepatitis B (CHB) progress to cirrhosis. Enhancement of autophagy was implicated in the proliferation of hepatitis B in hepatocytes. This study aimed to evaluate the potential role of autophagy in the progression of liver fibrosis in patients receiving antiviral treatments and having completely inhibited viral replication. This descriptive-analytical study was designed and conducted in 2020 at Mottahhari Hepatitis Clinic affiliated with Shiraz University of Medical Science (Shiraz, Iran). Patients who were on anti-hepatitis B nucleotide treatments for at least two years, and those who were not cirrhotic at baseline but later progressed to cirrhosis were identified to be included in the case group. Besides, for the control group, patients on the nucleotide regimens who did not have cirrhosis at baseline or during follow-up were randomly selected. Ultimately, 16 cases and 14 controls were included in the study. Data were analyzed using SPSS software, and $P<0.05$ was considered statistically significant. Serum Beclin-1 and LC3 levels were compared between the two groups using enzyme-linked immunosorbent assays. The *t* test was used to assess the statistical differences between the case and control groups. Beclin-1 level was significantly higher in cirrhosis patients than the control group (1283±244 vs. 1063±257, $P=0.024$). However, there was no statistical difference between the level of LC3 in the cirrhotic group (168±31) and the control group (150±16) ($P=0.065$). Autophagy may have a role in the progression of cirrhosis in patients with CHB. Future larger prospective studies are required to determine the effect of blocking on the progression of liver disease in this population.

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Keywords ● Autophagy ● Hepatitis B, chronic ● Fibrosis ● Beclin-1

What's Known

- The hepatitis C virus uses cellular autophagy in viral replication.
- Chloroquine was used as an autophagy suppressor.

What's New

- Cirrhosis emerged unexpectedly in a small subset of chronic hepatitis B patients treated with HBV DNA-negative nucleotide analogues. They had higher levels of Beclin-1 (an autophagy marker), suggesting that viral suppression is insufficient in liver disease and autophagy could be a possible mechanism.

Introduction

Hepatitis B virus (HBV) is a double-stranded DNA virus, which is one of the leading causes of hepatocellular carcinoma and liver cirrhosis in the world.[1] It is estimated that about 255 million people have chronic HBV infection worldwide.[2] The risk of liver cirrhosis

is higher in individuals with higher serum levels of HBV DNA. Moreover, suppressing viral replication was reported to reduce the risk of cirrhosis progression and hepatocellular carcinoma (HCC).[3]

Other risk factors, including co-infection with the hepatitis D virus, hepatitis B e-antigen positive, concomitant diabetes mellitus, and old age, increased the risk of progression to cirrhosis.[4] In somatic cells, autophagy is the primary degradation system. Eukaryotic autophagy involves several mechanisms to degenerate the short-lived protein.[5] The pathogenesis and progression of HCC are associated with decreased autophagy.[6]

A previous study reported numerous cases of chronic HBV infection-inducing autophagy.[7] This activation might be more prominent in HCC patients with chronic HBV infection.[8] Therefore, developing treatments that target autophagy might have a role in delaying or even retreating the liver injury induced by HBV, as well as fatty liver disease and HCC.[6, 9] Blockade of autophagy-mediated processes could provide new opportunities for preventing or reversing cirrhosis.

Beclin-1, a key autophagic gene, was found to be overexpressed in various human malignancies.[6] Furthermore, LC3-II was specifically associated with autophagosomes and autolysosomes.[10]

Beclin-1 is a crucial autophagic agent. The recruitment and activation of Beclin-1 are some of the initial steps in the construction of autophagosomes from pre-autophagic structures.[11] Beclin-1 acts as a tumor suppressor and is an essential autophagy mediator. Beclin-1 also interacts with Bcl-2 and can induce apoptosis.[9]

This study was designed to investigate the potential role of autophagy in the progression of liver fibrosis in chronic hepatitis B (CHB) patients who received antiviral treatment with complete viral replication suppression.

Patients and Methods

This descriptive-analytical study was designed and conducted in 2020 at Mottahhari Hepatitis Clinic, affiliated with Shiraz University of Medical Sciences (Shiraz, Iran). The present study had no selection or sampling procedure, because the study was conducted on all eligible patients who fulfilled the inclusion criteria and volunteered to participate. The inclusion criteria include being between the ages of 18 and 80 years, having at least two years of standard antiviral HBV treatment in the past, and having confirmed reduced viral load in the follow-up studies prior to the diagnosis of cirrhosis. In follow-up, cirrhosis was diagnosed using clinical examination, imaging, including transient elastography, and biochemical markers of fibrosis. The patients with concomitant comorbid diseases such as diabetes mellitus, those who used alcohol at any amount, those with HDV or HCV co-infections, hemochromatosis, and those with a BMI more than 30 at baseline or during follow-up were excluded.

For the control group, we randomly selected from the electronic health records those with the same characteristics but no evidence of cirrhosis in follow-up. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (code: IR.SUMS.MED.REC.1399.542). The participants were informed about the goals of the research, and written informed consent was obtained from all the participants. All the participants of the case group (n=16) were not cirrhotic at baseline, and the development of cirrhosis could not have been caused by any other pathologic condition or disease. The control group (n=14) was non-cirrhotic CHB individuals who were referred to Motahhari Hepatitis Clinic, Shiraz University of Medical Sciences, within the same period of time.

Blood samples (10 cc) were obtained from the patients by a venous puncture at the Hepatitis Clinic and centrifuged to be prepared. The separated serum was stored in a freezer at -20 °C until the analysis procedure. Serum Beclin-1 and LC3 levels were measured using an enzyme-linked immune-sorbent assay (Sunlong Biotech Co. Ltd, China) in the Research Laboratory of Shiraz University of Medical Sciences, School of Biochemistry (Shiraz, Iran). The serum assessment process was based on an intra-assay coefficient of variation (CV) of less than 10%, an inter-assay CV of less than 12%, and a lower detection limit of 1 pg/mL.

Statistical Analysis

All the data were analyzed using the SPSS software, version 21 (IBM Statistics, Chicago, USA). Data were expressed as mean±SD. The *t* test was used to compare the statistical differences between the case and control groups. Besides, covariance analysis was performed on age, length of the treatment, and transaminase levels. $P<0.05$ was considered statistically significant.

Results

All Cases admitted in this study were patients with CHB who received viral hepatitis B treatment for at least two years. Moreover, HBV DNA was negative in all cases.

Table 1: Comparison of demographic and biochemical factors between cirrhotic and non-cirrhotic chronic hepatitis B infected patients

Variable	Cirrhotic group (mean±SD)	Non-cirrhotic patients (mean ±SD)	P value*	P value**
Age (years)	60.9±8.5	49.1±10	0.002	-
Treatment duration (years)	11.3±4.8	10±4.2	0.446	-
AST (IU/L)	41.2±25.4	26.6±7.9	0.049	-
ALT (IU/L)	39.4±17.3	31.4±16.2	0.205	-
Plt (×1000/μL)	149±48	215±56	0.002	-
Hb (g/dL)	14.4±1.4	14.2±1.7	0.679	-
AFP (ng/mL)	4.6±1.8	2.4±1.9	0.005	-
Beclin level (pg/mL)	1283±244	1063±257	0.024	0.008
LC3 level (pg/mL)	168±31	150±16	0.065	0.290

*Based on independent *t* test; **Analysis of covariance adjusted by, age, and length of treatment; AST: Aspartate aminotransferase level of plasma; ALT: Alanine aminotransferase level of plasma; Plt: Platelet count; Hb: Hemoglobin; AFP: Alpha Fetoprotein level of plasma; P<0.05 was considered statistically significant.

In total, 30 patients participated in this study. Cirrhosis was diagnosed based on clinical and laboratory findings, as well as transient elastography. Despite treatment and satisfactory virologic response, 16 cases (53.3%) progressed to cirrhosis. The remaining 14 cases (46.6%) were not cirrhotic. The characteristics of the two groups are presented in table 1.

The mean age of cirrhotic and non-cirrhotic patients was 60.9±8.5 and 49.1±10, respectively. The mean age of the cirrhotic group was significantly higher than the non-cirrhotic group (P=0.002). The mean duration of antiviral treatment in the cirrhotic group (11 years) was not statistically different from the non-cirrhotic group (10 years).

Beclin concentration was higher in the cirrhotic patient group than the control group, which was statistically significant (P=0.024). Although the cirrhotic patient group had higher plasma concentrations of LC3, the difference was not statistically significant (P=0.065).

Treatment duration and age were supposed to be confounding variables. However, after adjusting for age, treatment duration, and level of serum transaminases levels using covariance analysis, the mean plasma concentration of Beclin was still significantly higher in the cirrhotic patients (P=0.008). As indicated in table 1, the covariance analysis revealed that mean LC3 concentration was not significantly different in both groups (P=0.290).

Discussion

The findings of the present study indicated that in this series of patients with CHB receiving long-term oral nucleotide analogues, who had negative HBV DNA as well as those who progressed to cirrhosis, had higher levels of Beclin-1. Moreover, the length of treatment and age were supposed to be the confounding variables. Thus, covariance analysis was used to account for them. However, the cirrhotic patients had significantly higher mean plasma concentrations of Beclin.

Despite antiviral treatment, the progression to cirrhosis in these patients might suggest that viral suppression with nucleotide analogues is insufficient for cirrhosis prevention. One of the potential mechanisms causing cirrhosis is autophagy. Beclin-1, one of the markers of early-phase autophagy, was higher in these patients, which could provide proof for this hypothesis.

HBV was found to utilize autophagy as a mechanism for cell proliferation.[12] Despite viral suppression, this mechanism might still be active and cause fibrosis and cirrhosis in some patients. Moreover, other etiologies for the development of cirrhosis was associated with autophagy. It could be a double-edged sword, enhancing pathogens and abnormal cell proliferation, such as malignancy. Therefore, it was implicated that autophagy contributes to the proliferation of HBV.[11]

Autophagy is involved in both the innate and adaptive immune responses to viral hepatitis, such as HBV.[8] Focusing on autophagy may introduce a new opportunity to prevent and even reverse fibrosis in patients with chronic liver disease from any cause, particularly CHB and CHC. Previous studies indicated that the use of chloroquine as a suppressor of autophagy inhibited HCV replication.[13, 14] However, the present study found that patients who developed cirrhosis after receiving antiviral medications had higher serum levels of Beclin-1, a marker of autophagy.

The present study had several limitations. Although none of the patients in these series had cirrhosis based on clinical, endoscopic, imaging findings as well as liver biopsy results, which were available at the time of initiation of anti-viral treatment, some might have had subclinical cirrhosis from the beginning. To address this

issue, a prospective study is required. Tissue studies may reveal further details about the events at the molecular level. Another limitation of our study was the small sample size of our series, because it was limited to a single center. Furthermore, one may argue that it is the effect of fibrosis rather than its cause.

Conclusion

The findings demonstrated that, at least in some patients with CHB, autophagy may play a role in cirrhosis progression despite adequate viral suppression. Moreover, even after controlling for confounding variables such as treatment duration and age, Beclin-1 concentration was significantly higher in the cirrhotic patient group than the control group. Therefore, Beclin-1 and other autophagy mediators might lead to new promising therapies to prevent or even reverse HBV-related liver fibrosis.

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Authors' Contribution

K.BL: Study concept, study design, and drafting; A.S: Performing the study, data management, and drafting; MR.F: Data gathering and data analysis, and critical revision; S.A: Laboratory data management and drafting; P.M: Laboratory data management and drafting; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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