Is There any Role for Autophagy in Progression of Liver Fibrosis in Chronic Hepatitis B Patients on Antiviral Treatment?

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Research Article

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Abstract

Introduction

Despite antiviral treatment, there are patients with chronic hepatitis B (CHB) who progress to cirrhosis. Enhancement of autophagy has been implicated in the proliferation of hepatitis B in hepatocytes. This study was designed to evaluate the possible role of autophagy in the progression of liver fibrosis in patients on antiviral therapies and fully suppressed viral replication .

Methods

Patients on anti-hepatitis B nucleotide treatments for at least two years and who were not cirrhotic at baseline but later developed cirrhosis were identified. For the control group, patients who were on the nucleotide regimens and did not have cirrhosis at baseline and on follow-up were randomly selected from our registry. Serum Beclin-1 and LC3 measured by enzyme-linked immunosorbent tests were compared between two groups.

Results

Beclin-1 level was higher in those with cirrhosis, but no statistical difference was seen for the level of LC3.

Conclusion

Autophagy may have a role in progression to cirrhosis in patients with CHB. Future prospective larger studies are required to find the effect of blockage of autophagy on liver disease progression in this group.

Introduction

Hepatitis B virus (HBV) is a double-stranded DNA virus that is one of the most common causes of hepatocellular carcinoma (HCC) and liver cirrhosis worldwide (1–6). It is estimated that more than 255 million people have chronic HBV (CHB) infection worldwide (7). In recent years, World Health Organization reported a silent HBV pandemic and estimated that only 1.7% of all eligible HBV cases receive treatment globally (8).

The risk of liver cirrhosis is higher in those with higher serum levels of the HBV DNA, and suppression of viral replication has been shown to reduce the risk of the development of cirrhosis and HCC in these patients (10).

Other factors, including coinfection with hepatitis D virus, hepatitis B e-antigen positivity, concomitant diabetes mellitus, and advanced age, increase the risk of progression to cirrhosis (10–13). Alternatively, there are patients with extremely high levels of HBV DNA who have no progression of liver disease through the so-called immune tolerance state (14).

The ductular reaction (DR) is a typical response to injury observed in various liver diseases. Autophagy activation is required for the mesenchymal transition during the DR and plays an essential role in the pathogenesis process of cirrhosis (15). Several studies revealed that DR is positively associated with an elevated autophagic marker during cirrhosis development (16). Blockade of autophagy-mediated processes could provide novel opportunities to prevent or reverse cirrhosis.

Autophagy is the primary degradation system in somatic cells. Eukaryotic autophagy comprises of several pathways to degenerate the short-lived protein (proteasome) and the plasma membrane and extracellular (lysosome) (17). Autophagy showed protective properties against infections, the physical stress of exercising, fasting, and cancers (18). Although autophagy demonstrated a valuable role in preventing the development of cancers at initial stages, this mechanism later provides the required energy for cancer cells and promotes tumor proliferation (19).

There are several reports of induction of autophagy by chronic HBV infection (20). This activation might be more prominent in HCC in the setting of chronic HBV infection (21). It is proposed that the X protein of HBV promotes viral proliferation and

resistance through the regulation of phosphatidylinositol-3-kinase class III and c-myc, which is an essential step in autophagy (22–25). Therefore, developing therapies targeting autophagy may have a role in delaying or even regressing the liver injury induced by HBV. These autophagy-based treatments may also have a role in treating fatty liver disease and HCC (9, 18, 26).

Beclin-1 is an important autophagic agent and plays a crucial role in autophagosome formation (27–29). Moreover, the association of Beclin-1 with multiple disorders, such as fatal Sindbis Virus pathogenesis, has been proved many years ago (30). Both in vivo and in vitro models demonstrated that hepatic stellate cells (HSC) promotion have an association with activation of autophagy-mediated by several molecules, including LC3-II

(31, 32). It has been shown that both defective basal levels of autophagy and stress-induced increases in autophagy are equally important in promoting liver fibrosis (33).

This study was designed to investigate the possible role of autophagy in the progression of liver fibrosis in CHB patients who received antiviral treatment with complete suppression of viral replication .

Methods

Patients

Among the patients with chronic hepatitis B in Mottahhari hepatitis clinic affiliated to Shiraz University of medical science who received antivirals and had no cirrhosis in baseline,

we identified those who developed cirrhosis despite at least two years of oral nucleotide treatment and negative HBV DNA at the time of the study.

Exclusion and later diagnosis of cirrhosis at baseline and then in follow-up were with clinical evaluation, imaging including transient elastography and biochemical markers of fibrosis. Excluded were patients with concomitant comorbid diseases including diabetes mellitus, those who used alcohol at any amount, patients with HDV or HCV coinfections, hemochromatosis, those who had BMI more than 30 at baseline or during follow up. Those who used any drug other than tenofovir in the past two months were also excluded.

All patients were on Tenofovir disoproxil fumarate at least for two years and had undetectable HBV DNA at the time of the study.

For the control group, we randomly selected from the digital files those who had the same characteristics but had not developed evidence of cirrhosis in their follow-up.

Ethics

This study is approved by the ethics committee of Shiraz University of Medical Sciences. (Ethic code: IR.SUMS.MED.REC.1399.542) All patients signed a standard informed consent and were aware of the purposes of the study.

Study Design

The case group was selected among cirrhotic patients (ultimately 16 cases). Cirrhosis diagnosis was confirmed with transient elastography and clinical, laboratory, or endoscopic evidence, including thrombocytopenia, peripheral cirrhosis stigmata, and esophageal varices. These patients were not cirrhotic at baseline, and any other pathologic condition or disease could not explain the progression to cirrhosis. The control group (ultimately 14 cases) were selected from non-cirrhotic CHB followed up in Motahhari hepatitis clinic, Shiraz University of Medical Sciences, during the same period.

Serum Sampling and Measurement

Blood samples of studied patients (10cc) were taken by a venous puncture at the hepatitis clinic and prepared by centrifuge. Separated serum was stored in a -20°C freezer until the analysis procedure. Serum Beclin-1 and LC3 levels were evaluated by

enzyme-linked immune-sorbent assay (Sunlong Biotech Co. Ltd, China) in the research laboratory of Shiraz medical university, faculty of biochemistry. The process of serum assessment was carried out based on the intra-assay coefficient of variation (CV) less than 10%, inter-assay CV less than 12%, and lower detection limit of 1 pg/mL.

Statistical Analysis:

All variables were expressed as the mean ± SD. The statistical differences between case and control groups were tested with the paired sample T-test to pairwise comparisons. Besides, covariance analysis was done for age, gender, treatment duration, and transaminases levels. All data were analyzed using SPSS software (version 21.0; SPSS, Chicago, IL, USA).

Results

All Cases admitted in this study were chronic hepatitis B patients who had been treated for viral hepatitis B for at least two years. In all cases, HBV DNA was negative.

Totally 30 patients were enrolled in this study. Diagnosis of cirrhosis was confirmed based on clinical and laboratory findings and transient elastography. Sixteen cases (53.3%) had developed cirrhosis despite treatment and proper virologic response. The other 14 cases (46.6%) were not cirrhotic. The characteristics of the two groups are shown in Table 1.

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

The platelet count was lower (P:0.002), AST and serum alpha-fetoprotein concentration both were higher in the cirrhotic patient's group (P:0.005). Hemoglobin concentration and alanine aminotransferase were lower in the cirrhotic group, but the difference was not

statistically significant.

Beclin concentration was higher in the cirrhotic patient's group compared to controls which was statistically significant (P:0.024). Plasma concentration of LC3 was higher in the cirrhotic patient's group, but the difference did not reach statistical significance (P:0.065)

After adjustment for gender, age, treatment duration, and level of serum transaminases based on covariance analysis, the mean plasma concentration of Beclin was still significantly higher in the cirrhotic patient group (0.008). Covariance analysis for adjustment for age, gender, treatment duration, and transaminases level revealed that mean LC3 concentration was not significantly different in both groups (P: 0.290) (Table 1).

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

		Age	Treatment duration	AST	ALT	PLT	Hemoglobin	AFP	BECLIN	LC3
Cirrhotic patients	Total number	16	16	16	16	16	16	16	16	16
	mean	60.9	11.3	41.2	39.4	149000	14.4	4.6	1283	168
	Std. Deviation	8.5	4.8	25.4	17.3	48000	1.4	1.8	244	31
Non cirrhotic patients	Total number	14	14	14	14	14	14	14	14	14
	Mean	49.1	10	26.6	31.4	215000	14.2	2.4	1063	150
	Std. Deviation	10	4.2	7.9	16.2	56000	1.7	1.9	257	16
Relationship between cirrhotic and non cirrhotics	P value†	0.002	0.446	0.049	0.205	0.002	0.679	0.005	0.024	0.065
	P value‡								0.008	0.290

† Based on independent t-test

‡ Analysis of covariance adjusted by gender, age, treatment duration and transaminases

Discussion

In this series of patients with CHB on long-term oral nucleotide analogues, all with negative HBV DNA, those who progressed to cirrhosis had higher levels of Beclin-1.

The progression to cirrhosis in these patients despite antiviral treatment, may indicate that viral suppression by nucleotide analogues is not sufficient in prevention of progression of liver disease. Autophagy may be one of the possible mechanisms driving this progression. Beclin-1, one of the markers of early phase autophagy, was higher in these patients, which may be a piece of evidence for this hypothesis.

It has been shown that HBV uses autophagy as a mechanism for its proliferation within cells. This mechanism may still be active despite viral suppression and lead to fibrosis and cirrhosis, at least in some patients.

Autophagy has been implicated in the development of cirrhosis due to other etiologies. This may act through activation of mesenchymal cells and enhancing collagen production and ultimately fibrosis.

Autophagy is a common mechanism in natural cells for providing required energy and materials, but in some cases, adverse effects of the autophagy system had been observed (34). It could be a double sword enhancing the proliferation of pathogens and abnormal cells, including malignancy. Autophagy has been implicated in the proliferation of HBV (40, 41) and hepatitis C virus infection (42, 43)

Several recent studies have shown enhanced autophagy as a mechanism for developing HCC (35, 36) and fatty liver disease (37–39). Autophagy is also implicated in innate and adaptive immune responses to viral hepatitis, including HBV (21). Studies on HCV pathogenesis showed that this hepatic virus uses cellular autophagy in favor of viral replication and noticeably increases LC3 (44). This may further modulate the course of chronic liver disease.

Focusing on autophagy may introduce a new opportunity to prevent and even reverse fibrosis in patients with chronic liver disease from any cause, especially CHB and CHC. Downregulation of autophagy in HSC has been linked to the attenuation of liver fibrosis (45, 46). We have previously shown that the use of chloroquine as a suppressor of autophagy suppresses HCV replication (47, 48).

Although this study has shown that serum Beclin-1 as a marker of autophagy was higher in patients who developed cirrhosis despite antiviral treatments, our study has several limitations.

Although none of the patients had not cirrhosis based on clinical, endoscopic, and imaging and liver biopsy when available at the time of initiation of anti viral treatment in this series, some may have had subclinical cirrhosis from the beginning. A prospective study is needed to resolve this issue. Tissue studies may further show the events at the molecular level. Our series was a small series from a single center which is another limitation of our study. Further one can argue this is the result rather than the cause of fibrosis. Cirrhosis is a complex and multifactorial state, and several mechanisms may act, which might be different in cirrhosis due to different etiologies. Therefore, further studies are required to investigate the precise role of autophagy in cirrhosis .

In conclusion, our study shows that at least in some patients with CHB, autophagy may have a role in the progression of cirrhosis despite adequate viral suppression. Targeting Beclin-1 and other autophagy mediators may lead to new promising therapies to prevent or even reverse HBV-related liver fibrosis.

Declarations

Ethics approval and consent to participate:

This study was approved by the local research ethics committee of Shiraz University of Medical Sciences. (Ethic code: IR.SUMS.MED.REC.1399.542), and all the patients signed a standard informed consent and were aware of the purposes of the study. All methods were performed in accordance with relevant guidelines and regulations.

Consent for publication:

Not applicable

Availability of data and materials:

The datasets generated and analysed during the current study are included in this published article and its supplementary information files

Competing interest:

Authors have no competing interest

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Author's Contributions:

Kamran Bagheri Lankarani led all aspects of the study, including defining the research question, overseeing the recruitment effort, finalizing the methodology, and writing the manuscript. Atefeh Sadidoost led the administration of the survey, data management and contributed to writing up the manuscript. Mohammadreza Fattahi contributed to data collection and interpretation of data. Saeid Amirzadehfard and Pooneh Mokarram contributed to laboratory data management and the writing of the manuscript. All authors provided final approval for publication.

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