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• VIRAL HEPATITIS •

Sequential changes of serum ferritin levels and their clinical significance in lamivudine-treated patients with chronic viral hepatitis B

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Abstract

AIM: To study the sequential changes of serum ferritin levels in lamivudine-treated patients with chronic viral hepatitis B and the clinical implications.

METHODS: Thirty-eight patients with chronic viral hepatitis B were prospectively studied during their treatment with lamivudine. Each patient received 100 mg oral lamivudine daily for 12 mo, and was observed and tested for blood biochemistry and hepatitis B virus (HBV) DNA levels and serum ferritin levels at baseline and at 3, 6 and 12 mo during the treatment. Serum HBV DNA levels were quantitatively determined using fluorescent quantitative polymerase chain reaction (FQ-PCR), and serum ferritin levels were measured by radioimmunoassay. The sequential changes of serum ferritin levels and their relationships with virological, serological and biochemical responses in the patients were analyzed.

RESULTS: All the patients had a baseline HBV DNA level higher than 1×10^7 copies/L as determined by FQ-PCR and positive HBsAg and HBeAg and abnormal ALT levels. At the end of the 12-mo treatment, 19 of the 38(50.00%) patients had undetectable serum HBV DNA levels by FQ-PCR, and 12(31.58%) became negative for serum HBeAg and 10(26.32%) had seroconversion from HBeAg to HBeAb. Nineteen out of the 38(50.00%) patients had biochemically normal ALT levels after 12-mo lamivudine treatment. Sequential determination showed that lamivudine treatment significantly reduced ferritin levels in chronic hepatitis B patients. When the patients were divided into different groups according to their post-treatment virological, serological and biochemical responses for analysis of the sequential changes of ferritin levels, it was found that the decrease of ferritin levels in HBV DNA-negative group was significantly more obvious than that in HBV DNA-positive group at 6 mo during the treatment (P=0.013). Consecutive comparisons showed that ferritin levels at 3 mo of treatment were obviously decreased as compared with the baseline levels (P<0.05) in HBeAg-negative group, and the decrease of serum ferritin levels in patients with normalized ALT was more significant than that in patients with abnormal ALT at the end of the 12-mo treatment (P=0.048).

CONCLUSION: Lamivudine treatment can reduce the serum ferritin levels in chronic viral hepatitis B patients and decreases of ferritin levels can be more significant in patients exhibiting virological, serological and biochemical responses, indicating that dynamic observation of serum ferritin levels in patients with chronic viral hepatitis B during lamivudine treatment might be helpful for monitoring and predicting patients' responses to the therapy.

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INTRODUCTION

Hepatitis B virus (HBV) is one of the major causes of liver diseases worldwide, which may progress into cirrhosis and hepatocellular carcinoma[1-5]. It is thus important to implement anti-viral therapy against chronic hepatitis B to minimize the liver damage[6]. Studies suggest that around half of all patients with chronic HBV infection respond to a 6- to 12-mo course of interferon (IFN) therapy, which may induce the elimination of serum hepatitis B viral DNA (HBV DNA) and hepatitis B e antigen (HBeAg), as well as normalization of serum alanine aminotransferase (ALT) activity. However, the response rate is still low and relapse occurs in about half of the responders[6-12]. Lamivudine has become a recent interest in the treatment of chronic viral hepatitis B[13-20] and it is suggested that high levels of pretreatment ALT and low levels of HBV DNA are predictive of response[8,21-23]. However, other predictors of response to lamivudine therapy are unclear. Studies indicated that serum ferritin levels could be used to assess the degree of hepatocyte lesion in chronic viral hepatitis B[24-31], but the role of serum ferritin determination in the treatment of viral hepatitis B with lamivudine remains uncertain. We therefore conducted the present study to investigate the possible role of sequential determination of serum ferritin levels in patients treated with lamivudine to explore the clinical implications.

MATERIALS AND METHODS

Patients and treatment

We prospectively studied 38 chronic hepatitis B patients with a complete clinical record, including 28 male and 10 female patients aged between 13 and 59 years (mean 29.32±10.97 years), and none of the patients received interferon or other anti-viral therapy 6 mo before this study. Chronic hepatitis B was defined as positive hepatitis B surface antigen (HBsAg), positive HBeAg, detectable HBV DNA and abnormal serum ALT levels (normal <40 IU/L) for more than 6 mo. All patients had at least three documented occasions of serum ALT levels higher than the upper normal limit measured at intervals of one mo, within 6 mo prior to the enrollment. Alcoholics and intravenous drug users or homosexual persons and patients

with use of hepatotoxic drugs, herbal medicine or immunosuppressive therapy within the past 6 mo were excluded, and none of the patients had decompensated liver function, cirrhosis, chronic renal failure, clotting abnormalities, hemophiliacs, serious neurological disorders, obesity, chronic viral hepatitis C or delta, human immunodeficiency virus (HIV) infection, autoimmune disease (anti-nuclear antibody titer >1:40), and/or inheritable disorders such as hemochromatosis, alpha-1-antitrypsin deficiency or Wilson's disease. All patients with peripheral white blood cells <4 000 per mm^3 and platelet < 80 000 per mm^3 were also excluded. Serum HBV DNA levels were quantitatively determined using hepatitis B virus fluorescence polymerase chain reaction (FQ-PCR) diagnostic kit manufactured by Daan Gene Corporation Limited of Zhongshan University (Shenzhen, China) according to the instruction. The lowest detectable HBV DNA level of FQ-PCR was 10^4 copies/mL. Serum ferritin levels were determined using a commercially available radioimmunoassay kit manufactured by China Atomic Energy Research Institute (Beijing, China). The reference values for normal serum ferritin level are 20-140 μg/L and 16-132 μg/L for male and female, respectively.

All patients received 100 mg oral lamivudine daily for 12 mo, and were observed and tested for blood biochemistry, HBV DNA levels, serological markers of HBV infection and serum ferritin levels at baseline and at 3, 6 and 12 mo during the treatment. Absence of serum HBV DNA and HBeAg, HBeAg seroconversion to HBeAb and normalization of serum ALT levels were assessed for the efficacy of treatment. Informed consent was obtained from all the patients before treatment.

Statistical analysis

Data in the text and tables are expressed as mean±SD and were analyzed with SPSS software. Statistical analysis was performed using two-tailed Fisher's exact test, two-tailed Student's *t* test and Chi-square test where appropriate. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Sequential changes of serum ferritin levels during treatment

All of the 38 patients completed the 12-mo treatment. When all the patients were analyzed for the changes of serum ferritin levels, it was shown that their serum ferritin levels gradually decreased as lamivudine treatment prolonged (Figure 1). Serum ALT and aspartate aminotransferase (AST) levels declined in correlation with the serum ferritin levels. Serum ferritin levels were significantly decreased within 6 mo of lamivudine treatment (baseline *vs* mo 3, P=0.018; mo 3 *vs* mo 6, P=0.027) and stabilized thereafter (mo 6 *vs* mo 12, P=0.593).

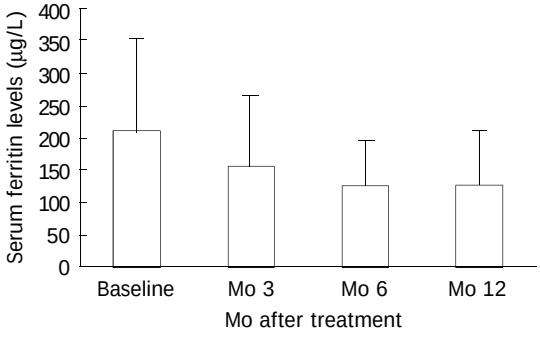

Figure 1 Sequential changes and comparisons of serum ferritin levels (μg/L) at different time points during lamivudine treatment.

Relationships between sequential changes of serum ferritin levels and virological responses to treatment

At the end of 12-mo treatment, 19 of the 38(50.00%) patients had undetectable serum HBV DNA levels and another half of the patients still had detectable HBV DNA by FQ-PCR. The ferritin levels and their sequential changes were analyzed between these two groups. The ages (28.58±11.47 years *vs* 30.05±10.71 years) and HBV DNA levels at baseline [$(6.3±8.6)×10^8$ copies/mL *vs* $(7.1±6.1)×10^8$ copies/mL] between two groups had no statistical difference. The mean baseline ALT and AST levels in the HBV DNA-negative group (141.68±105.99 IU/L and 101.00±71.85 IU/L, respectively) were higher than those in HBV DNA-positive group (99.94±73.00 IU/L and 63.80±37.99 IU/L, respectively), but not statistically different (P=0.166 and P=0.054, respectively) between two groups. The mean baseline ferritin levels between two groups were not statistically different (Table 1). After initiation of lamivudine treatment, serum ferritin levels were decreased in both HBV DNA-negative and -positive groups, but the decrease in the former group was more apparent, with a statistically significant difference at mo 6 (P=0.013, Table 1). Interestingly, the percentage of female patients was higher in HBV DNA-negative group than in HBV DNA-positive group (10/19 *vs* 0/19, P<0.01).

In HBV DNA-negative group, consecutive comparisons of the ferritin levels within this group showed that the mean ferritin level at mo 3 of treatment was significantly decreased as compared with the baseline level (P<0.01), and the level at mo 6 was not obviously decreased compared with that at mo 3 (P=0.140), as with the comparison between the level at mo 12 and at mo 6 (P=0.866). In HBV DNA-positive group, consecutive comparisons showed no significant decreases in ferritin levels when compared in the same way as in HBV DNA-negative group (P= 0.825, P=0.110 and P=0.349, respectively, Table 1).

Relationships between sequential changes of serum ferritin levels and serological responses to treatment

Of the 38 patients, 12(31.58%) were negative for serum HBeAg and 26 positive for serum HBeAg at the end of the 12-mo treatment. The ferritin levels and their changes were analyzed between these two groups. The gender (female/total, 4/12 *vs* 6/26), ages (28.33±12.67 years *vs* 29.77±10.33 years), baseline ALT (116.25±70.58 *vs* 122.92±101.88 IU/L), AST (91.92±59.13 *vs* 78.00±60.67 IU/L), ferritin (Table 2) and HBV DNA levels [$(8.85±9.83)×10^8$ copies/mL *vs* $(5.67±5.92)×10^8$ copies/mL] between two groups had no statistical difference. The comparisons of serum ferritin levels between the 2 groups showed no significant difference at any time point (Table 2).

Consecutive comparisons of ferritin levels within the two groups showed that, in HBeAg-negative group, ferritin level at mo 3 of treatment was obviously decreased as compared with the baseline levels (P<0.05), and the levels at mo 6 and 12 of treatment were not significantly decreased when compared with the level at month 3 (P=0.261) and mo 6 (P=0.373), respectively. In HBeAg-positive group, ferritin levels were not obviously decreased when compared in the same way as in HBeAg-negative group (P=0.228, P=0.051 and P=0.834, respectively, Table 2).

Of the 38 patients treated, 10(26.32%) had a seroconversion from HBeAg to HBeAb while 28 had not at the end of 12-mo treatment. The ferritin levels and their changes were also analyzed between these 2 groups of patients. The ages (29.77±10.33 years *vs* 28.33±12.67 years), ratio of female patients (3/10 *vs* 7/28) and baseline ALT(116.10±76.55 *vs* 122.50±98.43 IU/L), AST(96.50±64.12 *vs* 77.36±58.49 IU/L), ferritin (Table 3) and HBV DNA levels [$(10.2±1.03)×10^8$ copies/mL *vs* $(5.52±5.77)×10^8$ copies/mL] between the 2

groups were comparable. Statistically, no significant differences in serum ferritin levels between two groups were found at any time points although the decreases of ferritin levels in HBeAb-positive group after the initiation of treatment were more apparent than those in HBeAb-negative group (Table 3).

In HBeAb-positive group, consecutive comparisons of the ferritin levels showed that ferritin level at mo 3 of treatment was not obviously decreased as compared with the baseline level (P=0.077), and the levels at mo 6 and 12 of treatment were not obviously decreased compared with those at mo 3 (P=0.303) and 6 (P=0.370), respectively. In HBeAb-negative group, consecutive comparisons showed that the ferritin level at mo 6 was statistically decreased when compared with that at mo 3 (P= 0.041), while the ferritin levels at mo 3 and mo 12 of treatment were not decreased as compared with those at baseline (P=0.121) and mo 6 (P=0.836), respectively (Table 3).

Relationships between sequential changes of serum ferritin levels and biochemical responses to treatment

Nineteen out of the 38(50.00%) patients had biochemically normal ALT levels and another half of the patients still had elevated ALT levels at mo 12 of treatment. The ferritin levels and their changes were analyzed between these 2 groups of patients. The ages (29.16±11.71 years *vs* 29.47±10.49 years), ratio of female patients (5/19 *vs* 5/19) and baseline ALT (139.58±112.45 *vs* 102.05±63.98 IU/L), AST (94.58±77.00 *vs* 70.21±33.07 IU/L), and ferritin (Table 4) and HBV DNA levels [(7.34±9.12)×10^8 copies/mL *vs* (6.14±5.31)×10^8 copies/mL] between two groups were comparable. It was shown that the decrease of serum ferritin levels in patients with normalized ALT was more significant than that in those with abnormal ALT at mo 12 of treatment (P=0.048, Table 4).

In both groups, consecutive comparisons showed that the ferritin levels at mo 3, 6 and 12 of treatment were not significantly decreased when compared with those at baseline, mo 3 and 6 (P=0.120, P=0.145 and P=0.108 in patients with normalized ALT and P=0.062, P=0.088 and P=0.720 in those with still elevated ALT), respectively, although the ferritin levels at mo 12 were significantly decreased compared with the baseline levels in both groups (P=0.008 and P=0.020, respectively. Table 4).

DISCUSSION

Elimination of HBeAg could significantly improve the clinical outcome and survival in chronic hepatitis B patients[5-7]. A 6- to 12-mo course of IFN therapy could induce the elimination of serum HBV DNA and HBeAg, as well as normalization of serum ALT activity in about half of patients with chronic HBV infection at the end of treatment[6-8]. The usage of immune modulators, as the currently available thymosin-α1, seemed effective in part of patients[32,33]. However, the response rate was still low and relapse occurred in a high proportion of responders. Lamivudine has been increasingly administered to chronic viral hepatitis B patients in recent years and had a reportedly response with HBeAg seroconversion in about (15-30)% of patients after 1 to 2 years of therapy[13-20].

Table 1 Sequential changes and comparisons of serum ferritin levels (μg/L) at different time points during lamivudine treatment between patients negative and positive for HBV DNA at end of treatment

Group	Baseline	Months after treatment		
		3	6	12
HBV DNA (-) group (n=19)	231.20±182.50	130.51±110.21	96.25±68.03	110.78±83.37
HBV DNA (+) group (n=19)	187.42±103.60	182.44±107.15	154.92±66.62	141.28±86.48
P values	0.371	0.150	0.013	0.283

Table 2 Sequential changes and comparisons of serum ferritin levels (μg/L) at different time points during lamivudine treatment in HBeAg-negative and -positive groups at end of treatment

Group	Baseline	Months after treatment		
		3	6	12
HBeAg (-) group (n=12)	250.16±190.78	135.72±138.33	106.13±74.10	97.80±76.96
HBeAg (+) group (n=26)	190.45±123.50	166.05±96.51	135.31±71.60	140.18±87.40
P values	0.336	0.439	0.262	0.160

Table 3 Sequential changes and comparisons of serum ferritin levels (μg/L) at different time points during lamivudine treatment in HBeAb-negative and -positive groups at end of treatment

Group	Baseline	Months after treatment		
		3	6	12
HBeAb (+) group (n=10)	249.19±176.48	147.33±147.60	114.47±76.62	104.41±78.60
HBeAb (-) group (n=28)	195.07±137.31	159.74±96.94	129.85±72.26	134.61±87.51
P values	0.328	0.765	0.577	0.346

Table 4 Sequential changes and comparisons of serum ferritin levels (μg/L) at different time points during lamivudine treatment in patients with normalized serum ALT and those with abnormal ALT at end of treatment

Group	Baseline	Months after treatment		
		3	6	12
ALT normal group (n=19)	198.40±161.58	139.39±111.38	112.35±57.51	99.19±60.52
ALT elevated group (n=19)	220.22±136.72	173.56±109.68	140.37±86.07	155.21±98.94
P value	0.656	0.347	0.266	0.048

In this study, 19 of the 38 patients (50.00%) had undetectable serum HBV DNA levels after the 12-mo treatment, suggesting the potent inhibition of HBV replication in patients with chronic hepatitis B. In 12 of the 38(31.58%) patients, their serum HBeAg became negative and 10(26.32%) had HBeAg seroconversion to HBeAb at the end of treatment, indicating that lamivudine treatment can enhance the elimination of HBeAg and its seroconvertion to HBeAb in chronic hepatitis B patients through the inhibition of HBV replication. Biochemically, 19(50.00%) of the 38 patients had normalized ALT levels after lamivudine treatment, indicating that lamivudine treatment can also improve the biochemical abnormalities in the patients. The HBeAg seroconversion rate in the patients in this study was in accordance with other reports[13-20]. It can be obviously seen from our results and other reports that the response rate is still not satisfactory and further studies on new regimens, including lamivudine in combination with IFN and other agents, are needed[23,34-41].

The relationship between iron metabolism and liver diseases has long been a focus of study. More than two decades ago, it was found that a high serum ferritin level prior to HBV infection might increase the likelihood of persistent infection[24]. Correlations between an increase of both AST and ALT and a higher level of ferritin in patients with chronic hepatitis C and B and alcoholic hepatitis were also documented by previous observations[31]. Furthermore, Serum ferritin was significantly higher in cirrhotic patients in comparison to patients with chronic viral hepatitis and highly elevated ferritin levels were observed in hepatocellular carcinoma (HCC) patients[25-31]. Clinical observations also indicated that desferrioxamine infusion to achieve a normal serum ferritin level could enhance the likelihood of response by a chronic hepatitis B patient to IFN therapy[42].

The precise mechanisms associated with elevated ferritin levels in chronic hepatitis and other acute and chronic liver diseases have not been fully clarified. Firstly, it is implicated that HBV might infect the liver cells and then actively replicate with the propensity for increasing ferritin synthesis, resulting in increased liver iron storage[24,25]. The iron overload may enhance the hepatocyte damage induced by HBV. Secondly, serum AST activities correlated with serum ferritin levels in patients with liver disease and the severer the hepatocyte damage is, the higher the serum ferritin and serum iron are, which seems more obvious in fulminant hepatitis and liver cirrhosis. It is therefore suggested that ferritin and iron levels would increase in serum because of their release from hepatocellular storage in association with necrosis[29-31]. Thirdly, it was also indicated that tumor cells might produce ferritin[27], and lastly, iron-induced oxidative stress, in addition to iron storage and hepatocyte damage, has been believed to be another cause of increased serum ferritin levels in chronic liver diseases[43]. Our observations on the sequential changes of serum ferritin levels showed that the antiviral treatment with lamivudine could reduce the ferritin levels in patients with chronic hepatitis B and the decrease in HBV DNA-negative group at the end of 12-mo treatment could be more profound than that in HBV DNA-positive group, supporting the notion that virus replication could stimulate ferritin synthesis[24,25]. Our sequential determination also showed that the ferritin level in patients with normalized ALT at the end of treatment decreased more apparently than that in patients with abnormal ALT, and the decrease in ferritin levels paralleled that in ALT and AST levels, indicating that the improvement of necroinflammatory damage of hepatocytes also contributes to the decline of ferritin levels. In addition, the virological response in patients was associated with early decrease of ferritin levels while the biochemical response appeared to be associated with relatively gradual decreases of ferritin levels, indicating that the early decrease of ferritin levels might result mainly from the inhibition of HBV replication in hepatocytes and the reduction of ferritin synthesis while the subsequent changes might result from the improvement of necroinflammatory damage of liver tissues and the reduction of ferritin release from hepatocytes.

Many factors may affect the response to antiviral treatment for chronic hepatitis B and it was indicated that serum ferritin levels could be used for predicting the response to IFN treatment in patients with chronic hepatitis B. Interferon-α treatment of chronic hepatitis B showed that ferritin levels correlated well with the type of IFN response, as the serum ferritin level increased, the response rate to IFN declined. Serum ferritin level appeared to influence the type of IFN response achieved[8]. Observations in children with chronic hepatitis B found that in the group of patients with detectable seroconversion in the HBe system resulting from interferon-α therapy, lower serum levels of iron and ferritin could be present[44]. Therefore, to detect serum markers of iron metabolism was believed to be helpful for evaluating the curative effect and prognosis of hepatitis B[30]. So far, although it is suggested that higher levels of pretreatment ALT and lower levels of pretreatment HBV DNA levels were predictors of response[21-23], no data are currently available to address the value of serum ferritin detection in patients with chronic hepatitis B treated with lamivudine.

Our observation was focused mainly on serum ferritin levels with regard to their sequential changes and their relationships with the virological, serological and biochemical responses to lamivudine treatment in chronic hepatitis B patients. We found that lamivudine treatment could reduce serum ferritin levels in chronic hepatitis B patients and the effect was more significant during the first 6 mo of treatment. As the ages, gender, baseline ALT, AST and HBV DNA levels between the groups were comparable, our determination and analysis showed that statistically significant decreases of ferritin levels occurred in virologically and biochemically responding groups but not in nonresponding groups although female patients appeared to be more likely to have viral response. The decreases of ferritin levels in serologically responding groups (elimination of HBeAg and HBeAg seroconversion to HBeAb) were also more obvious than those in serologically nonresponding groups although they were not statistically significant between the two groups. Furthermore, we found that the decreases of serum ferritin levels paralleled the declines of serum ALT and AST levels during lamivudine treatment. Therefore, it indicates that sequential determination of serum ferritin levels, rather than only the detection of pretherapy ferritin levels, in chronic hepatitis B patients treated with lamivudine is helpful for monitoring and predicting the responses to treatment.

Contrary to other observations[22,23], we did not observe any statistical differences in the baseline ALT, AST and HBV DNA levels between any responding and nonresponding groups although, generally speaking, pretherapy ALT and AST levels in responding groups appeared to be higher than those in nonresponding groups. As the number of patients we observed in this study was relatively small, large-scale observations on the response predicting and monitoring factors in relation to lamivudine treatment are needed.

In conclusion, lamivudine treatment for patients with chronic hepatitis B can decrease serum ferritin levels, and the decreases are more profound in virologically and biochemically responsive patients. It is suggested that sequential determination of serum ferritin levels, rather than only the baseline ferritin levels, in patients with chronic hepatitis B may be useful for monitoring and predicting the virological and biochemical responses to the treatment of lamivudine.

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