

# Improvement of chronic hepatitis B by iron chelation therapy in a patient with iron overload

## A case report

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### Abstract

**Rationale:** This report describes seroconversion of hepatitis B surface antigen (HBsAg) in a patient with marked iron overload caused by chronic hepatitis B (CHB) after receiving iron chelation therapy and discusses the role of iron chelation therapy in CHB.

**Patient concerns:** Increased serum ferritin level for 2 months.

**Diagnosis:** Secondary iron overload and CHB.

**Intervention:** To relieve iron load of the body, the patient underwent regular phlebotomy therapy and deferoxamine (DFO) therapy. During the therapy, serum ferritin and hepatitis B virus (HBV) were monitored and the iron concentration of the liver and heart were followed by T2\* of magnetic resonance imaging (MRI) scan.

**Outcomes:** Serum ferritin gradually decreased. Approximately 1 year after the therapy, HBsAg turned persistently negative.

**Lessons:** Iron chelation therapy may attenuate HBV infection.

**Abbreviations:** ADV = adefovir dipivoxil, ALT = alanine aminotransferase, anti-HBc = antibody to HBV core antigen, anti-HBe = antibody to HBV e antigen, anti-HBs = HBV surface antigen, AST = aspartate transaminase, CHB = chronic hepatitis B, CHC = chronic hepatitis C, DFO = deferoxamine, DNA = deoxyribonucleic acid, HBeAg = HBV e antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, IL = interleukin-6, miR-122 = micro ribonucleic acid-122, MRI = magnetic resonance imaging, ROS = reactive oxygen species.

**Keywords:** hepatitis B virus, iron chelation therapy, iron overload

## 1. Introduction

Approximately 2 billion people worldwide are infected with hepatitis B virus (HBV), and more than 350 million have chronic hepatitis B (CHB).<sup>[1]</sup> In patients with CHB, increased iron status is common, with elevated serum iron and ferritin levels and iron accumulation in the liver.<sup>[2]</sup> Forty-two percent of patients with CHB were found to have positivity for hepatic iron through liver biopsy, whereas both hepatic iron concentrations and serum ferritin levels were normal.<sup>[2]</sup> Excessive iron will result in reactive oxygen species (ROS) overproduction,<sup>[1]</sup> which attacks components of cells and further induces hepatic, cardiovascular, and pancreatic dysfunctions, such as liver fibrosis, cirrhosis, and hepatic carcinoma.<sup>[3]</sup> Iron accumulation can even retard the

effect of antiviral therapy.<sup>[4]</sup> Mechanisms of iron overload in patients with CHB may involve hepcidin,<sup>[5]</sup> liver injury,<sup>[6]</sup> viral activity,<sup>[6,7]</sup> micro ribonucleic acid-122 (miR-122),<sup>[8]</sup> ROS,<sup>[9]</sup> interleukin-6 (IL-6), and other inflammatory factors.<sup>[5]</sup>

Many studies have explored the connection between iron overload and HBV lifecycle with differing results. Some found that iron promotes HBV lifecycle,<sup>[10,11]</sup> whereas others found the opposite results.<sup>[12,13]</sup> In addition, the evidence on the effect of iron chelation treatment on patients with CHB with iron overload is limited.

We describe the case of a middle-aged male patient diagnosed with iron overload caused by CHB. Regular phlebotomy therapy combined with intravenous infusion of deferoxamine (DFO) not only improves liver iron deposition but also facilitates seroconversion of hepatitis B surface antigen (HBsAg).

## 2. Case report

A 42-year-old man was referred to the Department of Hematology, Xuanwu Hospital, Capital Medical University, in August 30, 2010, because of increased serum ferritin level for 2 months.

Two months before the admission, he was followed up regularly at another hospital because of 20-year history of CHB. The liver showed high density on the computed tomography (CT) scan, with a CT value of 86HU, and serum ferritin level was >1500ng/mL (the upper limit of the detection threshold), whereas the liver showed normal density on the CT scan in 2005. Further biopsy examination of the liver showed marked iron deposition, and iron staining was positive in hepatocytes. Subsequently, the patient was transferred to Xuanwu Hospital.

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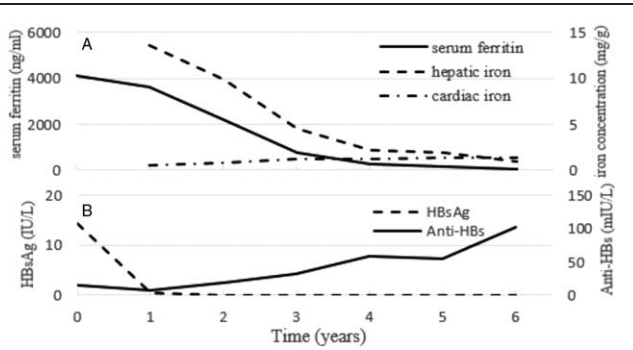
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**Figure 1.** Indexes changing trend during the iron chelation therapy. (A) Serum ferritin level gradually decreased to the normal level 4 years after therapy and continued to decrease. The hepatic iron concentration gradually decreased, whereas cardiac iron concentration increased first and then decreased. (B) The HBsAg level gradually decreased and became persistently negative approximately 1 year after the therapy, when anti-HBs became positive, and the titer gradually increased.

At that time, the patient had no symptom of discomfort, his HBsAg result had been continuously positive for 20 years; he had taken adefovir dipivoxil (ADV) regularly for 5 years, and the HBV deoxyribonucleic acid (DNA) result had already turned continuously negative for 3 years. No evidence of hepatic cirrhosis or hepatocellular carcinoma was seen. He had no other positive past medical history. His father was also an HBV carrier and had died because of hepatic carcinoma.

Upon physical examination at admission, no positive sign was detected, except for the obvious skin hyperpigmentation. Auxiliary examinations revealed normal complete cell count of the peripheral blood. Both alanine aminotransferase (ALT) and aspartate transaminase (AST) levels were slightly increased. Results for HBsAg, antibody to HBV core antigen (anti-HBc), and antibody to HBV e antigen (anti-HBe) were positive; and those for antibody to HBV surface antigen (anti-HBs) and HBV e antigen (HBeAg) were negative; HBV DNA result was quantitatively negative. Serum ferritin concentration, serum iron concentration, transferritin, saturation of transferritin, and total iron-binding capacity were 4235 ng/mL (23.9–336.2 ng/mL), 209  $\mu$ g/dL (50–150  $\mu$ g/dL), 1.5 g/L (2–3.6 g/L), 75% (25–35%), 266  $\mu$ g/dL (200–400  $\mu$ g/dL), respectively. Cytology of the bone marrow showed the normal plasia of the trilineage cells, and the iron staining of the bone marrow was strongly positive for extracellular iron and sideroblastosis without ring sideroblast.

All the data suggested iron overload condition. To exclude the primary hemochromatosis, mutations of 8 common related genes

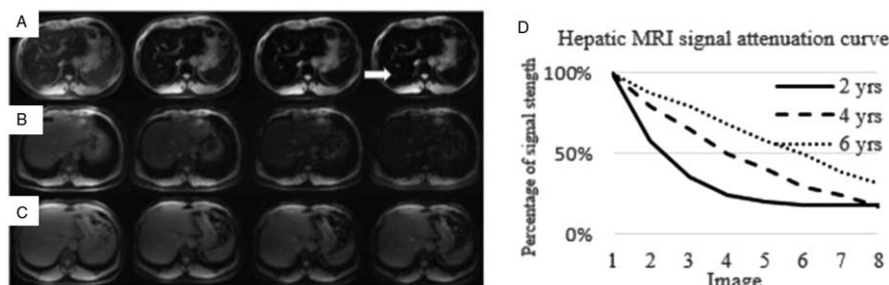
(*HFE*, *HFE2*, *FPN*, *HAMP*, *TRF2*, *BMP6*, *FTH*, and *FTL*) were detected, and the results were negative. Because the patient had normal growth and development, change in hepatic density occurred in recent years; the primary hemochromatosis-related gene mutations were negative, and the patient was diagnosed with secondary iron overload and CHB.

To relieve iron load of the body, the patient underwent phlebotomy therapy, under the condition of normal hemoglobin levels, from September 2010 until presently (300–400 mL each time, 0–2 times per month) and regular DFO therapy for approximately half a year from November 2011. There were no adverse effects. During the therapy, the following indexes were monitored: serum ferritin, ALT, AST, HBsAg, Anti-HBs, HBeAg, Anti-HBe, Anti-HBc, and HBV DNA quantification. One year after therapy, the iron concentration of the liver and heart were monitored by T2\* of magnetic resonance imaging (MRI) scan.

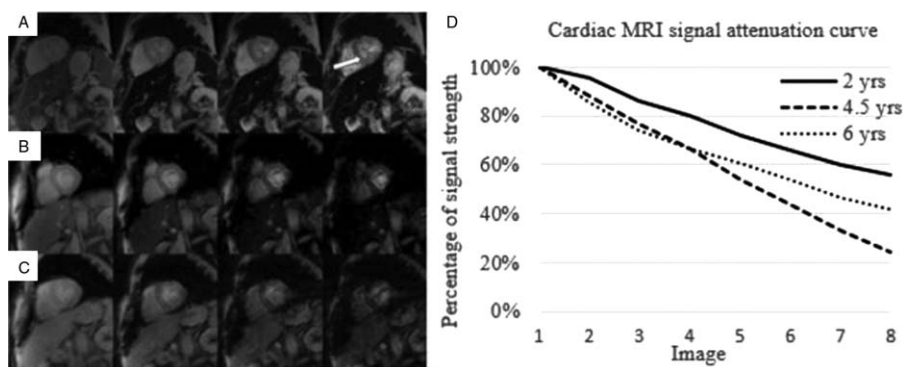
Along with iron chelation therapy, the hyperpigmentation of the patient's skin gradually disappeared, and the serum ferritin gradually decreased to the normal level 4 years after therapy and continued to attenuate (Fig. 1A). In MRI, the hepatic T2\* value became normal 5 years after the therapy. Subsequently, hepatic iron deposition continued to improve (Fig. 1A and Fig. 2). In contrast, cardiac iron concentration was normal at the beginning of iron chelation therapy; however, along with the therapy, heart iron overload gradually appeared. As the MRI examinations showed, cardiac T2\* value gradually decreased, and the patient had heart iron overload for the first time approximately 4 years after therapy, when cardiac T2\* value and cardiac iron concentration were 19.2 ms and 1.22 mg/g, respectively. Four and half years after therapy, when hepatic iron concentration was nearly normal, cardiac iron concentration reached the maximum, 1.44 mg/g, and cardiac T2\* value was 16.8 ms. Not until hepatic iron concentration normalized, did the cardiac iron concentration begin to decrease, with a speed much slower than in the liver (Fig. 1A and Fig. 3).

The most unexpected result is that CHB has greatly improved. The HBsAg level gradually decreased and turned persistently negative approximately 1 year after the therapy, when Anti-HBs turned positive, and the titer gradually increased (Fig. 1B). Besides, we also performed HBV DNA quantification, which showed persistently negative result. The liver function also decreased to normal levels approximately 1 year after the therapy. Therefore, the ADV treatment was stopped 4 years after the initiation of iron chelation treatment.

Currently, the patient is in a very good condition, without any antiviral therapy. To decrease the cardiac iron concentration, the



**Figure 2.** MRI images and signal attenuation curves of the liver during the iron chelation therapy. (A–C) The MRI images of hepatic parenchyma at the same level 2, 4, 6 years after therapy, respectively. The more the iron deposits in the liver, the weaker the signal in the first image, and the faster the signal attenuates, (D) the bigger the slope of the signal attenuation curve is. The arrow shows hepatic parenchyma.



**Figure 3.** MRI images and signal attenuation curves of the heart during the iron chelation therapy. (A–C) The MRI images of interventricular septum at the same level 2, 4.5, 6 years after therapy, respectively. The more the iron deposits in interventricular septum, the weaker the signal of the first image, the faster the signal attenuates, (D) the bigger the slope of the signal attenuation curve is. The arrow shows the interventricular septum.

patient still received regular phlebotomy therapy, 300 mL every 3 months, without occurrence of anemia.

### 3. Discussion

The most common factors causing iron overload are transfusion<sup>[14]</sup> and primary hemochromatosis.<sup>[15]</sup> Besides, iron overload can also be caused by steatohepatitis, alcoholic liver disease, and chronic viral hepatitis,<sup>[15]</sup> including CHB and chronic hepatitis C (CHC). On the basis of the diagnosis of hemochromatosis by the American Association for the Study of Liver Diseases in 2011 and the EASL clinical practice guidelines for HFE hemochromatosis in 2010,<sup>[16,17]</sup> diagnosis of primary hemochromatosis should be based on mutation of related genes, including *C282Y*, *H63D*, *HAMP*, *HJV*, *TRF2*, or *FPN*, and evidence of increased iron stores. This patient had increased iron stores, but did not have gene mutation; hence, we excluded the diagnosis of primary hemochromatosis. Besides, the patient had no transfusion history, no history of alcohol consumption, and no other liver diseases except CHB, and the liver density markedly increased more than 15 years after his diagnosis of CHB; thus, we diagnosed the patient with secondary iron overload, and suspected that the iron overload was caused by CHB.

Similar to common iron overload found in patients with CHC,<sup>[18,19]</sup> iron overload is also a common phenomenon in patients with CHB, particularly in the male sex, who does not have menstrual blood loss to excrete iron, and more severe in patients coinfecting with hepatitis D virus.<sup>[20]</sup> Before Dibisceglie et al<sup>[19]</sup> first found elevated serum ferritin and iron levels in patients with CHC virus in 1992, elevated serum iron in patients with CHB had already been observed by Sutnick et al<sup>[7]</sup> as early as 1974. Subsequently, Felton et al<sup>[6,21]</sup> described increased serum iron and iron saturation in HBsAg(+) patients and decreased ones after serum conversion. Subsequently, Kageyama et al<sup>[2]</sup> in 2000 confirmed liver iron overload in patients with CHB and CHC based on liver biopsy examination. Thereafter, more studies began to find iron overload in patients with CHB, whereas successful antiviral therapy can reverse it.<sup>[22]</sup>

To date, many studies have been devoted to exploring the mechanism on how HBV leads to iron overload. As an important trace element in the human body, iron is controlled in a relatively narrow range by hepcidin, which is a peptide expressed predominantly in the liver.<sup>[3,23]</sup> When the hepcidin levels are altered, iron metabolism will be perturbed.<sup>[23]</sup> Non-hepcidin

mechanisms also affect iron metabolism.<sup>[24]</sup> In contrast to the uniform perspective that reduced hepcidin level<sup>[25]</sup> leads to iron overload in patients with CHC, the mean hepcidin level in patients with CHB is higher than that in healthy controls.<sup>[5,26]</sup> Hepcidin level in patients with CHB is different from that in patients with CHC, which suggests that a non-hepcidin dependent pathway leading to iron overload exists, which further induces the upregulation of hepcidin, in patients with CHB. In addition, liver injury,<sup>[6]</sup> viral activity,<sup>[6,7]</sup> miR-122,<sup>[8]</sup> ROS,<sup>[9]</sup> IL-6, and other inflammatory factors<sup>[5]</sup> were found to be involved in iron overload in patients with CHB.

In turn, iron can also affect HBV proliferation. Many studies have suggested the positive role of iron on HCV translation, whereas the role of iron in promoting or suppressing HCV replication is debatable.<sup>[25]</sup> However, studies regarding the effect of iron on HBV life cycle are relatively less, with disputable results. In 1983, a study with 44 HBV patients found that higher levels of serum ferritin before HBV infection increased the likelihood that the infection will be persistent,<sup>[27]</sup> suggesting that iron may promote HBV infection. Other studies using cell models also found that iron promotes HBsAg release,<sup>[10]</sup> mRNA and DNA expression,<sup>[11]</sup> and HBV DNA secretion.<sup>[28]</sup> In this case, HBsAg result became negative after iron chelation therapy, along with decreased iron load. Thus, we speculate that iron chelation therapy may promote seroconversion of HBsAg, and that iron promotes HBV infection. However, some other studies using cell models found that iron inhibits HBV replication<sup>[28]</sup> and release.<sup>[10,13]</sup> Those disputable results may be due to different methods. Besides, whether these results from in vitro research could be suggestive to understand the effect of iron on HBV in human body is questionable.

Studies regarding HBsAg result becoming negative after antiviral therapy have been reported. Studies regarding the curative effect of ADV used singly for patients with HBeAg-negative CHB showed that the 1-, 2-, and 5-year cumulative rates of HBsAg <100 IU/mL were 0, <3%, <5%, respectively, and that 1-year cumulative rate of HBV DNA becoming negative was 65%.<sup>[29,30]</sup> The patient in this case used ADV regularly, and achieved negative HBV DNA and negative HBsAg 2 and 6 years after using ADV, respectively. The possibility of seroconversion is very low if using ADV singly. Thus, we speculate that iron chelation therapy may increase the possibility of seroconversion.

There is a lag in the loading and unloading of heart iron with respect to liver iron in this case, which has been observed by

Noetzli et al.<sup>[31]</sup> and Chen et al.<sup>[32]</sup> Jensen et al.<sup>[33]</sup> also points that liver iron concentration threshold (approximately 350  $\mu\text{mol/g}$ ) exists, after which chelatable iron pool is allowed to expand to this critical size and iron begins to be deposited in the heart. Noetzli et al.<sup>[31]</sup> explained that elevated liver iron may damage the liver, inhibiting liver non-transferrin bound iron (NTBI) uptake and increasing heart NTBI exposure. Maybe the ability of liver NTBI uptake can recover only when it is completely free of the damage by iron overload. High liver iron levels seem to increase the risk of heart iron deposition.<sup>[32]</sup> Furthermore, it is slower and more difficult to remove myocardial iron than hepatic iron,<sup>[31]</sup> which is obvious for this patient, whose heart iron concentration was only reduced by 0.02 mg/g after bloodletting 1500 mL, whereas liver iron concentration was reduced by 1.03 mg/g.

#### 4. Conclusion

The relationship between HBV and iron overload is complicated. Iron overload is common in patients with CHB, in which the mechanism is unclear. Whether iron promotes or inhibits HBV infection is disputable. It is easy for iron to be deposited and removed in the liver, whereas the speed of cardiac iron deposition and removal is much slower. This case shows the importance of monitoring liver iron status, and beginning iron chelation therapy once liver iron overload occurs and before heart iron overload occurs in patients with CHB. Iron chelation therapy can even increase the possibility of seroconversion.

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