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ORIGINAL ARTICLE

Hepatic iron status and response to therapy in chronic viral hepatitis B and C: A preliminary report

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ABSTRACT

Aim: To investigate if hepatic iron content influences the response to therapy in patients with chronic hepatitis C or B.

Background: It seems that the presence of elevated body iron stores and, in particular, elevated hepatic iron levels, is one of the strongest predictors of resistance to interferon treatment for chronic hepatitis C or B.

Patients and methods: Two hundred and one patients with chronic hepatitis C or B who were referred to Tehran Hepatitis Center were enrolled in this study. Histological quantification of hepatic iron was carried out by scoring iron separately within hepatocytes, sinusoidal cells, and portal triads. To estimate a cut off point for iron deposit as a predictor of response to treatment, Odd´s ratio with 95% confidence interval was calculated for each level of iron deposit.

Results: Iron scores were divided into two levels of high and low at several cut-off points, but the most significant differences were found when score 2 was considered as the cut-off point. Odd's ratios were not significant in any of the hepatic zones. The lowest P-values were related to zone II sinusoidal cells and the total of sinusoidal cells and portal iron score (P= 0.08) in which the Odd's ratios were 6 (95% CI, 0.77 to 46.6) and 3.9 (95% CI, 0.88 to 17.4) respectively.

Conclusion: In our society, liver iron content cannot be considered as an important risk factor of resistance to treatment in chronic hepatitis patients because of the very small prevalence. So, hepatic iron measurement and scoring do not seem cost-effective and valuable for all patients with chronic hepatitis in our population.

Keywords: *Iron, Chronic Hepatitis, Response to therapy*.

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INTRODUCTION

Liver iron accumulation in patients with chronic hepatitis has received increasing attention in recent years. A great number of hepatitis virus infected patients develop chronic, slowly progressive liver disease that may eventually result in cirrhosis and hepatocellular carcinoma (1). Several factors have been proposed to explain this unfavorable evolution such as gender, race, age at infection and alcohol abuse (2–5). Recently, the role of iron has been pointed out as an important element affecting the natural history of chronic hepatitis.

In fact, serum iron stores are frequently increased in chronic hepatitis patients (6-8) and hepatic iron deposition is also even more common among patients with end-stage liver disease due to hepatitis (9, 10). In experimental settings, excess hepatic iron deposition is known to be hepatotoxic

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and may exacerbate liver injury in several ways (11, 12). First, iron, which is essential for the growth of all organisms, may facilitate HCV replication (13–15). Second, iron may worsen liver tissue injury by increasing the formation of highly toxic hydroxyl radicals by the Fenton reaction, leading to progressive liver fibrosis (16) and increased risk of developing liver carcinoma (17). But there is another interpretation: iron deposition in the liver of patients with chronic hepatitis may simply be the result of iron released from damaged hepatocytes (18). As yet, little is known about the fundamental mechanism involved in the process of iron accumulation and it's precise clinical significance in the course of chronic hepatitis.

Iron overload seems to be associated with a poor response to therapy (6, 19-20), and removal of excess iron by repeated phlebotomy in combination with IFN may be of therapeutic benefit for patients with chronic hepatitis (21, 22). It seems that the presence of elevated body iron stores and, in particular, elevated hepatic iron levels, is one of the strongest predictors of resistance to interferon treatment for HCV (23-31). However, contradicting results have been previously reported (23, 24). They are lacking the data regarding iron in the liver and response to therapy in chronic hepatitis B.

This study was performed to investigate if hepatic iron content influences the response to therapy in patients with chronic hepatitis C or B.

PATIENTS and METHODS

Two hundred and one patients with chronic hepatitis C or B who had underwent needle liver biopsy at Tehran Hepatitis Center were enrolled in this historical cohort study.

Diagnosis of chronic hepatitis C (CHC) infection was determined by the detection of serum anti- HCV antibody (enzyme immunoassay and recombinant immuno blot assay) or serum HCV RNA titer (polymerase chain reaction; PCR) or elevated serum alanine aminotransferase (ALT) level for at least 6 months. Diagnosis of chronic hepatitis B (CHB) was also based on serum HBV RNA titer (PCR) or elevated serum ALT level for at least 6 months or positive HBS and HBe antigens.

Liver tissue was obtained by percutaneous needle biopsy and the liver samples were fixed in buffered formalin and embedded in paraffin for routine histological examination. Slides were then stained with Perl's Prussian blue to assess iron deposition.

Histological quantification of hepatic iron was carried out according to a recent study [4] by scoring iron separately within hepatocytes (hepatic iron score [HIS], 0–3), sinusoidal cells (sinusoidal iron score [SIS], 0–3) and portal triads (portal iron score [PIS], 0–3). The total iron score (TIS, 0–60) was defined by the sum of these scores. In order to evaluate more accurate hepatic iron deposits, we also considered 3 microscopic zones; a high power field around the portal space as zone I (periportal zone), a high power field around the central lobular vein as zone II, and the intermediate zone as zone III. So iron deposition scoring was performed for all patients in hepatocytes, sinusoidal cells, and portal tracts separately in each zone.

Patients were divided into 4 treatment groups; CHC patients received IFN-α or a combination of IFN-α and Ribavirin, and CHB patients were treated by IFN-α or Lamivudine. After the treatment course, virological exams were done and serum enzyme levels were measured. Response to treatment was defined as serum virus less than 200000 copy/ml (quantitative PCR) or a negative PCR (qualitative PCR).

To estimate a cut off point for iron deposit as a predictor of response to treatment, Odd´s ratio with 95% confidence interval was calculated for each level of iron deposit. Logistic regression method was used to control the effect of other

factors such as virus types and treatment groups. P-values less than 0.05 were considered statistically significant. Statistical analysis was performed using the software Stat-View version 8 (SAS Institute, Cary, NC, USA).

RESULTS

Among 201 hepatitis patients participated in the study, 29 patients (14.42%) were female. The mean age of the subjects was 36.04 (SD=11.92) years. Of the subjects, 68 patients were CHC and 133 were CHB. Fifty patients received IFN-α, 48

Table 1. Iron score of different hepatic zones in resistant to treatment and responder patients.

hepatic zones	Resistants	Respondents
Zone I hepatocyte iron score	0.52 (1.23)*	0.29 (0.96)
Zone II hepatocyte iron score	0.13 (0.72)	0.18 (0.75)
Zone III hepatocyte iron score	0.06 (0.36)	0.11 (0.58)
Total hepatocytes iron score	0.58 (1.93)	0.58 (2.03)
Zone I sinusoidal iron score	0.19 (0.79)	0.10 (0.57)
Zone II sinusoidal iron score	0.26 (1.00)	0.06 (0.46)
Zone III sinusoidal iron score	0.23 (0.80)	0.05 (0.43)
Total sinusoidal iron score	0.68 (2.55)	0.21 (1.37)
Portal connective tissue iron score	0.16 (0.73)	0.07 (0.47)
Portal lymphatics iron score	-	0.03 (0.32)
Portal vascular wall iron score	0.06 (0.36)	0.04 (0.35)
Total portal iron score	0.23 (0.80)	0.14 (1.01)
Total zone I hepatocyte and sinusoidal iron score	0.58 (1.78)	0.39 (1.36)
Total zone II hepatocyte and sinusoidal iron score	0.39 (1.58)	0.24 (1.07)
Total zone III hepatocyte and sinusoidal iron score	0.29 (1.01)	0.15 (0.94)
Total hepatocyte and sinusoidal iron score	1.13 (3.75)	0.79 (3.11)
Total portal and sinusoidal iron score	0.90 (3.27)	0.35 (2.18)
Total hepatocyte, portal and sinusoidal iron score	1.35 (4.43)	0.93 (3.87)

* Mean (Standard deviation)

Table 2. Odd's ratios and P-values of iron scores in different hepatic zones.

hepatic zones	Odd's ratio	Adjusted Odd's ratio	95% Confidence Interval	P-value
Zone I hepatocyte iron score	1.71	1.83	0.46 – 7.3	0.39
Zone II hepatocyte iron score	0.91	0.99	0.11 – 8.7	0.99
Zone III hepatocyte iron score	-	-	-	-
Total hepatocytes iron score	0.91	0.89	0.19 – 4.3	0.88
Zone I sinusoidal iron score	1.86	1.82	0.18 – 18.9	0.62
Zone II sinusoidal iron score	5.8	6	0.77 – 46.6	0.08
Zone III sinusoidal iron score	2. 8	2.7	0.22 – 34.3	0.44
Total sinusoidal iron score	2.9	3	0.51 – 17.1	0.23
Portal connective tissue iron score	1.03	2.8	0.24 – 32.7	0.41
Portal lymphatics iron score	-	-	-	-
Portal vascular wall iron score	-	-	-	-
Total portal iron score	1.86	1.9	0.19 – 19.1	0.59
Total zone I hepatocyte and sinusoidal iron score	1.1	1.2	0.24 – 5.7	0.85
Total zone II hepatocyte and sinusoidal iron score	1.89	2	0.39 – 10.8	0.40
Total zone III hepatocyte and sinusoidal iron score	3.8	3.9	0.62 – 25.2	0.14
Total hepatocyte and sinusoidal iron score	0.83	0.83	0.17 – 3.9	0.81
Total portal and sinusoidal iron score	3.5	3.9	0.86 – 17.4	0.08
Total hepatocyte, portal and sinusoidal iron score	1.19	1.24	0.33 – 4.7	0.75

patients received a combination of IFN-α and Ribavirin and 103 were treated by Lamivudin. After the treatment course, 170 patients responded to treatment but 31 subjects were considered resistant to treatment.

Table 1 demonstrates the scores in patients who are resistant or respondent to treatment, separately. Iron scores were divided into two levels of high and low at several cut-off points, but the most significant differences were found when score 2 was considered as the cut-off point. So, this cut-off point was used to calculate Odd's ratio for resistance to treatment in the patients.

As shown in table 2, Odd's ratios were not significant in any of the hepatic zones. The lowest P-values were related to zone II sinusoidal cells and the total of sinusoidal cells and portal iron score (P= 0.08) in which the Odd's ratios were 6 (95% CI, 0.77 to 46.6) and 3.9 (95% CI, 0.88 to 17.4) respectively. P-value could not be calculated for zone III hepatocytes, portal vessels and lymphatics since there was no resistant to treatment patient with iron score above 2 in these zones.

Table 3 demonstrates the diagnostic value of zone II sinusoidal iron score to determine resistance to treatment, using different cut-off points.

Table 3. Diagnostic value of zone II sinusoidal iron score to determine resistance to treatment.

Cut-off point	sensitivity	specificity	Positive predictive value	Negative predictive value
> 0	6%	98%	33%	85%
> 1	6%	98%	40%	85%
> 2	6%	99%	50%	86%
> 3	6%	99%	50%	86%

DISCUSSION

Previous studies demonstrated that patients with chronic hepatitis commonly have elevated serum and liver iron levels in various degrees (6–10). The present study was a histological evaluation of liver iron content in chronic hepatitis patients and the effect of hepatic iron accumulation on their response to treatment. In our study, patients with higher liver iron content specially zone II sinusoidal and also total sinusoidal and portal iron accumulation were more commonly resistant to treatment, although no significant difference was detected.

The result was the same as a previous study which reported more resistance to treatment in patients with higher zone II sinusoidal and portal iron content (4). Of course in our study, iron score of hepatocytes zone II and III and also lymphatic portal zone were higher in the group of respondents and total hepatocytes iron score was equal in both groups. The previous study reported total hepatic iron score of 10 in patients resistant to treatment and 5 in respondents, but in this study the scores were 1.35 and 0.93 respectively, which shows the lower total hepatic iron content of our patients.

Considering the studies suggesting that alcohol causes an increase in transferrin receptors on hepatocytes which leads to higher hepatic iron accumulation (32-35), the low liver iron content in our patients might be due to less alcohol consumption in this population. Low hepatic iron scores and high prevalence of score 0 demonstrates its small statistical importance. It seems that, in our society, liver iron content can not be considered as an important risk factor of resistance to treatment in chronic hepatitis patients because of the very small prevalence. So, hepatic iron measurement and scoring do not seem cost-effective and valuable for all patients with chronic hepatitis in our population.

Definitely, further studies with bigger sample sizes are suggested for determination of the effect of liver iron on response to treatment in chronic hepatitis patients. Also, we need studies to evaluate the relationship between hepatic iron and

alcohol consumption or other factors which might be responsible for lower liver iron content.

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