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Association of Serum Iron Parameters with Activity of Liver Enzymes in Mongolian Patients with Chronic Hepatitis "C"

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Objectives: The purpose of this study was to investigate the relationship between serum iron parameters with the activity of liver enzymes among patients with HCV in Mongolia. **Methods:** Twenty patients with HCV, who were registered at the National Center for Communicable Diseases, Mongolia, participated in our study. Serum iron and serum ferritin levels were determined using immunoassay. Hepcidin levels were analyzed using ELISA. **Results:** Liver enzymes, aspartate aminotransferase (ASAT) and alanine transaminase (ALAT), were 65.79 ± 0.52u/l and 51.22 ± 0.54 u/l, respectively. Serum iron levels were high (>180μg/dl) in 20% of patients, while serum ferritin levels were high in (>300ng/ml) in 25% of patients. Serum ferritin levels were strongly correlated with elevated ASAT ($r = 0.62$, $p = 0.005$) and ALAT ($r = 0.53$, $p = 0.02$). Additionally, serum iron was strongly correlated with ASAT ($r = 0.61$, $p = 0.002$) and ALAT ($r = 0.53$, $p = 0.02$). No correlation was identified between ASAT or ALAT and hepcidin. **Conclusion:** ALAT and ASAT levels were directly associated with increased levels of serum iron, but not serum ferritin. Serum hepcidin levels may affect ASAT levels.

Keywords: Chronic Hepatitis C, Transaminases, Hepcidins, Ferritins, Iron

Introduction

Hepatitis C virus (HCV) is a globally prevalent pathogen. Over the last 15 years, 2.8% or 185 million people worldwide have become infected with HCV [1, 2]. Although many subjects carrying the virus remain asymptomatic, chronicity is often accompanied by the alteration of liver function, progressive liver disease, and the culmination of cirrhosis or hepatocellular carcinoma in up to 20% of infected individuals [3]. The prevalence of the HCV in Mongolia is about 10%, and 97-98% of all of the incidences carry hepatitis C subtype 1b [4,5].

Iron is stored mostly in the liver as ferritin or hemosiderin. Moreover, the liver regulates iron traffic by producing a peptide-hormone named hepcidin, which is the site of synthesis of major

proteins of iron metabolism such as transferrin and ceruloplasmin [6, 7]. Liver injury, including liver cirrhosis, alcoholic liver disease, and chronic hepatitis B and C, leads to disturbance of iron metabolism. Hepatic iron overload is present 10-60% of patients with HCV, as a result of abnormally expressed hepcidin and ensuing disruption of iron metabolism [8-11].

Several clinical observations have shown complicated crosstalk between iron metabolism and HCV infection. The outcome of HCV infection depends on the host genetic background, the viral subtypes, and other external factors [12]. Iron overload, which has been correlated with a poor response to antiviral therapy, has been shown to be a common cause of complications in patients with HCV [13-15]. Serum iron removal by phlebotomy improves liver function tests and histology, increasing the probability of sustained HCV eradication with antiviral therapy [15-19]. The majority of similar studies have performed monthly or bimonthly phlebotomy of 400-500 ml of whole blood, until the development of iron deficiency anemia or serum ferritin levels of 35 ng/ml [20].

According to Mongolian traditional medicine theory, phlebotomy combined with specific herbal prescriptions, such as "three seed prescription" and "Barbad-10", are a very useful tool for eliminating "sick or bad blood" from "good or healthy blood". To eliminate "bad blood", we used the above herbal prescription for 5-7 days, then a 30-100ml phlebotomy was performed during summer [21, 22]. It was unknown whether a blood purifying herbal prescription in combination with phlebotomy would affect in HCV patients.

There have been no reports on iron metabolism in liver diseases damaged by hepatitis viruses in Mongolia. Therefore, it is necessary to study the iron status in Mongolian patients with HCV. The objective of this study was to investigate the serum iron parameters and their correlation with liver enzyme activation in patients with HCV.

Materials and Methods

1. Study subject

Twenty HCV patients from the National Center for Communicable Diseases, Mongolia participated in this study in November 2016. Criteria for inclusion was detection of the anti-HCV antibody carrying subtype 1b of HCV. All patients had received Mongolian traditional medicine herbal"three seed prescription" and "Barbad-10" prescription for 5-7 days, then a 30-100ml phlebotomy. Criteria for exclusion were hepatitis B surface antigen (HBsAg) and human immunodeficiency virus antibody (HIVAb) positivity. None of the patients were treated with antiviral therapy. Patients with clinical laboratory findings of acute liver failure, active systemic infection, cirrhosis, hepatocellular carcinoma, or those who received blood transfusions were not included in this study.

2. Laboratory tests

A 3.0 ml sample of venous blood was drawn in an EDTA tube from each participant and immediately centrifuged. After separating the serum, hepcidin levels were detected using ELISA (Sunlong Biotech, China) according to the manufacturer's instructions. Ferritin levels were determined using electrochemiluminescence immunoassay (ECLIA) on the Cobas e411 analyzer (Roche, USA). Aspartate aminotransferase (ASAT), alanine transaminase (ALAT), total protein, and serum iron concentrations were detected on the Cobas c311 analyzer (Roche, USA).

3. Data analysis

The analysis was performed using SPSS, version 20.0 (SPSS Inc., USA). Descriptive statistics was applied to observe data distribution. Normality of distribution was tested for all variables using the Shapiro-Wilk test, and differences between groups were calculated using the Mann-Whitney U test for data with non-normal distribution. Correlation was assessed using Spearman's rank correlation coefficient. Non-normally distributed parameters were transformed into normal distributions using log transformation. For the association of baseline predictors of HCV with serum indicators, a multiple linear regression was applied. p-value < 0.05 was considered statistically significance.

4. Ethical statement

This study was approved by the Research Ethics Committee of the Mongolian National University of Medical Sciences. All patients provided written consent before participating in this study.

Results

1. Biochemical Levels

Twenty newly diagnosed patients (2 men and 18 women) were recruited in this study. The mean age of patients was 56.16±19

Table 1. Biochemical levels in patients with HCV

Variables	Normal Levels (n (%))	Above Normal Levels (n (%))
ASAT (u/l)	13 (65%)	7 (35%)
ALAT (u/l)	15 (75%)	5 (25%)
Total protein (g/l)	17 (85%)	3 (15%)
Serum iron (µg/dl)	16 (80%)	4 (20%)
Serum ferritin (ng/ml)	15 (75%)	5 (25%)

years (34 to 69 years). All participants carried the anti-HCV antibody subtype 1b.

Liver enzymes, ASAT and ALAT, in the HCV patients were 65.79±0.52u/l and 51.22±0.54u/l, respectively. Thirteen patients (65%) had a normal serum ASAT levels (<40u/l), while seven patients (35%) had 2 - 4 times higher than the upper limit of normal serum ASAT (>40u/l) (Table 1). Fifteen patients (75%) had normal levels of serum ALAT (<40u/l) , while five patients (25%) had high levels of ALAT (>40u/l) (Table 1). Sixteen patients (80%) presented normal serum iron levels of 42.6 - 159.8µg/dl, while four patients (20%) had serum iron levels greater than 180µg/dl. Fifteen patients (75%) had normal serum ferritin levels of 27.84 - 205.7ng/ml, while five patients (25%) had serum ferritin levels exceeding 301 ng/ml. Because increased plasma and stored iron stimulate the production of hepcidin, we also analyzed serum hepcidin levels [17]. Hepcidin levels were 8.27 ± 1.95 ng/ml in our HCV patients.

2. Correlation of Iron Parameters and Liver Enzymes

We analyzed the correlation between iron parameters and the activation of liver enzymes in our HCV patients. Serum ferritin levels were strongly correlated with elevated ASAT ($r = 0.62$, $p = 0.005$) and ALAT levels ($r = 0.53$, $p = 0.02$; Table 2, Figure 1A). Additionally, serum iron was strongly correlated with ASAT ($r = 0.61$, $p = 0.002$) and ALAT ($r = 0.53$, $p = 0.02$; Table 2, Figure 1B). However, no correlation was seen between the activation of liver enzymes and serum hepcidin (Table 2, Figure 1C).

3. Normal Versus High Serum Iron and Ferritin Level Groups

For further analysis, we grouped the HCV patients by serum iron levels. The normal iron group had serum iron levels of <99µg/dl, and the high group had levels of >180µg/dl. Compared to the normal iron group, ALAT level was significantly higher in the high iron group ($p = 0.03$, Table 3). ASAT, serum ferritin, and serum hepcidin levels were marginally increased in the high iron group (Table 3).

Iron is stored mostly in the liver as ferritin or hemosiderin. Therefore, we grouped the HCV patients by serum ferritin level, normal (<300 ng/ml) and high (>301 ng/ml). Compared to the normal ferritin group, the high ferritin group had significantly higher levels of ASAT ($p = 0.042$, Table 4, Figure 2). The average serum iron concentration was 76 mg/dl in the normal ferritin group but was markedly increased to 145.3 mg/dl in the high ferritin group.Serum iron was strongly correlated with ALAT ($r = 0.8$, $p = 0.04$) and ASAT ($r = 0.9$, $p = 0.03$; Figure 3A and 3B)

Table 2. Correlation of iron parameters with activation of liver enzymes

Variables	Spearman's correlation coefficient	p-value
Ferritin - ASAT	0.62 **	0.005
Ferritin - ALAT	0.53*	0.02
Ferritin - Iron	0.54*	0.01
Ferritin - Hepcidin	0.4	0.08
Iron - ASAT	0.61**	0.002
Iron - ALAT	0.53*	0.02
Iron - Hepcidin	0.2	0.3

** p-value <0.005 is significant, *p-value < 0.05 significant

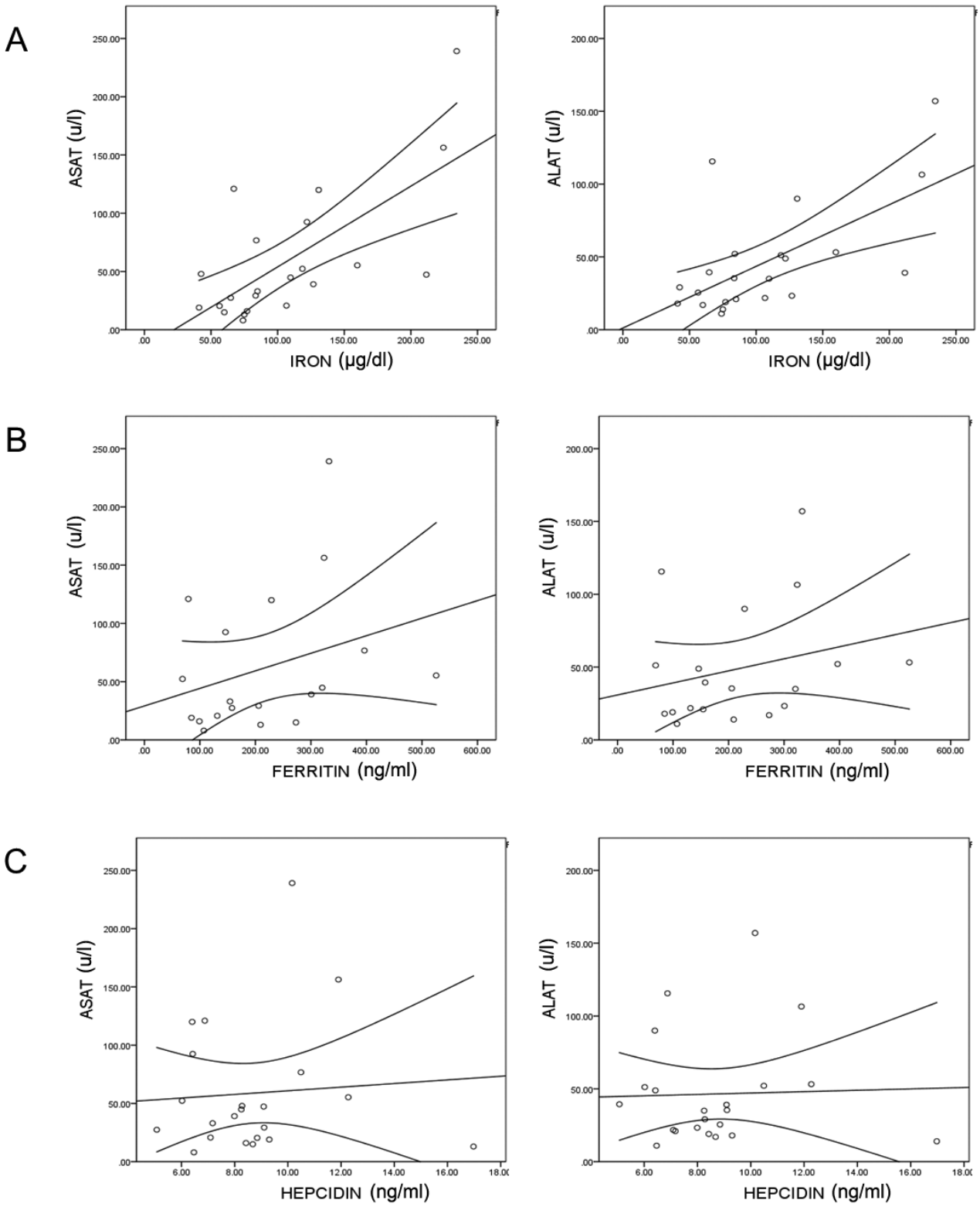

Figure 1. Correlation of iron parameters with activation of liver enzymes

(A) Correlation of serum iron with ASAT and ALAT; (B) Correlation of serum ferritin with ASAT and ALAT; (C) Correlation of serum hepcidin with ASAT and ALAT.

Table 3. Biochemical parameters of HCV patients with normal versus high serum iron levels

	Group		
Variables	**Normal Iron (n=16)**	**High Iron (n=4)**	**p-value**
ASAT (u/l)	32.0 (22.3 - 50.5)	80.0 (42.5 - 144.5)	0.07
ALAT (u/l)	22.5 (31.0 - 51.5)	105.0 (49.1 - 218.3)	0.03*
Total protein (g/l)	75.5 (71.5 - 84.3)	79.5 (71.5 - 83.5)	0.78
Serum Ferritin (ng/ml)	119.9 (49.5 - 157.0)	328 (127.5 - 447.8)	0.078
Serum Hepcidin (ng/ml)	7.0 (6.0 - 7.8)	9 (6.7 - 11.3)	0.17

*p-value < 0.05 significant

in the high ferritin group, but no correlation was identified in the normal ferritin group (Figure 3C, 3D). Compared to the normal ferritin group, the serum hepcidin level was slightly elevated in the high ferritin group (p=0.170, Table 4). Thus, high ferritin levels were accompanied by an elevation in ASAT and serum iron in patients with HCV.

4. Linear Regression Analysis

A linear regression analysis showed that elevated ASAT and ALAT levels were significantly associated with increased levels of serum iron (p = 0.004 and p = 0.03, respectively, Table 5). Serum hepcidin levels were significantly associated with ASAT levels (p<0.001).

Discussion

The major findings of this study were that (1) ALAT and ASAT levels are directly corrected with serum ferritin and iron levels, but not with hepcidin levels; (2) the serum levels of ASAT were significantly higher in high serum ferritin patients, and levels of ALAT were significantly higher in high serum iron patients; (3) ALAT and ASAT levels were directly associated with increased levels of serum iron, but not serum ferritin, and serum hepcidin levels were associated with ASAT levels.

Iron is a crucial factor for cellular metabolism, and the disturbance of iron homeostasis aggravates clinical outcomes of HCV infection. Iron overload and HCV infection cause liver damage, which leads to liver inflammation and fibrosis [23, 24].

Table 4. Biochemical parameters of HCV patients with normal versus high serum ferritin levels

	Group		
Variables	**Normal Ferritin (n=15)**	**High Ferritin (n=5)**	**p-value**
ASAT (u/l)	28.4 (16.8: 51.2)	66.0 (43.4 : 177.0)	0.042*
ALAT (u/l)	27.3 (18.25 :46.52)	52.7 (32.1 :118.2)	0.262
Total protein (g/l)	74.0 (70.1 : 78.6)	82.0 (79.3 : 85.5)	0.058
Serum Iron (μg/dl)	76 (61 :115.6)	143.3 (103.3 : 226.9)	0.004*
Serum Hepcidin (ng/ml)	7.72 (6.43 : 9.02)	10.32 (8.19 :11.99)	0.170

*p-value < 0.05 significant

Table 5. Linear regression analysis for association of serum iron, ferritin, and heparin with ASAT and ALAT

Variables	**Dependent variables**					
	ASAT			**ALAT**		
	β-coefficient	**95% CI**	**p-value**	**β-coefficient**	**95% CI**	**p-value**
Iron	0.02	0.25 to1.08	0.03	0.67	0.94 to 1.01	0.004*
Ferritin	0.03	-1.16 to 0.71	0.427	0.017	- 0.25 to 0.26	0.95
Hepcidin	0.84	6.91 to 15.14	0.000	0.05	- 4.9 to 6.04	0.83

*p-value < 0.05 significant

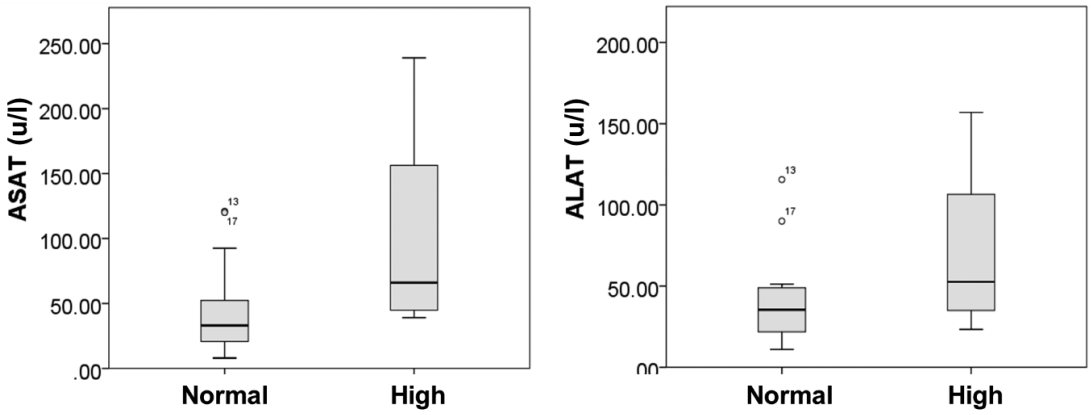

Figure 2. ASAT and ALAT in HCV patients with normal versus high serum ferritin levels

HCV patients were grouped by serum ferritin levels to two groups: normal ferritin group (<300ng/ml) and high ferritin group (>301ng/ml).

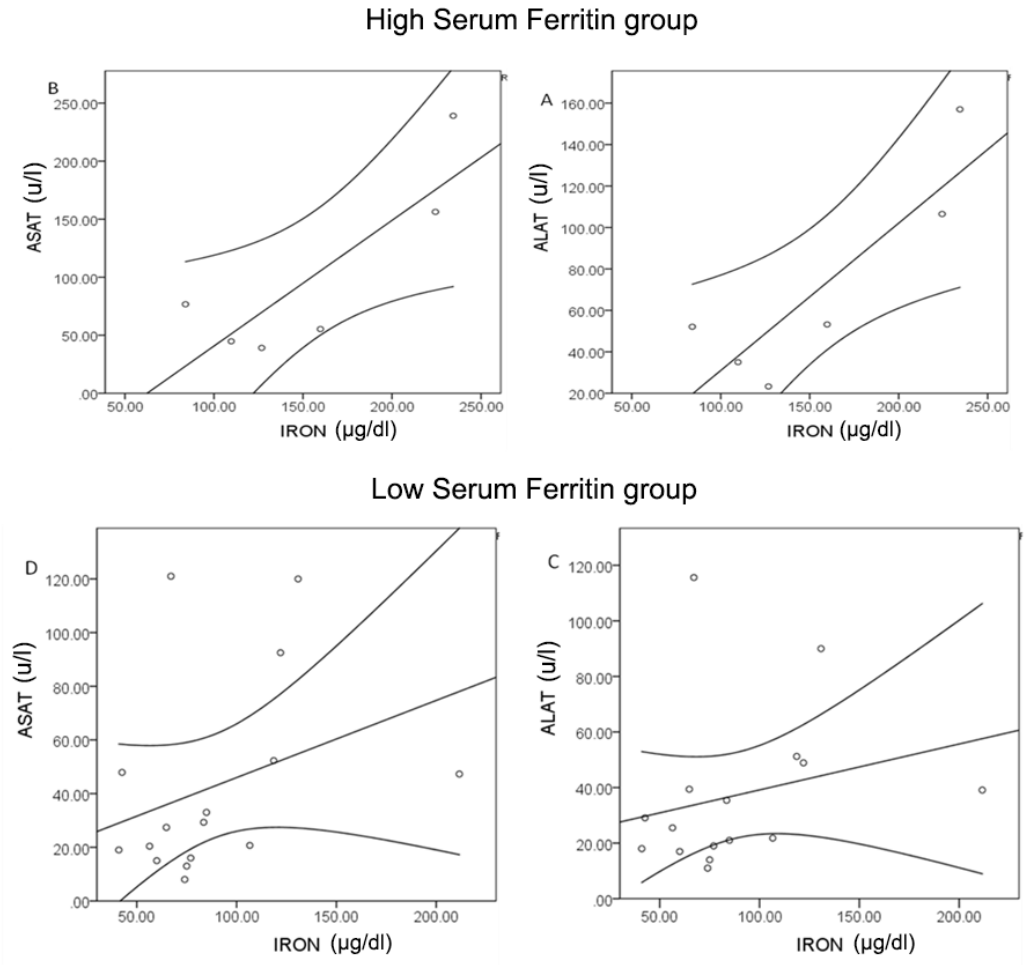

Figure 3. Correlation between serum iron and ASAT and ALAT in HCV patients with normal and high serum ferritin levels

HCV patients were grouped by ferritin level, normal ferritin (< 300 ng/ml) and high ferritin (> 301 ng/ml). There was a strong correlation between serum iron levels and liver enzyme elevation (p = 0.008) in the high ferritin group (A and B) but not in the low ferritin group (C and D).

Studies have found that 30-40% of all patients with HCV have elevated serum iron, ferritin, and transferrin saturations [2, 25, 26]. Elevated iron accumulation has been identified as a co-morbidity factor for disease progression following HCV infection and is associated with a decreased response to antiviral therapy [26, 27].

In our study, 20% of our patients had high serum iron levels, above 180μg/dl. There may be higher levels of iron metabolism among Mongolians versus other ethnicities due to lifestyle and geographical differences, but there was no data on iron metabolism among Mongolians and thus, no comparisons could be made.

Measurement of serum ferritin is a convenient method for assessing the iron storage [28]. Furthermore, ferritin is the most predictive laboratory parameter to show the degree of liver damage in patients with HCV [24, 29, 30]. In our study, serum ferritin was high (>301 ng/ml) in 25% of patients with HCV.

Hepcidin is a promising diagnostic test for iron status [31]. During a systemic inflammatory state, hepcidin is downregulated among patients with HCV and contributes to iron-loading in the liver [32-34]. Some evidence has shown hepcidin downregulation at peak viremia after HCV infection [35]. This may be because the knockdown of hepcidin by interference RNA significantly reduces HCV replication [31]. Our data showed that hepcidin levels among HCV patients differed, depending on the serum iron and ferritin levels.

Serum ferritin has been shown to be correlated with ALAT, iron, and transferrin levels and hepatic iron stains [32]. We analyzed the correlation of serum ferritin, iron, and hepcidin levels with ALAT and ASAT levels and found that serum iron and ferritin levels were significantly and directly correlated with serum ALAT and ASAT levels. However, no correlation was observed between hepcidin and ALAT or ASAT.

Kano et al. have reported that phlebotomy, combined with an iron-restricted diet, significantly reduces the development of HCV-associated hepatocarcinoma [33]. Further observational and interventional studies should be performed to investigate how phlebotomy combined with Mongolian traditional herbal prescriptions can purify the blood of patients with HCV.

The limitation of our study was our small sample size. Despite this, our study showed a reasonable correlation between iron parameters and liver enzymes activity in patients with HCV in Mongolia. In conclusion, ALAT and ASAT levels were directly associated with increased levels of serum iron, but not serum ferritin, and serum hepcidin levels may affect ASAT levels.

Conflict of Interest

The authors state no conflict of interest.

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