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RESEARCH ARTICLE

Is Serum Ferritin a Suitable Predictor of Treatment Outcomes in Chronic Hepatitis C Patients Treated with Interferon–Ribavirin Combined Therapy?

Rodalia M. Makhlouf[1], Mohamed M. Refaey[2], Hoda F. Ebian[3], Hebatallah H. Atteia[4], Amany M Ibrahim[1]

1) Internal Medicine department, Faculty of Medicine, Zagazig University, Egypt
2) Tropical Medicine department, Faculty of Medicine, Zagazig University, Egypt
3) Clinical Pathology department, Faculty of Medicine, Zagazig University, Egypt
4) Biochemistry department, Faculty of Pharmacy, Zagazig University, Egypt

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Corresponding Author

Mohamed M. Refaey
Tropical Medicine department, Faculty of Medicine, Zagazig University, 44519, Egypt.

Abstract

Introduction: This study was designed to find out if serum ferritin can be used as a predictor of treatment outcomes in chronic hepatitis C patients treated with interferon–ribavirin combination therapy.
Patients and Methods: The study included 15 chronic hepatitis C patients treated with pegylated interferon and ribavirin with sustained virological response and nine patients ending treatment without sustained virological response. Laboratory investigations of liver functions, kidney functions, complete blood picture, fasting blood glucose, hepatitis B surface antigen, alpha fetoprotein, pregnancy test, ANA and Thyroid-stimulating hormone were carried out for these patients. Serum hepatitis C Virus (HCV)-RNA was measured after starting the antiviral therapy and 24 weeks later. The levels of serum ferritin were measured by ELISA in sera of all patients.
Results: Before treatment serum ferritin levels were higher in group 2 patients. The pre- and on- treatment serum ferritin levels were positively linked with body weight and hepatic iron score. Upon treatment, serum ferritin levels increased in group 1 more than group 2 patients. Post-treatment, serum ferritin levels were lower in group 1 than those in group 2 patients. Serum ferritin levels from this period were associated only with hepatic fibrosis score on pre-treatment liver biopsy. During the 5th month post-treatment a further decline in serum ferritin levels occurred and serum ferritin levels returned towards baseline (pre-treatment) values. The degree of decline during this period was slightly higher in group 2 than in group 1.
Conclusions: Measurement of serum ferritin level has a role in prediction of sustained virological response in chronic HCV patients during treatment with antiviral agents. This role might help in monitoring HCV patients' response to treatment.

Introduction:

Hepatitis C virus (HCV) remains a large health care burden to the world. All over the world, 130–170 million persons are living with chronic HCV infection **[1],** which, if left without treatment, can lead to cirrhosis and liver cancer. Egypt has the greatest burden of HCV infection in the world, with a 10% prevalence of chronic HCV infection (CHC) among persons aged 15–59 years **[2].** Most (70–80%) HCV infections persist and about 30% of

individuals with persistent infection develop severe liver disease, comprising cirrhosis and hepatocellular carcinoma **[3].**

Currently the standard of care in treatment for HCV infection is the combination of pegylated interferon (INF)-α and ribavirin with the goal of achieving a sustained viral response (SVR). However, this treatment has variable cure rates and considerable side effects **[4, 5].** It has been reported that the response to combined INF–ribavirin in patients infected with HCV in clinical practice is quite low and not as good as reported in the phase-3 studies **[6,7].** Besides limited efficacy, some patients can not complete treatment because of side effects. Therefore, the main concern of HCV therapy is to identify the determinants of response to treatment.

Variability in virological response depends on diverse patient factors as well as virological and histological factors and on the interaction between these factors **[6-8].** These factors include age, gender, ethnicity, duration of infection, mode of acquisition, the degree of fibrosis of the liver, HCV genotype, viral load **[6-9],** and the degree of hepatic iron overload **[10-14].** Studies evaluating peginterferon α-2a and ribavirin combination therapy identify several predictive factors of SVR including low baseline viral load, body weight ≤ 75 kg, genotype other than 1, age less than 40 years, and absence of advanced fibrosis or cirrhosis **[15, 16].**

Serum ferritin (SF) is an established marker for liver iron deposition and has been widely used as a relatively low-cost and noninvasive tool to monitor iron status **[17]** and an indirect marker for the estimation of hepatic iron concentration, since hepatic iron controls the production of SF **[18].** However, SF is an acute phase reactant, and, as such, is affected by a variety of pathophysiologic states. In fact, hyperferritinemia has been also reported in patients with steatosis, type 2 diabetes, and metabolic syndrome **[19, 20],** which are often found in CHC patients **[21- 23].**

An increase in SF levels during combined INF–ribavirin therapy in patients infected chronically with HCV has been observed **[24- 28].** In addition, the degree of increase in SF during therapy was found to be an independent predictor of sustained virological response **[26,28].** The consistency and clinical significance of this phenomenon have not been investigated so far. A perturbation of circulatory and tissue iron pools during antiviral therapy may have important clinical implications as it may influence, indirectly, the activity of the drugs, the immunological response and, eventually, therapeutic outcome **[29, 30].**

This study was designed to find out if SF can be used as a predictor of treatment outcomes in patients with chronic hepatitis C treated with combined INF–ribavirin therapy.

Subjects And Methods:

Patients

This study included twenty four patients diagnosed as chronic active hepatitis C recruited from the hepatology clinics and Internal and Tropical medicine out-patient clinics, Zagazig University Hospitals, Egyptduring the period from September 2012 till October 2013. The enrolled patients were fulfilling the criteria for treatment with interferon and ribavirin. They were 18 males (75%) and 6 females (25%).Their age ranged from 28 to 56 years (at the beginning of therapy). An informed consent was obtained form all patients for participation in the current study. We followed the ethical guidelines of the institutional international review board (IRB) Committee, Faculty of Medicine, Zagazig University.

Inclusion criteria:

- All patients included in the study had active chronic HCV infection which was verified by the presence of significant HCV viremia and active liver disease on liver biopsy.
- All patients were receiving combination therapy that included subcutaneous injection of pegylated interferon and oral ribavirin, both administered at standard doses **[31]**. Eleven patients received a combination of PEG-INF a 2a, 180 ug/week and ribavirin (1,000 or 1,200 mg/day depending on whether body weight was below or above 75 kg). Thirteen patients received a combination of PEG-INF a 2b, 1.5 ug/kg/week and ribavirin, 10.6 mg/kg/day. Dose modifications of INF and/or ribavirin were performed in patients of both treatment groups, as indicated by the presence of adverse effects or hematological abnormalities.
- Only patients with early virological response, defined as a 2 log reduction in the viral load or HCV-RNA clearance at week 12, were included in the study. All patients included were treatment-naive and adherent to the antiviral regimen.

Exclusion criteria:

- Patients younger than 18 years or older than 60 years.
- If patients had been found to have additional causes for liver disease, infection with either hepatitis B virus, or HIV-1.

- If patients did not have at least four SF measurements (before, during, and at two time points after concluding the antiviral treatment).

All patients were subjected to complete history taking and clinical examination with special stress on abdominal examination, signs of liver failure, eye examination (fundus examination) and body mass index (BMI).

Laboratory investigations were carried out in Clinical Pathology Department, Zagazig University Hospitals. Assays of liver functions (serum levels of aspartate amino aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gama glutamyl transferase (GGT), total bilirubin, direct bilirubin, prothrombin time (PT), total protein and albumin), renal functions (serum creatinine and blood urea), complete blood picture, fasting blood sugar, hepatitis B surface antigen (HBsAg), alpha fetoprotein, pregnancy test (qualitative B-HCG in serum), ANA, Thyroid-stimulating hormone (TSH) were done for all patients.

Quantitative determination of serum HCV-RNA was performed at two additional points; just after concluding antiviral therapy and 24 weeks later. Absence of serum HCVRNA just after the end of the antiviral therapy was defined as end of treatment response. Absence of serum HCV-RNA at 24 weeks post-treatment was defined as sustained virological response. HCV-RNA was measured by quantitative real time PCR technology using COBAS® AmpliPrep/COBAS® TaqMan® HBV Test (Roche Diagnostics, Switzerland). HCV genotype was determined in all patients before treatment with the INNO-LiPA HCV II KIT (Bayer Diagnostics, Emeryville, CA).

Level of ferritin was estimated quantitatively in serum using DRG® Ferritin ELISA Kit (EIA-1872) for human. The test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA) **[32, 33].** The assay system utilized rabbit anti-ferritin for the solid phase (microtiter wells) immobilization and mouse monoclonal anti-ferritin in the antibody-enzyme (horseradish peroxidase) conjugate solution. The serum samples were allowed to react with the antibodies, and the ferritin molecules became sandwiched between the solid phase and enzyme-linked antibodies. After incubation for 45 minutes at room temperature, the wells were washed to remove unbound labeled antibodies. 3,3',5,5'-Tetramethylbenzidine (TMB) solution was added and incubated for 20 minutes, with the development of a blue color. The development of color was stopped with the addition of 1N HCl, and the produced yellow color is measured at 450 nm. The concentration of ferritin was directly proportional to the color intensity of the sample. The minimum detectable concentration of ferritin is estimated to be at least 5 ng/ml, with the assay range from 0 to 1000 ng/ml. The optical density (O.D.) was read at 450 nm in a microplate reader within 15 minutes after adding the stop solution. The standard curve was plotted as the relative O.D.450 of each standard solution (Y-axis) vs. the respective concentration of each standard solution (X-axis). Ferritin concentration of the serum samples was interpolated from the standard curve.

Histopathological evaluation of liver biopsies was performed blindly by a liver pathologist (O.P.). Histological grading of necroinflammatory changes and staging of architectural changes and fibrosis were done using the modified Histological Activity Index **[34].** Changes in fat distribution were reported using the steatosis score **[35].** Changes in iron staining were reported using an iron score **[36].**

Statistical analysis

Statistical presentation and analysis were conducted by SPSS version 17. Numerical values were presented as means±standard deviation. Cross tabulation was used to depict the relations between variables by using the contingency coefficient. Unpaired student t- test was utilized for comparison between two groups in quantitative data, Mann-Whitney test was used to evaluate the statistical difference between the two patients' groups regarding the levels of SF. Spearman's correlation test was used to assess relationships between two variables in one group. P value was considered non-significant if $P > 0.05$ and significant if $P < 0.05$.

Results

According to the results of viral RNA at 24 weeks post-completion of treatment, we divided our patients into two groups:

1. The first (group 1) consisted of fifteen patients who achieved sustained virological response.
2. The second (group 2) consisted of nine patients, only three (33.3%) had end-of-treatment response, and none had sustained virological response.

Table 1 shows the demographic and clinical characters of the patients:

- The two groups of patients did not differ in any of the demographic parameters collected: age, male/female ratio, height, weight, and BMI.
- Ninteen patients were infected with HCV genotype 1 and five patients were infected with non-genotype 1 viruses.
- The distribution of HCV genotypes between both groups was similar.
- The pretreatment ALT levels were also similar.

Table 2 indicated that there was no statistically significant difference between the two groups as regard the degree of hepatic fibrosis, portal and lobular inflammation, hepatic steatosis, and iron stain.

Antiviral therapy induced a decline in hemoglobin, platelet, and white blood cell counts. The degree of the lowest hemoglobin levels, platelet, and white blood cell counts did not differ between the groups. There was no difference between the two groups as regard the week of occurrence of the lowest hemoglobin levels, platelet, and white blood cell counts (Table 3).

Pre-treatment SF levels were higher in group 2 patients. Correlation analysis revealed that pre-treatment SF levels are linked with body weight ($r = 0.413$, $P < 0.05$) and with hepatic iron score ($r = 0.440$, $P < 0.05$) (Figure 1, Tables 4 and 5).

During active antiviral therapy (on-treatment), SF levels increased in both groups of patients. In most patients during the on-treatment period, multiple measurements of SF level were taken. Only the highest (peak) SF levels are reported in this study. The degree of increase in SF levels on-treatment (compared to baseline pre-treatment levels) was higher in group 1 patients (418.6% vs. 224.67 %, $P< 0.05$) (Figure 2, Table 4).

Correlation analysis revealed that on-treatment SF levels associated with body weight ($r= 0.545$, $P < 0.05$) and with hepatic fibrosis in pre-treatment liver biopsy ($r = 0.412$, $P = 0.05$) (Table 5).

Peak on-treatment SF levels occurred earlier in group 1 patients; however, the difference was not significant (Table 4). The calculated rate of the increase for on-treatment SF levels was higher in group 1 patients than in group 2 patients (34.57%/week vs. 13.5%/week, P =0.001) (Figure 3).

First month post-treatment SF levels in group 1 patients were lower than those measured in group 2 patients. In addition the degree of decline in SF levels (from peak serum ferritin levels) in group 1 patients was higher (70.94% vs. 59.16%, $P < 0.0.01$) (Table 4, Figure 2). Correlation analysis revealed that SF levels from this period were associated only with hepatic fibrosis score on pre-treatment liver biopsy ($r = 0. 521$, $P < 0.01$) (Table 5).

During the 5th month post-treatment a further decline in SF levels occurred and SF levels returned towards baseline (pre-treatment) values. The degree of decline (from 1st month post-treatment SF levels) during this period was slightly higher in group 2 patients than in group 1 patients (23.77% vs. 18.48%, respectively. P = Not Significant) (Table 4).

Table 1. Demographic and Laboratory Characteristics of the Study Population

	Group 1 (n=15)	**Group 2 (n=9)**	**P-value**
Age (years)	41.33±7.34(30-54)	43.11±8.04(32-56)	NS*
Sex • **Male** • **Female**	 11(61%) 4 (67%)	 7 (39%) 2 (33%)	NS#
Height (cm)	166.87±4.34 (159-174)	166.22±5.24(157-174)	NS*
Weight (kg)	74.07±4.79 (68-83)	72.56±5.25(68-82)	NS*
BMI (kg/m^2)	26.66±2.26 (22.44-30.51)	26.11±1.89 (22.59-29.39)	NS*
Baseline ALT (IU)	69.4±18.49 (43-98)	104.22±67.57 (37-183)	NS*
Genotype (n) • **1** • **2** • **3** • **4**	 12 0 2 1	 7 0 1 1	NS##
Duration of treatment (weeks)	43.2±6.09(36-48)	42.67±8.72(24-48)	NS*
End of treatment response (%)	100	33.3	0.001#

Data are presented as Mean±SD, range or percentage, and evaluated by independent sample t test (*), chi-squared tests (#), or chi-squared test with df = 3 (##), respectively. NS; Not Significant.

Table 2. Grading and Staging of Fibrosis, Steatosis, and Iron Stain Scores of Liver Biopsies of the Study Population

	Group 1 (n= 15)	Group 2 (n=9)	P-value
Periportal or periseptal interface hepatitis score	1(1-2)	2(0-4)	NS
Focal lytic, necrosis, apoptosis and focal inflammation score	1(1-2)	2(1-3)	NS
Portal inflammation score	2(1-3)	2(1-3)	NS
Hepatic fibrosis score	3(2-5)	4(0-5)	NS
Hepatic steatosis score	1(0-2)	0(0-2)	NS
Iron stain score	1(0-3)	2(0-3)	NS

Data are presented as median (range) and evaluated by independent samples t tests, NS; Not Significant.

Table 3. Hematological Parameters of the Study Population during the Various Stages of Anti-HCV Therapy

	group 1 (n=15)	group 2 (n=9)	P-value
Baseline hemoglobin (g/dl)	13.8±1.04(12.6-15.3)	13.7±0.82(12.7-15.3)	NS
The lowest hemoglobin level (g/dl)	10.5±0.73(9.3-11.5)	10.2±1.05(9.1-11.8)	NS
The week of the lowest hemoglobin level	25.7±4 (20-32)	28.6±2.55(24-32)	NS
The lowest platelet count ($x10^3$/ul)	118.1±25.78(82-157)	133.9±29.73(87-167)	NS
The week of the lowest platelet count (week)	18.9±4.93(10-26)	16.1±5.9(10-26)	NS
The lowest white blood cell count ($x10^3$/ul)	2.30±0.62(1.32-2.36)	2.45±0.41(1.32-3.36)	NS
The week of the lowest white blood cell count	19.3±3.86(13-26)	18.1±5.67(12-27)	NS

Data are presented as Mean±SD (range) and evaluated by independent samples t tests, NS; Not Significant.

Table 4. Serum Ferritin Levels and percent of change in the studied groups during Various Stages of Anti-HCV Therapy

	Group 1 (n=15)	Group 2 (n=9)	P-value
Pre-treatment serum ferritin levels (ng/ml)	109.47±37.65(30-154)	151.89±50.95(93-214)	0.029
On-treatment peak serum ferritin levels (ng/ml)	502.67±129.98(285-701)	495.67±128.07(304-752)	NS
Percent of increase in on-treatment serum ferritin levels (%)	418.6±217.84(157-896)	224.67±73.85(100-328)	0.018
rate of increase in on-treatment serum ferritin levels (%/week)	34.57±12.97(15.7-70.83)	13.5±7.25(5.56-27.27)	0.001
Peak serum ferritin levels (week)	11* (7-21)	14*(24-46)	NS
1st month, post-treatment serum ferritin levels (ng/ml)	141.53±35.16(57-183)	185.89±50.66(119-247)	0.019
1st month, post-treatment degree of decline in serum ferritin levels (%)	70.94±8.33(53.4-81.47)	59.16±10.02(45.04-70.48)	0.005
5th month, post-treatment serum ferritin levels (ng/ml)	115.07±31.64(46-157)	142.67±38.1(39-198)	NS
5th month, post-treatment degree of decline in serum ferritin levels (%)	18.49±6.02(12.62-31.74)	23.77±6.79(11.66-32.97)	NS

Data are presented as Median*, Mean±SD (range) and evaluated by independent samples t tests, NS; Not Significant.

Table 5. Spearman Correlations of Various Serum Ferritin Levels with Weight, Iron Staining Score, and Hepatic Fibrosis Score

	Weight		Iron stain score		Hepatic fibrosis score	
	rho	P-value	rho	P-value	rho	P-value
Pre-treatment ferritin	0.413	0.045	0.440	0.031	0.356	0.087
On-treatment ferritin	0.545	0.006	0.157	0.463	0.412	0.046
1st month post-treatment ferritin	0.371	0.074	0.330	0.115	0.521	0.009

Figure 1: Serum ferritin (SF) levels (ng/ml) at various stages of anti-HCV treatment according to long-term virological response in the two studied groups.

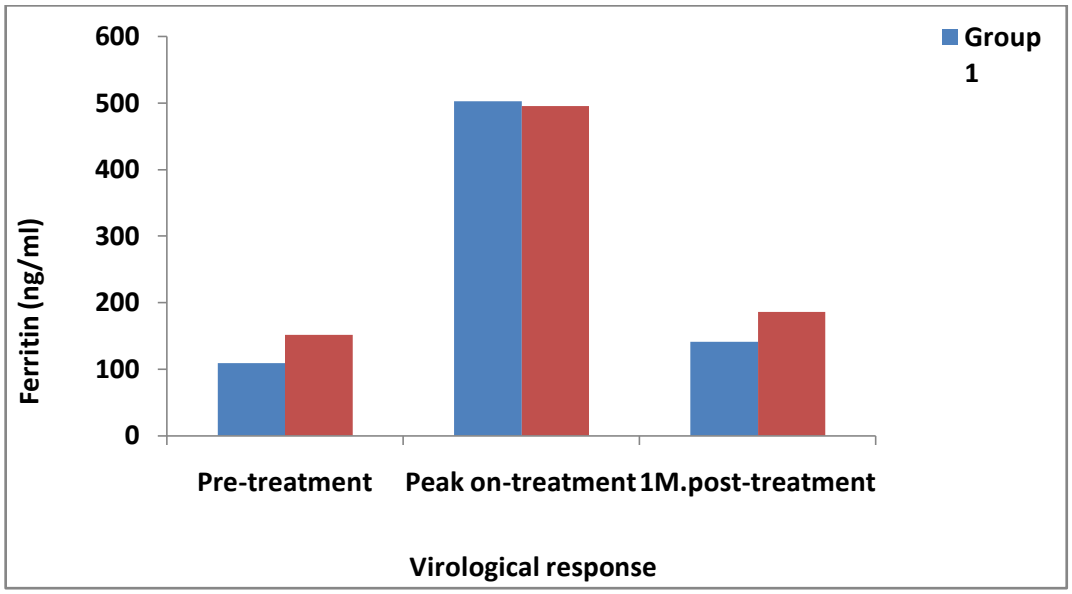


Figure 2: Percent of change in serum ferritin (SF) (%) levels at various stages of HCV treatment according to long-term virological response in the two studied groups.

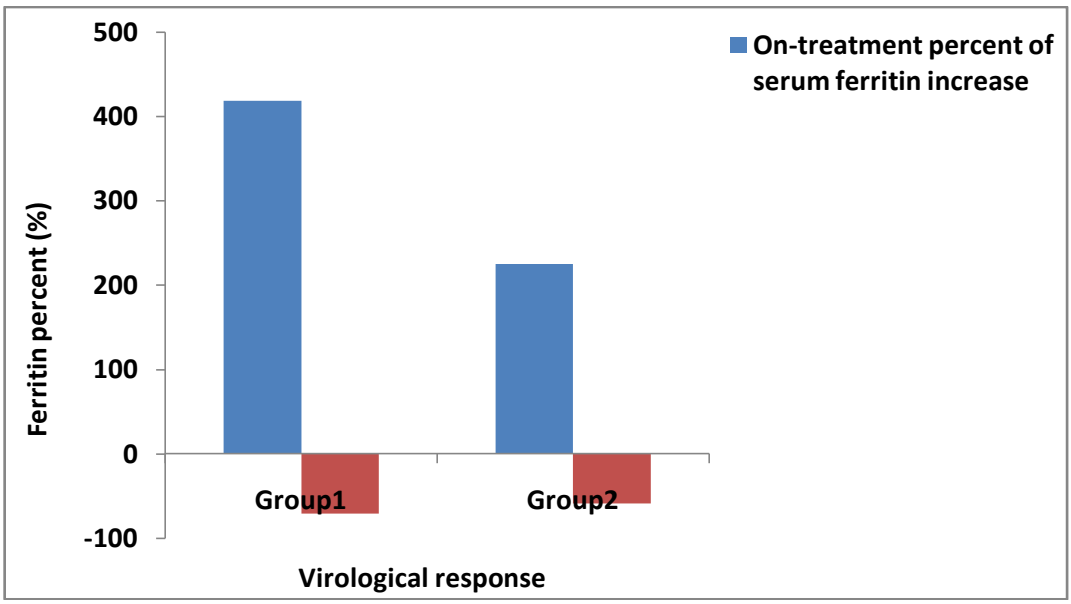
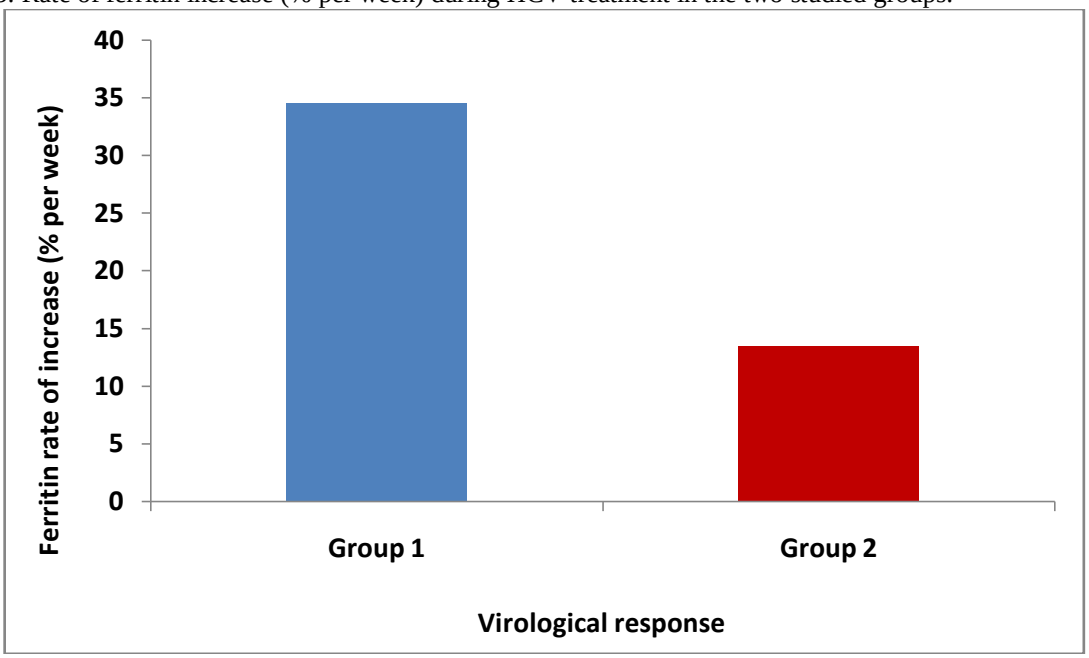


Figure 3: Rate of ferritin increase (% per week) during HCV treatment in the two studied groups.



Discussion

It has been postulated that disturbances in hepatic iron regulatory hormone (hepcidin) production may be a possible mechanism responsible for iron overload in chronic HCV **[37].** Elevated SF levels may reveal systemic inflammation and elevated iron storage which may play a role in an unfavorable outcome of chronic HCV. It has been also independently linked to advanced hepatic fibrosis, steatosis, and poor response to IFN-α-based treatment **[38].** This may be attributed to increased iron deposition in the liver promoting liver oxidative stress and inflammation as well as mitochondrial dysfunction **[39].** Supporting this notion is that repeated phlebotomy reduced necroinflammatory activity and risk of hepatocellular carcinoma progression in chronic HCV patients **[40-42].**

The major findings of the present study were that during the course of active antiviral treatment SF levels showed a marked increase. This is may be due to INF exposure where it may stimulate numerous interferon-activated genes and enhance the synthesis and release of several pro-inflammatory cytokines that probably up-regulate ferritin synthesis **[43, 44].** Accordingly, the present study showed that chronic HCV patients with sustained virological response displayed a greater increase in SF level than those without sustained virological response throughout treatment period. We reported also a decline in hemoglobin levels in HCV patients during antiviral therapy. This might be attributed to the hemolytic effects of ribavirin **[45].** Hemolysis can also increase hepatic iron deposition increasing SF levels **[46].**

On-treatment SF levels were positively correlated with body weight and hepatic fibrosis in pre-treatment liver biopsy. Similar results were reported before where baseline SF level was correlated with body weight and the stage of hepatic fibrosis **[26,27].** In confirm, obese subjects may have higher serum ferritin levels than non-obese subjects that may be related to the inflammatory state that accompanies obesity **[47].** It has been also reported that low SF levels may be an independent marker for the absence or reduced level of hepatic fibrosis **[48].** The current study demonstrated also that baseline SF level was positively correlated with hepatic iron score and hence it can indirectly reflect the concentration of iron in the liver of HCV patients.

The level of SF can be considered as an indirect marker of INF exposure. Ferritin may also alter the immune function. Before starting antiviral therapy, higher baseline levels of SF are associated with chronic stimulation of the endogenous INF system. Thus patients having higher levels of SF prior to antiviral treatment may have a reduced response to antiviral therapy **[49].** The immunomodulatory effects of ferritin in humans can be mediated by inhibition of lymphocyte function, delayed hypersensitivity response and by induction of myelosuppression **[50].** Therefore, high SF level in patients with limited response to antiviral therapy seems to be a

result of immunosuppression **[51].** Our results further reinforced these findings since antiviral therapy induced a decrease in white blood cells count.

One month after treatment the level of SF decreased. The rate of decrease was higher in group 1 having sustained response than in group 2 patients having no SVR. This suggests the prognostic impact of SF levels at this early stage post-treatment as a marker to help physicians to predict the prolonged virological response of chronic HCV patients.

Conclusion

In conclusion, higher SF is strongly correlated with reduced rate of virological response and liver fibrosis in HCV. Overall, measuring SF for chronic HCV patients during treatment is useful in determination of patients' virological response. This may help clinicians to determine the dose and length of treatment with antiviral agents for each patient.

References

1- The Global Burden of Hepatitis C Working Group (2004). Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol;44:20–9.
2- El-Zanaty F, Way A. Egypt demographic and health survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International; 2009. Available at http://www.measuredhs.com/pubs/ pdf/fr220/fr220.pdf. Accessed July 18, 2012.
3- David L , Chloe L , Maureen P, Ying Qi, Dongliang Ge, et al (2002). Genetic variation in IL28B and spontaneous clearance of hepatitis C virus.Nature 461, 798-801 (8 October 2009).
4- National Institutes of Health. (2002). National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C. Hepatology 36:S3–S20.
5- Poynard T, Yuen MF, Ratziu V, Lai CL. (2003). Viral hepatitis C. Lancet 362:2095–2100.
6- Backus LI, Boothroyd DB, Phillips BR, Mole LA.(2007). Predictors of response of US Veterans to treatment for the hepatitis C virus. Hepatology 46:37–47.
7- Feuerstadt P, Bunim AL, Garcia H, Karlitz JJ, Massoumi H, Thosani AJ, Pellecchia A, Wolkoff AW, Gaglio PJ, Reinus JF. (2010). Effectiveness of hepatitis C treatment with pegylated interferon and ribavirin in urban minority patients. Hepatology 51:1137–1143.
8- Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, Heathcote J, Edwards AM, McGilvray ID. (2005). Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 128:1437– 1444.
9- Thio CL, Thomas DL. (2010). Interleukin-28b: A key piece of the hepatitis C virus recovery puzzle. Gastroenterology 138:1240– 1243.
10- Sherrington CA, Olynyk JK. (2002). Iron as a cofactor in chronic hepatitis C infection. Liver 22:187–189.
11- Metwally MA, Zein CO, Zein NN. (2004). Clinical significance of hepatic iron deposition and serum iron values in patients with chronic hepatitis C infection. Am J Gastroenterol 99:286–291.
12- Alla V, Bonkovsky HL. (2005). Iron in nonhemochromatotic liver disorders. Semin Liver Dis 25:461–472.
13- Rulyak SJ, Eng SC, Patel K, McHutchison JG, Gordon SC, Kowdley KV.(2005). Relationships between hepatic iron content and virologic response in chronic hepatitis C patients treated with interferon and ribavirin. Am J Gastroenterol 100:332–337.
14- Fujita N, Sugimoto R, Urawa N, Araki J, Mifuji R, Yamamoto M, Horiike S, Tanaka H, Iwasa M, Kobayashi Y, Adachi Y, Kaito M. (2007). Hepatic iron accumulation is associated with disease progression and resistance to interferon/ribavirin combination therapy in chronic hepatitis C. J Gastroenterol Hepatol 22:1886– 1893.
15- Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med; 347: 975-982.
16- Rodriguez-Torres M, Sulkowski MS, Chung RT, Hamzeh FM, Jensen DM. (2010). Factors associated with rapid and early virologic response to peginterferon alfa-2a/ribavirin treatment in HCV genotype 1 patients representative of the general chronic hepatitis C population. J Viral Hepat; 17: 139-147.
17- Tavill AS. (2001). Diagnosis and management of hemochromatosis . Hepatology; 33 : 1321 – 8 .
18- Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. (2009). Ferritin for the clinician. Blood Rev 23:95–104.
19- Vari IS , Balkau B , Kettaneh A et al. (2007). Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) . Diabetes Care; 30 : 1795 – 801 .

20- Forouhi NG , Harding AH , Allison M e t al. (2007). Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study . Diabetologia; 50: 949 – 56.
21- Adinolfi LE, Gambardella M, Andreana A et al. (2001). Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with speciE c HCV genotype and visceral obesity. Hepatology; 33 : 1358 – 64.
22- Lecube A, Hernandez C , Genesca J e t al. (2004). Diabetes is the main factor accounting for the high ferritin levels detected in chronic hepatitis C virus infection . Diabetes Care; 27 : 2669 – 75.
23- Asselah T, Rubbia-Brandt L , Marcellin P et al. (2006). Steatosis in chronic hepatitis C: why does it really matter? Gut; 55 : 123 – 30 .
24- Nagashima M, Kudo M, Chung H, Ishikawa E, Inoue T, Nakatani T, Dote K.(2008). Elevated serum ALT levels during pegylated interferon monotherapy may be caused by hepatic iron overload. Intervirology 51:S76–S85.
25- Falasca K, Mancino P, Ucciferri C, Dalessandro M, Manzoli L, Pizzigallo E, Conti CM, Vecchiet J. (2009). Quality of life, depression, and cytokine patterns in patients with chronic hepatitis C treated with antiviral therapy. Clin Invest Med 32:E212–E218.
26- Ferrara F, Ventura P, Vegetti A, Guido M, Abbati G, Corradini E, Fattovich G, Ferrari C, Tagliazucchi M, Carbonieri A, Orlandini A, Fagiuoli S, Boninsegna S, Minola E, Rizzo G, Belussi F, Felder M, Massari M, Pozzato G, Bonetto S, Rovere P, Sardini C, Borghi A, Zeneroli ML, Toniutto P, Rossi E, Pietrangelo A. (2009). Serum ferritin as a predictor of treatment outcome in patients with chronic hepatitis C. Am J Gastroenterol 104:605–616.
27- Ladero JM, Lopez-Alonso G, Devesa MJ, Cuenca F, Ortega L, Agreda M, Suarez A, Ropero P, Dıaz-Rubio M. (2009). Oscillations in serum ferritin associated with antiviral therapy in chronic hepatitis C. Rev Esp Enferm Dig 101:31–40.
28- Yada N, Kudo M, Chung H, Hayaishi S, Takita M, Ueda T, Tatsumi C, Hatanaka K, Kitai S, Ishikawa E, Inoue T, Hagiwara S, Ueshima K. (2010). PEG-IF alpha/RBV combination therapy for chronic hepatitis C patients increases serum ferritin level while it improves sustained viral response rate. Intervirology 53:60–65.
29- Pietrangelo A (2003). Hemochromatosis gene modiE es course of hepatitis C viral infection. Gastroenterology ; 124 : 1509 – 23 .
30- Weiss G, Umlau' F, Urbanek M et al. (1999). Associations between cellular immune eA ector function, iron metabolism, and disease activity in patients with chronic hepatitis C virus infection. J Infect Dis; 180: 1452 – 8.
31- Dienstag JL, McHutchison JG. (2006). American Gastroenterological Association medical position statement on the management of hepatitis C. Gastroenterology 130:225–230.
32- Siimes MA, Addiego JE Jr, Dallman PR. (1974). Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. Blood.;43(4):581-90.
33- Uotila M, Ruoslahti E, Engvall E. (1981). Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. J Immunol Methods.;42(1):11-5.
34- Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN, Phillips JM, Portmann BG, Poulsen H, Scheuer PJ, Schmid M, Thaler H. (1995). Histological grading and staging of chronic hepatitis. J Hepatol 22:696–699.
35- Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA,Bacon BR. (1999). Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474.
36- Rowe JW, Wands JR, Mezey E, Waterbury LA, Wright JR, Tobin J, Andres R. (1997). Familial hemochromatosis: Characteristics of the precirrhotic stage in large kindred. Medicine (Baltimore) 56:197–211.
37- Nishina, S., Hino, K., Korenaga, M., (2008). Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. Gastroenterology, 134(1): 226-38.
38- Lange CM1, Kutalik Z, Morikawa K, Bibert S, Cerny A, Dollenmaier G, Dufour JF, Gerlach TJ, Heim MH, Malinverni R, Müllhaupt B, Negro F, Moradpour D, Bochud PY; Swiss Hepatitis C Cohort Study Group. (2012). Serum ferritin levels are associated with a distinct phenotype of chronic hepatitis C poorly responding to pegylated interferon-alpha and ribavirin therapy. Hepatology.;55(4):1038-47.
39- Drakesmith H, Prentice A. (2008). Viral infection and iron metabolism. Nat Rev Microbiol;6:541-552.
40- Di Bisceglie AM, Bonkovsky HL, Chopra S, Flamm S, Reddy RK, Grace N, Killenberg P, Hunt C, Tamburro C, Tavill AS, Ferguson R, Krawitt E, Banner B, Bacon BR. (2000). Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who have previously not responded to interferon: a multicenter, prospective, randomized, controlled trial. Hepatology;32:135-138.
41- Fontana RJ, Israel J, Le Clair P, Banner BF, Tortorelli K, Grace N, Levine RA, Fiarman G, Thiim M, Tavill AS, Bonkovsky HL. (2000). Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. Hepatology;31:730-736.

42- Kato J, Miyanishi K, Kobune M, Nakamura T, Takada K, Takimoto R, Kawano Y, Takahashi S, Takahashi M, Sato Y, Takayama T, Niitsu Y. (2007). Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol;42:830-836.
43- Stam TC, Swaak AJ, Kruit WH, Eggermont AM. (2002). Regulation of ferritin: A specific role for interferon-alpha (IFN-alpha)? The acute phase response in patients treated with IFN-alpha-2b. Eur J Clin Invest 32:S79–S83.
44- Chevaliez S, Pawlotsky JM. (2007). Interferon-based therapy of hepatitis C. Adv Drug Deliv Rev 59:1222–1241.
45- Kowdley KV. (2005). Hematologic side effects of interferon and ribavirin therapy. J Clin Gastroenterol 39:S3–S8.
46- Fiel MI, Schiano TD, Guido M, Thung SN, Lindsay KL, Davis GL, Lewis JH, Seeff LB, Bodenheimer HC Jr. (2000). Increased hepatic iron deposition resulting from treatment of chronic hepatitis C with ribavirin. Am J Clin Pathol 113:35–39.
47- Yanoff LB, Menzie CM, Denkinger B, Sebring NG, McHugh T, Remaley AT, Yanovski JA. (2007). Inflammation and iron deficiency in the hypoferremia of obesity. Int J Obes (Lond) 31:1412–1419.
48- Ladero JM, Delkader J, Ortega L, Fernandez C, Devesa MJ, Lopez-Alonso G, Mayol J, Cuenca F, Suarez A, Taxonera C, Dıaz-Rubio M. (2010). Non-invasive evaluation of the fibrosis stage in chronic hepatitis C: A comparative analysis of nine scoring methods. Scand J Gastroenterol 45:51–59.
49- Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, Theodore D, Zacks SL, Liang TJ, Fried MW. (2007). Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. Hepatology 46:1548–1563.
50- Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. (2010). Serum ferritin: Past present and future. Biochim Biophys Acta 1800:760–769.
51- Ackerman Z, Pappo O, Ben-Dov IZ. (2011). The prognostic value of changes in serum ferritin levels during therapy for hepatitis C virus infection. Journal of Medical Virology 83:1262–1268.