Value of Pre- and on Treatment Serum Ferritin as Predictors of Treatment Response in Egyptian Patients with Chronic Hepatitis C Infection

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Abstract: Background and aim: An increase in serum ferritin levels during combined interferon–ribavirin treatment in chronic patients with hepatitis C virus (HCV) has been established. This study was conducted to determine whether pre treatment serum ferritin and the changes of ferritin during antiviral therapy, may assist in predicting sustained virological response (SVR). **Patients and methods:** seventy patients with chronic HCV received the combination therapy were included. Serum ferritin and iron levels were measured pretreatment, 4 and 12 weeks during therapy. **Results:** thirty nine patients didn't achieve SVR (non SVR group) and 31 patients achieved SVR (SVR group). The median pre-treatment serum ferritin and iron were significantly higher in non SVR group compare to SVR group (171.7 vs 156 ng/ml and 138.8 vs 118 µg /dl) respectively. During antiviral therapy, ferritin levels increased in both groups, but the median increase (compared to baseline) and the calculated rate of the increase in serum ferritin levels was higher in SVR group patients compare to non SVR (0.07 vs. 0.031 at week 4 and 0.1923 vs 0.087 at week 12 of therapy respectively with p value < 0.05). Both Baseline ferritin and the increase of ferritin during therapy didn't correlate with baseline hemoglobin or with rate of hemoglobin drop. Also, higher rates of increase in serum ferritin during treatment together with lower fibrosis stage and rapid virological response (RVR) are strong independent predictors of SVR. **Conclusion:** Measuring serum ferritin levels during antiviral therapy in HCV patients may predict SVR

[Ahmed Abbas El Khateeb, Ashraf AlBreedy and Sherif Monier Mohamed. **Value of Pre- and on Treatment Serum Ferritin as Predictors of Treatment Response in Egyptian Patients with Chronic Hepatitis C Infection** *Life Sci J* 2014;11(1s):334-339]. (ISSN:1097-8135). http://www.lifesciencesite.com. 59

Key Words: Chronic hepatitis C; Combination therapy; Serum ferritin; Sustained viral response

1. Introduction:

The prevalence of hepatitis C virus (HCV) in Egypt exceeds 18% of the general population. Thus, with a population of more than 80 million, Egypt is considered to have the highest prevalence of hepatitis C in the world and consequently a high frequency of hepatocellular carcinoma (HCC).[(1-2)]

Before appearance of the new directly acting antiviral drugs (DAAs) as sofosbuvir, semiprevir and others [(3)], the most effective and recommended treatment for chronic HCV infection was the combination of pegylated interferon alpha (INF) and ribavirin. However, this treatment has variable cure rates and considerable side effects [(4)]. 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. [(5-6).]

The high cost of therapy and need for close follow up essentially restrict treatment and requires evaluation of reliable predictors for sustained virological response [(7)].

The natural history and the response to drug therapy of patients with HCV may depend on many host genetic factors and viral factors and on the interaction between these factors [(5-6)]. Included among these factors are age, gender, body mass index, ethnicity, duration of infection, mode of acquisition, the degree of fibrosis of the liver, HCV genotype, viral load, the degree of hepatic iron overload. [(8-9).]

Serum ferritin is an established marker for liver iron deposition; since hepatic iron controls the production of serum ferritin thus it has been widely used as a relatively low-cost and noninvasive tool to monitor iron status [(10)]. In patients with chronic hepatitis C (CHC) a rise of serum ferritin is common, whereas marked hepatic iron overload, in the absence of a genetic predisposition to iron overload, is rare. Hyperferritinemia in CHC may reflect ongoing necro-inflammatory events, and it's often accompanied with iron deposits in hepatic mesenchymal cells [(11)]. However, others have suggested that the increase in serum ferritin levels in patients infected with HCV may also be due to the presence of glucose intolerance, insulin resistance, or overt type 2 diabetes mellitus and fatty liver that co-exist in a substantial number of these patients [(12-13)].An increase in serum ferritin levels during combined IFN–Ribavirin therapy in patients infected chronically with HCV has been also observed [(14-16)].

Aim of the work:

To evaluate the role of pre- and on-treatment serum ferritin levels as predictor for sustained virological response (SVR) in chronic hepatitis C (CHC) patients receiving combined pegylated Interferon and Ribavirin therapy.

2. Patients and methods:

This prospective study was conducted in the Tropical and Internal Medicine department, Ain Shams University Hospitals and in Agouza police Forces Hospital (Gastroenterology and Hepatology department). Patients were recruited and selected from the outpatient clinic during the period from June 2011 to December 2011 and were followed from June 2011 to June 2013.

All patients included in the study had active chronic HCV infection which was verified by the presence of significant HCV viremia by PCR and active liver disease on liver biopsy. All patients were receiving combination therapy that included subcutaneous injection of pegylated interferon alpha once / week (180 µg for 2a and 1.5 µg/kg for2b) and daily oral ribavirin (15 mg/kg daily).

All patients included were treatment-naive and adherent to the antiviral regimen. Patients were excluded from the study if they had been found to have additional causes for liver disease, infection with either hepatitis B virus or HIV-1 and patients who stopped treatment due to side effects. The enrolled Patients were classified as responders if they achieved SVR (defined as undetectable HCV RNA at 24 weeks after the completion of therapy). The remaining patients were categorized as non-SVR.

Written informed consent was obtained from all patients before treatment and the protocol was approved by the ethics committee of Ain Shams University School of Medicine.

Before starting treatment, all enrolled patients were subjected to full history taking; thorough clinical examination; quantitative PCR for HCV-RNA(using Taqman method, Q1A amp viral RNA, Mini Kit 50, Cat No 52904, Beckman Caulter, USA); serum Ferritin and serum Iron levels (using Beckman CaulterIron Kit, USA); complete blood picture (CBC); erythrocyte sedimentation rate (ESR); liver profile including ALT, AST, serum billirubin, serum albumin, serum alkaline phosphatase and prothrombin time (PT) & INR; renal profile; fasting blood sugar and if the patient is diabetic, glycosylated hemoglobin level was measured; alpha feto-protein level; antinuclear antibodies titer; thyroid function tests and pregnancy test for married female patients. In addition to fundus examination and ECG were performed.

During the treatment period the patients were followed by quantitative PCR for HCV-RNA at 4, 12, 24 and 48 weeks then 24 weeks after treatment stoppage in patients who achieved end treatment response (ETR) i.e. undetectable HCV RNA at 48 weeks of therapy. The serum ferritin and serum Iron levels were measured at 4 and 12 weeks during therapy. CBC, bilirubin, creatinine, ALT and AST were measured every month during therapy, then 3 and 6 months after therapy in patients with ETR.

Statistical analysis

IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) was used for data analysis. Data were expressed as Mean± SD for quantitative parametric measures in addition to Median Percentiles for quantitative non-parametric measures and both number and percentage for categorized data. Student t - test was used for Comparison between two independent mean groups for parametric data. Mann-Whitney U test was used for Comparison between two independent groups for non-parametric data. Correlation between data was tested using the non-parametric Spearman rank correlation analysis. Chi-square test was used to compare between 2 independent groups. Differences were considered statistically significant at $p < 0.05$.The ROC was constructed to obtain the most sensitive and specific cutoff value for serum ferritin for identifying patients who achieved SVR from those who didn't. HCC Logistic stepwise multi-regression analysis was used to search for independently significant risk factors that predict the dependant variable.

The degree of change during follow-up study is called [delta change (dC)] and it could be calculated for each patient and from which, the mean delta change can be calculated. It is used for follow-up study and for comparison between the subgroups, also for correlation with other variables. It is defined as: dC = (Post-Pre)/Pre values for each patient.

3. Results:

This study was conducted on 70 patients with chronic HCV infection receiving combined pegylated interferon and ribavirin therapy. Thirty nine patients (55.7%) didn't achieve SVR (non SVR group) and thirty one patients (44.3%) achieved SVR (SVR group). Seven patients relapsed after having ETR and 7 patients had detectable PCR at end of treatment after having undetectable viremia at 12 and 24 weeks of treatment.

Twenty one patients (9 in non SVR group and 12 in SVR group) received Pegylated interferon alpha 2a and 49 patients (30 in non SVR group and 19 in SVR group) received Pegylated interferon alpha 2b with no significant statistical difference between the 2 groups **(Table 1)**.

The demographic and clinical, biochemical characteristics of the patients are shown in **Table 1**.

The two groups of patients did not differ in any of the demographic parameters collected: age, sex, weight, height, and BMI. Also, the pretreatment levels of ALT, AST, alkaline phosphatase, hemoglobin, platelets, white blood cells, alpha feto protein and degree of viremia didn't show any significant difference between both groups. According to Ishak score, patients who achieved SVR had much significant lower median fibrosis stage than that in patients who didn't achieved SVR (median 1 vs 3). In addition, 23 out of 31 patients (74.2%) who achieved SVR had RVR, while only 14 patients in non SVR group (35.9%) achieved RVR and the difference between the 2 groups reached statistical significance regarding this point (p<0.05). **(Table 1)**

Patients in non SVR group had significant higher baseline serum ferritin and iron when compared to those for patients in SVR group (171.7 vs 156 and 138.8 vs 118) respectively. Otherwise, no significant difference between both groups regarding median serum ferritin or iron at week 4 or 12 of therapy (p<0.05). **(Table 2)**

During active antiviral therapy (on-treatment) serum ferritin levels increased in both groups of patients. The degree of increase in serum ferritin levels on-treatment (compared to baseline pre-treatment levels) was much higher in SVR group patients (median increased 0.03 vs. 0.07% at week 4 and 0.087 vs 0.192 at week 12 $P < 0.05$). Antiviral therapy induced a decrease in hemoglobin at week 4 and 12, but the degree of decrease did not differ significantly between the 2 groups (P>0.05). **(Table 3)**

No significant correlation was found between baseline serum ferritin and delta changes of serum ferritin at week 4, 12 of treatment with weight, BMI, fibrosis stage, degree of changes in iron or hemoglobin in the first 4 and 12 weeks of treatment. **(Table 4)**

Step wise Multi-regression analysis shows that rapid virological response, fibrosis stage ≤ 3 and on treatment serum ferritin increase are the most sensitive discriminators for prediction of sustained virological response from those non-responder (P<0.05) **(Table 5)**.

Table 1: Univariate analysis for SVR in the Study Population

		Non SVR n=39 (55.7%)		SVR n=31 (44.3%)		*P* value
Sex: Female: No (%) Male: No (%)		2 (5.1%) 37 (94.9 %)		3 (9.7%) 28 (90.3%)		0.097‡
Age (years): Mean ±SD		38.92 ±7.01		40.71 ±8.79		0.089*
Weight (Kg): Mean ±SD		82.50 ±9.84		83.15 ±8.75		0.773*
Height (cm): Mean ±SD		162.41 ±47.53		175.77 ±7.08		0.126*
BMI: Mean ±SD (kg/cm²)		26.84 ±2.74		26.80 ±2.21		0.952*
AST(0): Median (3rd-1st quartile) (IU/L)		39 (66.25-32.5)		43 (55.3-32.35)		0.849†
ALT(0): Median (3rd-1st quartile) (IU/L)		47 (78.4-35)		62 (84.6-39.35)		0.307†
WBC(0): Median (3rd-1st quartile) (10³/µL)		6 (7.3-4.75)		6.2 (7.5-4.8)		0.638†
Hb(0): Median (3rd-1st quartile) (gm/dl)		15.3 (15.8-14.25)		15.5 (15.95-14.2)		0.942†
Plts(0): Median (3rd-1st quartile) (10³/µL)		196 (233.5-177)		199 (238-155)		0.515†
Alk. Ph(0): Median (3rd-1st quartile) (IU/L)		75.5 (90.1-59)		75.7 (89.3-61)		0.960†
AFP(0): Median (3rd-1st quartile) (ng/ml)		4.4 (9.65-3.45)		4.1 (8.8-2.85		0.475†
HCVRNA(0): Median (3rd-1st quartile) (IU/ml)		121026 (684541-26343)		87303 (201289-10062)		0.089†
Fibrosis stage: Median (3rd-1st quartile)		3 (3.5-2)		1 (3-1)		**0.001**†
HAI: Median (3rd-1st quartile)		5 (9-4)		5 (7-5)		0.726†
RVR	Yes	14	35.9%	23	74.2%	**0.001**‡
	No	25	64.1%	8	25.8%	
Type of IFN: Alpha 2a Alpha 2b		9 30	23% 77%	12 19	39% 61%	0.156‡

*Student's *t* test. †Mann – Whitney *U* test. ‡ chi square test.
(0) means values at baseline; BMI: body mass index; AST: aspartate transaminases; ALT: alanine transaminases; WBC: white blood cells; Hb: hemoglobin; Plts: platelets; Alk ph.: alkaline phosphatase; AFP: alpha fetoprotein; HAI: histology activity index; RVR: rapid virological response (undetectable PCR at week 4 of therapy); IFN: interferon

Table 2: Comparison of Serum ferritin and iron in the studied groups at baseline, 4 and 12 weeks of therapy

Parameter	Non-SVR patients n=39	SVR patients n=31	*P* value
	Median(3^{rd}-1^{st} quartile)	Median(3^{rd}-1^{st} quartile)	
Ferritin(0) (ng/mL)	171.7 (178.3-156.5)	156 (167.5-115.4)	**0.002**
Ferritin(4) (ng/mL)	172.1 (182.1-164.8)	169 (174.45-160.05)	0.289
Ferritin(12) (ng/ml)	177.2 (187.2-170)	171.45 (180.15-164.5)	0.147
Iron(0) (µg/dL)	138.8 (173.1-106.8)	118 (132-97)	**0.027**
Iron(4) (µg/dL)	145.1 (168-105)	130 (146.6-98.7)	0.246
Iron(12) (µg/dL)	139 (160.35-107.25)	115.5 (149.7-86.65)	0.068

(0) means values at baseline; (4) at 4 weeks of therapy; (12) at 12 weeks of therapy
Mann – Whitney *U* test

Table 3: Comparison of changes in serum ferritin, iron and hemoglobin during the first 12 weeks of therapy between the groups

Parameter	Non-SVR patients, n=39	SVR patients, n=31	*P* value
	Median	*Median*	
Ferritin dC04(ng/mL)	0.03143	0.070	**0.0423**
Ferritin dC012 (ng/ml)	0.08731	0.1923	**0.0348**
S. Iron dC04 (µg/dL)	0.04466	0.04560	0.6312
S.Iron dC012 (µg/dL)	-0.0201	0.03209	0.4593
dC Hb04	-0.1369	-0.1419	0.5485
dC Hb012	-0.2023	-0.2147	0.4715

dC04: delta change from baseline to week 4; dC012: delta change from baseline to week 12; Hb: hemoglobin

Table 4: Spearman correlations of various serum ferritin levels with weight, BMI, hepatic fibrosis stage, change of hemoglobin and serum iron

Parameter	*Ferritin-0*		*Ferritin dC04*		*Ferritin_dC012*	
	r	*P*	*r*	*P*	*r*	*P*
Weight	0.172	0.153	-0.151	0.210	-0.128	0.287
BMI	0.056	0.640	-0.095	0.432	-0.088	0.465
Fibrosis stage	0.109	0.368	-0.082	0.496	-0.034	0.776
dC Hb04	-0.042	0.725	0.037	0.761	0.071	0.555
dC Hb012	-0.121	0.315	0.125	0.301	0.137	0.258
dC iron 04	-0.202	0.092	0.191	0.113	0.103	0.394
dC iron 012	-0.142	0.238	0.215	0.073	0.218	0.068

BMI: body mass index; dC04: delta change from baseline to week 4; dC012: delta change from baseline to week 12; Hb: hemoglobin

Table 5: factors predicting SVR at multiple regression analysis

Dependent variable: SVR	B	S.E.	OR	95% CI	Sig.
RVR	1.630	0.555	4.349	1.46-14.2	**0.009**
Fibrosis stage ≤ 3	1.204	0.545	3.777	1.06-9.01	**0.006**
Serum ferritin increase	0.039	0.019	1.04	1.01- 1.09	**0.019**

RVR: rapid virological response; SVR: sustained virological response; OR: odds ratio

4. Discussion:

Measuring viral load kinetics during the early phases of combined interferon and ribavirin therapy has been reported to be a powerful predictor for SVR [(17)]. However, implementation of treatment strategies based only on the results of viral kinetics early in the course of combined INF–ribavirin therapy may not result in improved SVR rates [(18)]. Thus, it is suggested that utilizing a combination of markers, measured before and during the antiviral therapy, may be more powerful for predicting the response to antiviral therapy. In this study we tried to evaluate the role of pretreatment and on treatment serum ferritin in predicting SVR in patients receiving combined Pegylated interferon and ribavirin therapy for chronic HCV infection.

Most of studies reported SVR rates to combined IFN and ribavirin therapy for chronic HCV

infection genotype 4 ranging between 50, 55 and 69% [19-21]. Our study showed the SVR rate was 44.3% which is not far from most of other studies.

During active antiviral therapy serum ferritin levels increased significantly in our study. This is probably related to exposure to INF [22]. Administration of INF may activate multiple interferon-stimulated genes and induce the synthesis and liberation of various pro-inflammatory cytokines that may up regulate the synthesis of ferritin [23-24]. As a result of these findings, it is speculated that serum ferritin levels are an indirect marker for INF effects: the greater the serum ferritin response, the greater the INF effect. And indeed in the present study it was demonstrated that patients who had SVR had a greater slope in the increase of serum ferritin level during antiviral treatment. Although there was no difference between the 2 groups as regards the values of serum ferritin and iron at week 4 and 12 of therapy, the median rate of increase of serum ferritin from baseline to week 4 and 12 was significantly higher in SVR group when compared to non SVR group (ferritin dC 0/4 and 0/12:7% vs 3% and 19% vs 8.7%) respectively. Our results are in accordance with those by ***Ferrara et al***.[14]; ***Yada et al***.[16]; ***Ackerman et al***.[25] who found that serum ferritin level increased during PEG-IFN and RBV combination therapy and higher rate of increase of serum ferritin levels during combination therapy appeared to be associated with favorable therapeutic response.

In this study, higher baseline serum ferritin levels associated with lower response rate to antiviral therapy. Similar results were reported by others [14, 15, 25, and 26]. Two possible explanations for the reduced response rate to antiviral therapy in this group of patients can be suggested. First, as mentioned earlier, serum ferritin levels may be considered as an indirect marker of exposure to INF. In the patients with higher baseline ferritin levels, chronic activation of the endogenous INF system appears to present, prior to the antiviral treatment. Therefore, these patients may have a limited ability to mount a further response to treatment with INF [27]. Second, in addition to its role in iron metabolism, ferritin may have immunomodulatory activities. Several studies have indicated that ferritin can modulate the immune function in humans by inhibiting lymphocyte function, by inhibiting delayed type hypersensitivity response and by inducing myelosuppression [28]. Thus, it is possible that the chronic high serum ferritin levels in this group of patients caused some immunosuppression and hampered the response of these patients to the antiviral treatment.

In the current study, no significant correlations were found between baseline, pre-treatment serum ferritin, delta changes of serum ferritin from baseline to week 4 and 12 during therapy with body weight, BMI, pre treatment liver fibrosis stage or delta changes in hemoglobin and iron during the first 12 weeks of therapy. Correlation between body weight and baseline serum ferritin levels was reported in some studies [15-25] but not in the Italian cohort infected with HCV [14]. No correlation between serum ferritin levels and fibrosis was reported in patients from Britain [13]. Other groups found correlation between baseline serum ferritin levels and the stage of fibrosis [14-15]. On the other hand, other study reported that on-treatment serum ferritin levels were found to correlate with the degree of hepatic fibrosis in the pre-treatment liver biopsy. However, pre-treatment serum ferritin levels did not correlate with the degree of hepatic fibrosis [25]. The cause for this discrepancy is not clear.

During the antiviral treatment a decrease in hemoglobin levels occurred in patients in this study. This was probably due to the hemolytic effects of ribavirin [29]. Hemolysis from ribavirin can increase hepatic iron deposition and thus may cause an increase in serum ferritin levels [30]. In the current study the correlation between the degree of hemolysis and on-treatment serum ferritin levels was insignificant. Other investigators reported similar results [14-16].

In this study, univariate analysis showed that factors significantly associated with SVR were low serum baseline ferritin and iron, lower fibrosis stage, RVR and higher rates of increase in serum ferritin during therapy. However, at multivariate analysis, the correlation between serum baseline ferritin and iron and SVR was lost whereas lower fibrosis stage, RVR and higher rates of increase in serum ferritin during treatment are strong independent predictors of SVR. A study by **Ferrara and colleagues** showed that at multivariate analysis, factors that independently predicted SVR were only genotype 2 / 3, absence of mesenchymal iron deposits and extent of serum ferritin increase during treatment [14]. In our study, we didn't measure mesenchymal iron deposits in addition all our patients were genotype 4.

In conclusion, the higher the rate of increase of serum ferritin during combined Pegylated interferon and ribavirin therapy, the better the response. Therefore, serum ferritin could be included in the biochemical workup of CHC patients as it may represent an indirect index of disease duration and progression at baseline, whereas it may express a positive response to interferon activity during treatment. In addition, the ratio between baseline serum ferritin and serum ferritin rise during therapy (e.g., at week 4, 12) may be a useful surrogate for predicting response to therapy. Yet we don't know whether these findings could be useful in the near era of interferon free regimens.

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