Prognostic value of serum ferritin and leptin in prediction of sustained virological response in chronic HCV patients under peglated interferon and ribavirin therapy

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Abstract: Background and study aim: Hansenula derived interferon was recently accepted in the Egypt as a peglated interferon and used in Egypt few years ago in treatment of HCV infection as a standard of care therapy (SOCT). Sustained virological response is difficult to predict in patients receiving SOCT. We aimed in this study to evaluate the potential prognostic value of serum leptin and ferritin in patients under Interferon therapy. **Methods**: We prospectively assessed 100 chronic HCV patients who were eligible for interferon therapy (according to the National Egyptian Treatment Program for treatment of chronic HCV infection), additionally serum leptin and ferritin were assessed before treatment, after 12 weeks during treatment, end of treatment (48 weeks) after start of treatment and 6 months after the end of treatment. *Hansenula polymorpha* derived interferon was given in 160 microgram once weekly by subcaunteous route plus Ribavirin tablets 3 times divided daily doses according to the body weight of the patients. Cirrhotic patients and those with other associated causes of chronic liver disease were excluded. **Results**: The overall SVR was 50 % in the studied group of patients. The pre-treatment serum ferritin levels in patients achieved SVR was (167.5±121.8) and in non-responders (166.3±101.1), (P>0.05). The ferritin levels post treatment in responders was 278.2±108.8 and in non-responders 247.6±108.5. There was no statistical significant difference between responders and non-responders either before, 12 weeks on treatment, 24 weeks, at the end of treatment and 24 weeks post treatment. However the ferritin levels increased significantly post treatment in responders (P< 0.01) and non-responders (< 0.01). The pretreatment leptin concentrations in patients achieved SVR was 11.78±8.2 and in non-responders 19.7±26.3, (P>0.05). The leptin levels post treatment in responders was 7.6±5.8 and in non-responders was 13±18.2. There was no statistical significant difference between responders and non-responders either before, on treatment, or 24 weeks post treatment. The leptin levels decreased significantly among responders (P< 0.01) and non-responders (P< 0.01). **Conclusion**: Over all SVR was 50%. There was no significant association between SVR and changes of serum ferritin or leptin levels. The SOCT significantly increases serum ferritin and decreases serum leptin whatever SVR status of the studied patients.
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1. Introduction

Chronic hepatitis C (CHC) is one of the most significant infectious liver related diseases by being a leading cause of liver-related morbidity and liver transplantation worldwide. Egypt has the highest prevalence worldwide and 14.7% of populations between 15-59 years are chronically HCV infected (1). The current slandered of care treatment of chronic HCV infection is PEG interferon alpha and weight based ribavirin. Reiferon retard ®is 20-kDa linear PEG interferon α-2a. (Reiferon Retard®) derived from *Hansenula polymorpha* expression system. It was registered in Egypt and available in Egyptian market since 2004 (2).

HCV interferes with the host's iron metabolism, and hepatic iron measures were correlated with the grade and stage, as well as with the treatment outcome, of CHC (3-7). Infection with HCV leads to iron accumulation in the liver and increased serum ferritin levels, which can be, at least partially, explained by down-regulation of hepcidin, a key regulator of iron homeostasis (6,8). However, serum ferritin is also frequently elevated in any inflammatory conditions. Excess iron in the liver promotes liver inflammation, oxidative stress, and mitochondrial dysfunction (9). However, the role of serum ferritin as an independent predictor of sustained virologic response (SVR) to IFN-a-based therapy remains controversial (7, 10-14)

Leptin, the product of the obese (ob) gene, is mainly expressed by adipose tissue, although it is expressed in other organs, including the liver (15). Leptin plays an important role in the regulation and metabolism of body fat and may induce insulin

resistance, increase fatty acid concentrations in the liver, and enhance lipid peroxidation (16-18). Leptin may act as an immuno-modulator, inducing the release of cytokines, such as tumor necrosis factor (TNF)-α, interferon (INF)-γ, interleukin (IL)-18, and tumor growth factor (TGF)-β1, thus promoting liver steatosis and fibrosis (17).

Leptin contributes to the pathogenesis of liver steatosis (17, 18). In patients with CHC, higher serum leptin concentrations have been associated with the presence of steatosis (19).

2. Patients and Methods

Patients

This study was conducted on 100 patients with chronic hepatitis C, they were selected from patients eligible for interferon therapy, they aged from 23-59 years, they were 63 males and 37 females. The patients were selected from the outpatient clinics of National Liver Institute-Menoufiya University between January 2010 to December 2012. *Hansenula polymorpha* derived interferon (IFN) and Ribavirin combination therapy was given for all patients. Inclusion criteria included: patients who were positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emeryville, CA, USA), positive for HCV RNA qualitative analysis using PCR (nested polymerase chain reaction or Amplicor, Roche Diagnostic Systems, CA, USA), liver biopsy consistent with chronic hepatitis examined within 6 months of enrolment, all patients were naïve to IFN therapy. Exclusion criteria for treatment were as follows: patients with liver cirrhosis and/or HCC; positivity for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan); autoimmune liver disease; hemochromatosis; Wilson's disease; primary biliary cirrhosis; history of uncontrolled depression or psychosis; and patients with uncontrolled diabetes.

The data of patients' sex, age, body mass index [BMI: weight (kg)/height (m2)], HCV qualitative analysis with PCR, fasting blood glucose, serum leptin at initiation of treatment, and serum ferritin. Serum leptin was measured by a radioimmunoassay (RIA, Linco Research Inc., St. Louis, MO, USA). Serum was collected at the initiation of treatment following an overnight fast for 8 h. Assessment of serum leptin and ferritin were done on 4, 12 end of treatment and 24 weeks after treatment.

Liver Histopathological examination

Liver biopsy specimens were obtained percutaneously. Liver biopsy specimens were reviewed by a single pathologist, who was blinded to the clinical information of the subjects. For each liver biopsy specimen, hematoxylin-eosin and silver impregnation for collagen were available. Chronic hepatitis was diagnosed based on histo-pathological assessment according to a scoring system that includes semi-quantitative assessment of liver disease grading and staging (20).

IFN therapy

Patients received (*Hansenula polymorpha*) PEG IFN α-2a 160 µg once weekly and weight based ribavirin in 3 daily divided doses. Assessment of HCV RNA by PCR was done at baseline, 12, 48 weeks and 24 week after end of treatment. Patients had negative viremia at 12, 24, 48 weeks and 24 weeks after end of treatment were considered to have rapid virological response (RVR), early virological response (EVR), end of treatment response (ETR) and sustained virological reponse (SVR).

Patients achieved at least 2 log drop of the pre-treatment PCR after 12 weeks of therapy were allowed to continuo to 24 weeks of therapy, patients who were negative to HCV PCR at 24 weeks were allowed to continue to 48 weeks of therapy.

The study protocol was approved by the Ethics Committee of National Liver Institute (NLI) Menoufiya University.

Evaluation of patients

Patients were divided into two groups: responders and non-responders. All were classified by age, sex, BMI, leptin level, serum ferritin and pathological findings of biopsy samples. Assessment of complete blood count, AST, ALT, albumin, prothrombin time, total and direct bilirubin, and HCV RNA by PCR. Serum leptin and ferritin were assessed at baseline before treatment, 4, 12,48 weeks and 24 week after end of treatment and 6 months after end of treatment.

Statistical analysis

Comparisons between the two groups regarding these factors were performed using the $\chi 2$ test. Parameters that had an influence on IFN-response were compared by the univariate Cox's proportional hazard model analysis. Variables that achieved statistical significance by univariate analysis were subsequently included in a multivariate proportional hazard model analysis. All analyses were carried out using SAS program (SAS Institute, Cary, NC, USA). Differences were considered significant if $P < 0.05$. Results were expressed as mean±SD unless otherwise stated.

3. Results

At end of study 50 patients achieved SVR (6 months end of treatment) while 50 failed to achieve

SVR. The mean age of responders was (41.05±9.21) vs (39.23±10.62) in non-responders. The difference was not statistically significant (P> 0.05). Also BMI had no significant difference between responders and non-responders [(44.87±7.82) vs (45.61±7.19)] respectively, P> 0.05.

The pretreatment viral load didn't differ significantly between responders and non-responders (576,087±885,407 IU) Vs (3,322,885±20,982,900 IU) respectively, P >0.05. Also TSH showed no significant difference (1.37±0.45 in responders vs 1.61±0.82 in non-responders), P> 0.05. Serum createnine showed no difference between responders (0.85±0.11) and non-responders (0.81±0.15), p> 0.05. AST had no significant difference (60.09±28.66 in responders VS 57.91±28.37 in non-responders), P> 0.05. ALT also had no significant difference (63.14±25.49 in responders VS 59.59±23.54 in non-responders), P> 0.05. Serum albumin showed no difference (4.29±0.40in responders vs 4.43±0.31 in non-responders), P> 0.05. WBC count did not differ between responders (5.69±1.09) and non-responders (5.86±1.53), P>0.05. Also Hb concentration showed no difference (13.89±1.61 in responders vs 13.32±1.32 in non-responders), P>0.05.

The basal serum ferritin level in patients achieved SVR ranged from 48- 508 (167.5±121.8) and in non-responders ranged from 22-484 (166.3±101.1). And there was no statistical significant difference between responders and non-responders.

The serum level of ferritin after end of study in patients achieved SVR ranged from 74- 501 (278.2±108.8) and in non-responders ranged from 74-519 (247.6±108.5). There was no statistical significant difference between responders and non-responders.

Early virological response was assessed after end of first 12 weeks from the start of treatment. The serum ferritin level in responders was (268.58±109.88) vs (248.21±107.93) in non-responders. There was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal ferritin levels in either responders or non-responders.

After the end of 24 weeks from the start of treatment the virological response was assessed: Assessment of 79 patients (66 responders and 13 non responders) was done. The serum ferritin level in responders was (278.04±105.39) vs (236.14±122.61) in non-responders. There was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal ferritin levels in either responders or non-responders.

After the End of treatment at 48 weeks. Assessment of 66 patients (57 responders and 9 non responders) was done. The serum ferritin level in responders was (278.16±108.83) vs (277.00±78.36) in non-responders. There was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal ferritin levels in either responders or non-responders.

6 months after end of treatment, we assessed 57 patients (50 responders and 7 non responders). The serum ferritin level in responders was (281.47±111.27) vs (268.55±105.95) in non-responders. There was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal ferritin levels in either responders or non-responders.

The basal serum Leptin level in patients achieved SVR ranged from 1.70- 36,6 (11.78±8.2) and in non-responders ranged from1.8-101.00 (19.7±26.3). And there was no statistical significant difference between responders and non-responders.

The serum level of leptin after end of study in patients achieved SVR ranged from 1.5- 28 (7.6±5.8) and in non-responders ranged from 2-77 (13±18.2). And there was no statistical significant difference between responders and non-responders.

After 12 weeks, assessment of 100 (79 responders and 21 non responders) patients was done. The serum Leptin level in responders was (9.74±11.65) vs (11.63±18.11) in non-responders. And there was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal Leptin levels in either responders or non-responders.

After 24 weeks, assessment of 79 patients (66 responders and 13 non responders) was done. The serum Leptin level in responders was (9.39±11.73) vs (10.9±11.74) in non-responders. And there was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal Leptin levels in either responders or non-responders.

After 48 weeks, assessment of 66 patients (57 responders and 9 non responders) was done. The serum Leptin level in responders was (7.59±5.76) vs (24.88±30.53) in non-responders. And there was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal Leptin levels in either responders or non-responders.

At 24th weeks after end of treatment (SVR), assessment of 57 patients (50 responders and 7 non responders) was done. The serum Leptin level in responders was (8.10±6.39) vs (6.10±3.08) in non-

responders. And there was no statistical significant difference between responders and non-responders. Also there was no statistical significant difference from the basal Leptin levels in either responders or non-responders.

Table 1. Descriptive statistics of ferritin before and after and leptin before and after.

Response_to_interferon		Minimum	Maximum	Mean	Std. Deviation
Non responder	Ferritin_before_ttt	22.00	484.00	166.2558	101.08061
	Ferritin_after_ttt	47.00	519.00	247.6279	108.46461
	Leptin_before_ttt	1.80	101.00	19.7372	26.33261
	Leptin_after_ttt	2.00	77.00	12.9512	18.17407
Responder	Ferritin_before_ttt	48.00	508.00	167.5349	121.84482
	Ferritin_after_ttt	74.00	501.00	278.1628	108.83100
	Leptin_before_ttt	1.70	36.30	11.7767	8.23705
	Leptin_after_ttt	1.50	28.00	7.5884	5.75951

Table 2. Association between demographic data and interferon response.

	Response to interferon	Mean ± SD	t- test	p- value
Age	Responder	41.05±9.21	0.89	> 0.05
	Non responder	39.23±10.62		
BMI	Responder	44.87±7.82	0.49	> 0.05
	Non responder	45.61±7.19		

There is no statistical significant association between response to interferon and both age and BMI p- value > 0.05).

Table 3. Association between ANA, alkaline phosphatase, PCR HCV, TSH, glucose and creatinine and interferon response.

	Response to interferon	Mean ± SD	Mann Whitney test	p- value
ANA	Non responder	0.00±0.00	0.92	> 0.05
	Responder	0.02±0.15		
PCR_HCV	Non responder	3322885.58±20982900.89	0.01	> 0.05
	Responder	576087.98±885407.81		
TSH	Non responder	1.61±0.82	0.97	> 0.05
	Responder	1.37±0.45		
Glucose	Non responder	94.81±20.03	2.12*	**< 0.05**
	Responder	106.77±32.75		
Creatinin	Non responder	0.81±0.15	1.35*	> 0.05
	Responder	0.85±0.11		

* t- test. There is statistical significant association between response to interferon and fasting glucose level (p- value < 0.05). There is no statistical significant association between response to interferon and ANA, PCR HCV, TSH and creatinine (p- value > 0.05).

Table 4. Association between liver functions and interferon response.

	Response_to_interferon	Mean ± SD	t- test	p- value
AST	Responder	60.09±28.66	0.3*	> 0.05
	Non responder	57.91±28.37		
ALT	Responder	63.14±25.49	0.56*	> 0.05
	Non responder	59.59±23.54		
Albumin	Responder	4.29±0.40	1.85	> 0.05
	Non responder	4.43±0.31		
Total_bilirubin	Responder	1.07±0.13	2.31	**< 0.05**
	Non responder	1.00±0.22		
ALP	Responder	110.21±18.77	4.55	**< 0.01**
	Non responder	146.36±43.16		
PT	Responder	13.06±0.39	4.69	**< 0.01**
	Non responder	13.54±0.46		

* Mann Whitney test. There is highly statistical significant association between response to interferon and ALP, PT (p- value < 0.01) and total bilirubin (p- value < 0.05). There is no statistical significant association between response to interferon and AST, ALT and albumin (p- value > 0.05).

Table 5. Association between CBC and interferon response.

	Response_to_interferon	Mean ± SD	t- test	p- value
WBCs	Responder	5.69±1.09	0.65	> 0.05
	Non responder	5.86±1.53		
Hb	Responder	13.89±1.61	1.93	> 0.05
	Non responder	13.32±1.32		
Platelets	Responder	211.62±60.49	2.12	**< 0.05**
	Non responder	186.03±59.26		

There is no statistical significant association between response to interferon and both AST/ platelets ratio.

Table 6. Association between AST/ Platelets ratio and interferon response.

	Response_to_interferon	Mean ± SD	Mann Whitney test	p- value
AST_Platelets_ratio	Non responder	401.33±545.99	1.09	> 0.05
	Responder	308.14±164.12		

There is no statistical significant association between response to interferon and both AST/ platelets ratio (p- value > 0.05).

Table 7. Association between both ferritin and leptin before and after ttt and interferon response.

	Response_to_interferon	N	Mean ± SD	Mann Whitney test	p- value
Ferritin_before_ttt	Non responder	43	166.26±101.08	0.29	> 0.05
	Responder	57	167.53±121.84		
Ferritin_after_ttt	Non responder	43	247.63±108.46	1.06	> 0.05
	Responder	57	278.16±108.83		
Leptin_before_ttt	Non responder	43	19.74±26.33	0.06	> 0.05
	Responder	57	11.78±8.24		
Leptin_after_ttt	Non responder	43	12.95±18.17	0.43	> 0.05
	Responder	57	7.59±5.76		

There is no statistical significant association between response to interferon and both ferritin and leptin basal and follow up readings (p- value > 0.05).

Table 8. Association between both ferritin and leptin before and after ttt and rapid interferon response.

	Rapid_treatment_response_to_interferon	Mean ± SD	Mann Whitney test	p- value
Ferritin_before_ttt	Responder	172.58±119.69	0.51	> 0.05
	Non responder	152.21±86.39		
Ferritin_after_ttt	Responder	268.58±109.88	0.65	> 0.05
	Non responder	248.21±107.93		
Leptin_before_ttt	Responder	15.45±18.25	1.15	> 0.05
	Non responder	16.54±23.78		
Leptin_after_ttt	Responder	9.74±11.65	0.89	> 0.05
	Non responder	11.63±18.11		

There is no statistical significant association between rapid treatment response to interferon and ferritin and leptin basal and the follow up level (p- value > 0.05).

Table 9. Association between both ferritin and leptin before and after ttt and early treatment response.

	Early_treatment response_to_interferon	Mean ± SD	Mann Whitney test	p- value
Ferritin_before_ttt	Responder	178.19±126.67	0.49	> 0.05
	Non responder	153.36±93.27		
Ferritin_after_ttt	Responder	278.04±105.39	1.22	> 0.05
	Non responder	236.14±122.61		
Leptin_before_ttt	Responder	14.24±15.89	0.55	> 0.05
	Non responder	19.64±24.99		
Leptin_after_ttt	Responder	9.39±11.73	0.62	> 0.05
	Non responder	10.9±11.74		

There is no statistical significant association between early treatment response to interferon and ferritin and leptin basal and follow up level (p- value > 0.05).

Table 10. Association between both ferritin and leptin before and after ttt and end treatment response.

	End_treatment response_to_interferon	Mean ± SD	Mann Whitney test	p- value
Ferritin_before_ttt	Responder	167.53±121.84	1.96	> 0.05
	Non responder	269.80±144.67		
Ferritin_after_ttt	Responder	278.16±108.83	0.39	> 0.05
	Non responder	277.00±78.36		
Leptin_before_ttt	Responder	11.78±8.24	1.15	> 0.05
	Non responder	35.38±40.39		
Leptin_after_ttt	Responder	7.59±5.76	1.28	> 0.05
	Non responder	24.88±30.53		

There is no statistical significant association between end treatment response to interferon and ferritin and leptin basal and follow up level (p- value > 0.05).

Table 11. Association between both ferritin and leptin before and after ttt and sustained treatment response.

	Sustained_virological_response_to_interferon	Mean ± SD	Mann Whitney test	p- value
Ferritin_before_ttt	Responder	148.81±90.61	0.5	> 0.05
	Non responder	222.00±180.32		
Ferritin_after_ttt	Responder	281.47±111.27	0.04	> 0.05
	Non responder	268.55±105.95		
Leptin_before_ttt	Responder	12.49±9.03	0.68	> 0.05
	Non responder	9.69±5.07		
Leptin_after_ttt	Responder	8.10±6.39	0.59	> 0.05
	Non responder	6.10±3.08		

There is no statistical significant association between sustained virological response and ferritin and leptin basal or follow up readings (p- value > 0.05).

Table 12. Differences between ferritin and leptin before and after ttt in each responder and non-responder.

Response_to_interferon		Mean ± SD	Wilcoxon test	p- value
Non responder	Ferritin_before_ttt	166.26±101.08	4.22	**< 0.01**
	Ferritin_after_ttt	247.63±108.46		
	Leptin_before_ttt	19.74±26.33	5.16	**< 0.01**
	Leptin_after_ttt	12.95±18.17		
Responder	Ferritin_before_ttt	167.53±121.84	4.31	**< 0.01**
	Ferritin_after_ttt	278.16±108.83		
	Leptin_before_ttt	11.78±8.24	5.22	**< 0.01**
	Leptin_after_ttt	7.59±5.76		

There is highly statistical significant difference between ferritin and leptin basal and follow up readings in each interferon response group (p- value < 0.01).

Table 13. Pearson's correlation matrix between leptin and ferritin before treatment and PCR HCV, AST and ALT pretreatment in non-responder group.

		Ferritin before ttt	Leptin before ttt	PCR_HCV	AST
Leptin before ttt	r	- 0.197			
	p- value	> 0.05			
PCR_HCV	r	0.144	- 0.113		
	p- value	> 0.05	> 0.05		
AST	r	0.161	- 0.198	- 0.106	
	p- value	> 0.05	> 0.05	> 0.05	
ALT	r	0.057	- 0.182	- 0.090	0.859
	p- value	> 0.05	> 0.05	> 0.05	**.< 0.01**

There is strong positive correlation between AST and ALT (p- value < 0.01). But there was no correlation between ferritin, leptin and PCR HCV and AST and all other studied variables (p- value > 0.05).

Table 14. Pearson's correlation matrix between leptin and ferritin before treatment and PCR HCV, AST and ALT pretreatment in responder group.

		Ferritin before ttt	Leptin before ttt	PCR_HCV	AST
Leptin before ttt	r	- 0.139			
	p- value	> 0.05			
PCR_HCV	r	0.291	- 0.239		
	p- value	> 0.05	> 0.05		
AST	r	- 0.067	- 0.002	- 0.171	
	p- value	> 0.05	> 0.05	> 0.05	
ALT	r	0.004	- 0.060	- 0.096	0.918
	p- value	> 0.05	> 0.05	> 0.05	.> 0.05

There is no correlation between ferritin, leptin, PCR HCV and AST and other studied variables (p- value > 0.05).

4. Discussion

Many authors investigated the role of serum ferritin in liver disease; Musallam et al, 2012 evaluated the longitudinal changes in serum ferritin levels and transient elastography values as a measurement of hepatic stiffness, in patient with thalamsemia major as a predictive of fibrosis. They observed a significant increase in both serum ferritin levels and transient elastography values (20).

Additionally serum ferritin was studied as an independent prognostic factor in subjects awaiting liver transplantation in a cohort of 131 patients, subjects with higher serum ferritin greater than 500 mg/L had increased frequency of liver-related mortality. The addition of serum ferritin to MELD increased the area under the ROC curve to predict 1 year mortality by 7.5% (21).

HCV infection is significantly associated with higher serum levels of ferritin and iron (22). In the current study we evaluated serum ferritin in our patient at baseline, at end of 12 week, 24 week and end of treatment and 6 month after end of treatment by the SOCT. We found no significant differences between responders and non-responders at each evaluation time. The serum ferritin levels were statistically not different at baseline, 12 weeks, 24 weeks, 48 weeks and 24 weeks after treatment. However the serum levels changed significantly in responders and non-responders before and after treatment.

At every evaluation time point serum ferritin levels in responders were higher than in non-responders. However these changes were not statistically significant. The serum ferritin increased significantly in both responders and non-responders before and after treatment. This rise in serum ferritin can be explained on light of the stimulatory effect of IFN alpha on ferritin secretion as a component of acute phase reactions. Stam studied the effect of IFN alpha on acute phase reactants including ferritin in melanoma patients receiving interferon alpha. He found association between IFN treatment and rise in serum ferritin in treated patients and lack of this association in untreated patients (23).

However Oguz et al found that the acute reactant including Ferritin levels were significantly higher in the patient group ($p<0.05$). Levels ferritin, were significantly increased from baseline to the 4th week ($p<0.05$). The responders and non-responders to antiviral therapy had insignificantly but remarkably different, But they concluded that the variations were not alternatives to virological and biochemical parameters for predicting an early response to therapy in patients with CHC (24).

Distante et al, found a significantly higher median serum ferritin values in non-responders compared to SVR patients (130 microg/L vs. 75 micro g/L $P < 0.001$) (25).

Similarly, Pereira et al 2009, found no significant changes between responders and non-responders as regards iron indices including serum iron, ferritin, and hepatic iron concentration (26).

Additionally Ladero et al, found a significant higher baseline serum ferritin in non-responders. On follow up the authors found no significant changes between responders and non-responders. However after 24 weeks off treatment the serum ferritin in responders decreased below baseline in responders and to baseline in non-responders (27).

Also Barut et al, found that serum ferritin levels increased during PEG-IFN-based therapy for CHC. A lower serum ferritin level before starting treatment and higher serum ferritin levels during therapy appear to be associated with a favorable treatment response (28).

Lange et al, found that high serum ferritin levels to be highly predictors to treatment failure to peglated interferon-alpha and ribavirin therapy. Lange and colleagues found the association more significant in HCV genotype 3 and to a lesser extent in genotype 1 (29).

Chiou et al, investigated the changes of thiobarbituric acid reactive substances (TBARS; indicated oxidative stress), total antioxidant status (TAS) and ferritin in CHC patients during therapy

with SOCT as potential predictor factors for effectiveness of therapy. The levels of ferritin and ALT were significantly lower inpatients achieved SVR (30).

Similarly Younossi et al, concluded from large population study that low serum ferritin is independently associated with HCV clearance (31).

The variation in the results of these data might argued to many reason including the ethenity of the patients, gender distribution, HCV genotype difference between the studied subjects, degree of liver injures or the presence of associated causes of liver injuries that can lead to variation in serum ferritin and were not rolled out.

Leptin is secreted in the adipose tissue in a regulated manner as a product of the obese (OB) gene, (32). It interferes with hepatic injury associated with fatty infiltration, differentially modulating steatosis, inflammation, and fibrosis (33). The correlation of adipokines -including leptin- with liver disease was best described in hepatitis C virus (HCV) infection, where leptin may represent a link between viral infection, steatosis and metabolic disturbances (34, 35). The serum concentrations vary considerably among individuals and serum leptin is mainly correlated with body fat content and sex, (higher in obese than lean subjects and in females than in males).

Obesity, a modifiable risk factor, may be an important co-factor in accelerating fibrosis and increasing liver necro-inflammatory activity in chronic hepatitis C and add more challenges to SOCT (36, 37). The circulating leptin concentration correlates with fat mass size and distribution. The high serum leptin in obese patients has a pro-fibrogenic effect. At concentrations (30-50 ng/mL), leptin increased collagen I and III messenger RNA (mRNA) transcript levels in HSC leading to liver fibrosis (38). Activated rat hepatic stellate cells express leptin, and rat sinusoidal endothelial and Kupffer cells express the signaling-component isoform of the leptin receptor. Exposure of these cells to leptin results in an increased expression of transforming growth factor-β (TGF-β), which, in turn, stimulates fibro-genesis in hepatic stellate cells (39). Recent reports have indicated that inflammatory cytokines, including TNF-α or IL-1, determine both leptin expression and circulation (40).

Leptin has a pleio-tropic role in hematopoiesis, immune response, fibrogenesis, and might be involved in the process of hepatocarcinogenesis.

Testa et al, studied the mean serum leptin levels in patients with chronic hepatitis C, HCV-associated cirrhosis, HCV associated HCC and control groups was (6.13±3.94), (5.25±4.21), (4.17±0.28), and (3.59±3.44) ng/mL, respectively. The serum leptin level in patients with CHC was significantly higher than in controls. However the serum leptin levels between cirrhotic patients and controls and between male and female cirrhotic patients had no significant difference (41).

In 2006 Stefanou et al, observed significantly lower leptin receptors in peripheral blood mononuclear cells in patients with HBV and HCV patients compared to healthy controls and non-viral liver disease patients. Expression of leptin and leptin receptors was significantly increased in viral hepatitis liver tissues compared with healthy tissues. The authors concluded that leptin system may involved in the immuno-pathology of chronic viral hepatitis (42).

So from these data, besides viral factors, those related to individual patients including leptin and/or obesity might have important effects on IFN therapy.

In this study, we evaluated serum leptin in patients received SOCT before and after course of treatment. Despite the serum leptin levels were lower in responders than non-responders; they were not statistically significant at baseline, 12 weeks, 24 weeks, 48 weeks and 24 weeks off treatment. However the Leptin level decreased significantly in both responders and non-responders before and after treatment.

The lowering effect of interferon on leptin was demonstrated by Kaser et al on chronic HCV patients treated by interferon (43).

However Pavlidis et al, found that the baseline leptin concentrations didn't differ between patients who got and didn't achieve SVR, but with successful clearance of HCV with SVR, serum leptin levels were significantly lower. Among patients with genotype-1, non-responders had significantly higher serum leptin concentrations, both at baseline and at the end of follow-up than those who attained SVR. Among patients infected with HCV genotype-3, however, there were no significant differences in leptin concentrations at baseline and at end of follow-up between those who did and did not achieve SVR (44).

Additionally Eguchi et al studied the relation between serum leptin and response to standard interferon. They found that low serum leptin levels (< 8 μg/L) significantly associated with a good IFN-response, whereas the low viremia group with a high serum leptin level (≥ 8 μg/L) (44). However in the current study we utilized PEG interferon instead of standered interferon. The use of PEG interferon raised the SVR significantly and may explain the lack of association between pretreatment Leptin and response (45).

The variation in the above shown data may explained by many factors as patients related factors (BMI, gender distribution of the studied subjects, associated diseases as DM or genetic roles) and viral related as Genotype of the virus or associated causes of liver injury that were not rolled out from the studied subjects.

4. Conclusion

From our data the serum ferritin levels raised significantly in both responders and non-responders when compared to pretreatment levels. Also the serum leptin decreased significantly in both responders and non-responders when compared to pretreatment levels. But there was no significant difference between responders and non-responders as regards the serum concentrations of either ferritin or leptin at any time of assessment. And the pretreatment serum concentration of either leptin or ferritin cannot be utilized to prognosticate the response of chronic HCV patients receiving PEG interferon and ribavirin.

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