

Estimation of Serum Leptin Level in Egyptian Patients With Chronic HCV After Antiviral Treatment

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ABSTRACT

Hepatitis C virus usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Mediator factors named adipocytokines influence metabolic alterations (i.e. insulin resistance) as well as hepatocellular damage. Among them leptin seems to play important role in pathogenesis of liver damage.

AIM: We aimed in this study to evaluate the potential prognostic value of serum leptin in chronic hepatitis C patients under interferon therapy.

METHODS: A prospective study was performed in 90 patients with chronic hepatitis C treated with IFN and weight based ribavirin from January 2012 to May 2014. Sustained response was defined as negative by hepatitis C virus RNA analysis using PCR at 24 weeks after cessation of IFN therapy. Non responders, who were positive to HCV RNA at the end of 12 weeks of treatment. Serum leptin was assessed using EIA.

RESULTS: Sustained response was achieved in 52 patients (58%). Overall, the factors associated with sustained antiviral effects were HCV-RNA load, serum leptin level, and stage of liver fibrosis

evaluated by univariate analysis. BMI was not associated with any therapeutic effect of IFN. Multivariate analysis indicated that HCV-RNA load was a significant risk factor and leptin level was an independent risk factor for IFN resistance. Namely, a high level of serum leptin attenuated the effect of IFN in patients with chronic hepatitis C.

CONCLUSION: High serum leptin level is a negative predictor of response to antiviral treatment in chronic hepatitis C. In chronic hepatitis C patients, we considered serum leptin level ≤ 7 ng/mL is a predictor of sustained virological response.

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Key words: Chronic Hepatitis C; Leptin, IFN; Steatosis; Liver Cirrhosis

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INTRODUCTION

Chronic hepatitis C is one of the most significant infectious liver related diseases by being a leading cause of liver-related morbidity and liver transplantation world-wide. Egypt has the highest prevalence worldwide and 14.7% of populations between 15-59 years are chronically HCV infected^[1]. The current standard of care treatment of chronic HCV infection is PEG interferon alpha and weight based ribavirin. It was registered in Egypt and available in Egyptian market since 2004^[2]. Leptin, the product of the obese (ob) gene, is mainly expressed by adipose tissue, although it is expressed in other organs, including the liver^[3]. 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^[4-6]. 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 transforming growth factor (TGF)- β 1, thus promoting liver steatosis and fibrosis^[5]. Leptin contributes to the pathogenesis of liver steatosis^[5-6]. In patients with CHC, higher serum leptin concentrations have been associated with the presence of steatosis^[7]. We aimed in this study to evaluate the potential prognostic value of serum leptin in chronic hepatitis C patients under interferon therapy additionally; we evaluate the relationship between the serum leptin concentrations with histopathological parameters (necroinflammation, fibrosis, steatosis) of these patient.

METHODS

The setting for this study was the Hepatology outpatient clinic of Tropical Medicine Department, Minia University, Minia, Egypt from January 2012 to May 2014. The study was approved by the institutional review board of the Minia Faculty of Medicine, Minia University, Egypt. Written, informed consent was obtained from every participant. Patients with histologically confirmed chronic hepatitis C by liver biopsy and who fulfilled the criteria for IFN treatment were enrolled in this study.

Chronic hepatitis C was diagnosed based on histopathological assessment according to a METAVIR scoring system^[8-9]. Steatosis was evaluated by Adinolfi grading system in which steatosis graded from 0 to 4 based on the percentage of hepatocytes involved as follows: 0=none involved; 1=up to 10%; 2=up to 30%; 3=up to 60%; and 4=more than 60%^[4].

All patients were treated weekly with 180 mg subcutaneous IFN- α 2a and oral ribavirin at a total daily dose of 1,000-1,400 mg according to body weight.

Serum leptin was measured by a enzyme immunoassay (EIA, using human reader Germany using kits supplied by Quantikian USA R&D system inc). Serum was collected at the initiation of treatment following an overnight fast for 8 h. Assessment of serum leptin was done firstly at the start of treatment for all the studied patients and at the time of stoppage of treatment for the non-responders and at 6 months of the end of treatment for the responders.

HCV RNA was monitored in the serum by real-time PCR. Patients with undetectable plasma HCV RNA after the six-month follow up were classified as sustained responders (SR), patients who cleared the virus from their serum but with HCV RNA reappearance during follow-up were classified as relapse responders (RR). For undetectable HCV RNA there was a cut-off value of 50 IU/mL.

Statistical analysis

Comparisons between the two groups regarding these factors were performed using the χ^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 SPSS program 20. ROC analysis was carried out using Med Calc program. Differences were considered significant if $p=0.05$. Results were expressed as mean \pm SD unless otherwise stated.

RESULTS

At end of study 52 patients achieved SVR (six months end of treatment) while 38 failed to achieve SVR. The mean age of

responders was (39.5 \pm 10.4) vs (43.6 \pm 10.6) in non-responders. The difference was statistically insignificant $p=0.3$. Also, BMI had insignificant difference between responders and non-responders (37.7 \pm 8.3) vs (42.2 \pm 7.5), respectively, $p=0.4$. The pre treatment viral load was significantly lower in responders than the non-responders (57.860 IU Vs 99.210 IU respectively), $p=0.01$.

The base line serum leptin concentration was significantly high in non responders than the responders (28.4 \pm 2.3 and 15.6 \pm 10.5; respectively; $P=0.001$); Also at the end of treatment; the serum leptin level was significantly high in non responder than the responders (19.8 \pm 1.5 and 8.3 \pm 4.7; respectively $p=0.03$).

Liver histopathological examination revealed that the non-responders significantly had higher fibrosis and steatosis stages than the responders group ($P=0.02$; $P=0.003$; respectively) (Table 1).

By examining the relation between the serum leptin level and the histopathological parameters it was found that there were significant relations between the leptin level and activity grading also with steatosis ($P=0.05$, 0.03 respectively), on the other hand there was no significant association between liver fibrosis staging and serum leptin level (Table 2).

Using multivariate logistic regression analysis, we found that high serum leptin level at baseline and high viral load were significantly associated with non-response to therapy. No significant associations between response to treatment and histopathological findings (Table 3). ROC curve analysis of leptin in prediction of interferon response among the studied patients. The figure showed that the curve was significant with $p=0.01$ and area under the curve=0.79 \pm 0.07. In chronic hepatitis C patients, we considered interferon response when leptin \leq 7ng/mL (Figure 1).

DISCUSSION

This study demonstrated the possibility that among the host factors, serum leptin level can be a significant negative predictor for IFN-response in chronic hepatitis C. We evaluated 90 naive patients with

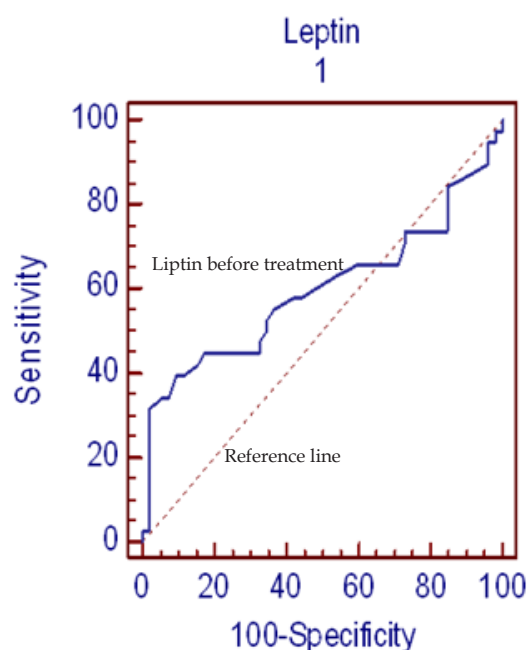


Figure 1 ROC curve analysis of leptin in prediction of interferon response among the studied patients. Sensitivity= 39.5%; Specificity=90.4%; Cut off value \leq 7; Positive predictive value=79.2; Negative predictive value=94.3.

Table 1 Sociodemographic and histopathological characteristics of chronic hepatitis C patients.

	SR (n=52)	NR (n=38)	P value
Age years, mean± SD	39.5±10.4	43.6±10.6	0.3
Sex			0.2
Male	41 (78.8%)	34 (89.5%)	
Female	11 (21.2%)	4 (10.5%)	
HCV RNA (IU)			
Median (IQR) (Before treatment)	57.860 (12.230-210.423)	99.210 (60.230-480.222)	0.01*
BMI (kg/m ²)	37.7±8.3	42.2±7.5	0.4
Leptin (ng/L)			
At the base line	15.6±10.5	28.4±2.3	0.001*
End of follow up	8.3±4.7	19.8 ±1.5	0.03*
Pathological findings			
Activity grading			0.6
A1	24 (42.3%)	18 (23.7%)	
A2-A3	28 (57.7%)	20 (76.3%)	
Fibrosis staging			0.02*
F1	32 (61.5%)	10 (26.3%)	
F2-F3	20 (38.5%)	28 (73.7%)	
Steatosis			0.003*
Mild	25 (48.1)	7 (18.4%)	
Mmoderate	17 (32.7)	10 (26.3%)	
Sever	10 (19.2%)	21 (55.3%)	

NR: Non-responder; SR: Sustained responder; Normal BMI less than 25kg/m²; * statistically significant.

Table 2 Relation between serum leptin level and histopathological parameters among the studied patients.

level	Serum leptin median (rang)	P value
Necroinflammatory activity		^a 0.05
A1	1.01 (0.05-1.47)	
A2-A3	3.98 (0.04-2.79)	
Fibrosis		^b NS
F1	1.46 (0.04-2.25)	
F2-F3	1.59 (0.04-2.79)	
Seatosis		^c 0.03
S1	4.85 (0.04-1.76)	
S2-S3	2.16 (0.04-2.79)	

^a P<0.05; activity; A1 vs A 2 & A3; ^b P; NS: not significant; ^c P<0.03; seatosis; S1 vs S2 & S3; * Kruskal-Wallis test.

Table 3 Logistic regression analysis of factors associated with interferon resistance among the studied patients.

Variables	Odds ratio	95% CI	P-value
Fibrosis	5.25	0.4-70.3	0.2
Steatosis	2.5	0.6-10.6	0.2
Activity grading	0.16	0.02-1.5	0.1
Leptin	0.96	0.9-1.03	0.03*
PCR HCV	0.45	0.2-0.9	0.04*

* Statistically significant.

chronic hepatitis C who had received pegylated IFN and weight based ribavirin. We showed the response rate and pre treatment predictive factors associated with IFN response in these patients. It was suggested that serum leptin level was an important factor for IFN response, although viral load was most critical for IFN therapy. Overall, the SR rate in our study was 58%. Subjects with a low serum leptin level were significantly associated with a good IFN response. The high viral load markedly influenced on IFN therapy as has been indicated in several previous studies^[5-6]. Multivariate analysis revealed a significantly independent association between high serum leptin level and low IFN-response. A histopathological approach indicated

that serum leptin level was significantly associated with worsening steatosis and inflammatory activity, these factors in liver samples were independent risk factors for IFN therapy in this study. Esam *et al* concluded that the serum leptin decreased significantly in both responders and non-responders when compared to pre treatment levels. But there was no significant difference between responders and non-responders as regards the serum concentrations of leptin at any time of assessment. And the pre treatment serum concentration of either leptin cannot be utilized to prognosticate the response of chronic HCV patients receiving IFN and ribavirin^[10].

Włodzimierz *et al* observed that serum concentrations of leptin differs among subgroups of patients with varied grades of necro inflammatory activities, but did not find any correlation with intensity of steatosis and progression of fibrosis^[11]. Piche *et al* reported that leptin is an independent metabolic factor associated with the severity of liver fibrosis in patients with chronic hepatitis C and higher BMI^[12].

Previous study demonstrated that overweight patients with chronic hepatitis C had significantly more steatosis and increased serum insulin and leptin levels^[13]. On the other hand, Giannini *et al* demonstrated that there was no relationship between serum leptin levels and the severity of steatosis^[14]. The relationship between serum leptin levels and the severity of steatosis in patients with chronic hepatitis C remains controversial, because serum leptin levels can be affected by the progression of chronic hepatitis and host factors, such as visceral adiposity, insulin resistance and other adipokines. The mechanism of leptin in the antiviral response to IFN treatment was not determined in this study, but several studies might support the possibility of a relationship between the two factors. Serum leptin concentration increased in liver steatosis and cirrhosis in addition to obesity^[15].

Another study also has shown that liver fibrosis correlates with steatosis and obesity^[16]. From these studies, it could be judged that lipid deposits within the hepatocytes might cause functional disturbances by increasing the architectural distortion of the hepatic lobule caused by fibrosis, and decrease the contact area between drugs (e.g., IFN) and hepatocytes. While the expression of leptin in adipose tissue increased in response to feeding and energy repletion, leptin had a pro inflammatory and/or profibrogenic role that might regulate cytokine-influenced repair and fibrogenesis of liver^[17]. Our data suggests that low serum leptin might be a predictor for sustained viral response following a course of IFN therapy for chronic hepatitis C with leptin level less than 7 ng/mL. However, high serum leptin level is a remarkable risk factor for antiviral therapy in chronic hepatitis C, because this study showed that serum leptin levels over 7 ng/mL or less than 7 ng/mL were able to be the threshold factor about the efficacy of antiviral therapy. This study does not address the issue if pre-treatment intervention to decrease serum leptin level before IFN therapy would compensate for the low response rate in patients with a high serum leptin level.

CONCLUSION

High serum leptin level is a negative predictor of response to antiviral treatment in chronic hepatitis C. Serum leptin levels were found to be associated with the necroinflammatory grades, and intensity of steatosis.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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