IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 1 Ver. IX. (Feb. 2014), PP 21-29
www.iosrjournals.org

Serum leptin and plasma ghrelin concentrations in patients with chronic hepatitis C virus with steatosis: Their effect on the response to antiviral therapy

Abdelnaser Badawy[1], Emad El-Masry[2], Awwad Alenezy[3] Mohamed Sherif [4], Hazem Hakim[4] , Mohamed Elrakhawy[5] and Al-Dhafeeri Obaid[6]

[1]*Biochemistry Department, Faculty of Medicine, Mansoura University, Egypt.*
[2]*Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt.*
[3]*Department of Family Medicine , Faculty of Medicine, Northern Border University, Saudia Arabia*
[4]*Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt*
[5]*Diagnostic Radiology department, Faculty of Medicine, Mansoura University, Egypt*
[6]*Radiology department, Dammam Medical Hospital, Ministry of Health, Saudia Arabia*

Abstract: Objective*: To assess serum leptin and plasma ghrelin concentrations and their roles in the response to antiviral therapy in patients with chronic hepatitis C virus (HCV) and steatosis*
Methods*: This study was conducted on 50 chronic HCV patients with steatosis, and 50 healthy participants of matched age as a control group. Serum leptin, plasma ghrelin, serum HCV-RNA concentrations and insulin resistance were measured in patients with HCV before and after antiviral treatment using Peg-IFN plus ribavirin for 48 weeks.*
Results: *We showed high insulin resistance and serum leptin level, while. low plasma ghrelin level at base line in patients with HCV compared to healthy controls. Leptin level increase (P=0.023) while ghrelin levels tend to decrease (P=0.004) as the grade of steatosis worsens. Sustained virological response (SVR) as achieved in 28 patients (56%) and was associated with a lower grade of liver steatosis (P=0.013), milder fibrosis (P=0.002), low value of insulin resistance (P=0.001), lower leptin levels (P=0.005) and higher ghrelin levels (P=0.001).*
Conclusions*: Decreased serum leptin whereas Increased plasma ghrelin before treatment may predict Sustained virological response. Ghrelin exerts antifibrotic effects in the liver and may represent a novel antifibrotic therapy.*
Key words: *Ghrelin, Steatosis, HCV, Leptin , SVR*

I. Introduction

Persistent Hepatitis C virus (HCV) infection is widespread; it affects millions of people worldwide and induces a range of chronic liver disease [1]. Egypt has the highest countrywide prevalence of HCV in the world; about 12 to 15% of the total population are infected [2], with HCV Genotype-4 (HCV-4) accounting for the overwhelming majority of HCV infections [1].

HCV infection increases oxidative stress, tissue damage, and pro-inflammatory cytokine secretion, all of which contribute to progressive fibrosis, cirrhosis, cancer, and liver failure [3]. Recent data suggest a close relationship between HCV infection and metabolic syndrome [4]. It is possible that HCV infection causes fatty liver disease, a precursor of hepatic steatosis, which is a recognized component of metabolic syndrome, causing wide adipocytokines changes and impairs glucose metabolism leading to increased prevalence of insulin resistance (IR) and type 2 diabetes [5]. This association is important, because several studies have shown that the presence of IR is associated with increased rates of fibrosis [6] and lower rates of rapid and sustained response to antiviral therapy. However, the mechanisms of metabolic syndrome-induced interferon (IFN) resistance are not completely understood [1].

Leptin is an adipokine that contributes to the pathogenesis of liver steatosis [7]. In patients with chronic HCV, higher serum leptin concentrations have been associated with the presence of steatosis [8]. Although no clear correlation has been observed between leptin concentrations and the extent of steatosis [9], a recent study reported that high serum leptin concentrations correlated with more severe steatosis, lower viremia, and a lower antiviral response [10].

Leptin, the product of the obese (ob) gene, is mainly expressed by adipose tissue, although it is expressed in other organs, including the liver. 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 [7,11]. Leptin may act as an immunomodulator, 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 [7].

Ghrelin is a peptide that acts as an endogenous ligand of the growth hormone secretatog receptor [12]. Ghrelin is involved in energy metabolism, food intake, and glucose homeostasis [12,13] . Recent studies have assessed whether ghrelin acts as an independent signal of adiposity or as a downstream mediator of leptin, affecting energy balance[14] .

Little is known about plasma ghrelin concentrations in patients with chronic HCV and steatosis, or on the effects of ghrelin concentration on treatment response. We therefore assessed whether pretreatment serum leptin and ghrelin concentrations differ in steatotic patients infected with HCV , and whether these concentrations are associated with response to antiviral treatment.

II. Subjects and methods

2.1, Subjects

The investigated subjects in this study were randomly withdrawn from the Outpatient Clinics of Internal Medicine Hospital and Mansoura University Hospitals, Mansoura, Egypt. They included 50 patients with chronic hepatitis C virus (HCV), and 50 apparently healthy participants (who had donated blood) of matched age as a control group. All participants gave written informed consent to participate in the study and the investigations conformed to the principles outlined in the Declaration of Helsinki. The study protocol was approved by local ethics committee of the hospital.

Inclusion criteria: adult patients of both sexes (27-66 years old), diagnosed within the previous 6 months, positive for HCV RNA in serum (by RT-PCR assay), with evidence of chronic hepatitis with steatosis supported by liver biopsy and elevated alanine aminotransferase (ALT) activity (> 40 IU/L and < 400 IU/L). The control group was made from adults negative for HCV RNA.

Patients with decompensated cirrhosis; other causes of chronic liver disease; Schistosoma co-infection; autoimmune hepatitis; a history of intravenous drug abuse or alcohol consumption; use of hepatotoxic drugs, herbal medications or immunosuppressive agents; diabetes; thyroid disorders; chronic renal failure; serious psychiatric disorders; HIV or HBV co-infection; or hepatocellular carcinoma, were excluded. None of these patients had previously received antiviral treatment or steatosis-inducing therapy.

Patients were treated either with Peg-IFN-α-2a 180 mcg/week or Peg-IFN-α-2b 1.5 mcg/kg/week plus ribavirin (1000 mg or 1200 mg/day for body weight ≤ or > 75 kg, respectively) for 48 weeks independent of virologic response. Patients who did not achieve undetectable HCV-RNA or a decrease in 2 logs of HCV-RNA at week 12 as compared to baseline (early virological response or EVR), were considered non-responders but included in the study. All patients were clinically, hematologically and biochemically evaluated at weeks 2, 4, 8, 12, 24 and 48 after the start of treatment.

2.2, Methods:

In addition to investigations needed to fulfil the selection criteria, all individuals included in this study were subjected to the following:

2.2.1, Medical History

Full history was taken with special reference to risk factors for liver diseases such as previous HCV exposure in surgical wards, blood transfusions, dental therapy, needle stick injury, history of HCV in the spouse and i.v. injection.

2.2.2, Physical Examination

Complete medical examination with particular focus upon the manifestations of hepatitis such as jaundice, hepatomegaly, and tenderness in the right hypochondrium. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). Abdominal ultrasonography was performed for all patients.

2.2.3, Histopathological investigations

Liver biopsy specimens were formalin-fixed and paraffin embedded then sectioned and stained (hematoxylin and eosin) for routine histopathological examination. Grading and staging of chronic hepatitis was performed according to Modified Knodell's Score [15]. Steatosis was quantified as the percentage of hepatocytes that contained fat droplets and was graded using a three-tier scale: grade 1/mild (1%-33%), 2/moderate (33%-66%), and 3/severe (>66%). Fibrosis stage (F) was scored as F0 (absent), F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis) and F4 (cirrhosis).

2.2.4, Laboratory investigations

Venous blood samples were taken in the morning after 12-h overnight fast from all patients and controls. Each blood sample was divided into 3 aliquots, one aliquot was left to coagulate for 30 minutes, then

centrifuged at 3000 rpm for 15 minutes to separate serum and the other part was put in 2 tubes containing EDTA, one for complete blood count and the other centrifuged at 3000 rpm for 15 minutes to obtain plasma. Serum and plasma aliquots were immediately labeled and stored at -70°C until laboratory investigations were performed.

Plasma glucose, serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), albumin (Alb), total bilirubin levels (Bil), cholesterol (Chol), HDL-cholesterol (HDL-C) and triglycerides (TG) were measured by using Coobas Integra 400 plus, Roche Diagnostic, Germany. Platelet count (Plt) was measured for all patients and controls using Sysmex KX-21 Analyzer, Germany.

Serum insulin levels were estimated by enzyme immunoassay using Medgenix-Ins-EASIA kit (BioSource, Belgium). Insulin resistance (IR) was calculated on the basis of fasting levels of plasma glucose and serum insulin, according to the homeostasis model assessment (HOMA) method. Calculation for the HOMA model followed a standard protocol: insulin resistance (HOMA-IR) = fasting glucose (mmol/L) × fasting insulin (μIU/mL)/22.5 (16)

Serum leptin was measured using commercially available ELISA kit (Diagnostic Biochem, Canada). Human plasma ghrelin was measured with a commercially available EIA kit (Phoenix – Pharmaceuticals, Inc., Belmont, USA), which measures total circulating ghrelin concentrations.

2.2.5, Viral Markers

2.2.5.1, ELISA assays

Sera of all patients and controls were tested for HBsAg, anti-HBc and anti-HCV antibodies by ELISA, using third generation kits (DiaSorin, Italy) according to the manufacturer's instructions.

2.2.5.2, Quantitation of HCV-RNA in serum

HCV-RNA was quantitated in all patients' serum samples at baseline and then at weeks 12, 24 and 48 weeks after antiviral treatment using Real Time PCR (RT-PCR). Primers and RT-PCR reagents supplied from Stratagene, Qiagen, USA.

2.3, Statistical Analysis:

Symmetrically distributed continuous variables were summarized as a mean ± standard deviation (SD) or median and interquartile ranges. Categorical variables were presented as frequency and percentage. Comparisons between groups were made by using the Mann–Whitney U test or the Student *t* test for continuous variables and the χ^2 or Fisher exact probability test for categorical data. ANOVA test was used to compare leptin, ghrelin and HOMA-IR with different stage of hepatic steatosis and fibrosis. Spearman rank correlation was used to quantify the association between continuous or ordered categorical variables. Multiple linear regression analysis was used to model the association between baseline ghrelin and other covariates. A probability value of $P < 0.05$ was considered statistically significant. SPSS software for Windows version 17 (SPSS Inc., Chicago, IL, USA) was used to perform all analyses.

III. Results

Our study included 50 patients infected with HCV and 50 healthy subjects of matched age as a control group. The mean age was 44.8±10.4 years (range 27-66 years), the mean BMI was 25.1±4.53 kg/m^2 (range 19.4-35.2 kg/m^2), 37 (74%) patients were male and 13 (26%) were female. Their clinical, histological and baseline laboratory parameters are detailed in Table 1.

Table 2. Showed HOMA-IR, leptin and ghrelin in relation to hepatic fibrosis score and hepatic steatosis grade in HCV patients. Steatosis grade at base line was significantly higher as leptin concentrations and HOMA-IR increased (P=0.023 and 0.005, respectively) and ghrelin concentrations decreased (P=0.004) (fig. 1&2).

After treatment by combination therapy with PEGylated INF plus weight adjusted ribavirin for 48 weeks, the mean serum leptin levels and HOMA-IR were (at baseline 16.4±10.4 ng/ml; 2.67±1.49, respectively) significantly decreased (at the end of follow up 12.4±7.52 ng/ml, P=0.031; 2.13±1.02, P=0.037, respectively), while plasma ghreline levels (at base line 556±146 pg/ml) were significantly increased (at the end of follow up 616±139 pg/ml, P=0.046).

Twenty eight patients (56%) achieved sustained virological response (SVR =responder), they have significantly lower histological stage of fibrosis with liver disease (P=0.002) and lower grade of steatosis in liver biopsy (P=0.013), also they have lower HOMA-IR (P=0.001) compared to 22 (44%) patients did not achieve SVR (non responder). Leptin concentrations were significantly lower in responder compared to non responder both at baseline (P=0.005) and at end of follow up after treatment (P=0.000), but we did not observe a statistically significant difference between baseline and end of follow up leptin concentrations among responder (P=0.10), as well as among non responder (P=0.095). Serum ghrelin concentrations were significantly higher in

responder, both at base line (P=0.001) and at the end of follow up (P=0.000) than non responder. Also we observe higher ghrelin concentrations but not reach a statistically significant difference between baseline and end of follow up in responder (P=0.059), as well as among non responder (P=0.20) Table 3.

Serum leptin at baseline showed significant positive correlations with age and HOMA-IR (fig. 3). While, plasma ghrelin at baseline showed significant negative correlations with HOMA-IR (fig. 4) and serum leptin (Table 4). There were no any significant correlations between leptin (P=0.380), ghrelin (P=0.086), insulin resistance (P=0.095), hepatic fibrosis (P=0.560) and hepatic steatosis (p=0.280) with viral load. By multiple linear regression analysis, HOMA-IR was independently associated with plasma gherlin levels (P=0.000) Table 5.

Table 1: Clinical, Histological And Baseline Laboratory Parameters Of Chronic HCV And Healthy Controls

Parameter	HCV (n=50)	Controls (n=50)	P value
Age (year)	44.8±10.4	42.8±10.1	0.430
Male/female (%)	37 (74%)/13 (26%)	28 (70%)/14 (30%)	-
BMI (kg/m^2)	25.1±4.53	24.2±2.92	0.390
Platelets ($x10^9/L$)	201±55.4	241±66.5	0.007
Total bilirubin (mg/dl)	1.33±0.82	0.788±0.20	0.001
Albumin (g/dl)	3.97±0.56	4.67±0.46	0.000
ALT (IU/L)	83.4±40.8	25.3±9.2	0.000
AST (IU/L)	70.3±29.2	25.1±8.34	0.000
GGT (IU/L)	52.0±31.0	29.1±11.6	0.000
Cholesterol (mg/dl)	178.0±36.6	154.0±40.9	0.012
Triglycerides (mg/dl)	111.0±45.5	91.5±23.6	0.055
HDL-C (mg/dl)	44.4±5.97	47.0±5.21	0.070
LDL-C (mg/dl)	110.0±35.1	80.8±43.9	0.002
Fasting glucose (mmol/L)	4.99±0.98	4.44±0.71	0.014
Fasting insulin (uU/ml)	11.4±4.80	8.70±2.38	0.009
HOMA-IR	2.67±1.49	1.76±0.72	0.005
Leptin (ng/ml)	16.4±10.4	8.49±3.39	0.000
Ghrelin (pg/ml)	556±146	702±155	0.000
Viral load (log_{10})	712,315.5±382,454.7	-	-
Steatosis			
Mild	28 (56%)	-	-
Moderate	18 (36%)	-	-
Severe	4 (8%)	-	-
Fibrosis			
F1	24 (48%)	-	-
F2	17 (34%)	-	-
F3	7 (14%)	-	-
F4	2 (4%)	-	-

Significant p<0.05

Table 2: HOMA-IR, Leptin And Ghrelin In Relation To Hepatic Fibrosis Score And Hepatic Steatosis Grade In HCV Patients

	Fibrosis				Steatosis			
	Score	HCV Patients	F	P	Grade	HCV Patients	F	P
HOMA-IR	1	1.83±0.94	10..851	0.000	1	2.08±1.13	5.891	0.005
	2	2.99±1.21			2	3.39±1.71		
	3	3.99±1.70			3	3.52±0.93		
	4	5.34±0.90						
Leptin	1	9.95±6.15	11.283	0.000	1	12.9±8.26	4.116	0.023
	2	18.4±8.44			2	20.3±12.2		
	3	26.4±11.3			3	23.0±6.35		
	4	33.9±6.12						
Ghrelin	1	642±105	12.030	0.000	1	613±120	6.253	0.004
	2	521±108			2	492±155		
	3	409±154			3	441±87.3		
	4	324±89.0						

Significant p<0.05

Table 3: Clinical, Histological And Laboratory Parameters In Responders And Non Responders HCV Patients With Respect To Antiviral Therapy

Parameter	Responders (n=28)	Non Responders (n=22)	P value
Age (year)	43.2±10.5	46.9±10.2	0.220
Male/female	19 /9	18/4	0.263
BMI (kg/m^2)	24.9 ±4.97	25.3±3.98	0.790
HOMA-IR	2.10±1.31	3.39±1.41	0.001
Leptin-baseline (ng/ml)	12.9±9.41	20.9±9.93	0.005
Leptin end of follow-up (ng/ml)	9.39±5.76	16.3±7.81	0.000
Ghrelin-baseline (pg/ml)	611±137	485±128	0.001
Ghrelin end of follow-up (pg/ml)	678±123	529±93.2	0.000
Steatosis (1-3)	1.32±0.54	1.77±0.68	0.013
-Mild	20 (71.43%)	8 (36.36%)	0.040
-Moderate	7 (25.0%)	11 (50%)	
-Severe	1 (3.57%)	3 (13.64%)	
Fibrosis (1-4)	1.43±0.74	2.14±0.83	0.002
-F1	19 (67.86%)	5 (22.73%)	0.008
-F2	7 (25.0%)	10 (45.45%)	
-F3	1 (3.57%)	6 (27.27%)	
-F4	1 (3.57%)	1 (4.55%)	

Significant p<0.05

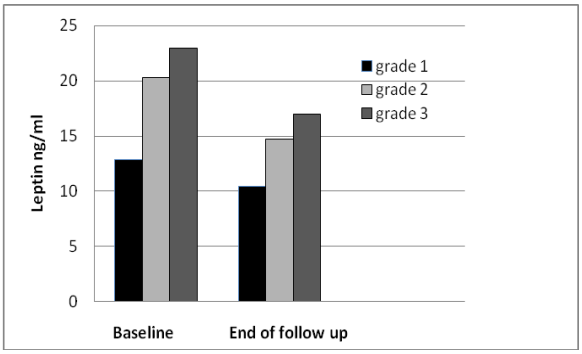

Figure 1: Serum leptin baseline and end of follow up according to to steatosis grade

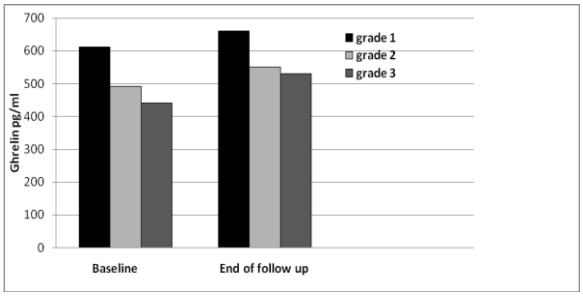

Figure 2: Plasma ghrelin baseline and end of follow up according to steatosis grade

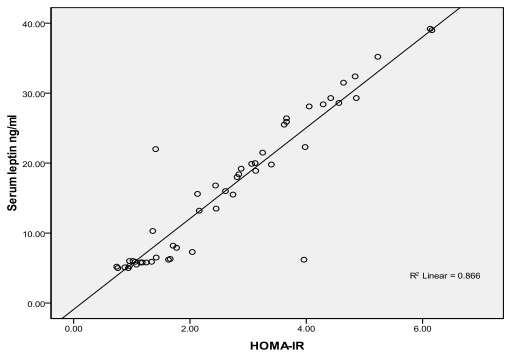

Figure 3: Correlations between serum leptin and HOMA-IR

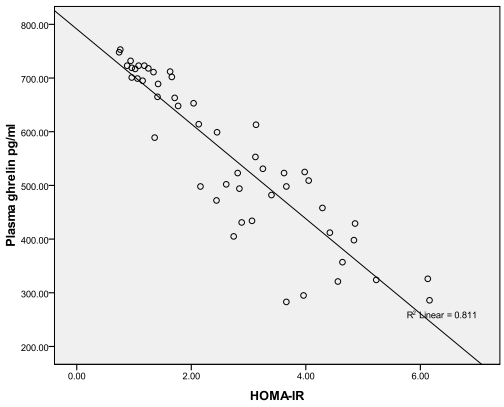

Figure 4: Correlations between plasma ghrelin and HOMA-IR

Table 4: Correlations Of HOMA-IR, Leptin, Ghrelin With Clinical, Histological And Laboratory Parameters In Chronic Hepatitis C Patients

	Sig	HMOA-IR	Leptin	Ghrelin	Fibrosis	Steatosis
Age	r p	0.362** 0.010	0.458** 0.001	-0.247- 0.084	0.233 0.104	0.307* 0.030
BMI	r p	-0.010- 0.944	0.116 0.424	-0.136- 0.345	0.292* 0.040	0.181 0.207
ALT	r p	0-.016- 0.911	0.017 0.906	0.066 0.647	-0.012- 0.934	0.051 0.725
GGT	r p	0.001 0.995	0.102 0.480	0.057 0.696	-0.116- 0.424	0.066 0.648
AST	r p	-0.066- 0.647	-0.023- 0.876	0.090 0.532	-0.063- 0.661	0.006 0.969
Albumin	r p	0.005 0.970	0.016 0.912	-0.088- 0.542	-0.032- 0.824	-0.149- 0.302
Bilirubin	r p	0.050 0.731	0.053 0.714	0.089 0.541	-0.019 0.895	0.159 0.271
Cholesterol	r p	-0.177- 0.218	-0.245- 0.086	0.274 0.054	-0.471-** 0.001	0.004 0.978
TG	r p	-0.286-* 0.044	-0.363-** 0.010	0.350* 0.013	-0.339-* 0.016	-0.026- 0.859
HDL-C	r p	0.056 0.698	0.074 0.609	-0.134- 0.353	0.083 0.567	0.006 0.965
LDL-C	r p	-0.146- 0.313	-0.210- 0.143	0.232 0.104	-0.428-** 0.002	0.041 0.779
Platelets	r p	0.233 0.104	0.267 0.061	-0.075- 0.605	0.270 0.058	0.075 0.606
HOMA-IR	r p	1	0.931** 0.000	-0.901-** 0.000	0.643** 0.000	0.419** 0.002
Leptin	r p	0.931** 0.000	1	-0.824-** 0.000	0.651** 0.000	0.375** 0.007
Ghrelin	r p	-0.901-** 0.000	-0.824-** 0.000	1	-0.662-** 0.000	-0.448-** 0.001

Significant p<0.05

Table 5: Multiple Linear Regression Analysis For Factors Associated With Plasma Ghrelin In Patients With HCV

	Unstandardized Coefficients		Standardized Coefficients		
Model	B	Std. Error	Beta	T	Sig.
(Constant)	729.489	151.236		4.824	0.000
HOMA-IR	-92.831-	18.736	-0.947-	-4.955-	0.000
Fibrosis	-15.828-	16.177	-0.093-	-0.978-	0.335

Steatosis	-26.920-	15.661	-0.119-	-1.719-	0.095
Leptin	1.776	2.772	0.126	0.641	0.526

Dependent Variable: Ghrelin Significant $p<0.05$

IV. Discussion

Although the precise pathogenetic mechanisms of steatosis among the patients with chronic hepatitis C virus (HCV) still remains largely unknown. Recent evidences appeared elucidating some of the cellular and molecular mechanisms linking obesity and insulin resistance with liver fibrosis progression, identifying various adipokines and gut hormones as relevant modulators of the pathophysiology of liver injury and repair [17].

We assessed whether pretreatment serum leptin and plasma ghrelin concentrations differ in steatotic patients infected with HCV, and whether these concentrations are associated with response to antiviral treatment. We also evaluated the correlations between pretreatment serum leptin and ghrelin concentrations and liver histology and metabolic factors. Our results are similar to observations made by other investigators [18], suggesting a possible involvement of adipocytokines like leptin and ghrelin in disease progression among the patients with chronic HCV [19, 20] .

Leptin is a putative link between HCV infection and steatosis [21]. Although a high incidence of hyperleptinemia has been observed in HCV infected patients with liver steatosis [22], the underlying mechanism promoting this effect remains undefined. Leptin may increase insulin resistance and fatty acid concentrations in the liver, leading to enhanced lipid peroxidation and promoting steatosis. Leptin may also induce the release of cytokines, such as TNF-α, INF-γ, IL-18, and TGF-β1, which are involved in the pathogenesis of both liver steatosis and fibrosis [20]. In steatosis, activated hepatic stellate cells, but not quiescent cells, can express leptin [23] . However the results of involvement of leptin in hepatic steatosis and fibrosis are not equivocal [18].

Although, many studies were demonstrated profibrogenic role of leptin [24,25], others did not reveal any role of leptin in determining severity of steatosis and fibrosis in patients with HCV [26]. Similarly to other investigators [18,24], we found high serum leptin concentrations and high insulin resistance at base line in patients with HCV compared to healthy controls. We also observe that there is difference in serum concentrations of leptin in relation to liver fibrosis and steatosis severity and progression, as serum leptin levels tend to increase as the grade of steatosis worsens, suggesting that leptin increases during infection as a part of the host immune response, and may contribute to the development of steatosis.

However, Cua et al. [27] found that insulin resistance and liver injury in HCV are not associated with virus-specific changes in adipocytokines. Nkontchou et al. [17] found that IR but not serum leptin levels predicts outcome of viral hepatitis C. In addition, other studies reported that, the structural and nonstructural proteins of HCV may directly cause steatosis by provoking oxidative stress [28,29]. Alternatively, the viral core protein may target microsomal triglyceride transfer protein activity, modifying very low density lipoprotein assembly in, and secretion by hepatocytes [30]. The core protein may also affect the cytoplasmic domain of members of the TNF receptor family or act directly on the mitochondria, leading to increased oxidative stress and lipid peroxidation. They also found that the grade of steatosis was correlated with higher viral load at baseline and this in agreement with the direct "steatogenic" effect of HCV[28]. However in this study, we did not observe any correlations between viral load and the grade of steatosis.

Although ghrelin is important in food intake, energy balance, and the regulation of the growth hormone releasing mechanism [13], its role in hepatic disease has not been extensively evaluated to date. Increased serum ghrelin concentrations have been reported in patients with cirrhosis and hepatocellular carcinoma, suggesting that this adipokine may be involved in the anorexia-cachexia syndrome during the terminal stages of liver diseases [31]. Data on ghrelin concentrations in patients with HCV are limited [32].

This study demonstrated low plasma ghrelin concentrations in patients with HCV, and negative correlations between plasma ghrelin and hepatic fibrosis and steatosis. Also we found negative correlations between ghrelin with HOMA-IR and leptin. These results are in agreement with the results of previous studies [20,33] that demonstrated low plasma ghrelin concentrations that tend to decrease more as the grade of steatosis increase in patients with HCV, indicating that ghrelin may prevent or reduce steatosis by negatively regulating leptin [20]. In addition Moreno et al. [33] found that, in patients with chronic liver diseases and chronic HCV, plasma ghrelin levels decreased in those with advanced fibrosis, and ghrelin gene hepatic expression correlated with expression of fibrogenic genes, and polymorphisms of the ghrelin gene (-994CT and -604GA) influenced the progression of liver fibrosis, thus Ghrelin exerts antifibrotic effects in the liver and may represent a novel antifibrotic therapy. In contrast to our results, other investigators did not find any significant difference in plasma ghrelin concentrations and no any correlations with the grade of steatosis among patients with HCV [18].

In this study, similarly to the large clinical trials of anti-viral therapy using Peg-IFN and ribavirin [34], Sustained virological response (SVR) was achieved in 56% of patients. Also we found that antiviral therapy

produces significant changes in HOMA-IR, leptin and ghrelin levels. At base line, HOMA-IR and serum leptin were significantly lower in patients with SVR compared to non- SVR and continued to decrease but not reach statistically significant levels at the end of follow up. In addition responder had significantly lower hepatic fibrosis score and steatosis grade than non responder. While plasma ghrelin concentrations were significantly higher in responders than non responders and continued to increase but not reach statistically significant levels at the end of treatment.

We confirmed previous observation of the role of HOMA-IR and fibrosis score as predictive factors of therapy-induced viral eradication [35]. These findings are in accordance with data that showed insulin sensitivity improvement during IFN treatment restricted to patients with SVR [36]. Similarly, two papers demonstrated, respectively, leptin levels reduction during antiviral therapy[37] and leptin decrease during IFN treatment only in virological responders [38]. Khattab et al., showed that antiviral therapy produces significant changes in IR and leptin levels. At the end of follow-up, HOMA-IR and leptin were lower in patients with SVR, but remained unchanged in patients who did not response or relapsed [37].

In addition, Pavlidis et al. [20] found that responders had lower serum leptin and higher plasma ghrelin concentrations at baseline than non-responders, and that ghrelin concentration increased to reach significant levels at the end of treatment, indicating that ghrelin may prevent or reduce steatosis by negatively regulating leptin. This may enhance the likelihood of SVR, since responders also have lower baseline leptin concentrations. However, ghrelin may be also considered as an independently acting factor, based on their finding that responders with moderate and severe steatosis had low ghrelin concentrations at baseline and that these concentrations were increased significantly after treatment. In contrast, no significant differences were observed in non-responders and there were no correlations with leptin concentrations.

V. Conclusion:

The degree of hepatic steatosis and fibrosis can be known by noninvasive techniques as measurement of plasma ghrelin and Serum leptin levels. They can be useful for screening because it is detectable in early stages, also, their levels can be used for prediction of the response to antiviral therapy in patients with HCV. We should decrease insulin resistance and leptin levels as well as increase ghrelin level before starting antiviral treatment to obtain good response. More researches can be done about ghrelin and its use for treatment of fibrotic liver. It may be necessary to enlarge the study groups in order to obtain more significant conclusions and to evaluate the role of these parameters in diagnosis and follow up of fibrosis and steatosis in HCV patients to prevent further complications of the disease.

References

[1] Mohamed AA**,** Loutfy SA**,** Craik JD**,** Hashem AM, Sia I, Chronic hepatitis C genotype-4 infection: role of insulin resistance in hepatocellular carcinoma, Virology Journal, 2011, **8**:496. doi:10.1186/1743 -422X-8-496.

[2] Zekri AR, Bahnassy AA, Abdel-Wahab SA, Khafagy MM, Loutfy SA, Radwan H, and Shaarawy SM, Expression of pro- and anti-inflammatory cytokines in relation to apoptotic genes in Egyptian liver disease patients associated with HCV-genotype-4, J Gastroenterol Hepatol, 2009, 24:416-28.

[3] Loguercio C, Federico A, Oxidative stress in viral and alcoholic hepatitis, Free Radic. Biol. Med., 2003; 34(1):1–10.

[4] Negro F and Alaei M, Hepatitis C virus and type 2 diabetes,World J Gastroenterol, 2009; 15(13):1537–47.

[5] Eslam M, Khattab MA and Harrison SA, Insulin resistance and hepatitis C: an evolving story, Gut, 2011; 60:1139-1151.

[6] Moucari R, Asselah T, Cazals-Hatem D, et al., Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis, Gastroenterology, 2008; 134:416-423.

[7] Giannini E, Barreca T and Testa R, Leptin in nonalcoholic steatohepatitis: is it one of the "hits"?, Am. J. Gastroenterol., 2001; 96:2519-2520.

[8] Romero-Gómez M, Castellano-Megias VM, Grande L, Irles JA, Cruz M, Nogales MC, Alcón JC and Robles A, Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C, Am. J. Gastroenterol., 2003; 98:1135-1141.

[9] Gwak GY, Kim TH, Yu SJ, Yoon JH, Yong JJ, Park SC and Lee HS, Lack of association between serum leptin levels and hepatic steatosis, fibrosis or response to antiviral therapy in Korean chronic hepatitis C patients, Hepato-Gastroenterology, 2007; 54:844-848.

[10] Eguchi Y, Mizuta T, Yasutake T, Hisatomi A, Iwakiri R, Ozaki I and Fujimoto K, High serum leptin is an independent risk factor for non-response patients with low viremia to antiviral treatment in chronic hepatitis C, World J. Gastroenterol., 2006; 12:556-560.

[11] Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R and Ruggiero G, Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity, Hepatology, 2001; 33:1358-1364.

[12] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H and Kangawa K, Ghrelin is a growth-hormone-releasing acylated peptide from stomach, Nature, 1999; 402:656-660.

[13] Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG and Ghatei MA, The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion, Endocrinology, 2000; 141:4325-4328.

[14] Cummings DE and Foster KE, Ghrelin-leptin tango in body-weight regulation, Gastroenterology, 2003; 124:1532-1535.

[15] Ishak K, Baptista A, Bianchi L, Callea F, Degroote J and Denk F, Histopathological staging and grading of chronic hepatitis, J. Heptol., 1995; 22: 696-699.

[16] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia, 1985, 28:412-9.

[17] Nkontchu G, Bastard J-P, Ziol M, et al., Insulin resistance, serum leptin and adiponectin levels and outcomes of viral hepatitis C cirrhosis, J. Hepatol., 2010; 53(2): 827-833.

[18] Mazur W, Kukla M, Logiewa-Bazger B, Beniowski M, Kwiatkowski J, Wagner G, Jurzak M, Berdowska A and Zwirska-Korczala K, Resistin, leptin and ghrelin serum levels among the patients with chronic hepatitis C, Hepatology, 2010; 6(3-4): 17-24.
[19] Bertolani C and Marra F, The role of adipokines in liver fibrosis, Pathophysiology, 2008; 15: 91–101.
[20] Pavlidis C, Panoutsopoulos GI, Tiniakos D, Koutsounas S, Vlachogiannakos J and Zouboulis-Vafiadis I, Serum leptin and ghrelin in chronic hepatitis C patients with steatosis, World J. Gastroenterol., 2011; 17(46): 5097-5104.
[21] Ellidokuz E, Cömlekçi A, Ellidokuz H, Akpinar H, Gökçe C, Tankurt E, Sagol O, Simsek I and Gönen O, The role of serum leptin levels in chronic hepatitis C with steatosis, Hepatogastroenterology, 2003; 50 Suppl : cclxix–cclxxii.
[22] Manolakopoulos S, Bethanis S, Liapi C, Stripeli F, Sklavos P, Margeli A, Christidou A, Katsanika A, Vogiatzakis E, Tzourmakliotis D, et al., An assessment of serum leptin levels in patients with chronic viral hepatitis: a prospective study, BMC Gastroenterol., 2007; 7: 17.
[23] Clouston AD, Jonsson JR, Purdie DM, Macdonald GA, Pandeya N, Shorthouse C and Powell EE, Steatosis and chronic hepatitis C: analysis of fibrosis and stellate cell activation, J. Hepatol., 2001; 34:314–320.
[24] Leclerq IA, Farrell GC, Schriemer R and Robertson GR, Leptin is essential for the hepatic fibrogenic response to chronic liver injury, J. Hepatol., 2002; 37: 206-213.
[25] Lo Iacono O, Venezia G, Petta S, et al., The impact of insulin resistance, serum adipocytokines and visceral obesity on steatosis and fibrosis in patients with chronic hepatitis C, Aliment. Pharmacol.Ther., 2007; 10: 1181-1189.
[26] Giannini E, Ceppa P, Botta F, et al., Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C, Am. J. Gastroentrol., 2000; 11: 3211-3217.
[27] Cua IH, Hui JM, Bandara P, et al., Insulin resistance and liver injury in hepatitis C is not associated with virus-specific changes in adipocytokines, Hepatology, 2007; 46: 66-73.
[28] Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G and Day CP, Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease, Gastroenterology, 2004; 126:586–597.
[29] Matos CA, Perez RM, Pacheco MS, Figueiredo-Mendes CG, Lopes-Neto E, Oliveira EB, Lanzoni VP, Silva AE and Ferraz ML, Steatosis in chronic hepatitis C: relationship to the virus and host risk factors, J. Gastroenterol. Hepatol., 2006; 21:1236–1239.
[30] Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Malé PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, et al., Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3, J. Hepatol., 2000; 33: 106–115.
[31] Tacke F, Brabant G, Kruck E, Horn R, Schöffski P, Hecker H, Manns MP and Trautwein C, Ghrelin in chronic liver disease, J. Hepatol., 2003; 38: 447–454.
[32] Watanabe H, Saito T, Karasawa T, Kudo S, Nakano K, Ito JI, Sugahara K, Saito K, Togashi H and Kawata S, Reduction of serum ghrelin concentration during interferon-alpha therapy in patients with chronic hepatitis C, Hepatol. Res., 2005;33:14–18.
[33] Moreno M, Chaves JF, Sancho-Bru P, et al., Ghrelin attenuates hepatocellular injury and liver fibrogenesis in rodents and influences fibrosis progression in humans., Hepatology, 2010; 51(3): 974-985. doi: 10.1002/hep.23421.
[34] Hadziyannis SJ, Sette H, Morgan TR, et al., Peg interferon alpha 2 a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose, Ann. Intern. Med., 2004; 140:346–355.
[35] Khattab M, Eslam M, Sharwae MA, Shatat M, Ali A and Hamdy L., Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients, Am. J. Gastroenterol., 2010; 105:1970-1977.
[36] Conjeevaram HS, Wahed AS, Afdhal N, Howell CD, Everhart JE and Hoofnagle JH, Viral hepatitis-C Study Group: Changes in insulin sensitivity and body weight during and after peg-interferon and ribavirin therapy for hepatitis C, Gastroenterology, 2011; 140:469-77.
[37] Khattab M, Eslam M, Shatat M, et al., Changes in adipocytokines and insulin sensitivity during and after therapy for hepatitis C genotype 4, J. Gastrointest. in Liver Dis., 2012; 21(1): 59-65.
[38] Zografos TA, Rigopoulou EI, Liaskos C, et al, Alterations of leptin during IFN-alpha therapy in patients with chronic viral hepatitis, J. Hepatol., 2006; 44:848–855