Adipokines and insulin resistance, predictors of response to therapy in Egyptian patients with chronic hepatitis C virus genotype 4

Yasmin Saad[a], Amal Ahmed[c], Doa'a A. Saleh[b] and Wahid Doss[a,d]

Background **Hepatitis C virus (HCV) infection has major health impact worldwide and is a significant cause of chronic liver disease. In Egypt, HCV is highly endemic (up to 15% of the population); 91% of the patients are infected with genotype 4. Searching for new predictors of response to therapy is mandatory to decrease the cost and the adverse effects of current therapy.**

Aim **The aim of this study was to clarify the usefulness of serum leptin, adiponectin, and insulin resistance (IR) as predictors of response to treatment in hepatitis C virus genotype 4 (HCVG4).**

Methods **One hundred patients with chronic HCVG4 who were candidates for treatment with pegylated interferon α and ribavirin were included in the study. Age, sex, and BMI were determined, and quantitative HCV PCR, assessment of serum leptin, adiponectin, IR, and pretreatment liver profile, and liver biopsy were performed.**

Results **The male to female ratio was 68/32; the mean age of the patients was 40.9±7.8 years and BMI was 28.3±10 kg/m^2. Sustained virological response (SVR) was achieved by 56% of the patients. On performing logistic regression, BMI [odds ratio (OR) 6.5; $P=0.004$], serum leptin (OR 27.8; $P \leq 0.001$), aspartate aminotransferase (OR 1.06; $P \leq 0.001$), IR (OR 1.15; $P \leq 0.001$), histological activity index (OR 1.77; $P=0.006$), and fibrosis (OR 2.93; $P=0.001$) were found to be independent negative predictors of SVR, whereas serum adiponectin (OR 0.74; $P \leq 0.001$) was found to be an independent positive predictor of SVR. Pretreatment adiponectin (cutoff 13.75; sensitivity 92.86%; specificity 86.86%) shows area under the curve of 0.879 (95% confidence interval 0.802–0.956; $P<0.001$) and insignificant area under the curve for leptin or IR.**

Conclusion **BMI, pretreatment high leptin levels, and IR are negative predictors for SVR and pretreatment low adiponectin levels are an independent positive predictor for SVR in HCVG4. *Eur J Gastroenterol Hepatol* 25:920–925 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

European Journal of Gastroenterology & Hepatology 2013, **25**:920–925

Keywords: adiponectin, hepatitis C virus genotype 4, insulin resistance, leptin, sustained virological response

Departments of [a]Endemic Medicine and Hepatology, [b]Community Medicine, Faculty of Medicine, Cairo University, [c]Department of Medical Biochemistry and [d]Tropical Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt

Correspondence to Yasmin Saad, MD, Department of Endemic Medicine and Hepatology, Faculty of Medicine, Cairo University, Cairo 11520, Egypt
Tel: +20 223 593 594/20 223 647 524;
fax: +20 233 688 400/20 233 682 774; e-mail: dr_ysaad99@yahoo.com

Received 13 October 2012 **Accepted** 18 January 2013

Introduction

Hepatitis C virus (HCV) is the cause of a significant proportion of cases of chronic liver disease, hepatocellular carcinoma, and death because of liver disease. On the basis of the current prevalence of infection and anticipated rates of progression, morbidity and mortality as well as the costs of treatment for HCV infection are expected to increase significantly in the next two decades [1].

Searching for new predictors of response to therapy is mandatory to decrease the cost and the adverse effects of current therapy [2]. Currently, the existing association among the different virus genotypes and the level of fat upload in the liver as well as the various levels of response to the antiviral conventional treatment are well known [3–5]. Different links with lipidic profile alterations have generated particular interest as they have offered, at least partially, explanations on the mechanism of viral operation [6,7].

Insulin resistance (IR) is associated with the presence of HCV and it determines a low rate of response to the standard antiviral therapy [8]. IR expressed in hepatocytes is a result of alterations in a group of serologic and local factors as well as an altered adiponectin profile. Adiponectin is the most abundant adipokine in plasma and its production decreases in the context of obesity and IR. It has anti-inflammatory properties and plays a role in increasing hepatocyte sensitivity to the gluconeogenesis inhibition mediated by insulin [9]. Adipokines and hyperinsulinemia have been considered as determining factors of the extent of fibrosis and of the unresponsiveness to pegylated interferon (PEG IFN), the result and duration of the antiviral treatment depending on the patient's metabolic and nutritional status and the viral genotype [10,11]. However, recently, attention has been focused on adiponectin and its changes in different types of chronic liver disease. Its relation to hepatic fibrosis and IR in posthepatitis liver disease is not clear [12].

Leptin is a circulating 16-kDa nonglycosylated protein [13]; its levels are related to the adipose tissue mass [14]. Leptin has been implicated in many actions

0954-691X © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/MEG.0b013e32835f2726

including liver fibrogenesis [15–18]. Serum leptin levels have been found to be higher in patients with chronic hepatitis C (CHC) and in particular in those with more severe fibrosis or cirrhosis [19]; however, the results are conflicting [20–22].

The aim of this study was to clarify the usefulness of the clinical use of serum leptin, adiponectin, and IR as a predictor of response to treatment in hepatitis C virus genotype 4 (HCVG4).

Methods

Population samples

This study was carried out on 100 consecutive patients with chronic HCVG4 infection who were candidates for treatment with PEG IFN-α and ribavirin. The inclusion criteria were as follows: naive patients 18–60 years of age, HCV RNA positive with abnormal alanine aminotransferase (ALT), liver biopsy performed within 6 months before enrollment, and a diagnosis of CHC without cirrhosis. The exclusion criteria were as follows: those who are not fit for IFN therapy (coinfection with hepatitis B virus, alcohol intake, clinically evident liver cirrhosis, any end organ failure, hematological diseases, major psychiatric disorder, pregnant, and breast-feeding women). Informed consent was obtained from all participants before enrollment in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki, and its appendices, and local and national laws.

All the patients were subjected to a clinical assessment. Height and weight were determined at baseline and BMI was calculated as weight in kg divided by height in m^2. Before starting therapy, laboratory investigations included complete liver profile, kidney function, international Ratio, and complete blood count. HCV PCR was quantified in all patients' sera using real time PCR (Stratagene, Foster City, California, USA); accordingly, they were classified into low (< 100 000 IU/ml), medium (100 000–1 000 000 IU/ml), and high (> 1 000 000 IU/ml), and as known more than 90% of Egyptian patients have HCVG4 [23,24]. α-Fetoprotein and HCV antibody were determined using Axyam (Abbot, New York, USA). Serum levels of adiponectin, leptin, and fasting insulin were determined using the ELISA test. Blood samples were obtained in the morning after 12 h of fasting. They were centrifuged and serum was separated, and then stored at −80°C before use. IR was determined by homeostasis model assessment (HOMA) using the following equation [25]: HOMA = fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5. HOMA values more than 2.7 were considered to indicate IR; this cutoff corresponds to the upper quartile of a previously published control Italian population [26]. Abdominal ultrasound was performed for all the enrolled patients using a Toshiba (Japan) machine with a 3.5 MHz convex probe; patients were examined after at least 8 h of fasting. Percutaneous liver biopsy was performed under ultrasound guidance using 16-G needles. Specimens at least 2.5 cm in length, including a minimum of 12 portal tracts, were considered reliable for adequate grading and staging using a modified Knodell's score. Liver biopsies were assessed by a single pathologist who was blinded to the clinical data; the Metavir classification was used for the assessment of necroinflammation and stage of fibrosis. All the patients were treated with PEG IFN-α-2a, 180 μg/week subcutaneously, plus ribavirin (1000–1200 mg orally/day on the basis of body weight).

Statistical methods

Continuous, normally distributed variables are summarized as mean ± SD. Categorical and ordinal data are presented as frequencies and percentages. Differences between the means of continuous variables were assessed using Student's *t*-test. The association of a sustained virological response (SVR) with potential risk factors was assessed using the χ^2 and Fisher's exact tests where appropriate. Those factors showing a significant association in bivariate analysis or considered to be important factors irrespective of bivariate analysis results were included in multivariate logistic regression analysis. A ROC was constructed and the area under the curve (AUC) was calculated to determine whether adiponectin is capable of predicting a SVR. *P* values of less than 0.05 were considered statistically significant. Analyses were carried out using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA).

Results

The baseline characteristics of 100 chronic HCVG4 patients enrolled in the study are shown in Table 1; there were 68 men and 32 women, mean ± SD age 40.9 ± 7.8 years and mean ± SD BMI 28.3 ± 10 kg/m^2. The mean value of transaminases ± SD was: aspartate aminotransferase (AST) = 63.5 ± 20.1, ALT = 55.8 ± 15.1); the mean ± SD of α-fetoprotein was 10.3 ± 4.9. Number of patients with low, medium, and high HCV viral load were 17, 51, and 32, respectively. The mean ± SD serum leptin level was 9.4 ± 5.5, that of adiponectin was 17.4 ± 7.0, and IR was 35.0 ± 17.2. The overall hepatic grade of inflammation was 5.9 ± 1.1 and stage of fibrosis was 3.3 ± 0.8. Of 100 patients, 56 showed a SVR, whereas 44 showed no response or relapse; no patient withdrew from therapy because of side-effects and no patients received less than 80% of the therapeutic schedule. Their demographic, laboratory, and histological parameters are shown in Table 2. Both groups were age, sex, and HCV viral load matched; BMI was significantly higher in the nonresponder group compared with the SVR group (35.3 ± 8.1 vs. 22.8 ± 7.6; $P < 0.001$). High serum leptin was statistically significant in the nonresponder group compared with the SVR group (13.4 ± 4.7 vs. 6.2 ± 3.7; $P = 0.012$), whereas low serum adiponectin was statistically significant in the nonresponder group versus the SVR group (11.6 ± 5.2 vs. 22.0 ± 4.4; $P < 0.001$). IR was significantly higher in the nonresponder group (48.0 ± 15.1 vs. 24.8 ± 10.8; $P < 0.001$).

Table 1 Demographic, laboratory, and histological characteristics of a total of 100 chronic hepatitis C virus genotype 4 patients

Variables	Value
Age (years)	40.9±7.8
Sex (M : F)	68 : 32
BMI (kg/m^2)	28.3±10.0
AST (IU/l)	63.5±20.1
ALT (IU/l)	55.8±15.1
Total bilirubin	1.1±0.4
Alb	3.8±0.3
AFP	10.3±4.9
TSH	3.6±0.7
HCV viral load (number of patients) (low : medium : high)	17 : 51 : 32
Leptin (ng/ml)	9.4±5.5
Adiponectin (μg/ml)	17.4±7.0
F-insulin (μU/ml)	7.9±3.7
FBG (mg/dl)	100.9±17.8
IR	35.0±17.2
Fibrosis score	3.3±0.8
HAI	5.9±1.1

Data are reported as mean±SD except in HCV viral load represented as number of patients.
AFP, α-fetoprotein; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBG, fasting blood glucose; HAI, histological activity index; HCV, hepatitis C virus; IR, insulin resistance; TSH, thyroid stimulating hormone.

Table 2 Demographic, laboratory, and histological characteristics of responder and nonresponder chronic hepatitis C virus genotype 4 patients to combined pegylated and ribavirin

	SVR (N=56)	NR (N=44)	P value
Age (years)	40.4±8.1	42.2±7.5	0.16
Sex (M : F)	35 : 21	33 : 11	0.18
BMI (kg/m^2)	22.8±7.6	35.3±8.1	<0.001
AST (IU/l)	54.5±17.1	74.9±17.8	<0.001
ALT (IU/l)	54.6±15.8	57.3±14.3	0.38
Total bilirubin	1.1±0.4	1.1±0.5	0.56
Alb	3.9±0.3	3.8±0.3	0.11
AFP	10.2±5.0	10.29.9±4.8	0.85
TSH	3.7±0.8	3.4±0.6	0.04
PCR (low : medium : high)	11 : 30 : 15	6 : 21 : 17	0.413
Leptin (ng/ml)	6.2±3.7	13.4±4.7	0.012
Adiponectin (μg/ml)	22.0±4.4	11.6±5.2	<0.001
F-insulin (μU/ml)	5.5±2.4	10.9±2.9	<0.001
FBG (mg/dl)	101.8±17.1	99.8±18.8	0.59
IR	24.8±10.8	48.0±15.1	<0.\001
HAI	5.6±1.0	6.2±1.2	0.005
Fibrosis stage	3.0±0.8	3.6±0.7	<0.001

Data are reported as mean±SD.
AFP, α-fetoprotein; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBG, fasting blood glucose; HAI, histological activity index; IR, insulin resistance; SVR, sustained virological response; TSH, thyroid stimulating hormone.
*P<0.05 is significant.

In terms of the histological features, there were significantly higher grades of inflammation and stages of fibrosis in nonresponders compared with those who achieved an SVR (6.2±1.2 and 3.6±0.7 vs. 5.6±1.0 and 3.0±0.8; $P = 0.005$ and $P < 0.001$, respectively).

A multivariate comparison of variables between patients with and without an SVR is shown in Tables 3 and 4. By logistic regression, BMI [odds ratio (OR) 6.5; 95% confidence interval (CI) 1.7–23.4; $P = 0.004$], serum leptin (OR 27.8; 95% CI 6.3–123.9; $P \leq 0.001$), AST (OR 1.06; 95% CI 1.04–1.09, $P \leq 0.001$), IR (OR 1.15; 95% CI 1.09–1.21, $P \leq 0.001$), histological activity index (OR 1.77; 95% CI 1.18–2.65; $P = 0.006$), and fibrosis (OR 2.93; 95% CI 1.56–5.49; $P = 0.001$) were found to be independent negative predictors of SVR, whereas serum adiponectin (OR 0.74; 95% CI 0.68–0.82; $P \leq 0.001$) was found to be an independent positive predictor of SVR.

Table 3 Multivariate analysis of host and viral factors associated with sustained virological response

Variables	OR (95% CI)	P value
Age	0.94 (0.86–1.02)	0.18
Sex (male vs. female)	2.8 (0.73–10.91)	0.133
BMI (≥ 25 vs. <25)	6.5 (1.7–23.4)	0.004
Leptin (≥ 8 vs. <8)	27.8 (6.3–123.9)	<0.001
Adiponectin	0.74 (0.68–0.82)	<0.001
AST	1.06 (1.04–1.09)	<0.001
ALT	1.01 (0.99–1.04)	0.38
AFP	0.99 (0.92–1.08)	0.85
IR	1.15 (1.09–1.21)	<0.001
HAI	1.77 (1.18–2.65)	0.006
Fibrosis	2.93 (1.56–5.49)	0.001

Values are presented as mean±SD unless indicated otherwise.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; IR, insulin resistance; OR, odds ratio.
*P<0.05 is significant.

Table 4 Multivariate analysis of (leptin, adiponectin, insulin resistance, body mass index, and fibrosis) associated with sustained virological response

Variables	OR (95% CI)	P value
BMI (≥ 25 vs. <25)	6.5 (1.7–23.4)	0.004
Leptin (≥ 8 vs. <8)	27.8 (6.3–123.9)	<0.001
Adiponectin	0.74 (0.68–0.82)	<0.001
IR	1.15 (1.09–1.21)	<0.001
Fibrosis	2.93 (1.56–5.49)	0.001

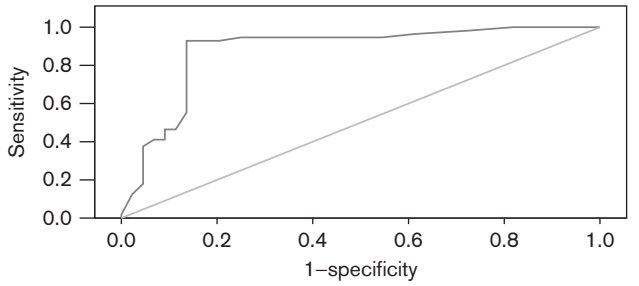
CI, confidence interval; IR, insulin resistance; OR, odds ratio

To evaluate the suitability of pretreatment levels of leptin, adiponectin, and IR to predict patients with a SVR, it was found that at a cutoff point of 13.75 for pretreatment adiponectin, the sensitivity and specificity of adiponectin for the SVR were 92.86 and 86.86%, respectively. The AUC for adiponectin was 0.879 (95% CI 0.802–0.956; $P < 0.001$) as shown in Fig. 1. For the serum leptin and IR, there were no significant AUCs with no sensitivity or specificity (AUC for leptin was 0.10, 95% CI 0.03–0.17 and AUC for IR was 0.09, 95% CI 0.03–0.14).

Discussion

SVR, defined as undetectable HCV RNA in serum 24 weeks after the end of therapy [27], varies primarily according to the HCV genotype, which is the single most important parameter affecting the rate of cure. In our study, 56% of the patients with chronic HCVG4 showed an SVR, which is in agreement with other studies in which SVR rates of 55–60% have been reported [28,29]; in some studies, it reaches 69% [30,31]. However, studies

Fig.1



ROC of adiponectin in relation to sustained virological response. Diagonal segments are produced by ties.

carried out in Europe [32–34] have shown that the SVR rates in Africans or Europeans with chronic HCVG4 treated with PEG IFN-α plus ribavirin are lower than those obtained in studies carried out in the Middle East. This may be influenced by multiple factors; some are related to HCV itself as patients infected with HCV-G4a had higher SVR rates than those infected with HCV-G4d [34]. Other factors are unrelated to HCV: genetic factors, age, sex, BMI, stage of liver disease, and some comorbidities such as IR, which affect both liver fibrosis progression and response to therapy [35]. The results of our study further confirm that in patients with CHC, high BMI has been associated with a lower SVR rate; furtheremore, certain studies have found that BMI more than 30 is an independent risk factor for nonresponse to antiviral therapy in chronic HCV [36], and this can be attributed to the following: first, BMI has been shown to correlate with the degree of steatosis found in hepatitis C [37]. Steatosis leads to an increase in lipid deposits within cells [38], which may cause a functional disturbance by decreasing the contact area between the drugs and the hepatocytes containing the virus [39]. Second, in obese individuals, there may be a reduction in the initial absorption of a drug administered subcutaneously because of an increase in subcutaneous fat. Third, the structural property of PEG IFNs is such that its size precludes rapid uptake into the vascular system, but large proteins (> 15 kDa) injected subcutaneously are primarily taken up by the lymphatic system, and obese individuals are known to have poor lymphatic circulation [40]; this could potentially lead to lower serum levels of PEG IFN, thus reducing the likelihood of a successful antiviral response.

Leptin concentration, which is a proinflammatory adipokine, is increased in obese patients. Leptin stimulates Th1 responses [41], which favors an SVR in CHC patients receiving antiviral therapy [42]. This is not contradictory to the presence of a high baseline leptin level in our CHCG4 patients without SVR, because upregulation of leptin secretion may result from peripheral leptin resistance, thus suggesting that leptin resistance (rather than increased effects) may underlie hyporesponsiveness to IFN-α [43]. Leptin resistance in these patients may be caused by abnormal leptin transport and disturbances in ObR signaling, including overexpression of the suppressor of cytokine signaling-3 (SOCS3), an agent that inhibits leptin signaling [44]. Univariate regression analysis of our patients showed statistically significant high baseline serum leptin levels in nonresponder chronic HCVG4 patients; however, in multivariate logistic regression analysis, high baseline leptin level was a negative prognostic factor for response to antiviral therapy [9], but with small AUC. Another factor that may be related to the reduced response to IFN-α is IR; increasing levels of IR are associated with reduced rates of an initial virological response [45–47] and SVR [48–52]. Our study also provides further evidence that IR impairs sustained response to antiviral treatment in genotype 4-infected patients. This negative association has been reported to occur both in patients infected with the genotype 1 [48,49,52] and in those with genotypes 2 and 3 [51]. A reasonable explanation might be the involvement of HCV in the development of IR, a theory supported by many previous reports [53]. The levels of the SOCS3, a factor promoting the degradation of the insulin receptor substrate-1, thus leading to impaired insulin signaling and IR, have been reported to be increased in cells expressing the HCV core protein [53] as well as in the liver of CHC patients not responding to antiviral therapy [54]. SOCS3 is also a negative regulator of IFN-α signaling [55]. Thus, SOCS3 upregulation by HCV may represent a first molecular link between a reduced response to IFN-α and IR. Other evidence suggests that IR is associated with severe fibrosis and poor response to treatment in patients with CHC [48,56,57], which was proved in our study. Moreover, there may be an interaction between serum leptin and IR on hepatic fibrogenesis and poor response to IFN-ribavirin therapy in genotype 4, as documented in previous reports on genotype 1 [58], and as already proposed by Romero-Gomez *et al.* [48], HOMA-IR could represent the best host marker to predict an SVR in genotype 1-infected patients.

Another adipokine that plays a role in chronic hepatitis is adiponectin; it has hepatoprotective and antifibrogenic effects in cases of hepatic injury and protects against liver steatosis [59]. In the current study, there was a significant decrease in serum adiponectin in CHCG4 patients with SVR and it represent a positive prognostic factor for response to IFN-ribavirin therapy in CHCG4 in multivariate regression analysis, which can be explained by the fact that adiponectin, both total and high molecular weight isoforms, increased the production of antiviral cytokine IFN-γ by polymorph nuclear cells from HCV-infected patients, in addition to attenuation of IL-6 production [60]. Although IFN-γ is a proinflammatory cytokine, it is crucial in establishment of the antiviral state both by inhibition of viral replication [61] and by induction of proteins involved in regulating the innate

and adaptive immune response [62]. In conclusion, BMI, pretreatment high leptin, and IR are negative predictors for SVR and pretreatment low adiponectin is an independent positive predictor for SVR in HCVG4.

Acknowledgements

The authors thank Egyptian National Committee for Control of Viral Hepatitis and National Hepatology and Tropical Medicine Research Institute, Egypt.

Conflicts of interest

There are no conflicts of interest.

References

1 Dienstag JL, McHutchison JG. American gastroenterological association medical position statement on the management of hepatitis C. *Gastroenterology* 2006; **130**:225–230.
2 Hamamoto S, Uchida Y, Wada T, Moritani M, Sato S, Hamamoto N, *et al*. Changes in serum lipid concentrations in patients with chronic hepatitis C virus positive hepatitis responsive or non-responsive to interferon therapy. *J Gastroenterol Hepatol* 2005; **20**:204–208.
3 Abid K, Pazienza V, de Gottardi A, Rubbia-Brandt L, Conne B, Pugnale P, *et al*. An *in vitro* model of hepatitis C virus genotype 3a-associated triglycerides accumulation. *J Hepatol* 2005; **42**:744–751.
4 Solis-Herruzo JA, Pérez-Carreras M, Rivas E, Fernández-Vázquez I, Garfia C, Bernardos E, *et al*. Factors associated with the presence of nonalcoholic steatohepatitis in patients with chronic hepatitis C. *Am J Gastroenterol* 2005; **100**:1091–1098.
5 Lonardo A, Loria P, Adinolfi LE, Carulli N, Ruggiero G. Hepatitis C and steatosis: a reappraisal. *J Viral Hepat* 2006; **13**:73–80.
6 Gupte P, Dudhade A, Desai HG. Acquired apolipoprotein B deficiency with chronic hepatitis C virus infection. *Indian J Gastroenterol* 2006; **25**:311–312.
7 Iacono O, Venezia G, Petta S. The impact of insulin resistance, serum adipocytokines and visceral obesity on steatosis and fibrosis in patients with chronic hepatitis C. *Aliment Pharmacol Ther* 2007; **25**:1181–1119.
8 Leclercq IA, Da Silva Morais A, Schroyen B, Van Hul N, Geerts A. Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. *J Hepatol* 2007; **47**:142–156.
9 Eguchi Y, Mizuta T, Yasutake T, Hisatomi A, Iwakiri R, Ozaki I, *et al*. 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.
10 Saxena NK, Titus MA, Ding X, Floyd J, Srinivasan S, Sitaraman SV, Anania FA. Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation. *FASEB J* 2004; **18**:1612–1614.
11 Ding X, Saxena NK, Lin S, Xu A, Srinivasan S, Anania FA. The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. *Am J Pathol* 2005; **166**:1655–1669.
12 Derbala M, Rizk N, Al-Kaabi S, Amer A, Shebl F, Al Marri A, *et al*. Adiponectin changes in HCV-genotype 4: relation to liver histology and response to treatment. *J Viral Hepat* 2009; **16**:689–696.
13 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Position cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**:425–432.
14 Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell* 2001; **104**:531–543.
15 Potter JJ, Womack L, Mezey E, Anania FA. Transdifferentiation of rat hepatic stellate cells results in leptin expression. *Biochem Biophys Res Commun* 1998; **244**:178–182.
16 Leclercq IA, Farrell GC, Schriemer R, Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 2002; **37**:206–213.
17 Bethanis S, Theocharis S. Leptin in the field of hepatic fibrosis: a pivotal or incidental player? *Dig Dis Sci* 2006; **51**:1685–1696.
18 Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. *Am J Gastroenterol* 2006; **101**:2629–2640.
19 Bolukbas FF, Bolukbas C, Horoz M, Gumus M, Erdogan M, Zeyrek F, *et al*. Child-Pugh classification dependent alterations in serum leptin levels among cirrhotic patients: a case controlled study. *BMC Gastroenterol* 2004; **4**:23.
20 Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, *et al*. Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2000; **95**:3584–3588.
21 Ben-Ari Z, Schaffer Z, Sulkes J, Manhaim V, Tur-Kaspa R, Fainaru M. Alterations in serum leptin in chronic liver disease. *Dig Dis Sci* 2002; **47**:183–189.
22 Comlekci A, Akpinar H, Yesil S, Okan I, Ellidokuz E, Okan A, *et al*. Serum leptin levels in patients with liver cirrhosis and chronic viral hepatitis. *Scand J Gastroenterol* 2003; **38**:779–786.
23 Zainab DM, Ed Wakil A. Hepatitis C in Egypt. *Am J Gastroenterol* 2006; **94**:1018–1022.
24 Egyptian Ministry of Health. Egyptian Ministry of Health Annual Report, 2007. Available at: *http://www.mohp.gov.eg/Main.asp* [Accessed 10 August 2007].
25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985; **28**:412–419.
26 Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, De Iasio R. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 2005; **90**:3498–3504.
27 Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, *et al*. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**:346–355.
28 Esmat G, Abouzied AM, Abdel-Aziz F, Strickland T. Subjects with chronic hepatitis C and genotype 4 have a similarly effective response to standard or pegylated interferon in combination with ribavirin [abstract]. *Hepatology* 2003; **38 (Suppl)**:324A.
29 Shobokshi OA, Serebour FE, Skakni L, Al Khalifa M. Combination therapy of peginterferon alfa-2a and ribavirin significantly enhance sustained virological and biochemical response rate in chronic hepatitis C genotype 4 patients in Saudi Arabia [abstract]. *Hepatology* 2003; **38 (Suppl)**:996A.
30 Thakeb FAI, Omar MM, El Awadi MM, Isshak SY. Randomized controlled trial of peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus genotype 4 among Egyptian patients [abstract]. *Hepatology* 2003; **38**:252A.
31 Hasan F, Asker H, Al-Khaldi J, Siddique I, Al-Ajmi M, Owaid S, *et al*. Peginterferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C genotype 4. *Am J Gastroenterol* 2004; **99**:1733–1737.
32 Diago M, Hassanein T, Rodes J, Ackrill AM, Sedarati F. Optimized virologic response in hepatitis C virus genotype 4 with peginterferonalpha2a and ribavirin. *Ann Intern Med* 2004; **140**:72–73.
33 Soriano V, Nunez M, Sanchez-Conde M, Barreiro P, Garcia-Samaniego A, Martin-Carbonero L, *et al*. Response to interferon-based therapies in HIV-infected patients with chronic hepatitis C due to genotype 4. *Antivir Ther* 2005; **10**:167–170.
34 Roulot D, Bourcier V, Grando V, Deny P, Baazia Y, Fontaine H, *et al*. Observational VHC4 Study Group. Epidemiological characteristics and response to peginterferon plus ribavirin treatment of hepatitis C virus genotype 4 infection. *J Viral Hepat* 2007; **14**:460–467.
35 Alberti A. What are the comorbidities influencing the management of patients and the response to therapy in chronic hepatitis C? *Liver Int* 2009; **29 (Suppl 1)**:15–18.
36 Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**:639–644.
37 Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; **29**:1215–1219.
38 Giannini E, Ceppa P, Botta F, Fasoli A, Romagnoli P, Cresta E, *et al*. Steatosis and bile duct damage in chronic hepatitis C: distribution and relationships in a group of Northern Italian patients. *Liver* 1999; **19**:432–437.
39 Giannini E, Ceppa P, Testa R. Steatosis in chronic hepatitis C: can weight reduction improve therapeutic efficacy? *J Hepatol* 2001; **35**:432–433.
40 Banerjee D, Williams EV, Ilott J, Monypenny IJ, Webster DJ. Obesity predisposes to increased drainage following axillary node clearance: a prospective audit. *Ann R Coll Surg Engl* 2001; **83**:268–271.
41 Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**:897–901.

42 Kamal SM, Fehr J, Roesler B, Peters T, Rasenack JW. Peginterferon alone or with ribavirin enhances HCV-specific CD4 T-helper 1 responses in patients with chronic hepatitis C. *Gastroenterology* 2002; **123**:1070–1083.

43 Martin SS, Qasim A, Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *J Am Coll Cardiol* 2008; **52**:1201–1210.

44 Myers MG, Cowley MA, Munzberg H. Mechanisms of leptin action and leptin resistance. *Ann Rev Physiol* 2008; **70**:537–556.

45 Huang JF, Yu ML, Dai CY, Hsieh MY, Hwang SJ, Hsiao PJ, *et al*. Pretreatment insulin sensitivity contributes to the treatment response to peginterferon plus ribavirin combination therapy for patients with chronic hepatitis C. *Hepatology* 2007; **46**:349A.

46 Bortoletto G, Realdon S, Dal Pero F, Gerotto M, Scribano L., Boninsegna S. Insulin resistance (IR) defined by the homeostasis model of assessment insulin resistance (HOMA-IR) index has a direct effect on early viral kinetics during pegylated-interferon therapy for chronic hepatitis C. *Hepatology* 2007; **46**:361A.

47 Nasta P, Gatti F, Puoti M, Cologni G, Bergamaschi V, Borghi F, *et al*. Insulin resistance impairs rapid virologic response in HIV/hepatitis C virus coinfected patients on peginterferon-alfa-2a. *AIDS* 2008; **22**:857–861.

48 Romero-Gomez M, Del Mar Viloria M, Andrade RJ, Salmeron J, Diago M, Fernandez-Rodriguez CM, *et al*. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**:636–641.

49 Conjeevaram HS, Kleiner DE, Everhart JE, Hoofnagle JH, Zacks S, Afdhal NH, *et al*. Race, insulin resistance and hepatic steatosis in chronic hepatitis C. *Hepatology* 2007; **45**:80–87.

50 Persico M, Capasso M, Persico E, Svelto M, Russo R, Spano D, *et al*. Suppressor of cytokine signaling 3 (SOCS3) expression and hepatitis C virus-related chronic hepatitis: insulin resistance and response to antiviral therapy. *Hepatology* 2007; **46**:1009–1015.

51 Poustchi H, Negro F, Hui J, Cua IH, Brandt LR, Kench JG, *et al*. Insulin resistance and response to therapy in patients infected with chronic hepatitis C virus genotypes 2 and 3. *J Hepatol* 2008; **48**:28–34.

52 Chu CJ, Lee SD, Hung TH, Lin HC, Hwang SJ, Lee FY, *et al*. Insulin resistance is a major determinant of sustained virological response in genotype 1 chronic hepatitis C patients receiving peginterferon alpha-2b plus ribavirin. *Aliment Pharmacol Ther* 2009; **29**:46–54.

53 Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, *et al*. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**:1499–1508.

54 Walsh MJ, Jonsson JR, Richardson MM, Lipka GM, Purdie DM, Clouston AD, *et al*. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. *Gut* 2006; **55**:529–535.

55 Gadina M, Hilton D, Johnston JA, Morinobu A, Lighvani A, Zhou YJ, *et al*. Signalling by type I and II cytokine receptors: ten years after. *Curr Opin Immunol* 2001; **13**:363–373.

56 Fartoux L, Poujol-Robert A, Guechot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 2005; **54**:1003–1008.

57 Romero-Gomez M. Insulin resistance and chronic hepatitis C. *World J Gastroenterol* 2006; **28**:7075–7080.

58 Manolakopoulos S, Bethanis S, Liapi C, Stripeli F, Sklavos P, Margeli A, *et al*. An assessment of serum leptin levels in patients with chronic viral hepatitis: a prospective study BMC. *Gastroenterology* 2007; **7**:17.

59 Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, *et al*. Recent advances in the relationship between obesity, inflammation and insulin resistance. *Eur Cytokine Netw* 2006; **17**:4–12.

60 Palmer C, Hampartzoumian T, Lloyd A, Zekry A. A novel role for adiponectin in regulating the immune responses in chronic hepatitis C virus. *Hepatology* 2008; **48 (No. 2)**:374–384.

61 Frese M, Schwarzle V, Barth K, Krieger N, Lohmann V, Mihm S, *et al*. Interferon-gamma inhibits replication of subgenomic and genomic hepatitis C virus RNAs. *Hepatology* 2002; **35**:694–703.

62 Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; **19**:65–91.