

## Improvement of steatosis after interferon therapy in HCV genotype 4 is related to weight loss

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### Abstract

**Introduction** Hepatic steatosis is common in patients with chronic hepatitis C virus (HCV) infection, and its occurrence may be related to both host and viral factors. Relationship between improvement in steatosis and response to anti-viral treatment remains unclear. This study assessed the factors associated with steatosis in patients infected with genotype 4 HCV, and to correlate degree of changes in steatosis with host factors and response to treatment.

**Methods** Records of 175 patients with chronic genotype 4 HCV infection, who had received interferon and ribavirin combination therapy, were reviewed retrospectively to extract data on body mass index (BMI), presence of diabetes mellitus, and liver histology findings. Paired BMI data and liver biopsies (pre- and 24-weeks post-treatment) were available in 86 patients. Baseline steatosis and its changes (before and after treatment) were the dependent variables in a univariate and multivariate analyses.

**Results** Steatosis was found in 88/175 (50.3%) of baseline biopsies. Its presence was related to baseline BMI ( $r=0.33$ ,  $P<0.01$ ), but not with viral load, or grade of liver inflammation or fibrosis. On follow up, improvement in steatosis was significantly associated with degree of weight loss but not with response to anti-viral treatment.

**Conclusion** Steatosis is common in genotype 4 HCV infection, and its presence appears to be related to high BMI, but not to viral load or degree of liver injury.

**Keywords** Combination interferon therapy · HCV genotype 4 · Obesity · Steatosis · Sustained viral response

### Introduction

Hepatic steatosis is a common feature of chronic hepatitis C virus (HCV) infection. It may be caused directly by the virus, as occurs in genotype 3 HCV infection, or may be associated with host factors like genetic (sex, HLA type, cytokine polymorphism),<sup>1–3</sup> obesity, diabetes mellitus with insulin resistance.<sup>4</sup> Some studies show that degree of steatosis is related to body mass index (BMI),<sup>5</sup> but others have failed to find such a relation.<sup>6</sup>

Though the results of antiviral therapy in chronic hepatitis C have improved during the last decade, these remain suboptimal.<sup>7</sup> Role of hepatocyte steatosis in interferon (IFN) resistance is still unclear. Steatosis was found to predict failure to achieve sustained virologic response (SVR) in patients with genotype 3 HCV infection,<sup>8</sup> but not in those with genotype 2a HCV infection.<sup>9</sup>

In this study, we looked at factors associated with the presence of steatosis, and its association with response to treatment in patients with genotype 4 HCV infection.

### Methods

This retrospective study included 175 patients who had been treated for genotype 4 HCV infection at our National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt with combined IFN–ribavirin therapy.

The study included previously untreated patients with a serologic, virological and histological diagnosis of chronic

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HCV (genotype 4) infection, and ALT level above the upper limit of normal. Patients with decompensated liver disease, low hemoglobin (<13 g/dL for men and <12 g/dL for women), white blood cell count <3000/ $\mu$ L, neutrophil count <1500/ $\mu$ L, platelet count <100,000/ $\mu$ L, HBsAg seropositivity, active schistosomiasis, elevated serum creatinine, poorly-controlled diabetes mellitus, hypertension, or psychiatric diseases were excluded. BMI (weight [Kg]/height [m]<sup>2</sup>) was calculated at weeks 0, 24, 48, 60, and 72. The study protocol conformed to the ethical guidelines of 1975 Declaration of Helsinki.

The patients had received standard or pegylated IFN $\alpha$ -2b (3 MIU thrice weekly or 100  $\mu$ g/week, respectively), along with ribavirin (800–1000 mg/day) for 48 weeks. If HCV RNA was detectable at 24 weeks, the treatment was stopped. Sustained viral response (SVR) was defined as loss of detectable HCV-RNA by qualitative RT-PCR at week 72 (24 weeks post-treatment).

Paired percutaneous liver biopsies (baseline and 24 weeks post-treatment) were available for 86 patients. Cores of at least 1–1.5 cm length or encompassing three portal areas in minimum were considered suitable for interpretation. The pathologist was unaware of clinical and biochemical data. Scoring system of Ishak *et al.*<sup>10</sup> (modified HAI and modified staging) was used for the assessment of necroinflammatory injury and fibrosis stage. Histological activity was considered as minimal (score 1–3), mild (4–8), moder-

ate (9–12) and severe (13–18). Fibrosis was staged separately on a scale 0–6, corresponding to no fibrosis (0), mild (1–2), moderate (3–4), and severe or cirrhosis (5–6).<sup>10</sup> Steatosis was graded based on the proportion of hepatocytes involved: mild (<33%), moderate (33–66%) and severe (>66%).<sup>11</sup> Histologic response was defined as a decrease of at least 1 point in the fibrosis (staging) or steatosis scores or of at least 2 points in the activity score (grading), relative to the baseline biopsy score.<sup>12,13</sup>

#### Statistical analysis

Data from patients with and without steatosis were compared using chi-squared test or Student's *t*-test. Multivariate regression analysis using steatosis and improvement of steatosis as dependent variables was done. Analysis of variance with repeated measures was used to compare BMI changes with difference in steatosis at histology. P value of <0.05 was considered significant.

#### Results

Table 1 shows the baseline characteristics of the 175 study patients (137 [78%] men). Thirteen (6.5%) patients had diabetes, and all had good control of blood sugar before and during the treatment. SVR was seen in 75 (43%) patients. Patients with steatosis were older and had higher BMI than those without steatosis. Presence of steatosis at baseline

**Table 1** Baseline characteristic features of the studied patients

		All cases (n=175)	No steatosis (n=87, 49.7%)	Steatosis (n=88, 50.3%)	P value	
		Range				
Age (years) (mean [SD])		39.8 (8.5)	21–58	38.3 (9.2)	41.2 (7.5)	0.02
Sex (M/F)		137/38		65/22	72/16	0.25
BMI kg/m <sup>2</sup> s (mean [SD])		27.8 (3.6)	20–36	26.8 (4.1)	29.1 (3.3)	<0.01
ALT (xULN) (mean [SD])		1.9 (1.0)	0.8–7	1.9 (1.1)	1.9 (1.0)	0.85
HCV-RNA load (IU $\times$ 10 <sup>6</sup> ) (mean [SD])		0.459 (0.56)	0.01–5	0.384 (0.36)	0.531 (0.70)	0.08
HAI (n [%])	Minimal	18 (10.3)		13 (15)	5 (6)	0.13
	Mild	116 (66.3)		54 (62)	62 (71)	
	Moderate/severe	41 (23.4)		20 (23)	21 (23)	
Fibrosis (n [%])	No or minimal	18 (10)		11 (13)	7 (8)	0.19
	Mild	133 (76)		61 (70)	72 (82)	
	Marked	24 (14)		15 (17)	9 (10)	
Interferon therapy (n [%])	PEG	91 (52)		46 (53)	45 (51)	0.81
	STD	84 (48)		41 (47)	43 (49)	
Response to treatment (n [%])	Failure	100 (57.1)		45 (52)	55 (63)	0.15
	SVR	75 (42.9)		42 (48)	33 (37)	

ALT – Alanine transferase; HAI – Histological activity index; PEG – Pegylated; STD – Standard; SVR – Sustained virological response

**Table 2** Changes in steatosis grade in paired liver biopsies in relation to response to combined IFN therapy

Steatosis changes	Failure of treatment (n=35)	SVR (n=51)	P value
Worse	4 (11.4)	6 (11.8)	0.77
Same	16 (45.7)	27 (52.9)	
Improved	15 (42.9)	18 (35.3)	

Data are as n (%)

SVR = Sustained viral response

biopsy had a significant correlation with BMI ( $r=0.33$ ,  $P<0.01$ ), but not with viral load ( $r=0.13$ ,  $P=0.08$ ), HAI ( $r=0.08$ ,  $P=0.31$ ) or fibrosis score ( $r=-0.01$ ,  $P=0.87$ ). In a multivariate regression analysis that included age and baseline BMI as possible predictors, baseline BMI was the only predictor of steatosis grade. ( $\beta=0.29$ ,  $P<0.01$ ).

All patients lost weight during IFN treatment as compared to baseline ( $P<0.05$  in each group separately by repeated measures), and gained weight during the follow up period. Weight reduction was associated with significant improvement of steatosis in patients with paired liver biopsies irrespective of SVR. Improvement in steatosis was associated with more weight loss as compared to the other two groups with stationary or worsened level of steatosis ( $P=0.03$ ).

## Discussion

Steatosis is common in patients with HCV infection and may be a major determinant of progression of liver injury in these patients.<sup>4</sup> The steatosis in this condition may be related either to presence of risk factors such as obesity, diabetes mellitus, alcohol intake, or to HCV infection itself.<sup>14</sup>

A significant correlation between steatosis grade and baseline BMI in our patients with genotype 4 HCV infection suggests that steatosis may be related to obesity. Similar results have been reported in patients with genotype 1 HCV infection.<sup>5</sup> In contrast, other workers have raised the possibility of a direct effect of specific viral sequences on the pathogenesis of lipid accumulation in genotype 3. Probably, the interaction of HCV core protein with the lipoprotein secretion pathways causes the characteristic alterations of lipid metabolism observed in HCV-related steatosis.<sup>4</sup>

In our study, IFN-based therapy was associated with weight loss. This finding has been reported previously and is mostly related to anorexia, a known side effect of IFN.<sup>10</sup>

Improvement of steatosis occurred in 42% of our patients; these results are similar to those reported by Castera *et al.*,<sup>14</sup> who reported improvement in 36% of genotype 3 patients with steatosis. The mechanism by which obesity may affect the viral response to treatment is not understood. Steatosis may cause a functional disturbance by decreasing the contact area between the antiviral drugs and the hepa-

cytes containing the virus,<sup>15,16</sup> thus causing limitation in antiviral drug efficacy. In addition, the degree of steatosis has been shown to correlate with the severity of fibrosis in previous studies.<sup>5</sup>

Although steatosis negatively affected the response rate to IFN-based anti-viral treatment in a previous study,<sup>17</sup> our results did not confirm this finding. Bressler *et al.*<sup>7</sup> included patients with genotype 3a in whom steatosis was evident at histology, and reported excellent response to antiviral therapy. Castera *et al.*<sup>14</sup> also reported similar finding in patients with genotype 3. This provides evidence for direct involvement of HCV genotype 3 in the pathogenesis of hepatic steatosis.

Reduction in BMI in our patients seems to be the possible explanation of improvement of steatosis in patients with genotype 4. Seyam *et al.*<sup>10</sup> reported that patients with genotype I infection with greater weight loss during therapy did not show higher success rate in therapy. In addition, they did not find any association of increased weight loss with age, gender, pre-treatment weight, ethnicity, pre-treatment histologic stage, cumulative IFN dose, HCV genotype, or treatment outcome. In contrast, weight reduction was irrelevant in patients with genotype 3, who had resolution of steatosis after successful antiviral treatment.<sup>18</sup>

The resolution of steatosis after successful antiviral treatment adds convincing evidence that steatosis is related to viral infection in genotype 3.<sup>4,18</sup> This does not seem to be the case in genotype 4 patients. Viral load in our patients did not correlate with baseline hepatic steatosis or with changes in steatosis in paired liver biopsies.

In conclusion, steatosis is common in chronic hepatitis C genotype 4. It is significantly correlated with baseline BMI, but not with inflammation, fibrosis or viral load at baseline. Steatosis improvement is related to weight loss, but not to response to therapy.

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