



The role of Adiponectin and Tumor necrosis factor in Hepatic Steatosis in patients with Chronic Hepatitis C

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Abstract

Objective: Hepatic steatosis is a common histological feature in chronic hepatitis C (CHC), but its pathogenic mechanisms are not completely understood. We aimed to evaluate the metabolic factors associated with hepatic steatosis and fibrosis in CHC patients, and the relation between metabolic syndrome (Met S) and CHC.

Patients and Methods: This study included 71 patients with CHC infection who underwent clinical, BMI, biochemical (assessment of insulin resistance, serum adiponectin, TNF- α , cholesterol and triglycerides), virological and histological assessments in addition to 24 age and sex matched healthy individual serve as a control group. All patients were referred to the Department of internal Medicine Al-Azhar Assiut University Hospital and to Sohag Specialized Liver Institute (in the period from April 2015 to May 2016).

Results: Significant steatosis (>33%) was detected in 54% of the patients, while 21.12% of the patients had stage 3/4 fibrosis. Higher degree of steatosis was significantly associated with higher Body Mass Index (BMI), serum insulin, Homeostasis Model of Assessment (HOMA) index and Tumor Necrosis Factor- α (TNF- α) (P <0.0001, P <0.0006, P <0.0001, and P <0.01 respectively). Higher stages of fibrosis were significantly associated with higher BMI and serum triglycerides (P <0.04, P <0.02 respectively). Multivariate analysis of the metabolic factors showed that HOMA index (P <0.001) and TNF- α (P <0.03) were the factors mostly predicting higher degree of steatosis. While, BMI index (P <0.01) and serum triglycerides (P <0.03) were the factors mostly predicting higher stage of fibrosis. We also found that CHC is closely related to Met S, and we recognized that older age (P <0.011), higher BMI (p <0.0001), lower serum adiponectin (P <0.0001), higher TNF- α (p <0.0001) and higher steatosis degree (p <0.04) were significantly associated with Met S in these patients.

Conclusions: Inpatients with CHC in Upper Egypt, steatosis is of metabolic origin as it is closely associated with the presence of metabolic syndrome. Higher BMI, HOMA-IR, lower serum adiponectin and higher serum TNF- α and triglycerides were associated with HCV hepatic steatosis and metabolic syndrome, while higher BMI and serum triglycerides are the predictors of more advanced fibrosis stage in CHC patients.

Keywords: chronic hepatitis C, body mass index, adiponectin, tumor necrosis factor- α and homeostasis model of assessment

Introduction

Hepatitis C virus (HCV) infection is an important public health problem because approximately 170 million people are infected worldwide. Chronic liver disease results from persistent infection in the majority of patients infected with the virus [1]. The Egyptian Demographic Health Survey (EDHS), a cross sectional survey including (HCV) biomarkers, was conducted in 2008 on a large nationally representative sample [2]. It estimated HCV prevalence among the 15-59 years age group to be 14.7%. Accordingly, Egypt has the highest HCV prevalence in the world [3]. Liver steatosis is a common finding in patients infected with hepatitis C virus (HCV) [4]. HCV steatosis was recently identified as a risk factor for progression to extensive fibrosis [5]. Chronic HCV infection is closely related to the metabolic syndrome (Met S). Accordingly, CHC should be classified into CHC with and CHC without Met S. Insulin resistance (IR) is the main feature of the Met S. In CHC, there is a close association between IR, hepatic steatosis [6], and progression of fibrosis [7].

The adipocytokine profile seems to play a distinct role, together with IR, in the pathogenesis of CHC [8]. Adiponectin modulates hepatic fat content and has an anti-steatotic effect on the liver [9]. Adiponectin is also a hepatic insulin sensitizer and has the opposite effect in comparison with tumor necrotic factor (TNF- α) on lipid metabolism, insulin sensitivity and inflammation [10]. In CHC, adiponectin levels are also associated with the degree of steatosis and insulin resistance [11]. Abnormalities of lipid metabolism such as the increase of serum triglyceride, cholesterol and LDL-cholesterol level and decrease in HDL-cholesterol may be the contributing factors in the development of NASH [12].

Aim of the work

We aimed to assess which host metabolic factors influence the degree of hepatic steatosis and fibrosis in patient with chronic HCV and to assess which metabolic factors related to virological parameters of HCV.

Patients and Methods

The present study included 71 patients (49 males and 22 females) with (CHC). Their ages ranged from 21-57 years. All patients were referred to the Department of Internal Medicine Al-Azhar Assiut University Hospital and to Sohag Specialized Liver Institute for doing liver biopsies and the complementary laboratory tests before starting treatment with pegylated interferon and ribavirin therapy at Sohag Specialized Liver Institute. Before inclusion in the study, all participants gave informed consents and the study protocol was approved by the Local Ethics Committee.

In addition, 24 age and sex matched healthy individuals were also included to serve as a control group.

Patients were diagnosed as chronic hepatitis C based on clinical data, positive anti-HCV by ELISA test and HCV RNA by PCR for more than 6 months.

Exclusion criteria

Patients were excluded if they have any of the following:

- Hepatitis B virus co-infection,
- Autoimmune disease.
- Alcohol consumption.
- Decompensated liver disease.
- Pregnancy or breast feeding.
- Medical diseases as renal or ischaemic heart disease, significant retinal abnormality, uncontrolled neuropsychiatric disease, immunologically mediated disease, or organ transplantation

Methods

The following were done to all patients:
 Complete history taking and full clinical examination.
 Body mass index was calculated from patients' weight: patients' weight (Kg)/ patients' height (meter)².

Investigations

1) Laboratory

- a) Liver function tests: serum albumin, ALT, AST, total bilirubin, direct & indirect bilirubin, prothrombin time and concentration by standard methods.
- b) Fasting serum glucose, creatinine, and complete blood picture.
- c) Polymerase chain reaction (PCR) for HCV.

- d) Serum cholesterol and triglycerides.
- e) Measurement of serum adiponectin and serum TNF- α .
- f) Fasting serum insulin to calculate HOMA index.

2) Abdominal ultrasonography

3) Histopathological assessment: Seventy one liver biopsies were included in the study and submitted to histopathological examination. Hematoxylin and eosin (H&E) stained sections were done to assess both the grade and the stage of chronic viral hepatitis, in addition to the degree of steatosis. Metavir grading and staging systems were used.

Statistical analysis

Data were computed and analyzed using STATA intercooled version 9.2. Quantitative data were analyzed using ANOVA test and Post Hoc Bonferroni test for comparison of the means of three groups. When the data were not normally distributed Kruskal–Wallis rank test and Mann-Whitney test were used. Qualitative data were compared using Chi square test. P value was considered significant if it was less than 0.05. Both univariate and multivariate analyses were used to determine risk factors (predictors) of significant steatosis (moderate and severe), and the risk factors (predictors) of advanced fibrosis (stage 3 and 4).

Results

This study was conducted on 71 patients with chronic hepatitis C who fulfilled the study's inclusion criteria. The patients were predominantly males (49 males and 22 females), with ages ranging from 20 - 57 years and a mean age of 41.86 \pm 9.70. Besides, 24 persons served as controls (18 males and 6 females), with ages ranging from 22-50 years and a mean age of 41.75 \pm 7.66. According to the histopathology reports, the studied patients were classified according to the degree of steatosis into 2 groups: Steatosis group 1: includes 33 patients (46.5%) with chronic hepatitis C and steatosis < 33%. Steatosis group 2: includes 38 patients (53.5%) with chronic hepatitis C and steatosis > 33%.

The same patients were also categorized according to the fibrosis score into 3 groups: Fibrosis group 1: includes 40 patients (56.3%) with fibrosis score F 0/1. Fibrosis group 2: includes 16 patients (22.5%) with fibrosis score F2. Fibrosis group 3: includes 15 patients (21.1%) with fibrosis score F $\frac{3}{4}$.

Table 1: Comparison between each steatosis group and controls

Characteristic		Steatosis group 1 N=33	Steatosis group 2 N=38	Controls N=24	Steatosis group 1 versus group 2	Steatosis group 1 versus controls	Steatosis group 2 versus controls
Age	Mean \pm SD	39.88 \pm 10.35	43.62 \pm 8.85	41.75 \pm 7.66	0.11	0.52	0.09
Gender (n,%)	Males	25 (75.76%)	24 (64.86%)	16 (66.6%)	0.32	0.09	0.36
	Females	8 (24.24%)	13 (35.14%)	8 (33.3%)			
DM	No (n = 47)	27 (81.81%)	20 (52.6%)		0.17		
	Yes (n = 24)	6 (18.19%)	18 (47.4%)				
Body mass index (BMI) Mean \pm SD		25.60 \pm 3.42	29.2 \pm 3.57	24.33 \pm 1.82	0.0001	0.23	0.0001
ALT (IU/L) Mean \pm SD		62.45 \pm 41.49	72.89 \pm 79.01	32.58 \pm 5.14	0.83	0.001	0.0002
AST (IU/L) Mean \pm SD		47.45 \pm 22.03	63.89 \pm 67.06	29.17 \pm 5.89	0.24	0.004	0.0001
Triglyceride (mg/dl) Mean \pm SD		115.06 \pm 30.82	139.86 \pm 34.72	96.25 \pm 11.69	0.003	0.046	0.0001
Cholesterol (mg/ dl) Mean \pm SD		142.55 \pm 29.85	153.68 \pm 39.78	130 \pm 21.85	0.19	0.19	0.06
Adiponectin (ng/ml) Mean \pm SD		26.45 \pm 4.50	20.49 \pm 5.01	28.45 \pm 4.03	0.0001	0.19	0.0001
TNF- α (pg/ml) Mean \pm SD		100.29 \pm 41.25	128.01 \pm 47.21	49.47 \pm 9.58	0.01	0.0001	0.0001
Insulin (μ U/ml) Mean \pm SD		1.83 \pm 1.51	3.44 \pm 2.11	1.84 \pm 1.86	0.0006	0.99	0.02
HOMA Mean \pm SD		1.67 \pm 0.66	3.44 \pm 0.77	1.42 \pm 0.40	0.0001	0.32	0.0001
Viral load Mean \pm SD		760591.5 \pm 1205421	523987.7 \pm 992435.9		0.71		

N=number

When steatosis groups were compared regarding their laboratory data, we found that BMI, serum insulin and HOMA index were significantly higher in steatosis group 2 than group 1 (P= 0.0001, P= 0.0006 and P= 0.0001, respectively). Serum adiponectin was significantly higher in steatosis group 1 (P= 0.0001), while serum TNF- α was higher in steatosis group 2 (P= 0.01). Serum lipogram shows significantly higher triglycerides in steatosis group 2 (P= 0.003). While no significant difference was found between the two groups as regards age, gender, ALT, AST, serum cholesterol and viral

load. Patients in steatosis group 1 have significantly higher serum ALT and AST levels (P= 0.001, P= 0.004 respectively), TNF- α (P= 0.0001), and serum triglycerides (P= 0.046), compared to the controls. Patients in steatosis group 2 have higher BMI (P= 0.0001), ALT, AST levels (P= 0.0002,P= 0.0001 respectively), serum triglycerides, serum TNF- α , HOMA index, (P= 0.0001 for each), and higher serum insulin (P= 0.02) compared to the controls. On the other hand, serum adiponectin was significantly lower in steatosis group 2 than the controls (P= 0.0001) (Table 1).

Table 2: Laboratory and clinical results of studied groups within each stage of fibrosis

Characteristic	Stage 0/1 N=40	Stage 2 N=15	Stage 3/4 N=15	Comparison Between different stages of fibrosis	Stage 0/1 versus stage 2	Stage0/1 versus stage 3/4	Stage 2 versus stage 3/4
Age Mean \pm SD	39.72 \pm 10.09	43.2 \pm 8.18	46.2 \pm 8.84	0.07	0.69	0.08	1.00
Body mass index (BMI) Mean \pm SD	25.02 \pm 3.45	25.69 \pm 3.20	29.07 \pm 3.93	0.04	1.00	0.01	0.01
ALT (IU/L) Mean \pm SD	56.05 \pm 31.27	66.4 \pm 42.39	101.33 \pm 118.33	0.27	0.26	0.16	0.68
AST (IU/L) Mean \pm SD	46.73 \pm 18.82	49.4 \pm 19.56	88 \pm 101.25	0.19	0.56	0.07	0.31
Triglyceride (mg/dl) Mean \pm SD	119.08 \pm 32.14	133.87 \pm 35.25	146.73 \pm 35.74	0.02	0.45	0.03	0.89
Cholesterol (mg/dl) Mean \pm SD	147.38 \pm 34.59	163.67 \pm 40.21	136 \pm 29.89	0.09	0.39	0.86	0.10
Adiponectin (ng/dl) Mean \pm SD	23.19 \pm 4.45	23.25 \pm 5.52	24.19 \pm 5.38	0.79	1.00	1.00	1.00
TNF- α (pg/dl) Mean \pm SD	106.21 \pm 44.09	117.89 \pm 54.85	135.27 \pm 38.51	0.11	1.00	0.12	0.90
HOMA Mean \pm SD	2.49 \pm 1.14	3.03 \pm 1.23	2.50 \pm 1.04	0.31	0.14	1.00	0.24
Viral load (copies/ml) Mean \pm SD	679725.9 \pm 1325751	478240.5 \pm 724249.2	674961.4 \pm 684944.6	0.44	0.56	0.30	0.27

The clinical characteristics of studied population within each stage of fibrosis were shown in Table 2. We did not find any significant difference between the 3 groups of the studied population as regards serum ALT, AST, adiponectin, TNF- α

and HOMA index. While a significant difference was found in BMI, and serum triglycerides being highest in stage 3/4 fibrosis (P= 0.04,P= 0.02 respectively).

Table 3: Multivariate analysis for the factors predicting higher degree of steatosis

Variables	Odds ratio (95% Confidence Interval)	P value
TNF- α	0.97 (0.94-0.0.99)	0.03
HOMA index	0.004 (0.00-0.13)	0.001

Table 4: Multivariate analysis for the factors predicting higher stage of fibrosis

Variables	Odds ratio (95% Confidence Interval)	P value
BMI	0.91 (0.83-0.99)	0.01
Triglycerides	32.04 (1.45-706.02)	0.03

Multivariate analysis of the factors mostly predicting higher degree of steatosis, showed that TNF- α (P= 0.03) and HOMA index (P= 0.001) are the factors mostly predicting higher degree of steatosis (Table 3). Multivariate analysis of the

factors mostly predicting higher degree of fibrosis showed that BMI index (P= 0.01) and serum triglycerides (P= 0.03) are the factors mostly predicting higher stage of fibrosis (Table 4).

Table 5: Characteristics of CHC patients according to the presence or absence of metabolic syndrome

Variables	Metabolic syndrome		
	Absent	Present	P
Number	46	25	
Age	46.08 \pm 4.32	48.68 \pm 3.84	0.011
Gender	Males: (n, %)	32 (65%)	17 (35%)
	Females: (n, %)	14 (64%)	8 (36%)
BMI	24.24 \pm 1.74	28.8 \pm 3.5	0.0001
Blood pressure >130/85 mmHg (n,%)	22 (47.8)	21 (84)	0.003
ALT (IU/L)	57.56 \pm 44.23	74.41 \pm 76.22	0.21
AST (IU/L)	46.33 \pm 26.01	62.45 \pm 64.38	0.14
Serum triglycerides (<150 mg /dl)	118.25 \pm 78	178.85 \pm 45	0.003
Total serum cholesterol (mg/dl)	132 \pm 26.18	157 \pm 32.33	0.0007
HDL-cholesterol (mg/dl)	< 40 (M)	41.2 \pm 2.1	38.5 \pm 2.45
	< 50 (F)	52.34 \pm 6.23	50.58 \pm 5.66

Adiponectin (ng/dl)		27.33 ± 4.7	21.02 ± 3.7	0.0001
TNF- α (pg/dl)		98.54 ± 31.29	130.05 ± 39.65	0.0001
Fasting glucose (mg/dl)		99.04 ± 2.41	116.23 ± 3.65	0.0001
Fasting insulin (μ U/ml)		1.78 ± 1.4	3.18 ± 2.2	0.002
HOMA-IR		1.4 ± 0.54	3.66 ± 0.78	0.0001
Steatosis degree: (n, %)	Group1:	26/33 (78.9%)	7/33 (21.2%)	0.04
	Group2:	20/38 (52.63%)	18/38 (47.4)	
Fibrosis: (n, %)	F 0/1: (n= 40)	30 (75%)	10 (25%)	0.64
	F 2: (n= 16)	10 (63%)	6 (37%)	
	F 3/4: (n= 15)	11 (73%)	4 (27%)	

To assess whether hepatic steatosis in the current series is related to the presence of metabolic syndrome, patients were re-categorized into a group without Met S (n=46) and a group with Met S (n=25). We found 12 variables; patients with older age, higher BMI, elevated blood pressure more than 130/85 mmHg, higher serum triglycerides, total serum cholesterol, HDL-cholesterol in males, serum TNF- α , fasting blood glucose, fasting serum insulin, HOMA-IR, steatosis degree and lower serum adiponectin were significantly related to the Met S (Table 5).

Discussion

Chronic hepatitis C has many features which suggest that this disease must be viewed not only as a viral disease, but also as a metabolic liver disease which implies insulin resistance [13], and high prevalence of steatosis [14]. Hourigan *et al* [15] found a significant relationship between hepatic fibrosis and steatosis, suggesting that in chronic HCV infection steatosis may play a role in disease progression.

Our results showed that significant hepatic steatosis (affecting >33% of cells) was present in 53.5% of studied CHC patients. Hepatic steatosis in our study was significantly higher than that reported in Greek patients with CHC genotype 4 where 26.4 % of them showed significant steatosis [16].

In the current study, patients with different severity of steatosis did not show significant difference in their mean age. This was in agreement with El-Zayadi *et al* [17] who demonstrated that age is not significantly correlated with steatosis among HCV genotype 4 infected patients. In our study, we found no relation between hepatic fibrosis and age, unlike Hu *et al* [18] who declared that older patients had advanced stages of fibrosis.

Our study showed a significant association between BMI and the severity of steatosis. This agrees with Hu *et al* [18] and Negro and Sanyal [19] who found that BMI plays an important role in steatosis in patients with HCV, and disagrees with Adinolfi *et al* [8] who found that steatosis was not significantly associated with BMI in the overall cohort study of HCV infected patients.

In our study, we found a significant relation between BMI and stages of fibrosis. And this agrees with Hourigan *et al* [15] and Hu *et al* [18].

We found that type 2 DM was present more in steatosis group 2 (47.4%) than in steatosis group 1 (18.19%), but with no statistically significant difference. El-Zayadi *et al* [17] also declared that DM was not significantly associated with hepatic steatosis in HCV genotype 4 patients. We as well as others, Castera *et al* [19] and Negro and Sanyal [20] also found a significant association between levels of serum triglycerides

and the degree of steatosis in patients with CHC. This disagrees with Hu *et al* [21] who revealed in their cohort of patients with chronic hepatitis C, that hypertriglyceridemia was not significantly associated with hepatic steatosis.

In our study, we found a significant relationship between the serum triglycerides and fibrosis stage. This was in agreement with Hu *et al* [18] and in contrast to Solis-Herruzo *et al* [22] who failed to confirm this relation.

In the current study, mean serum adiponectin level was significantly lower in patients than controls. Also, it was significantly lower in steatosis group 2 than steatosis group 1. On the other hand, CHC patients showed significantly higher TNF- α level than the control. Also, TNF- α was significantly more raised in steatosis group 2 than steatosis group 1. The same result was also reported by Durante-Mangoni *et al* [23] who found that low level of adiponectin and elevated level of TNF- α were independently associated with grades of steatosis and HOMA-IR.

The elevated level of TNF- α had a direct relationship to the progression of fibrosis in CHC patients [24]. Lu *et al* [25] found that serum level of adiponectin did not differ significantly between healthy subjects and patients with HCV infection.

In the current study, higher serum insulin level was found in more advanced steatosis than in milder group and controls. Also, we found that insulin resistance (IR) measured by HOMA-IR was significantly higher in CHC patients than control, and it was significantly higher in steatosis group 2 than in steatosis group 1. This was in agreement with Cua *et al* [11] and Lawrence and Jacqueline [26] who demonstrated that patients with HCV have more insulin resistance than those without. This also agrees with Younossi *et al* [27] who demonstrated that patients with HCV have more insulin resistance and poor response to treatment. In contrast to our study, Muzzi *et al* [28] delineated in their cohort study that the level of insulin resistance was not correlated with hepatic steatosis in HCV infected patients.

In the present study, no correlation was found between insulin resistance as measured by HOMA-IR and hepatic fibrosis in CHC patients, and this conforms with Grigorscu *et al* [29] who revealed a lack of correlation between IR and fibrosis. In contrast to D'Souza *et al* [30] who reported that IR plays an important role in hepatic fibrosis in CHC patients, irrespective of the genotype.

Multivariate analysis of our data revealed that TNF- α (P= 0.03) and HOMA index (P= 0.001) are the most independent factors predicting hepatic steatosis in patients with CHC. Steatosis was not an independent factor associated with fibrosis, and only BMI (P= 0.01) and triglycerides (P= 0.03) were independently predict advanced fibrosis.

Our findings disagrees with Rubbia-Brandt *et al* [31] and

Cholet *et al* [4] who reported a significant association between steatosis and high stage of fibrosis.

Our results showed that steatosis was significantly associated with the presence of MS in CHC patients. This was also reported by Grigorscu *et al* [29] in patients with HCV genotype 1.

This together with the absence of a significant association between viral load and degree of steatosis in our study strongly suggest that steatosis in the current series is due to metabolic origin.

Conclusion

In patients with CHC, higher BMI, HOMA-IR, higher serum TNF- α , triglycerides and lower serum adiponectin were associated with HCV hepatic steatosis and metabolic syndrome, while higher BMI and serum triglycerides were associated with more advanced fibrosis stage.

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