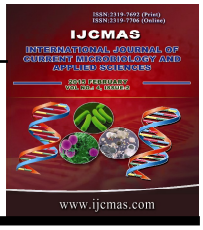
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 770-784

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 770-784**
http://www.ijcmas.com



Original Research Article

Assessment of host metabolic factors: Adiponectin, TNF-α and Insulin resistance influence on the degree of hepatic steatosis in patients infected with Hepatitis C virus

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Keywords

Adiponectin, Insulin resistance, Hepatic steatosis, Chronic hepatitis C.

A B S T R A C T

Hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide. Hepatic steatosis is a common histological feature of HCV infection where host factors , namely obesity and insulin resistance, seem to play the major role in HCV genotype non-3 infection. This work aimed to assess host metabolic factors (adiponectin, TNF-α and insulin resistance) influence on the degree of hepatic steatosis in HCV patients. Fifty chronic HCV patients and 25 healthy controls included in the study. To all studied subjects anthropometric measurements and laboratory investigations were done. In addition to estimation of serum levels of adiponectin and TNF-α . Liver biopsy was done for all patients. Lower significant levels of Adiponectin with high significant TNF-α levels were found in CHC patients than controls. Significant inverse correlations between adiponectin level and TNF-α, HOMA-IR, BMI, age and TG were found with Significant direct correlations of HOMA-IR with TNF-α, BMI, FBS, insulin level, TG and age were also found . Significantly higher levels of TNF-α , HOMA-IR index with significant lower adiponectin were found in patients with severe steatosis than mild steatosis . High prevalence of IR occurred in HCV patients. HOMA-IR index was more sensitive than adiponectin in distinguishing between patients with steatosis than without. Conclusion: The presence of steatosis in CHC patients is associated with the presence of host metabolic risk factors (high BMI, visceral obesity, HOMA-IR , low adiponectin and high inflammatory cytokine TNF-α).

Introduction

Hepatitis C virus (HCV) infection is a major global health issue. Estimates indicate that three to four million persons are newly infected each year, 170 million people are chronically infected and death in about 20%-25% of cirrhotic cases. (Perz *et al.*, 2006; Sangiovanni *et al*., 2006; Chen *and Morgan*, 2006) HCV infection represents a leading indication for liver transplantation. HCV-associated cirrhosis appears to be associated also with the development of HCC in 1%-5% of the cases. (Hanafiah *et al.*, 2013) Egypt has the highest prevalence of HCV in the world, estimated nationally at 14.7%. (Mohamoud *et al*., 2013)

Hepatic steatosis, defined as excessive lipid accumulation in the cytoplasm of hepatocytes, is a frequent histological feature and established risk factor for disease progression in patients with chronic hepatitis C (CHC) infection. The reported prevalence of steatosis in patients with CHC varies between 40% and 80%. (Vanni *et al*., 2009; Goodman *et al*., 1995) In CHC, there are two types of steatosis: A) Metabolic steatosis, which is consequence of metabolic factors like alcohol consumption and obesity. B) Viral steatosis that may result from a direct viral cytopathic effect. However, even "metabolic" steatosis can be partially an indirect consequence of viral infection, since HCV induces a metabolic deregulation with insulin resistance (IR). (Adinolfi *et al*., 2001; Monto *et al*., 2002) Obesity is a well-recognized risk factor for the development of steatosis (mainly nonalcoholic steatohepatitis (NASH), and of fibrosis in HCV infected patients. Visceral fat distribution rather than BMI proved to be associated with HCV related steatosis. (Lonardo *et al*., 2004).

Insulin resistance (IR) is a frequent feature of CHC. Whether IR could be the cause or consequence of steatosis and fibrosis is unknown. (Fartoux *et al.*, 2005) In CHC, there is a close association between IR, hepatic steatosis, progression of fibrosis, adipocytokine profile and a lower rate of sustained virological response (SVR). (Moucari *et al*., 2008; Camma *et al*., 2006; Peres *et al*., 2013) The presence of viral particles in the liver is regarded as the origin of the development of IR, through both direct and indirect pathways, affects the insulin signaling pathways, promoting IR at a cellular level. (El Zayadi *and Anis*, 2012) It has been also suggested that increased levels of pro-inflammatory cytokines such as interleukin 1, TNF-α, IL-6 and leptin, and reduced levels of adiponectin may directly contribute to the occurrence of HCV-related IR, thereby to promote hepatic inflammation and fibrosis and a lower SVR rate. (Harrison, 2008) Over the last decade, adipose tissue has been found to play role as an endocrine organ, where secretes variety of hormones including adiponectin and leptin, which may contribute to the development of metabolic abnormalities. (Adinolfi *et al*., 2001) Adiponectin was found to have a role in the development of steatosis in CHC, where it modulates hepatic fat content and has an anti-steatotic effect on liver. It is considered that adiponectin inhibits triglyceride accumulation in the hepatocytes by reducing their free fatty acids production and increasing beta-oxidation (You *et al*., 2005).

Moreover, adiponectin is a hepatic insulin sensitizer and exerts anti-inflammatory effects by opposing the synthesis and release of TNF-α from macrophages within adipose tissue. (Tsochatzis *et al*., 2006; Kamada *et al*., 2003) Tumour necrosis factor α (TNF-α) is another adipocytokine that has numerous effects on adipose tissue, the primary action of TNF-α is to increase insulin resistance in liver; muscle and adipose tissue. Additional actions on the liver include increased free fatty acids (FFA) production and cholesterol synthesis and decreased glucose uptake and FFA storage. (Prins *et al*.,1997; Poirier *et al*., 2006) TNF-α is reported to be raised in serum of patients with fatty liver diseases, it modulates IR and seems to play a proinflammatory role in NAFLD (Wigg *et al*., 2001; Crespo *et al*., 2001)

Adiponectin and TNF-α have opposed effects on lipid metabolism, insulin sensitivity and inflammation. Serum adiponectin level modulates that of TNF-α, thus their balanced activity is important for the metabolic homeostasis. (Diehl *et al.*, 2005) In addition, hypoadiponectinaemia is

independently associated with IR and thus, in turn, is strictly associated with the development of steatosis. It is likely that excess FFA flux due to peripheral IR may induce hepatic steatosis. On the other hand, excess fat deposition in the liver may render hepatocytes less sensitive to insulin action and lead to hepatic IR which occur in early stages of course of HCV infection before the development of cirrhosis (Dixon *et al*., 2001; Petit *et al*., 2001).

The objective of this study was to assess whether host metabolic factors influence the degree of hepatic steatosis in patients infected with HCV by investigating the role of adiponectin, TNF-α and insulin resistance.

Patients and methods

Fifty chronic hepatitis C patients (CHC) were enrolled in this study; they were admitted to Internal Medicine department in Medical Research Institute, Alexandria University.. Exclusion criteria included: (a) Other causes of chronic liver disease such as HBV (b) Decompensated liver disease (c) History of diabetes (d) Previous treatment with metformin or thiazolidinedione. The research study was approved by the Ethics Committee and written consent to participate in this study was taken from all patients.

Twenty-five healthy control males were also included in this study, they were age and sex matched.

All subjects were subjected to History taking and complete clinical evaluation. Baseline characteristics collected including age, sex, height, weight, waist circumference and body mass index (BMI; kg/m^2) was calculated (Hickman *et al*., 2003).

Laboratory Investigations

Assays were carried out on plasma and serum samples collected after overnight fasting and stored at a temperature less than-20°C until use.

-Lipid profile was done by determination of serum cholesterol, high density lipoprotein-cholesterol (HDL-C) , low density lipoprotein- cholesterol (LDL-C) and serum triglyceride (TG) levels using auto-analyzer Olympus AU400. (Mizoguchi *et al*., 2004; Warnick *et al*., 2001; McGowan *et al*., 1983).

- Liver enzymes were measured including serum levels of alanine and aspartate aminotransferases (ALT and AST respectively). (Bergmeyer *et al*., 1980; Rej *et al*., 1983).

- Serum glucose concentration was estimated without deproteinization using enzymatic colorimetric method. (Trinder *et al*., 1980).

Serum level of insulin was quantitatively determined using ELISA technique (DRG instruments GmbH, Germany EIA-2935) which is a solid phase ELISA based on the sandwich technique (Evena *et al*., 2007). The degree of insulin resistance was calculated according to the homeostasis model assessment for insulin resistance [HOMA-IR] measured by multiplying fasting serum insulin (microunits per milliliter) and fasting plasma glucose (micromoles per liter) divided by 22.5. (Mathews *et al*., 1985) Serum adiponectin concentration was measured by using commercial ELISA (human adiponectin ELISA kit; Quantikine DRP300, R&D Systems, Minneapolis, USA).

Plasma TNF-α was measured by using Quantikine ELISA kit. Manufactured by R&D systems Inc. Minneapolis, USA

ELISA for the presence of a hepatitis C virus antibody (anti-HCV using Murex anti-HCV (version III test).

- HCV RNA quantitation by Real Time PCR: All anti-HCV-positive samples were submitted to RNA extraction using Qiagen QIAamp viral RNA mini spin protocol. HCV-RNA was amplified using Artus HCV QS-RGQ PCR Kit reagents based on the amplification and simultaneous detection of a specific a 240 bp region of the 5' UTR of the HCV genome using real-time RT-PCR with TaqMan assay. A heterologous internal control (IC) is also amplified to check for possible PCR inhibition. The linear range of the test is from 15 IU/ml to 69,000,000 IU/ml. The following was used: Incubation at 50°C for 30 min to reverse transcribe viral RNA to Complementary DNA (cDNA) by RT. This was followed by AmpliTaq gold activation for 95° C for 10 min, followed by 45 cycles of three PCR-step amplification, denaturation for 95° C for 30 sec, followed by annealing at 50° C for 1 min and extension at 72° C for 30 sec, with end point fluorescence detection.

Liver biopsy was done for all patients for diagnostic purposes. Liver specimens were formalin-fixed and paraffin-embedded for histological evaluation. (Brunt *et al*., 1999)

Steatosis was graded by percentage of cells showing fatty changes. Grading was made according to macrovesicular steatosis as:

Grade 0: Absent or minimal (less than 5% of hepatocytes involved)
Grade 1: Mild (5-30% of hepatocytes involved)
Grade 2: Moderate (30-60% of hepatocytes involved)
Grade 3: Severe (60% of hepatocytes involved)

Statistical analysis

It was performed with SPSS software. All text and table values are expressed as means ± S.D. For analysis of parameters, analysis of variance (ANOVA) was used to address differences between groups. Univariate analysis was done by chi-square test for frequencies and by Mann–Whitney rank-sum test for means. For multivariable analysis, when steatosis was used as dependent variable (i.e. absence vs. presence of steatosis), we considered as possibly independent variables as body mass index (BMI), HOMA score, plasma adiponectin level, plasma TNF-□ level and triglyceride. The sensitivity (Sn), specificity (Sp), positive predicative value (PPV), negative predictive value (NPV) and accuracy calculated for adiponectin and HOMA by using ROC curves. Pearson's correlation coefficients were used to test the correlation between variables. (P values less than 0.05 were considered to be statistically significant). (Puri, 2002)

Result and Discussion

It was performed with SPSS software. All text and table values are expressed as means ± S.D. For analysis of parameters, analysis of variance (ANOVA) was used to address differences between groups. Univariate analysis was done by chi-square test for frequencies and by Mann–Whitney rank-sum test for means. For multivariable analysis, when steatosis was used as dependent variable (i.e. absence vs. presence of steatosis), we considered as possibly independent variables as body mass index (BMI), HOMA score, plasma adiponectin

level, plasma TNF- level and triglyceride. The sensitivity (Sn), specificity (Sp), positive predicative value (PPV), negative predictive value (NPV) and accuracy calculated for adiponectin and HOMA by using ROC curves. Pearson's correlation coefficients were used to test the correlation between variables. (P values less than 0.05 were considered to be statistically significant). (Puri, 2002)

Infection with HCV is a leading cause of chronic liver disease worldwide. HCV infection is not confined to the liver, but can induce disturbances in many other organs and systems. (Alter *et al*., 2000; Koike, 2005) CHC 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: IR, high prevalence of steatosis, increased prevalence of impaired glucose tolerance, type 2 DM, changes in lipid metabolism and high triglycerides level. These findings together suggest that chronic HCV infection is closely related to the metabolic syndrome (MS) (Grigorescu *et al*.,2008).

The aim of this work was to assess whether host metabolic factors influence the degree of hepatic steatosis in patients infected with HCV by investigating the role of adiponectin, TNF-α and insulin resistance.
Insulin resistance, the major feature of the metabolic syndrome, is also common in chronic HCV infection which could be caused by an interplay between viral and host factors. HCV infection per se generates multiple defects in hepatic insulin signaling pathways. The major role of HCV in IR development is also supported by the identification of IR in patients with normal BMI (Yoneda *et al.,* 2007). In the present study, IR was present significantly in the majority of HCV patients included. Moucari R *et al*. (2008) reported that in a large cohort study IR was specifically associated with HCV infection independently of metabolic factors and severity of liver disease which agrees with this study. There was no significant relation between HCV-RNA and HOMA-IR found in the present study, which was similar to the findings of several studies (Grigorescu *et al*., 2008; Durante-Mangoni *et al*., 2006; Lo Iacono *et al*., 2007) where they did not find an association between viremia and HOMA-IR as well.

However, Moucari R *et al.* (2008) and Hsu Cs *et al.* (2008) were able to demonstrate a direct role of viral replication in IR development, establishing a significant correlation between HOMA-IR and HCV-RNA levels, which oppose the results of the present study.

The result of the present study found a significant elevation of serum level of TNF-α and a significant decrease in plasma adiponectin level in CHC patients when compared to control. This was similar to previous findings Eguchi Y et al. (2009) whose hypothesis stated that serum TNF-α, TNF-α receptors and adiponectin may be partly related to the increase in IR in HCV infected patients, similar to the case in many previous studies. (Cua *et al*., 2007; Knobler *et al*., 2003) These results together with the present study indicate that the progression of IR owing to the increased TNF-α activity may be enhanced by a decrease in adiponectin in HCV-infected patients with visceral obesity. In fact, previous studies have reported that HCV infection itself induces insulin resistance, which in turn causes oxidative stress and accelerates progression of liver fibrosis (Fartoux *et al.,* 2005; Muzzi *et al*., 2005). Diehl AM *et al.* (2005) stated that Adiponectin antagonizes both production and activity of TNF-α and on the other hand, TNF-α itself inhibits adiponectin moreover, the combination of low adiponectin and high TNF-α levels results in hepatic steatosis and IR (Diehl *et*

al., 2005). This agrees with the present study where adiponectin inversely correlated with HOMA-IR. It is hypothesized that imbalanced production of fat derived cytokines, such as adiponectin and TNF-α, occurs in CHC that is associated with IR. The adipocytokine profile seems to play a distinct role, together with IR, in the pathogenesis of CHC infection (Adinolfi *et al*., 2001). The cytokines produced by adipose tissue have a pivotal role in modulating inflammatory response and insulin sensitivity and contribute to the development of metabolic abnormalities. The results of the present study strongly suggest that HCV infection is a risk factor for the development of IR, particularly in patients with visceral obesity.

Many studies have suggested that the development of IR is affected by direct interference of HCV with the insulin cascade; this functional impairment of the insulin cascade is enhanced through increased levels of pro-inflammatory cytokines, including TNF-α. IR occurs very early in HCV infection, in parallel with an elevation in TNF-α level. HCV infection promotes IR mainly through increased TNF-α that inhibit insulin receptor and IRS tyrosine phosphorylation (Kawaguchi *et al*., 2004; Shintani *et al*., 2004). The results of this study demonstrated that CHC patients with steatosis had reduced serum levels of adiponectin, with significant inverse correlation between adiponectin level and steatosis grade, HOMA index, BMI, waist circumference and TNF-□. These results were in agreement with Petit JM *et al* (2001) and Ashour E *et al.* (2010) who reported an association between serum levels of adiponectin and HCV related steatosis. CHC patients have the lowest levels of adiponectin that inversely correlated with severity of steatosis which lead to increased serum free fatty acids, which are then taken up by hepatocytes. In a study done by Lopez-Bermejo A *et al.* (2004) , they found that adiponectin is inversely correlated with BMI, intraabdominal fat and indices of insulin resistance. Growing evidence suggests that adiponectin can regulate lipid and glucose metabolism and lipid fat content in hepatocytes (Yamauchi *et al*., 2001). Tsochatzis E *et al.* (2007) declared that the actions of adiponectin on the liver are to oppose fatty acid synthesis, and promote mitochondrial oxidation; these actions are exerted through activation of the cyclic-AMP dependent protein kinase (AMPK). Adiponectin also exerts anti-inflammatory effects by opposing the synthesis and release of TNF-α from macrophages within adipose tissue. In addition, hypoadiponectinaemia is independently associated with IR and this, in turn, is strictly associated with the development of steatosis. Dixon JB *et al.* (2001) stated that whether hepatic steatosis is a consequence of hepatic or peripheral insulin resistance or whether hepatic steatosis causes hepatic insulin resistance remains unclear. It is likely that excess free fatty acid flux due to peripheral insulin resistance may induce hepatic steatosis. On the other hand, excess fat deposition in the liver may render hepatocytes less sensitive to insulin action and lead to hepatic insulin resistance which occur in early stages of course of HCV infection before the development of cirrhosis (Hickman *et al*., 2003).

In our study the result of ROC curve indicated greater ability of HOMA index for distinguishing steatosis from non-steatosis group, studies supported this association suggesting that IR enhances progression to fibrosis by inducing steatosis, implying a complex mechanism in which inflammatory activity and modified cytokine profile have a distinct role (Lo Iacono et al., 2007; Hsu *et al*., 2008), Papatheodoridis GV *et al.* (2006) were not able to demonstrate this association.

Table.1 Laboratory investigations of CHC patients

	No.	%
FBS (mg/dl)		
≤110	47	94%
>110	3	6%
Min. – Max.	61.0 – 161.0	
Median	87.0	
Insulin (µIU/ml)		
Normal	41	82.0
Abnormal	9	18.0
Min. – Max.	6.34 –31.20	
Median	13.35	
HOMA IR		
<2	11	22.0
≥2	39	78.0
Min. – Max.	1.14 – 6.80	
Median	2.85	
Cholesterol (mg/dl)		
≤200	47	94.0
>200	3	6.0
Min. – Max.	92.0 – 237.0	
Median	143.50	
TG(mg/dl)		
Normal (60 – 160)	35	70.0
Abnormal	15	30.0
Min. – Max.	38.0 – 206.0	
Median	101.50	
HDL-c (mg/dl)		
Normal (≥50)	12	24.0
Abnormal	38	76.0
Min. – Max.	13.0 – 72.0	
Median	38.0	
ALT(U/L)		
Normal	27	54.0
Abnormal	23	46.0
Min. – Max.	7.0 – 142.0	
Median	34.50	
AST(U/L)		
Normal	28	56.0
Abnormal	22	44.0
Min. – Max.	15.0 – 177.0	
Median	32.50	

CHC= Chronic hepatitis C; FBS= Fasting blood sugar; TG= Triglycerides; HDL-c= High density lipoprotein cholesterol; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; p: p value for comparing between the two studied group; □2: Chi square test; t: Student t-test; *: Statistically significant at $p \leq 0.05$

Table.2 Comparison between the studied patients according to gender regarding adiponectin, TNF-α and steatosis Grade

	Gender		Test of sig.
	Male (n = 31)	**Female (n = 19)**	
Adiponectin(µg/ml)			
Min. – Max.	1.50 – 20.40	2.70 – 13.0	$^{t}p = 0.052$
Median	9.40	7.10	
TNF-α (pg/ml)			
Min-Max	9.6 - 112.0	18.90-125.0	$^{MW}p = 0.353$
Median	24.96	27.53	
HOMA-IR			
Min-Max	1.14-6.80	1.50-6.60	$^{t}p=0.96$
Median	2.7	3	
Steatosis grade			
0	11 (35.5%)	3 (15.8%)	$^{MC}p = 0.421$
1	8 (25.8%)	8 (42.1%)	
2	10 (32.3%)	7 (36.8%)	
3	2 (6.5%)	1 (5.3%)	
Min. – Max.	0.0 – 3.0	0.0 – 3.0	$^{MW}p = 0.401$
Mean ± SD	1.10 ± 0.98	1.32 ± 0.82	
Median	1.0	1.0	

TNF-α = Tumour necrosis factor- α; p: p value for comparing between the two studied group
t: Student t-test; MW: Mann Whitney test; *: Statistically significant at $p \leq 0.05$

Table.3 Statistical correlations between adipocytokines and other studied parameters

	Adiponectin	
	Coff.	P
TNF-α	$r_s = -0.757^{*}$	<0.001
HOMA IR	r = -0.772	<0.001
BMI	$r = -0.420^{*}$	0.002
Waist circumference	$r = -0.480^{*}$	<0.001
HCV RNA	$r_s = -0.010$	0.943
ALT	$r_s = -0.521$	0.066
AST	$r_s = -0.493$	0.059
Age	$r = -0.391^{*}$	0.005
Cholesterol	$r_s = -0.017$	0.909
FBS	$r_s = -0.242$	0.090
HDL-c	$r_s = 0.069$	0.633
Insulin	$r_s = -0.725^{*}$	<0.001
TG	$r = -0.586^{*}$	<0.001

TNF-α= Tumour necrosis factor- α; BMI= Body mass index.; ALT= Alanine aminotransferase
AST= Aspartate aminotransferase; FBS= Fasting blood sugar; HDL -c= High density lipoprotein cholesterol
TG= Triglycerides; r_s: Spearman coefficient; *: Statistically significant at $p \leq 0.05$

Table.4 Statistical correlations between HOMA-IR and other parameters

	HOMA IR	
	Coffe.	**P**
TNF-α	$r_s = 0.878^*$	<0.001
BMI	$r = 0.508^*$	<0.001
FBS	$r= 0.316^*$	0.026
Insulin	$r_s = 0.939^*$	<0.001
Age	$r= 0.426^*$	0.002
TG	$r= 0.711^*$	<0.001
Cholesterol	r= 0.061	0.672
HDL-c	r= -0.053	0.713
HCV-RNA	r = 0.085	0.558

TNF-α= Tumour necrosis factor- α ; BMI= Body mass index.; FBS= Fasting blood sugar; TG= Triglycerides HDL -c= High density lipoprotein cholesterol; r_s: Spearman coefficient; *: Statistically significant at $p \leq 0.05$

Table.5 Statistical analysis of different parameters regarding the presence or the absence of visceral obesity among the CHC patients

	Visceral obesity		**Test of sig.**
	Absent (n = 18)	**Present (n = 32)**	
HOMA-IR			
Min. – Max.	1.14 – 4.80	1.50 – 6.80	$^t p <0.001^*$
Median	2.43	4.12	
Adiponectin (µg/ml)			
Min. – Max.	2.80 – 20.40	1.50 – 14.50	$^t p<0.001^*$
Median	11.30	6.80	
TNF-α (pg/ml)			
Min.-Max.	9.0 – 112.0	18.90 – 125.0	$^{MW} p = 0.001^*$
Median	20.6	28.33	
Steatosis grades			
0	12 (57.1%)	2 (7%)	$^{MC} p <0.001^*$
1	5 (38.1%)	11 (28%)	
2	1 (4.8%)	16 (55%)	
3	0 (0.0%)	3 (10%)	
Min. – Max.	0.0 – 2.0	0.0 – 3.0	$^{MW} p <0.001^*$
Median	0.0	2.0	

CHC= Chronic hepatitis C; TNF-α= Tumour necrosis factor- α; p: p value for comparing between the two studied group; t: Student t-test; MC: Monte Carlo test; MW: Mann Whitney test; *: Statistically significant at $p \leq 0.05$

Table.6 Comparison between the level of the studied cytokines regarding BMI among the studied groups

	Cases with BMI>25 kg/m^2 (n = 37)	**Cases with BMI≤25 kg/m^2 (n = 13)**	**Control with BMI≤25 kg/m^2 (n = 25)**	**Test of sig.**
Adiponectin(µg/ml)				
Min. – Max.	1.50 - 20.40	5.80 - 18.60	11.40 - 20.0	^{F}p <0.001*
Mean ± SD	8.44 ± 4.51	10.85 ± 4.13	16.31 ± 2.83	
Median	7.50	9.40	17.0	
$^{Scheffe}p_1$		0.176	<0.001*	
$^{Scheffe}p_2$		0.001*		
TNF-α (pg/ml)				
Min. – Max.	9.60 - 125.0	12.42 - 112.0	10.81 - 20.69	^{KW}p <0.001*
Mean ± SD	30.52 ± 18.49	28.64 ± 25.69	14.20 ± 2.99	
Median	26.13	20.66	13.87	
$^{MW}p_1$		0.109	<0.001*	
$^{MW}p_2$		<0.001*		

BMI= Body mass index; TNF-α= Tumour necrosis factor- α; p: p value for comparing between the two studied group; p1: p value for comparing between cases with BMI>25 kg/m2 and each other group; p2: p value for comparing between cases with BMI≤25 kg/m2 and control; F: F test (ANOVA); KW: Kruskal Wallis test
Sch: Post Hoc Test (Scheffe); MW: Mann Whitney test; *: Statistically significant at p ≤ 0.05

Figure.1 Receiver operating characteristic curve (ROC) for HOMA-IR and adiponectin

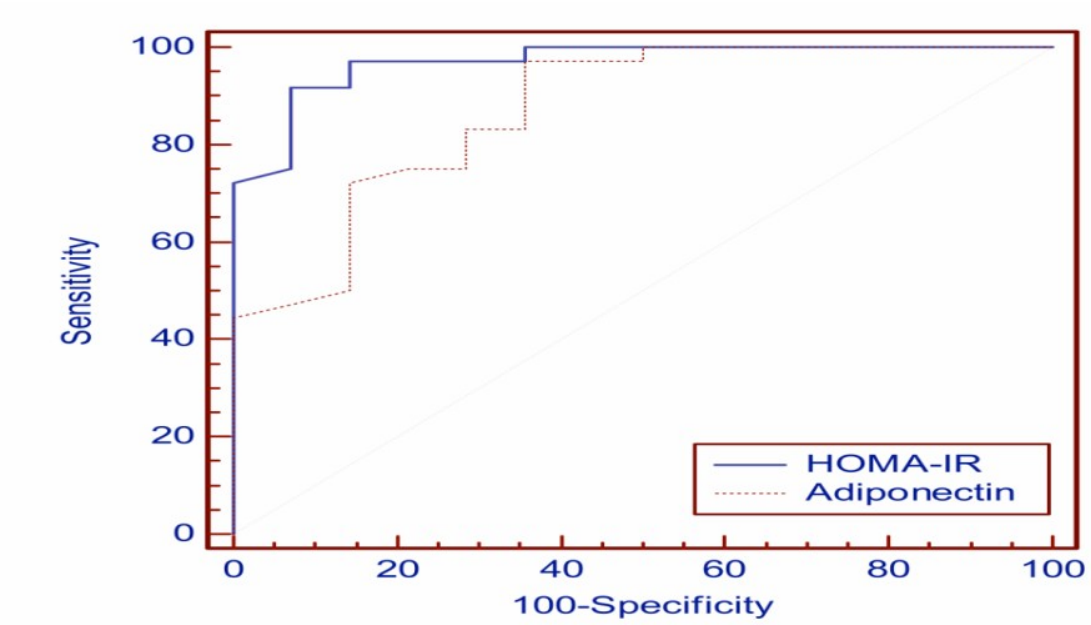

Table.7 Univariate analysis of the factors associated with the severity of liver steatosis in the studied CHC patients

	Mild steatosis (0-1)	Severe steatosis (2-3)	Test of sig.
Adiponectin(µg/ml)			
Min. – Max.	2.80 – 20.40	1.50 – 10.40	$^{t}p < 0.001^{*}$
Median	11.40	5.85	
TNF-α (pg/ml)			
Min. – Max.	9.60 – 112.0	23.40 – 125.0	$^{MW}p < 0.001^{*}$
Median	21.98	30.46	
HOMA-IR			
Min. – Max.	1.14 – 4.80	2.99 – 6.80	$^{t}p < 0.001^{*}$
Median	2.42	4.47	
TG			
Min. – Max.	38.0 – 156.0	94.0 – 206.0	$^{t}p < 0.001^{*}$
Median	83.0	162.0	
BMI (kg/m^2)			
Min. – Max.	18.0 – 34.0	28.0 – 36.0	$^{t}p < 0.001^{*}$
Median	26.50	32.0	
Visceral obesity			
Absent	17 (34%)	1 (2%)	$^{\chi 2}p < 0.001^{*}$
Present	13 (26%)	19 (38%)	
HDL-c			
Min. – Max.	15.0 – 72.0	13.0 – 62.0	$^{t}p = 0.332$
Median	38.0	36.50	
Cholesterol			
Min. – Max.	92.0 – 237.0	103.0 – 209.0	$^{t}p = 0.725$
Median	140.0	146.50	
Age			
Min. – Max.	24.0 – 64.0	37.0 – 60.0	$^{t}p = 0.016^{*}$
Median	47.0	52.0	
HCV-RNA ×10^3			
Min. – Max.	0.37 – 3400.0	0.26 – 416000.0	$^{MW}p = 0.898$
Median	345.0	300.0	
ALT			
Normal	17 (34%)	10 (20%)	$^{\chi 2}p = 0.642$
Abnormal	13 (26%)	10 (20%)	
AST			
Normal	16 (32%)	12 (24%)	$^{\chi 2}p = 0.643$
Abnormal	14 (28%)	8 (16%)	

CHC= Chronic hepatitis C; TNF-α= Tumour necrosis factor- α
TG= Triglycerides; BMI= Body mass index; HDL -c= High density lipoprotein cholesterol; ALT= Alanine aminotransferase;AST= Aspartate aminotransferase

Table.8 Agreement (sensitivity, specificity and accuracy) for HOMA-IR and Adiponectin

		Remission	Steroid resistant + relapse	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
HOMA-IR	**≤2.43**	13	3	0.969*	91.67	92.86	97.06	81.25	92.0
	>2.43	1	33	<0.001					
Adiponectin	**>13**	9	1	0.872*	97.22	64.29	87.50	90.0	88.0
	≤13	5	35	<0.001					

AUC: Area Under Curve.
PPV: Positive Predictive Value.
NPV: Negative Predictive value

In conclusion, lower adiponectin level were found among Egyptian patients with HCV suffering from steatosis and it is inversely correlated with insulin resistance and TNF- . These data support a role for adiponectin in protection against liver injury and that hypoadiponectinemia may contribute to hepatic steatosis progression. Further molecular and genetic studies with larger numbers of patients are required to confirm these results.

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