Original Article

The Study of the Relationship between Insulin Resistance Index and Serum Apelin Level in Patients with Chronic Hepatitis C Virus.

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Abstract:

Objective: Highlighting the apelin system would present a new therapeutic target for liver disease. Apelin; endogenous ligand for the orphan receptor APJ, was recently suggested to be associated with fibrosis progression and cirrhosis in addition to insulin resistance (IR) and inflammation. The present study was conducted to detect the relationships between apelin serum levels and insulin resistance in patient with HCV in comparison to patients without HCV and calculating the apelin serum level and HOMA 2- IR among 60 egyptian patients with HCV and 20 non HCV. Serum apelin levels were significantly lower in HCV patients with median value (167.69±221.70) when compared with control group (867.0±158.95), at P <0.001 with significant apelin variations among asymptomatic carriers (ASC), fibrosis, and cirrhosis patients .Multiple Linear Regression models depicated that HbA1c, S.insulin and HCV were found to be the best predictors affecting apelin levels, and FBS, S.insulin were found to be the best predictors affecting HOMA IR with a decreasing and increasing effect respectively and there were negative significant correlation between S.apelin with BMI, FBS and Hb while there were positive significant correlation between HOMA IR with S.insulin (P < 0.001). **Conclusion:** Apelin level varies in HCV patients, which may contribute to fibrosis progression. In addition, IR could act as comorbid factors affecting apelin level in patients with HCV.

Keywords: Insulin Resistance Index, Serum Apelin, Chronic Hepatitis C Virus

Introduction:

Hepatitis C virus (HCV) has been recognized as a major cause of chronic liver disease(s) (CLD) in Egypt[(1)]. The emerging role of apelin in CLD is complex, as described in a recent report linking apelin to the initiation and maintenance of the inflammatory and fibrogenic processes occurring in the fibrotic liver[(2)], as well as to the vascular and haemodynamic abnormalities in cirrhosis and its complications[(3,4)]. However, clinical data demonstrating the role of apelin in CLD is limited, as depicted by Bertolani and Marra.[(5)]

Hepatic insulin signaling is markedly impaired in HCV patients; in addition, down regulation of hepatic insulin mediators is associated with enhanced hepatocyte apoptosis and fibrogenesis[(6)]. Currently some evidence supports a relationship between insulin resistance (IR) and hepatitis C on one hand, playing role in progression of liver disease[(7-10)], and between IR and apelin level.

The receptor APJ remained orphan until 1998, when Tatemoto and his coworkers isolated its endogenous ligand from bovine stomach extract. They isolated a 36-amino-acid peptide which was named apelin (from APJ endogenous ligand)[(11)] .Apelin exists in at least three forms, consisting of 13, 17, or 36 amino acids, all originating from a common 77-amino-acid precursor[(12)]. Apelin has been shown to be involved in vessel formation, where it exerts a pro-angiogenic role [(13,14)], and

in the regulation of cardiovascular function, by reducing arterial blood pressure, via stimulation of nitric oxide-mediated vasorelaxation[15,16]. Moreover, apelin has recently been added to the family of adipokines[17,18], which are adipocytokines mainly derived from adipose tissue as well as endothelial cells (ECs) in various parts of the body [12]

Subjects and Methods:

Subjects:

Eighty age matched subjects selected from outpatient clinic of the diabetes and metabolism unit, Alexandria Main University Hospital were enrolled in this study divided into 60 patients with chronic hepatitis c virus (Child-Paugh A,B,C) and 20 patients without HCV as a control group. After protocol approval, the study was conducted in the period from September 2013 to April 2015. All subjects gave written informed consent prior to participation .The following exclusion criteria were used for all subjects: Other causes of chronic liver disease as alcoholic and autoimmune, treatment with interferon beta, pregnancy and lactation, conditions reported to be associated with altered serum apelin level as under-weight (B.M.I < 18.5 Kg/m2), morbid obesity (B.M.I > 40 Kg/m2), unstable body weight within last three months, patients receiving corticosteroids for any reason, diabetes mellitus, known concurrent malignancy, severe chronic renal disease and recent major surgery.

Methods:

Data collection:

A detailed drug treatment(s) history was collected, and physical examination of the patients was carried out with special emphasis on previous surgical procedures and prior parenteral therapy. Complete clinical examination was done, on the day of sample withdrawal which included the manifestations of hepatitis such as hepatomegaly, tenderness in the right hypochondrium, splenomegaly, and lower limb edema, or liver cell failure such as jaundice, hepatic encephalopathy, bleeding varices, and ascites. abdominal ultrasonography were also done side by side with routine laboratory investigations including complete blood picture, liver, kidney function tests, fasting blood sugar, serum insulin ,HbA1c, lipid profile and HOMA-IR. HCV was diagnosed by anti-HCV antibodies, and HCV RNA by real time PCR. Medical records indicated that 60 patients were of HCV .

Body mass index (BMI) was calculated as an index of the weight (in kilograms) divided by the square of the height (in meters) measured on the same day of sample withdrawal. Centers for Disease Control and Prevention (CDC) classify the normal range of BMI to be between 18.5–24.9kg/m^2, overweight BMI between 25–29.9kg/m^2, and the obese BMI > 30 kg/m^2[19] . Since BMI could be influenced by the presence of ascites in patients with cirrhosis, earlier weights taken prior to the diagnosis of ascites were used for the calculation of BMI.[20]

Sample Preparation, Collection, and Storage:

All subjects were advised to take no medication on the morning before blood sample collection. Fasting blood (5mL) was obtained from the antecubital vein, after an overnight fasting period (10–12 hours). Samples were divided into two parts; one containing trisodium citrate (final concentration 1mg/mL) for prothrombin time (PT) determination. The other part was taken into vacutainer clotted tubes, where sera were separated by centrifugation at 3000rpm for 10min for other lab measurements. Other sera aliquots were kept frozen at −70°C for measurement of serum apelin (with no need for aprotinin pretreatment step applied to plasma samples [21]) TNF-α, and insulin.

Laboratory Assessments:

Sera were obtained, aliquoted for the measurement of LFTs: aspartate transaminase (AST), ALT, bilirubin, albumin, fasting blood glucose (FBG), and lipids (total cholesterol (TC) and triacylglycerol (TAG)) by using standard enzymatic techniques using appropriate kits and

semiautomated photometer 5010. Determination of serum apelin level using DRG® Apelin C-Terminus Elisa (Human/Mouse/Rat) (EIA-5178)., procedures were carried out according to the manufacturers' instructions.

Insulin resistance was determined by the homeostasis model of assessment (HOMA) [22] using the formula: fasting insulin (μIU/mL) × fasting blood glucose (mg/dL)/405.

Statistical Analysis:

SPSS statistics (V. 19.0, IBM Corp., USA, 2010) was used for data analysis. Data was expressed as mean ± S.D for quantitative parametric measures, in addition to median and IQR for nonparametric data and percentiles for categorical data. Student's t test was used for comparison of two independent groups for parametric data and Wilcoxon Rank Sum for nonparametric data. However, for comparison between more than 2 patient groups for parametric data, we used analysis of variance (ANOVA). Multiple comparisons (Post hoc test: LSD (least significant difference)) were also followed to investigate the possible statistical significance between each 2 groups. Moreover, comparison between more than 2 patients' groups for nonparametric data Kruskall Wallis test was used. Finally, Spearman's ranked correlation test, to study the possible association between each two variables among each group for nonparametric data, using the probability of error at 0.05 was considered significant, while at 0.01 and 0.001 were highly significant. Multiple linear regression analysis was done on all measured parameters to allow for adjustment of apelin level.

Results:

No significant differences with regards to age or gender distribution existed among groups. The clinical and demographic characteristics as well as studied parameters of all participants (control and HCV patients) are shown in Table 1. Significant differences in median values of apelin and HOMA-IR levels existed between control and HCV patients as shown in Table I.

Comparison between the two studied groups according to HOMA IR were presented in Table 2, it showed that, HOMA IR ranged between 0.94 – 22.50 and 0.35-2.98 with the mean of 3.82±3.60 and 1.67±0.69 for HCV patients and control groups respectively, HCV group have values statistically higher than control group with statistically significanct difference. (P=<0.001) as shown in Table II.

Comparison between the two studied groups according to S.apelin were presented in Table (3), it showed that, S.apelin ranged between 7.0-860.0 and 400.0-1000.0 with the mean of 167.69±221.70 and 867.0±158.95 for HCV patients and control groups respectively, Control group have values statistically higher than HCV patients group with statistically significant differences. (P=0.001) as shown in Table III.

The correlation between HOMA IR and S.apelin with different studied parameters in HCV patients group was shown in Table 4 demonstrated that there were negative significant correlation between S.apelin with S.insulin (P= 0.001), while there were positive significant correlation between HOMA IR with FBS, S.insulin and Hb (P=0.003, 0.001 and 0.022 respectively), as shown in Table IV .

Correlation between HOMA IR and S.apelin with different studied parameters in control group demonstrated that, there were negative significant correlation between S.apelin with BMI , FBS and Hb while there were positive significant correlation between HOMA IR with S.insulin (P < 0.001) as shown in Table V.

Determining the best predictors which affect apelin and HOMA IR measurements was also evaluated by multiple linear regression analyses. All possible factors, which were defined in correlation analyses, were evaluated together in Multiple Linear Regression models. HbA1c, S.insulin and HCV were found to be the best predictors affecting apelin levels, and FBS, S.insulin were found to be the best predictors affecting HOMA IR with a decreasing and increasing effect respectively, Tables (VI,VII) shows the all possible factors that may affect apelin levels and HOMA IR with the Multiple Linear Regression model.

Table (I): Comparison between the two studied groups according to different parameters

	HCV Patients (n = 60)	Control (n = 20)	p
Sex			
Male	60 (100%)	20 (100.0%)	-
Female	0 (0.0%)	0 (0.0%)	
Age	37.67 ± 11.53	38.90 ± 6.22	0.547
Weight	82.03 ± 13.19	86.30 ± 5.96	0.052
Height	171.30 ± 6.86	174.25 ± 3.01	0.010*
BMI	27.97 ± 4.52	28.41 ± 1.74	0.527
SGOT	72.95 ± 25.71	21.25 ± 6.13	<0.001*
SGPT	92.82 ± 21.22	25.15 ± 6.45	<0.001*
S. albumin	2.93 ± 0.25	3.83 ± 0.17	<0.001*
Total Bilirubin	2.08 ± 0.45	1.10 ± 0.12	<0.001*
Direct bilirubin	1.34 ± 0.42	0.75 ± 0.09	<0.001*
PT	14.38 ± 0.64	14.0 ± 0.0	<0.001*
Hb	11.10 ± 1.45	14.17 ± 1.02	<0.001*
Creatinine	0.93 ± 0.22	0.85 ± 0.11	0.023*
Cholesterol	192.98 ± 43.42	173.85 ± 37.90	0.083
Triglyceride	163.30 ± 44.35	146.25 ± 46.41	0.145
FBS	92.10 ± 9.06	91.30 ± 10.71	0.745
S.insulin	9.55 (2.30 – 51.40)	7.55 (1.50 – 12.80)	0.090
HbA1c	6.07 ± 0.23	4.87 ± 0.52	<0.001*
HOMA IR	2.78 (0.94 – 22.50)	1.72 (0.35 – 2.98)	<0.001*
S. apelin	90.0 (7.0 – 860.0)	925.0 (400.0 – 1000.0)	<0.001*

Normally quantitative data was expressed in (Mean. ± SD) and was compared using t-student test while for abnormally quantitative data expressed in Median (Min. – Max.) and was compared using Mann Whitney test.

*: Statistically significant at p ≤ 0.05

Table (II): Comparison between the two studied groups according to HOMA IR

	HCV Patients (n = 60)	Control (n = 20)	Z	p
HOMA IR				
Min. – Max.	0.94 – 22.50	0.35 – 2.98		
Mean ± SD.	3.82 ± 3.60	1.67 ± 0.69	3.711*	<0.001*
Median	2.78	1.72		

Z: Z for Mann Whitney test

*: Statistically significant at p ≤ 0.05

Table (III): Comparison between the two studied groups according to S.apelin

	HCV Patients (n = 60)	Control (n = 20)	Z	p
S. apelin				
Min. – Max.	7.0 – 860.0	400.0 – 1000.0		
Mean ± SD.	167.69 ± 221.70	867.0 ± 158.95	6.394*	<0.001*
Median	90.0	925.0		

Table (IV): Correlation between HOMA IR and S.apelin with different studied parameters in HCV patients group.

	S. apelin		HOMA IR	
	r_s	p	r_s	p
S. apelin	-	-	-0.333*	0.009
Age	-0.039	0.768	-0.156	0.234
Weight	0.100	0.446	0.569*	<0.001
Height	0.029	0.826	0.099	0.452
BMI	-0.192	0.141	0.393*	0.002
PCR	-0.148	0.258	0.134	0.309
FBS	0.026	0.843	0.410*	0.001
HbA1c	-0.236	0.070	0.020	0.878
S.insulin	-0.402*	0.001	0.925*	<0.001
SGOT	-0.112	0.392	0.091	0.490
SGPT	-0.004	0.973	0.025	0.847
S.albumin	-0.194	0.138	-0.054	0.684
T.Bilirubin	0.053	0.689	-0.054	0.680
D.bilirubin	0.035	0.791	-0.082	0.533
PT	0.112	0.393	0.003	0.981
Hb	-0.069	0.599	0.316*	0.014
Creatinine	-0.136	0.302	0.139	0.291
Cholesterol	-0.025	0.847	0.122	0.353
Triglyceride	0.136	0.300	0.189	0.148

r_s: Spearman coefficient *: Statistically significant at $p \leq 0.05$

Table (V): Correlation between HOMA IR and S.apelin with different studied parameters in control group

	S. apelin		HOMA IR	
	r_s	p	r_s	p
S. apelin	-	-	0.488*	0.029
Age	0.039	0.870	0.263	0.262
Weight	0.044	0.854	0.100	0.673
Height	-0.005	0.982	0.242	0.305
BMI	-0.127	0.593	0.006	0.980
FBS	-0.134	0.573	0.243	0.302
HbA1c	-0.285	0.223	-0.398	0.082
S.insulin	0.545*	0.013	0.915*	<0.001
SGOT	0.308	0.186	0.166	0.485
SGPT	-0.266	0.257	0.184	0.438
S.albumin	-0.076	0.749	-0.261	0.265
T.Bilirubin	-0.450*	0.047	-0.157	0.508
D.bilirubin	-0.106	0.657	-0.160	0.502
Hb	-0.079	0.742	0.153	0.520
Creatinine	-0.343	0.139	-0.201	0.396
Cholesterol	0.281	0.229	0.037	0.879
Triglyceride	0.405	0.076	0.138	0.561

r_s: Spearman coefficient *: Statistically significant at $p \leq 0.05$

Table (VI): Linear regression for factors affecting S. apelin

	B	SE	t	p	95% CI	
					LL	UL
HbA1c	-143.998	69.375	2.076*	0.041*	-282.170	-5.826
S.insulin	-5.674	2.358	2.406*	0.019*	-10.372	-0.977
HCV	-494.107	99.967	4.943*	<0.001*	-693.209	-295.005

Table (VII): Linear regression for factors affecting HOMA IR

	B	SE	t	p	95% CI	
					LL	UL
FBS	0.077	0.026	2.914*	0.005*	0.024	0.129
S.insulin	0.206	0.026	7.839*	<0.001*	0.154	0.259
Hb	0.296	0.185	1.604	0.113	-0.072	0.664
HCV	1.335	0.828	1.612	0.111	-0.314	2.985

Discussion:

Recent emerging studies pointed to the possible multiple effects of the apelinergic system in the liver and related it to oxidative stress, inflammation[18], fibrosis[2], angiogenesis[23], as well as haemodynamic and vascular disturbances [3,4]

Hepatic insulin signaling is markedly impaired in HCV patients; in addition, down regulation of hepatic insulin mediators is associated with enhanced hepatocyte apoptosis and fibrogenesis[24]. Currently some evidence supports a relationship between insulin resistance (IR) and hepatitis C on one hand, playing role in progression of liver disease [1-5], and between IR and apelin level on the other hand in cases of obesity[25].

This study aims to detect the relationships between apelin serum levels and insulin resistance in patient with HCV in comparison to patients without HCV. 80 patients were selected from the out patient clinic of Main University Hospital, Alex, Egypt. Subjects will be divided into 2 main groups: Group A: 60 patients with chronic hepatitis c virus (Child-Paugh A,B,C). Group B: 20 patients without HCV as a control group.

Hala, et al., (2011) showed that, apelin was evaluated in all groups both before and after adjustment for BMI, TAG, and TC that acted as potential covariants. Studies assessing apelin-36 and apelin-12 levels in patients with nonalcoholic fatty liver disease (NAFLD) indicated that the elevation of this peptide did not persist after adjustment for potential confounders and rather attributed apelin elevation in these cases to obesity and IR that are closely associated with NAFLD [10,26].

In contrast to NAFLD models, the present study shows that serum apelin level is changed in patients with either fibrosis or cirrhosis due to HCV, with significant differences among the two groups being lower in the latter, even after apelin adjustment. This is in line with previous investigations demonstrating that patients with HCV showed significant increase in apelin circulating levels [3,27].

Moreover, recent emerging studies speculated that activated hepatic stellate cells (HSCs) represent a potential source for apelin in liver [3] and that apelin could be an important mediator of the profibrogenic gene induction that markedly stimulates collagen-I synthesis [2]. In addition, apelin contributes to platelet-derived growth factor-induced proliferation of HSC's in vitro [4], all of which are known to contribute

largely to fibrosis progression and extracellular matrix deposition [28,29]. In this setting, we also found apelin serum level to be significantly elevated in patients with HCV. Thus, apelin emerges as a major contributor to the fibrogenic process(es) occurring in liver disease[2] as well as playing role in disease progression.

On the other hand, this elevation in case of ASC disappeared after apelin adjustment to cofounders, which points to the interference of these cofactors in elevating apelin levels and may result in the early upset of the system in CHC patients.

Moreover, the current study demonstrated that this peptide is closely associated to ascites and portal hypertension complications. Tiani and his coworkers suggested that the expression of endogenous apelin/APJ signaling is associated with development of portal hypertension and contributes to the formation of portosystemic collateral blood vessels and splanchnic neovascularization in portal hypertensive rats [23].

In the last few years, several data have accumulated suggesting that obesity also plays role in development and progression of liver disease of well-defined etiology [30]. Recently, expression of both apelin and APJ has been described in adipocytes [17] and is suggested to stimulate blood vessel growth, due to its proangiogenic activity, thus leading to increased growth of adipose tissue[31]. Coinciding with that line, our study also demonstrated that obese subjects with CHC had significant increased circulating apelin levels than lean patients, regardless of the stage or grade of liver disease.

On the other hand, studies also demonstrated that apelin expression was higher in animal models of obesity associated with hyperinsulinemia [17], in addition to its role in adipogenesis[31] and steatosis[18]; all of which contribute largely to fibrosis progression, as well as higher degree of inflammation[30,32]. Besides, virus C infection may induce IR by blocking intracellular signaling [33]. Further insight in our study revealed that IR was significantly higher among all groups of CHC in comparison to the control group. Moreover, significant difference was also found between ASC and cirrhotic groups, being higher in the latter. Moreover, when comparing apelin levels in IR and non-IR groups, it was significantly higher in IR group. Additionally, there was significant positive correlation between IR and adjusted-apelin in patients with CHC. This is in agreement with a previous study conducted by Aktas et al., who depicted that apelin, the novel adipokine, was associated with the components of the metabolic syndrome (hyperlipidemia, obesity, and IR) in NAFLD patients[34]. However, our correlation was quite significant in ASC but was unexpectedly nonsignificant in the case of progressive stages of fibrosis and cirrhosis, suggesting that the upset of the apelin system in these stages may follow a unique different pattern irrelevant to IR.

Moreover, previous investigations had pointed to s.insulin as an inductor of apelin synthesis in adipocytes[35]. Hence, we sought to investigate such correlation in our CHC population. TNF-α was mostly elevated in cases of ASC, this was in agreement with Zekri and his coworkers[36] but was opposite to what was reported by Goyal et al.[37] and Toyoda et al.[38]. This could be attributed to the difference in the epitopes of the ELISA system used by the different investigating groups or to the difference in genotypes where all our patients were HCV genotype 4. Moreover, the striking elevation of this proinflammatory cytokine in carriers may reflect both insufficiencies of HCV elimination and/or a failure to control the cytokine cascade [36].

These findings point to the possible role of apelin in CLD progression. Moreover, this provides a rationale to investigate new drugs targeting the apelin/APJ signaling pathway to reduce fibrosis and to improve hemodynamics in those patients.

The strong correlation of apelin- with LDL and HDL-cholesterol is an interesting finding. Although apelin is secreted also from adipose tissue, this is not sufficient to explain the relationship. Malyszko et al.[39] found negative correlation between apelin and total cholesterol, LDL-cholesterol and triglyceride levels in HD patients. This difference from our study may be related with metabolic abnormalities like hyperglycemia, dyslipidemia and obesity that are more common in PD patients due to the glucose content of PD solutions. Moreover, Tasci et al.[40] found in their studies that apelin levels were lower in patients with high LDL-cholesterol in non-uremic population; and lowering LDL-cholesterol with life style changes and/or statins resulted in an increase in apelin levels. These different results between uremic and nonuremic population may be regarded as a clue for different lipid profile of uremic patients.

Conclusion:

From our results we can conclude the following:

HOMA IR in HCV patients group has values statistically higher than control group with statistically significanct difference, S.apelin level in control group has values statistically higher than HCV patients group with statistically significant differences, there were negative significant correlation between S.apelin with S.insulin, while there were positive significant correlation between HOMA IR with FBS, S.insulin and Hb and finaly Multiple Linear Regression models depicated that HbA1c, S.insulin and HCV were found to be the best predictors affecting apelin levels, and FBS, S.insulin were found to be the best predictors affecting HOMA IR with a decreasing and increasing effect respectively.

Conflict of Interests:

The authors declare that there is no Conflict of Interest.

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