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Study of Apelin Serum Levels in patients with Hepatitis C virus Chronic Liver Disease.

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ABSTRACT

Objective: The aim was to study the apelin serum levels and assessment of insulin resistance role in chronic liver diseased patients.

Background: Apelin is involved in many physiological and pathological function in human, it is thought to increase liver fibrosis progression with the aid of insulin resistance. There is a significant association of insulin resistance and apelin level and liver progression.

Materials and methods: This study was carried at Internal Medicine Department and National Liver Institute, Menoufia University Hospitals, from the period of January 2014 to October 2014. Subjects were classified into 5 groups each group contained 15 subjects: Group (I) served as healthy control of matched sex and age, Group (II) had liver steatosis without HCV infection, Group (III) had HCV-related liver steatosis, Group (IV) had HCV-related liver fibrosis and Group (V) had HCV-related liver cirrhosis.

Results: Serum apelin level was elevated in liver steatosis, fibrosis and it was significantly higher in cirrhotic patients. Insulin resistance correlate positively with serum apelin and liver disease progression.

Conclusion: Serum apelin is significantly higher in patients with HCV-liver cirrhosis compared to HCV-related liver fibrosis and steatosis that say, apelin plays a role in progression of liver disease and inhibition of the apelinergic system could offer new therapeutic opportunities against liver complication. A strong relation between insulin resistance and apelin on one side and other side between insulin resistance and liver fibrosis progression.

Key words: Apelin; Insulin resistance; Lliver fibrosis; Steatosis and hepatitis C virus (HCV);

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INTRODUCTION:

Apelin was first isolated from bovine stomach extracts (1), in a drug screen searching for endogenous ligands for a previously orphaned G protein coupled receptor named angiotensin-like receptor 1 (AGTRL1), or APJ (which itself was discovered in a search for isotypes of the vasopressin receptor) (2). Apelin exists in at least three forms, consisting of 13, 17, or 36 amino acids, all originating from a common 77 aminoacid precursor (3). Moreover, apelin has been recently added to the family of adipokines (4), which are adipocytokines mainly derived from adipose tissue as well as endothelial cells (ECs) in various parts of the body.

Apelin and APJ mRNA are found to be expressed in many rodent and human tissues as in the central nervous system and peripheral tissues (5). Apelin is involved in many physiological, pathological conditions and even in neoplastic growth. Apelin regulate cardiovascular system functions, blood pressure, vessel formation, fluid homeostasis, hypothalamic pituitary adrenal axis and bone physiology. Pathophysiological, apelin involved in heart failure, hypertension, obesity, glucose intolerance, diabetes mellitus type 2, gastric ulcer and osteoporosis (4,7).

There is a relationship between insulin resistance (IR) and hepatitis C on one hand, playing a role in progression of liver disease (9) and with apelin on the other hand (10). Hepatic insulin resistance is associated with increased apoptosis and fibrogenesis as impairment of hepatic insulin signaling and down regulation of hepatic insulin mediators in HCV infected patients (11). The emerging role of apelin in liver disease is complex, it is important for initiation and maintenance of the inflammatory and fibrogenic processes occurring in the fibrotic liver (12), as well as to the vascular and hemodynamic abnormalities in cirrhosis (13,15) and promotes HCV induced liver cell dysplasia and progression of carcinoma (HCC) (15,16). Thus, this study was designed to elucidate apelin and insulin resistance role in hepatic remodeling progression and in fatty liver which would present a new therapeutic target for liver disease.

SUBJECTS AND METHODS:

This study was performed on (75) subjects aged from 21 years to 48 years from inpatient wards and outpatient clinics at Internal Medicine Department and National liver institute, Menoufia university hospitals, from the period of January 2014 till October 2014. All subjects gave written informed consent prior to participation and the study was approved by the Committees on Medical Ethics of the Menoufia University.

Study groups: subjects were classified into 5 groups each group contained 15 subjects: Group (I) served as healthy control of matched sex and age, Group (II) subjects had liver steatosis without HCV infection, Group (III) subjects had HCV-related liver steatosis, Group (IV) subjects had HCV-related liver fibrosis and Group (V) had HCV-related liver cirrhosis.

Exclusion criteria: The following subjects were excluded from the study:

1-Other causes of chronic liver disease as causes of hepatitis: as Hepatitis B virus, autoimmune hepatitis, Wilson disease, Budd Chiari syndrome, hemochromatosis, hepatocellular carcinoma, and biliary disorders.2- Diabetic patients.3-Cardiovascular disorders.4- Neurological disorders.

All were subjected to: I- Thorough medical history taking. II- Complete physical examination. III- Routine investigations: CBC, Liver function tests, fasting blood glucose, fasting insulin, Hepatitis C virus antibodies, hepatitis B s antigen, HIV antibodies. IV- Estimation of body mass index (BMI). V- Abdominal ultrasound. VI- Specific Investigations: Serum apelin assay by ELISA technique.

Statistical analysis: SPSS statistics (V. 20) was used for data analysis. Data was expressed as mean±SD 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 independent groups for parametric data and qualitative data was analyzed by chi square and Fisher's exact test. 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 groups. Moreover, comparison between more than 2 patients' groups for nonparametric data Kruskall Wallis test and Mann-Whitney test (U) were 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 (6).

RESULTS:

In this study the distribution of the studied groups regarding their demographic data showed that: the mean age was (39.06±8.98) years in group I (control), (41.20±7.44) years in group II, (39.40± 7.09) years in group III, (42.27± 7.35) years in group IV and (46.00±2.13) years in group V. As regard sex: group I (control) included 7 males (46.7%) and 8 females (53.34%), group II included 5 males (33.4%) and 10 females (66.6%), group III included 9 males (60%) and 6 females (40%) and group IV included 11 males (73.3%) and 4 females (26.7%), group V included 11 males (73.3%) and 4 females

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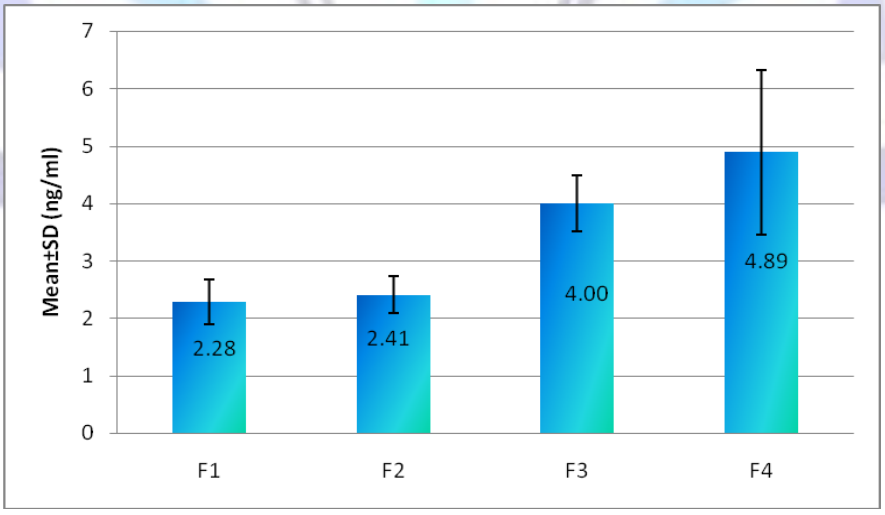
(26.7%) and these results showed that all groups had been matched with no significant differences between them as regard age or sex (P value > 0.05) (Table 1).

Table (1): Sociodemographic characteristics of the studied groups.

Socio-demographic characteristics	Studied groups										Test of significance	P value
	Controls		Liver disease (n=60)									
			Steatosis		Steatosis &HCV		Fibrosis &HCV		Cirrhosis &HCV			
	(n=15)		(n=15)		(n=15)		(n=15)		(n=15)			
Age (years)												
Mean ±SD	39.06±8.98		41.20±7.44		39.40± 7.09		42.27± 7.35		46.00±2.13		**F**= 2.39	0.06
Range	25.00 – 49.00		28.00 – 51.00		21.00 – 46.00		23.00 – 50.00		41.00 – 48.00			NS
Sex :	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**χ2** = 7.41	0.12 NS
Male	7	46.7	5	33.3	9	60.0	11	73.3	11	73.3		
Female	8	53.3	10	66.7	6	40.0	4	26.7	4	26.7		
BMI (kg/m²):												
Mean ±SD	24.03±3.08		27.09±1.12		26.80±1.61		26.46±1.29		26.13±3.66		**F=** 1.85	0.13
Range	19.40– 26.00		24.00– 28.40		23.00 –28.90		23.40 – 28.00		19.90 – 30.00			NS

Serum apelin shows highly significant statistical difference between exposed and control groups regarding serum apelin (P <0.001), as in F1 its mean level (2.28±0.39), F2 its mean level (2.41±0.32), in F3 its mean level (4.00±0.49), and in F4, (group V), its mean level (4.89±1.43), while in group II, its mean level (2.98±1.09), in group III, its mean level (3.29±1.33) and in control group (I) its mean level (0.87±0.25) (Figure 1), (Figure 2)

Figure (1): Relationship between liver fibrosis degree and serum apelin level among HCV-related fibrosis and cirrhotic patients.



Mean serum apelin level in group I was (0.87±0.25), in group II was (2.98±1.09), in group III was (3.29±1.33), in group IV was (3.57±1.02) and in group V was (4.89±1.43) as demonstrated in (Table 2).

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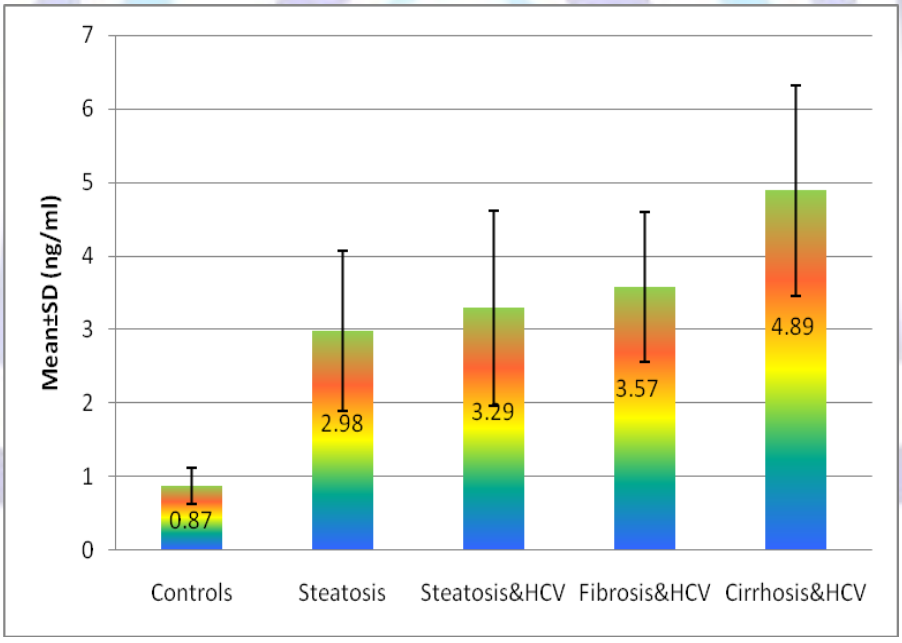
Table (2): Comparison between the studied groups as regards serum apelin level.

Serum apelin (ng/ml)	Studied groups					Kruskal Wallis test	P value
	Controls	Liver disease (n=60)					
		Steatosis	Steatosis &HCV	Fibrosis &HCV	Cirrhosis &HCV		
	(n=15)	(n=15)	(n=15)	(n=15)	(n=15)		
Mean ±SD	0.87±0.25	2.98±1.09	3.29±1.33	3.57±1.02	4.89±1.43		
Median	0.91	2.70	2.78	3.40	4.50	K=46.11	<0.001
Range	0.35–1.20	2.10–6.50	1.90–6.20	2.20–4.95	3.10–8.30		HS
Post Hoc test	——	<0.001 [a] HS	<0.001 [a] HS 0.41 [b] NS	<0.001 [a] HS 0.14 [b] NS 0.48 [c] NS	<0.001 [a] HS <0.001 [b] HS <0.001 [c] HS 0.002 [d] S		

Figure (2): Shows serum apelin level among the studied groups.



The cutoff point of serum apelin in case of HCV-related liver cirrhosis (3.95) by (ROC) curve (Table 3).

Table (3). Diagnostic validity serum apelin in case of HCV-related liver cirrhosis (n=15).

Optimal cutoff point	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Diagnostic accuracy (95%CI)	DOR (95%CI)
3.95	93% (66–100)	85% (73–92)	61% (39–80)	98% (88–100)	87% (76–93)	9.25 (4.42–31.66)

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As regards assessment of insulin resistance by HOMA-IR, mean level in group I was (0.96±0.15), in group II was (1.31±0.34), in group III was (1.84±1.04), in group IV was (1.48±0.89) and in group V was (3.05±0.87) as demonstrated in (Table 4).

Table (4): Comparison between the studied groups as regards insulin resistance**.**

Laboratory parameters	Studied groups					Test of significance	P value
	Controls	Liver disease (n=60)					
		Steatosis	Steatosis &HCV	Fibrosis &HCV	Cirrhosis &HCV		
	(n=15)	(n=15)	(n=15)	(n=15)	(n=15)		
HOMA-IR							
Mean ±SD	0.96±0.14	1.31±0.34	1.84±1.04	1.48±0.89	3.05±0.87	**K=**39.11	<0.001
Range	0.75–1.25	0.78–2.11	0.73–4.12	0.71–3.44	1.64–4.93		HS
Post Hoc test	——	0.09 [a] NS	0.002 [a] S	0.07 [a] NS	< 0.001 [a] HS		
			0.16 [b] HS	0.90 [b] NS	< 0.001 [b] HS		
				0.19 [c] NS	< 0.001 [c] HS		
					< 0.001 [d] HS		

In correlation between apelin level and laboratory parameters in the HCV-related groups; there was a highly significant negative correlation between serum apelin and Hb%, PT% and serum albumin (P<0.001) and reached a significant level for platelets (P <0.05). Also, shows a highly significant positive correlation between serum apelin and total bilirubin, fasting insulin and HOMA-IR (P<0.001) and non-significant correlation with fasting blood glucose and AST (Table 5). In correlation between apelin level and laboratory parameters in non HCV-related group; a significant positive correlation between serum apelin; fasting insulin and HOMA-IR (P<0.05) (Table 6).

Table (5): Correlation between serum apelin and other assessed laboratory parameters among studied patients with HCV-related CLD (n=45).

Laboratory parameters	Serum apelin (ng/ml)	
	r	P value
Hb%(gm/dl)	- 0.56	<0.001 HS
WBCs count (×10³)	- 0.17	0.26 NS
Platelets count(×10³)	- 0.42	0.005 S
PT (%)	- 0.61	<0.001 HS
ALT (IU/L)	- 0.07	0.65 NS
AST (IU/L)	0.14	0.34 NS
Serum Albumin (gm/dl)	- 0.59	<0.001 HS
Total bilirubin (mg/dl)	0.53	<0.001 HS
FBG (mg/dl)	0.31	0.06 NS
Fasting insulin (µU/ml)	0.81	<0.001 HS
HOMA-IR	0.59	<0.001 HS

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Table (6): Correlation between serum apelin and other assessed laboratory parameters among studied patients with non HCV-related CLD (steatosis) (n=15).

Laboratory parameters	Serum apelin (ng/ml)	
	r	P value
Hb%(gm/dl)	0.22	0.43 NS
WBCs count (×10³)	0.48	0.07 NS
Platelets count(×10³)	0.37	0.17 NS
PT (%)	- 0.18	0.53 NS
ALT (IU/L)	- 0.29	0.29 NS
AST (IU/L)	- 0.19	0.49 NS
Serum Albumin(gm/dl)	0.21	0.46 NS
Total bilirubin(mg/dl)	0.16	0.56 NS
FBS(mg/dl)	0.49	0.06 NS
Fasting insulin (µU/ml)	0.55	**0.03 S**
HOMA-IR	0.54	**0.04 S**

DISCUSSION:

Adipokines comprise bioactive substances which perform essential regulatory functions. They act in autocrine, paracrine or endocrine way to influence several biological functions; one of them is apelin (5). Apelinergic system in the liver is related it to oxidative stress, inflammation (11), fibrosis (12), angiogenesis (13,20), as well as hemodynamic and vascular disturbances (13,17). The role of Apelin during different stages of fibrogenesis has not been clarified (12,19). Elevated apelin levels in patients with nonalcoholic fatty liver disease (NAFLD) is related to insulin resistance as it disappeared after adjustment of IR and obesity (18,21). Apelin able to induce profibrogenic genes in the hepatic stellate cells, that markedly stimulates collagen-I synthesis (13) and contributed to its in vitro platelet derived growth factor induced proliferation (14). Apelin-APJ pathway may promote HCV-induced liver cell dysplasia and progression of carcinoma (16).

In the current study, serum apelin level correlated to the presence of insulin resistance and different stages of liver affection as steatosis and HCV related steatosis, fibrosis and cirrhosis, which revealed highly significant elevated serum apelin level in cirrhotic patients more than its level in other groups. Also, apelin correlates positively with insulin resistance in progression of liver remodeling.

That is explained by Principe et al, who found the hepatic apelin system is markedly and selectively activated in cirrhosis which result from an increased hepatic production and involved in the hepatic remodeling (15). That agreed with a study by Chen et al, which showed apelin protein and mRNA were overexpressed in human cirrhotic liver compared with normal liver, and the magnitude increased as cirrhosis progressed from early to advanced stage (26).

Farid et al, analyzed the pattern of apelin expression in different stages of human chronic liver disease. At the early stage of hepatic fibrosis (F1&F2), apelin was almost undetectable as in non-parenchymatous cells, sinusoidal endothelial cells/hepatic stellate cells, myofibroblasts (16).

Also, El-Mesallamy et al, concluded a significant increase in apelin circulating levels in cirrhosis more than fibrosis, this links apelin to the process of fibrogenesis (23).

Apelin has a role in steatosis and related to insulin resistance, our study showed a highly significant positive correlation between serum apelin and fasting blood sugar, fasting insulin and HOMA-IR.

This finding is in agreement with Hashim et al, study which show, NAFLD patients had increased circulating apelin and that increased levels were correlated with insulin resistance (25). That is in agree with Garica-Diaz et al, as apelin has an important role in adipogenesis and steatosis (10). Contrary, Ercin et al, showed that serum apelin levels were not changed in nondiabetic and normotensive male subjects with NAFLD (24) this may be due to limitations of the study as sample size and the strict inclusion criteria, the findings obtained were not representative for all subjects with NAFLD.

However, apelin is related to IR as it may contribute to hepatic steatosis by promoting IR and by altering insulin signaling in hepatocytes, so as to promote increased intra-cellular fatty acids (21). Moreover, at a later stage, apelin may cause hepatic steatosis to turn into steatohepatitis by amplifying selected proinflammatory responses (27).

CONCLUSION:

Serum apelin levels were continuously elevated with the advancement of liver disease which suggests that apelin plays a role in progression of liver disease and inhibition of the apelinergic system. This could offer new therapeutic opportunities against liver disease progression. A strong relation between IR and apelin on one side and other side between IR and liver fibrosis progression. Therefore, treatment which targets IR against liver fibrosis progression can be of a great value.

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