

Is serum apelin related to portal hemodynamics in patients with liver cirrhosis?

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Received 27 September 2017

Accepted 20 November 2017

The Egyptian Journal of Internal Medicine 2018, 30:8–12

Background

Apelin is the endogenous ligand of the angiotensin-like receptor. The expression of apelin–APJ (apelin receptor) signaling is associated with the development of portal hypertension (PH) and contributes toward the formation of Porto systemic collaterals and splanchnic neovascularization in PH.

Aims

We aimed to study the relationship between apelin and portal hemodynamics in cirrhotic patients.

Patients and methods

Thus study included 60 cirrhotic patients from Menoufia University Hospitals (Egypt) and 20 healthy volunteers as a control group. Participants underwent a physical examination and laboratory investigations [complete blood count, urea, creatinine, alanine transaminase, aspartate transaminase (AST), serum albumin, bilirubin, international normalized ratio, hepatitis C virus antibody, hepatitis B virus antigen, hepatitis C virus PCR, alpha feto protein (AFP), and serum level of apelin]. Abdominal ultrasonographic studies of portal vein diameter, splenic size, and portal hemodynamics were carried out for all participants. Child–Pugh score, model for end-stage liver disease score, and AST/platelet (PLT) index were calculated for all participants.

Results

Serum apelin was highly significantly elevated in cirrhotics than in controls, with a *P* value of 0.001. Serum apelin was significantly correlated to some laboratory parameters in cirrhotics such as PLT count, alanine transaminase, AST, γ -glutamyl transferase, and bilirubin, with *P* value less than 0.05. There was a positive correlation between serum apelin level and the degree of liver fibrosis estimated by the AST/PLT index. Serum apelin was significantly correlated to portal vein diameter and portal flow velocity, with a *P* value less than 0.05, and highly significantly correlated to splenomegaly, with a *P* value of 0.001. The optimal cut-off point of serum apelin for the prediction of PH in cirrhotics is 2550 ng/dl, with a sensitivity of 89%, a specificity of 65%, and an accuracy of 81%.

Conclusion

Serum apelin is elevated in patients with cirrhosis and PH, and a positive correlation is found between serum apelin and the degree of hepatic fibrosis. Measurement of serum apelin represents a rapid, noninvasive method for the prediction of PH in cirrhotics and can assess the degree of hepatic fibrosis.

Keywords:

apelin, cirrhosis, portal hemodynamics

Egypt J Intern Med 30:8–12

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1110-7782

Introduction

Portal hypertension (PH) is a hemodynamic outcome of liver cirrhosis in western countries. It causes severe alterations responsible for the onset of complications of cirrhosis. Hemodynamic alterations of PH involve portal–hepatic hemodynamics and also involve splanchnic and systemic circulation [1].

Apelin is a peptide isolated from bovine stomach extracts acts as an endogenous ligand for previously orphaned G protein-coupled receptors (APJ receptor). Now, putative receptor protein related to the type-1 angiotensin receptor [2]. Apelin receptors are expressed in vascular

endothelial cells during early embryogenesis and apelin in combination with vascular endothelial growth factor, leading to angiogenesis through induction of the proliferation of endothelial cells and the formation of new blood vessels [3,4].

Expression of endogenous apelin–APJ (apelin receptor) signaling is associated with the development of PH, and

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contributes toward the formation of porto systemic collaterals and splanchnic neovascularization in PH rats [5]. Apelin signaling has been known to contribute toward angiogenesis [6]; thus, apelin and its receptor APJ are essential for any embryonic angiogenesis [4,7,8] and are needed for retinal vessels' formation [3,9–12]. Furthermore, apelin pitch angiogenesis *in vivo* and *in vitro* [4,11–14]. Principe *et al.* [15] had shown that the endogenous apelin system could be involved in intrahepatic remodeling in cirrhotic rats. Thus, apelin signaling could represent a future therapeutic target during any pathological neovascularization associated with PH [16].

The role of apelin in the pathogenesis of liver cirrhosis is complex as described in a report linking apelin to the initiation and maintenance of the inflammatory and fibrogenic processes occurring in liver fibrosis [17] as well as the hemodynamic and vascular abnormalities in liver cirrhosis and its complications [15,18]. However, there are limited clinical data showing the role of apelin in liver cirrhosis, as reported by Bertolani and Marra [19]. Therefore, highlighting the apelin system would present a potential therapeutic target for liver disease. Thus, this study aimed to determine whether there is a relation between serum apelin and portal hemodynamics in liver cirrhosis.

Patients and methods

This study included 60 patients with liver cirrhosis and 20 healthy volunteers as a control group from Menoufia University Hospitals and the National Liver Institute in Menoufia University (Egypt) in the period from April 2014 to April 2015. They underwent physical examination and laboratory investigations such as complete blood count, urea, creatinine, liver profile [alanine transaminase (ALT), aspartate transaminase (AST), serum albumin, bilirubin, international normalized ratio (INR)], hepatitis C virus antibody, hepatitis B virus antigen, PCR for hepatitis C virus RNA, alpha feto protein (AFP), and serum level of apelin. Abdominal ultrasonographic studies of portal vein diameter, splenic size, and portal hemodynamics were carried out for all participants. Child–Pugh score, model for end-stage liver disease score, and the AST/PLT index were calculated for all patients. The exclusion criteria were as follows: patients with hypertension, diabetes mellitus, alcoholics, cardio-pulmonary disorders, hepatocellular carcinoma, renal disorders, and smokers.

Approval

The Menoufia Faculty of Medicine Committee for Medical Research Ethics reviewed and formally

approved the study before it was started and a written consent was obtained from all participants of the study.

Statistical analysis

The data collected were tabulated and analyzed using the SPSS statistical package version 11 (SPSS, version 11; SPSS Inc., Chicago, Illinois, USA) on IBM compatible computer. Descriptive statistics was presented as mean±SD, and number and percentage and analyzed using the χ^2 -test. Student's *t*-test was used to compare two groups of normally distributed variables; the Mann–Whitney *U*-test, the correlation co-efficient test (*r*-test), and regression analysis were also carried out whenever appropriate. Results were considered significant at a *P* value of less than 0.05 and highly significant at a *P* value of less than 0.001.

Results

Apelin level was highly significantly increased in cirrhotic patients than in controls, with a *P* value of 0.001 as shown in Table 1.

Apelin level was significantly correlated to some laboratory parameters in cirrhotic patients such as platelet (PLT) count, ALT, AST, γ -glutamyl transferase, and bilirubin, with a *P* value of less than 0.05 as shown in Table 2.

Apelin level was significantly correlated to portal vein diameter and portal flow velocity, with a *P* value of less than 0.05, and highly significantly correlated to splenomegaly, with a *P* value of 0.001, as shown in Table 3.

There was a positive correlation between serum apelin level and degree of liver fibrosis estimated by the AST/PLT index as shown in Table 4.

The optimal cut-off point of serum apelin in cirrhotic patients for the prediction of PH is 2550 ng/dl with a sensitivity of 89%, a specificity of 65%, and an accuracy of 81% as shown in Table 5.

Table 1 Comparison between the groups studied in apelin levels (n=80)

	Cirrhotic group (n=60) (mean±SD)	Control group (n=20) (mean±SD)	<i>U</i> test	<i>P</i> value
Age	52.2±6.4	48.7±10.4	0.22	0.07
Sex				
Male	41 (8)	11 (55)	0.11	0.3
Female	19 (32)	9 (45)		
Apelin (ng/dl)	4524.35±3642.16	2164.99 ±817.70	4.68	0.001**

**Highly significant.

Table 2 Correlation between apelin and laboratory parameters in the cirrhotic group

Laboratory parameters	Apelin	
	<i>r</i>	<i>P</i> value
Hemoglobin (g/dl)	0.03	0.81
WBCs (cell/cm ³ ×10 ³)	0.11	0.31
Platelets (cell/cm ³ ×10 ³)	+0.31	0.004*
Alanine transaminase (IU/l)	+0.22	0.04*
Aspartate transaminase (IU/l)	+0.41	0.001**
GGT (IU/l)	+0.22	0.04*
Albumin (g/dl)	+0.04	0.67
Bilirubin (mg/dl)	+0.28	0.01*
INR	0.01	0.92
Urea (mg/dl)	0.03	0.71
Creatinine (mg/dl)	0.02	0.81

GGT, γ -glutamyl transferase; INR, international normalized ratio; WBC, white blood cells. *Significant. **Highly significant.

Table 3 Correlation between apelin and ultrasound parameters in the cirrhotic group

U/S parameters	Apelin	
	<i>r</i>	<i>P</i> value
Portal vein diameter (mm)	+0.22	0.04*
Portal flow velocity (cm/s)	+0.28	0.01*
Splenic size (cm)	+0.41	0.001**

U/S, ultrasound. *Significant. **Highly significant.

Table 4 Correlation between apelin and the aspartate transaminase/platelet index, child score, and model for end-stage liver disease score in the cirrhotic group

Parameters	Apelin	
	<i>r</i>	<i>P</i> value
AST/PLT index	+0.39	0.001**
Child score	0.12	0.36
MELD score	-0.13	0.34

AST, aspartate transaminase; MELD, model for end-stage liver disease; PLT, platelet. **Highly significant.

Table 5 Validity of serum apelin for the prediction of portal hypertension

Optimal cut-off point	Sensitivity	Specificity	Accuracy
2550	89%	65%	81%

Discussion

Chronic liver disease (CLD) including cirrhosis can be induced by various etiologies, such as hepatitis viruses, alcohol, autoimmune, or metabolic causes. Through these stimulation and inflammatory injuries, intrahepatic fibrotic change occurs and is followed by intrahepatic vascular changes, angiogenesis, and development of PH [20].

Some emerging studies have pointed to the possible effects of the apelinergic system in the liver and related it to inflammation [21], fibrosis [17], angiogenesis as well as vascular and hemodynamic disturbances [15,18]. Other emerging studies

speculated that activated hepatic stellate cells represent a potential source for hepatic apelin [15] and that apelin contributes toward the proliferation of hepatic stellate cells induced by PLT-derived growth factors *in vitro* [18]. Furthermore, apelin could be an essential mediator of the profibrogenic gene induction that markedly initiates collagen-I synthesis [17], all of which are known to contribute largely toward deposition of extracellular matrix and progression of fibrosis [22,23]. Melgar-Lesmes *et al.* [17] pointed to the decrease in apelin expression induced by tumor necrosis factor- α in hematopoietic stem cell cultures and explained that it could represent a homeostatic protective response toward reducing the overactivated hepatic apelin system in advanced cirrhosis. These findings point to the possible role of apelin in CLD progression. In addition, this provides a rationale to investigate new drugs targeting the apelin-APJ signaling pathway to reduce fibrosis and to improve hemodynamics in these patients.

In the present study, the mean value of serum apelin was significantly higher in cirrhotic patients than in the control group. This result is in line with previous studies of Lim *et al.* [20], Principe *et al.* [15], and El-Mesallamy *et al.* [24], who reported that patients with cirrhosis showed a significant increase in apelin circulating levels than normal participants.

Another finding of the present study is the increased serum apelin level in patients with cirrhosis and PH and this is in agreement with Tiani *et al.* [5], who suggested that the expression of endogenous apelin-APJ signaling is associated with the development of PH and contributes toward the formation of Porto systemic collateral blood vessels and splanchnic neovascularization in PH rats. Moreover, Lim *et al.* [20] reported that serum apelin showed an increase with an increase in portal pressure. Yokomori *et al.* [18] reported that 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. In early-stage cirrhotic liver, apelin expression was increased in hepatic sinusoidal endothelial cells and in proliferated arterial capillaries directly connecting to the sinusoids. In end-stage cirrhosis, apelin was strongly expressed in proliferated arterial capillaries. These findings suggest a role of apelin in the capillarization of hepatic sinusoid and the proliferation of arterial capillaries in cirrhosis.

Tiani *et al.* [5] reported that the administration of apelin receptor blocker (F13A) effectively decreased

splanchnic neovascularization and the formation of Porto systemic collateral vessels as well as the expression of proangiogenic factors vascular endothelial growth factor, PLT-derived growth factor, and angiopoietin-2. These findings strongly suggested a possibility of the apelin antagonist (F13A) as a new therapeutic target in terms of both fibrosis and PH. Principe *et al.* [15] also reported that rats with cirrhosis treated with the apelin receptor antagonist showed decreased hepatic fibrosis and vessel density, improved cardiovascular performance, and renal function and lost ascites. These findings suggest that the apelin-APJ system could be a candidate for a therapeutic target of antifibrosis and antiportal hypertension treatment.

Our present data also found a significant positive correlation between serum apelin level and both AST and ALT, which is similar to the result of Sagiroglu *et al.* [25], who found that ALT and AST levels were significantly higher in rats injected with apelin intraperitoneally and then exposed to ischemia/reperfusion injury than the control group.

Also, our study found a significant positive correlation between serum apelin and liver fibrosis estimated by the AST/PLT index. These results are in agreement with Farid *et al.* [26], who analyzed the pattern of apelin expression in different stages of human CLD. In the early stage of hepatic fibrosis (F1 and F2), apelin was almost undetectable as in nonparenchymatous cells, such as sinusoidal endothelial cells/hepatic stellate cells, myofibroblasts, and endothelial cells. With progression of liver fibrosis (F3), apelin-positive cells were located in sinusoidal endothelial cells/hepatic stellate cells. In cirrhosis (F4), apelin-positive cells shifted to the fibrotic septa and spread as linear staining in the septa and on the proliferated capillary endothelial cells.

Conclusion

Serum apelin is elevated in patients with cirrhosis and PH, and a positive correlation was found between serum apelin and degree of hepatic fibrosis. Measurement of serum apelin levels can represent a rapid, noninvasive method for the prediction of PH in cirrhotic patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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