Usefulness of serum osteopontin level as a noninvasive parameter of portal hypertension

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Background
Osteopontin (OPN) is a multifunctional protein that is physiologically expressed in the kidney and bone. Plasma OPN levels were shown to predict liver fibrosis in various chronic liver diseases and could be related to the degree of portal hypertension. The aim of the study is to investigate the usefulness of OPN as a noninvasive biomarker of portal hypertension.
Patients and methods
A case–control study including 90 (45 patients with confirmed liver cirrhosis and 45 normal healthy individuals) patients were enrolled in the study. Laboratory investigations with abdominal ultrasound and duplex of the portal system were carried out for all patients. OPN was measured using the enzyme-linked immunosorbent assay in the plasma.
Results
Compared with controls, the plasma levels of OPN in cirrhotic patients were significantly high ($P<0.001$). Also, plasma levels of OPN were significantly high in patients with portal hypertension ($P<0.001$). The cutoff value of OPN to detect the presence of portal hypertension is 1.65 ng/ml with sensitivity and specificity of 80 and 95.6%, respectively).
Conclusion
Serum OPN is a good noninvasive parameter to detect portal hypertension.

Keywords:
cirrhosis, osteopontin, portal hypertension

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Introduction

Liver injury and fibrosis have a pathological relation to portal hypertension that leads to major complications of cirrhosis and has been evaluated by invasive measurement of portal pressure [1]. The hepatic venous pressure gradient (HVPG) is a prognostic factor for survival in case of cirrhosis and can reflect progression of the disease in the precirrhotic stage [2]. There is an association between the severity of hepatic inflammation and fibrosis and HVPG even before cirrhosis develops [3].

Recently, noninvasive biomarkers of cirrhosis have been suggested as substitutes for invasive measurement of portal pressure in some indications [4]. Osteopontin (OPN), a multifunctional protein, is involved in numerous pathological conditions including inflammation, immunity, angiogenesis, fibrogenesis, and carcinogenesis in various tissues.

The protein is produced in a variety of tissues: brain, liver, gastrointestinal tract, lung, bone, cardiac tissues, joints, and kidney and appears in a variety of biologic fluids including blood, urine, milk, and seminal fluid [5,6].

Plasma OPN levels were shown to predict liver fibrosis in various chronic liver diseases, such as nonalcoholic steatohepatitis [7], alcoholic liver disease [8], and chronic viral hepatitis B and C [9].

As OPN levels correlate significantly with the fibrosis stage in alcohol-induced liver disease [8], it follows that OPN levels could be related to the degree of portal hypertension and, hence, serve as a surrogate noninvasive marker of portal hypertension. Therefore, this study was carried out to investigate the usefulness of OPN as a noninvasive biomarker of portal hypertension.

Patients and methods

This study included 90 participants who were divided into two groups:

(1) Group 1: 45 (15 women and 30 men) patients with liver cirrhosis (LC) secondary to HCV, age ranged from 42 to 75 years with age median value of 58 years.
(2) Group 2: 45 (26 women and 19 men) normal healthy individuals as the control group, age ranged from 46 to 58 years with age median value of 35 years.

All patients were chosen from Kasr El Aini University Hospital, Internal Medicine Department and Clinic. The study protocol conformed to the ethical guidelines of the Declaration of Helesinki [10], approved by Internal Medicine Research Ethics Committee, Cairo University. A written informed consent was obtained from all patients participating in the study.

Inclusion criteria

All patients with LC are secondary to hepatitis C virus infection.

Exclusion criteria

(1) Patients with any malignant tumor including hepatocellular carcinoma.
(2) Concomitant antiviral treatment.
(3) The use of any drug affecting splanchnic hemodynamics or portal pressure within 2 weeks before measurement of portal venous pressure.
(4) Portal vein thrombosis.

Specimen collection and handling

Blood samples were collected under aseptic conditions by clean, venipuncture without frothing using disposable syringes. About 10 ml of blood was withdrawn after taking an informed consent from each patient and distributed into the following three portions:

(1) About 3 ml of blood was delivered into a test tube containing EDTA to perform the hemoglobin and platelet count.
(2) About 3 ml of blood was delivered into a test tube containing sodium citrate for prothrombin time.
(3) Four ml of blood was delivered in a clean dry test tube and allowed to clot at room temperature. The serum was separated by centrifugation at 2000 rpm for 10 min for chemistry investigations and OPN assay.

Methods

The patients were subjected to the following:

(1) Thorough history taking.
(2) Clinical examination.
(3) Laboratory investigations include:
    (a) Complete blood count.
    (b) Liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ glutamyltransferase, total protein, albumin, prothrombin time, prothrombin concentration, international normalized ratio, bilirubin total, and direct].
    (c) Kidney function tests: urea and creatinine.
    (d) Hepatitis markers for B and C virus: hepatitis B surface antigen and hepatitis C virus antibody.
(4) Measurement of serum OPN level.
(5) Abdominal ultrasound.
(6) Duplex on portal circulation.
(7) Upper gastrointestinal tract endoscopy.

Portal vein evaluation

Portal system was evaluated by ultrasound (gray scale), color form, and pulsate wave. The flow was confirmed by a power Doppler and B-flow modality using a curvilinear transducer of Philips IU 22 (Amsterdam, Netherlands) high-end machine by the operator for all patients.

Portal vein was measured at the hilum of the liver. The direction of flow was detected by Doppler wave form in addition to same settings and modules for splenic and superior mesenteric venous evaluation.

Portal vein thrombosis was excluded by gray scale evaluation.

Portal vein measurement and direction of the flow in addition to splenic and superior mesenteric calibers were used to assess portal hypertension.

So, presence of dilated portal vein of more than 13 mm, retrograde portal vein flow, splenic hypertension (evident by dilated tortuous splenic vein or presence of splenomegaly), and ascites can provide a clue to diagnose portal hypertension.

Measurement of serum osteopontin level

Serum OPN level was estimated by enzyme-linked immunosorbent assay kits (human OPN Quankine enzyme-linked immunosorbent assay kit DOS 100) that uses a double-antibody sandwich to assay the level of OPN in blood samples. The results are calculated by the straight line regression equation of the standard curve with the standard density and the OD value. With the sample OD value in the equation, the sample density is calculated.

Statistical methods

Data were coded and entered using the statistical package for the social sciences (SPSS) version 25. Data were summarized using mean, SD, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the nonparametric Mann–Whitney test. For comparing categorical data, χ^2-test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman's correlation coefficient. A receiver operating characteristic curve (ROC) curve was constructed with area under curve analysis to detect the best cutoff value of OPN for detection of portal hypertension. *P* values less than 0.05 were considered as statistically significant.

Results

In this study, there is significant difference between both groups regarding hemoglobin and platelet count. Albumin and prothrombin concentration were significantly low in patients. However, AST, ALT, ALP, γ glutamyltransferase, bilirubin, and international normalized ratio were significantly high in patients. Interestingly, OPN was statistically high in patients compared with the control group as shown in Table 1. From the data in Table 2, it is apparent that the majority of patients with LC had ascites. About half of cirrhotic patients had hepatic encephalopathy. And by abdominal ultra-sound (US) and duplex of the portal system, all patients had portal hypertension and this was statistically significant. We found that the mean of portal vein diameter and the mean of variceal size in cirrhotic patients are 1.47±0.44 and 2.2±0.81, respectively, as shown in Table 3.

Surprisingly, we found no significant correlation between serum OPN concentration and other noninvasive parameters for detection of portal hypertension as shown in Table 4, but OPN level was statistically very high in patients with portal hypertension as shown in

Table 2 Characteristic data of cases involved in the study

	Groups [count (%)]		*P* value
	Patient	Control	
Ultrasound			
Liver cirrhosis	45 (100.0)	0	<0.001
No	0	45 (100.0)	
Portal hypertension			
Present	45 (100.0)	0	<0.001
No	0	45 (100.0)	
Ascites			
Present	30 (66.7)	0	–
No	15 (33.3)	0	
Encephalopathy			
Positive history	25 (55.6)	0	–
No history	20 (44.4)	45 (100.0)	

Table 1 Demographic data of cases involved in the study

	Groups										
	Patient					Control					
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	*P* value
Age	58.96	7.79	58.00	42.00	75.00	34.76	10.28	35.00	20.00	56.00	<0.001
TLC	6.48	4.39	5.20	1.67	25.90	6.55	1.60	6.30	4.20	9.60	0.087
Hb	9.22	1.59	8.80	7.01	15.10	13.15	1.51	12.80	10.80	16.20	<0.001
PLT	101.78	52.18	95.00	30.00	243.00	241.27	60.72	244.00	151.00	392.00	<0.001
AST	77.47	79.77	58.00	20.00	533.00	22.93	9.49	22.00	8.00	45.00	<0.001
ALT	58.22	121.87	34.00	13.00	837.00	19.04	8.54	18.00	6.00	39.00	<0.001
ALB	2.68	0.61	2.60	1.50	3.90	4.23	0.50	4.20	3.50	5.20	<0.001
Total Protein	6.43	0.62	6.50	5.00	8.30	7.90	0.52	8.10	6.50	8.50	<0.001
ALP	105.56	46.18	101.00	32.00	215.00	59.24	17.14	55.00	33.00	99.00	<0.001
GGT	40.60	35.31	28.00	6.00	228.00	19.18	9.75	18.00	5.00	45.00	<0.001
Total bilirubin	2.10	2.06	1.50	0.30	12.70	0.82	0.25	0.84	0.33	1.25	<0.001
Direct bilirubin	0.78	0.99	0.40	0.00	6.10	0.11	0.08	0.10	0.00	0.30	<0.001
PT	17.78	3.13	17.80	11.80	25.80	12.95	0.74	13.00	11.20	14.30	<0.001
PC	58.73	17.67	55.00	31.00	99.00	84.91	10.22	83.00	70.00	110.00	<0.001
INR	1.50	0.34	1.50	1.01	2.61	1.07	0.16	1.09	0.10	1.20	<0.001
Urea	59.16	32.51	46.00	22.00	152.00	17.47	4.76	17.00	10.00	26.00	<0.001
Creatinine	1.39	1.11	1.12	0.40	7.27	0.84	0.17	0.82	0.52	1.23	<0.001
OPN level (ng/ml)	6.38	7.49	3.50	1.40	37.20	1.33	0.26	1.40	0.80	1.70	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ glutamyltransferase; Hb, hemoglobin; INR, international normalized ratio; LB, albumin; LP, alkaline phosphatase; OPN, osteopontin; PC, prothrombin concentration; PLT, platelets; PT, prothrombin time; TLC, total leukocytic count.

Table 3 Noninvasive parameters for detection of portal hypertension

	Patient				
	Mean	SD	Median	Minimum	Maximum
Diameter of portal vein (cm)	1.47	0.44	1.41	0.70	3.19
AST/ALT ratio	1.72	0.50	1.67	0.63	2.87
Platelet/spleen size	7.14	3.84	6.64	1.97	17.48
Varices size	2.20	0.81	2.00	1.00	4.00
Child score	9.02	2.06	9.00	5.00	14.00
MELD score	15.18	5.10	14.00	7.00	34.00

ALT, alanine aminotransferase; AST, aspartate aminotransferase; MELD, model for end-stage liver disease.

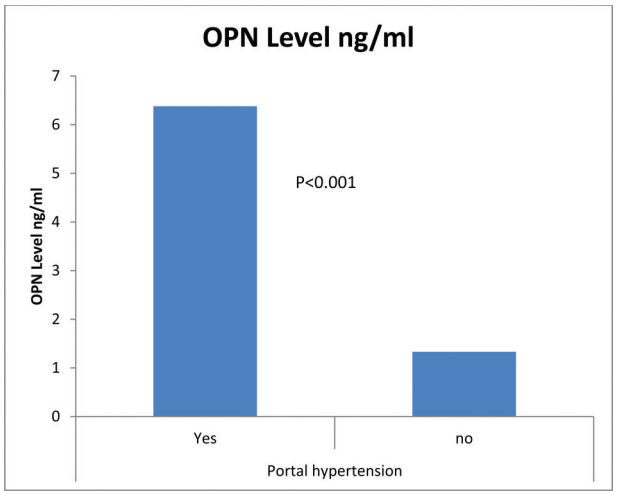
Table 4 Correlation between osteopontin and noninvasive parameters for detection of portal hypertension

Diameter of portal vein (cm)	
Correlation coefficient	**−0.123**
P value	**0.423 (NS)**
N	45
AST/ALT ratio	
Correlation coefficient	−0.020
P value	0.898 (NS)
N	45
Platelet/spleen size	
Correlation coefficient	−0.027
P value	0.860 (NS)
N	45
Varices size	
Correlation coefficient	0.142
P value	0.353 (NS)
N	45
Child score	
Correlation coefficient	−0.039
P value	0.798 (NS)
N	45
MELD score	
Correlation coefficient	−0.034
P value	0.827 (NS)
N	45

ALT, alanine aminotransferase; AST, aspartate aminotransferase MELD, model for end-stage liver disease; NS, non significant.

Fig. 1. The cutoff value of OPN for detection of portal hypertension is 1.65 ng/ml with a sensitivity of 80% and specificity of 95.6% as highlighted in Fig. 2.

Figure 1



Correlation between osteopontin and portal hypertension.

Discussion

OPN is highly expressed in many inflamed tissues and has a role in wound healing, being a proinflammatory cytokine and integrin-binding ligand [11].

Increased OPN gene expression has been reported in macrophages, Kupffer cells, stellate cells, biliary epithelial cells and in inflammatory cells of the necrotic areas in rodent liver fibrosis [12].

The observation that hepatic inflammation was associated with elevated serum OPN levels is in favor of a role for OPN in liver inflammation. Recent studies have highlighted the role of OPN in inflammatory liver diseases as alcoholic and nonalcoholic liver diseases and T-cell-mediated hepatitis [13].

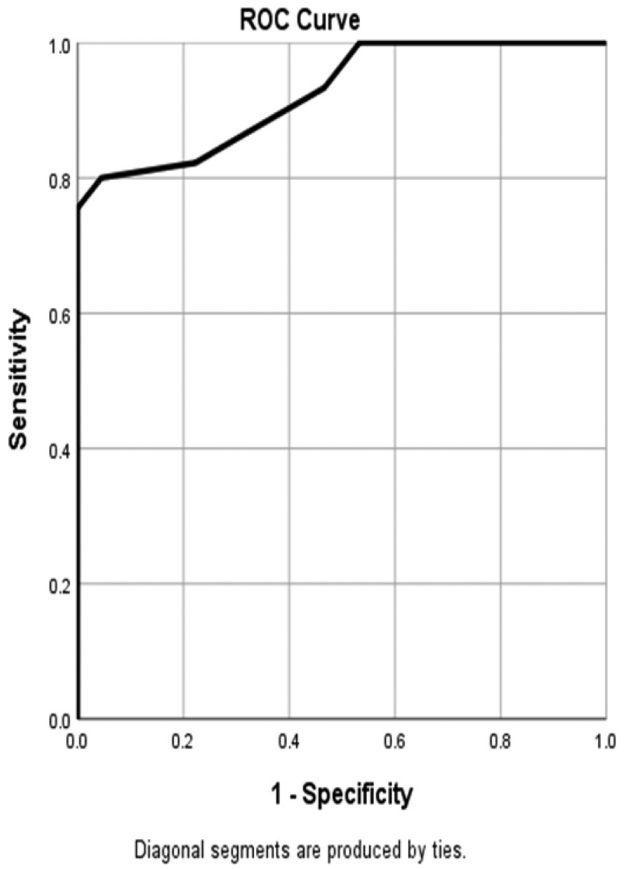
Furthermore, it has been suggested that OPN could have fibrogenic properties. OPN expression was increased in activated hepatic stellate cells [14] and was required for myofibroblast differentiation [15].

As OPN levels correlate significantly with the fibrosis stage in alcohol-induced liver disease, it follows that OPN levels could be related to the degree of portal hypertension and, hence, serve as a surrogate noninvasive marker of portal hypertension.

In this study, we tried to evaluate the usefulness of serum OPN as a noninvasive parameter of portal hypertension. To our knowledge, there is only one previous study which investigates the relation between OPN and portal hypertension.

For this purpose, 90 patients of LC due to hepatitis C virus were subjected to history taking, full clinical

Figure 2



A receiver operating characteristic curve (ROC) curve to detect portal hypertension using osteopontin.

examination, and thorough full laboratory assessment for liver function, abdominal ultrasound, and Doppler on portal circulation. We found that six patients have been classified as Child–Pugh A, 21 patients as Child–Pugh B, and 18 patients were classified as Child–Pugh C.

In this study, we found that the majority of cases with LC were men (66%). This agrees with the study by Guy and Peters [16] who found that the majority of cases of liver disease due to viral hepatitis are men.

This study showed that liver enzymes were high in cirrhotic patients with statistically significant difference by comparison between both groups. This result partially agrees with the study done by Khattab *et al.* [17] which stated that AST increases in patients with LC in comparison to controls. Total protein and albumin were low in cirrhotic patients with statistically significant difference in comparison with the control group ($P>0.001$). This finding supports previous research done by Nagao and Sata [18] which showed that albumin level decreases in patients with LC due to impairment of liver function and signifies albumin as a predictor of mortality in patients with chronic liver disease.

This study observed that serum OPN level was significantly high in our patients enrolled in the study when compared with the control group. This result agrees with the study by Bruha *et al.* [19]who showed that the plasma levels of OPN in cirrhotic patients were significantly high ($P<0.001$); moreover, this finding supports previous research done by Zhao *et al.* [20] who found that plasma levels of OPN were significantly increased in cirrhosis ($P<0.001$).

This finding also corroborates the ideas of Patouraux *et al.* [8] who found that the OPN level was increased in patients with moderate and severe fibrosis or cirrhosis compared with patients with mild fibrosis. This study showed that OPN was high in patient with ascites, but it was not statistically significant. In contrast to our study, Bruha *et al.* [19] and colleagues study showed a significant correlation between OPN level and presence of ascites ($P<0.001$); this difference can be partially explained by the difference in the number of patients involved in the study; our study was done on 90 participants but the study by Bruha *et al.* [19] included 154 participants.

There was no correlation between OPN serum level and different clinical and noninvasive parameters of portal hypertension such as diameter of the portal vein, platelet count, AST/ALT ratio, size of varices, and history of variceal bleeding. These results were concordant with the study by Bruha *et al.* [19] which stated that the OPN level only correlates with platelet count ($P = 0.009$).

This difference can be partially explained by the difference in platelet count between our study and the study by Bruha *et al.* [19] as the platelet count in our study was lower than the count in their study which is 101 (30–243) versus 107 (74–163), respectively.

The most interesting finding was that OPN level was significantly high in patients with portal hypertension when compared with controls ($P<0.001$).

It is encouraging to compare this result with that found by Pereira *et al.* [21], who concluded that patients with hepatointestinal schistosomiasis with portal hypertension have increased serum levels of OPN than whose without portal hypertension, suggesting that increased OPN levels may be indicative not only of liver fibrosis, but also of portal hypertension, this result

is also consistent with the study made by Honsawek *et al.* [22] who found that patients with biliary atresia and portal hypertension had higher plasma OPN levels compared with those without portal hypertension (116.7±31.1 vs. 19.5±9.3 ng/ml, P=0.01).

Moreover in this study, the cutoff OPN value of 1.65 ng/ml is sufficient to detect presence of portal hypertension with sensitivity 80% and specificity 95.6%. In contrast to the study made by Bruha *et al.* [19] which showed that plasma levels of OPN were closely and positively related to HVPG values (P=0.002) and plasma levels of OPN above 80 ng/ml distinguishes patients with HVPG of more than 10 mmHg with 75% sensitivity and 63% specificity.

This difference can be partially explained by the difference in methodology used for the assessment of portal hypertension; in our study portal hypertension was assessed by doing Doppler of the portal system, while Bruha *et al.* [19] assessed portal hypertension by doing catheterization of portal system and measurement of HVPG.

Conclusion

This study found that serum OPN is a good predictor of portal hypertension and act as a novel noninvasive parameter of portal hypertension.

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Conflicts of interest

There are no conflicts of interest.

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