Serum Paraoxonase-1 (PON1) Activity and Concentration: a New Biomarker in Cirrhotic and Hepatocellular Carcinoma Egyptian Patients

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Abbreviations:
HCC, Hepatocellular carcinoma, PON-1, paraoxnase-1, HDL, high density lipoprotein, AFP, alpha Fetoprotein, LC, liver cirrhosis, CLD, chronic liver disease, ALT, alanine transaminase, AST, aspartate transaminase

Background and study aim: Hepatoma is the most common cancer worldwide, being regarded as the fifth of all malignancies. Mandating for a new, sensitive and specific markers for HCC are critically needed. Human paraoxonase-1 (PON-1), a Ca^{+2} dependent esterase in the liver, with antioxidant functions, and binds to the HDL particles and hence discarding carcinogenic lipid radicals as byproduct of lipid peroxidation. The aim of this study was to estimate the importance of serum PON-1 activity and concentration in HCC in Egyptian patients as a more sensitive and specific biomarker compared to an established biomarker; Alpha-fetoprotein (AFP).

Patients and Methods: This study was conducted on 88 subjects: 64 patients; 40 patients with HCC, 24 cirrhotic patients and 24 healthy subjects who admitted in Internal Medicine Department, Suez Canal University Hospital, Ismailia, Egypt. Clinical and radiological investigations in those of the Cirrhotic, and HCC groups for viral hepatitis markers, liver function tests, serum assay of AFP and PON-1 activity and cconcentrations.

Results: The results showed that PON-1 concentration was found significantly lower in HCC patients than those of the control and Cirrhotic group ($p<0.001$).The sensitivity and specificity of PON-1 concentration for HCC were superior to those of AFP and PON-1 activity especially in recently detected HCC. PON-1 concentration had a sensitivity of 88.3% and specificity of 90.48% at the optimal cut-off value of 70.55 (ng/ml). AFP gives a sensitivity of 82.50% and specificity of 73.08 at a cut-off 20.44 ng/ml.

Conclusion: This study demonstrates that PON1 concentration and activity were superior to AFP in the recently detected HCC.

INTRODUCTION

Hepatoma is the commonest primary liver cancer. Major etiologic factors for HCC are longstanding infections with hepatotropic viruses; hepatitis B virus (HBV), hepatitis C virus (HCV), the exposure to aflatoxin-b and excessive alcohol consumption **[1]**.

Poor life expectancy in HCC patients with a less than 5% 5-year survival rate. Screening strategies every 6 month in Cirrhotic patients including AFP and ultrasound**[3]**. However, Diagnostic AFP is a marker with poor sensitivity and specificity **[2]** and the ultrasound is dependent on the experience of the operator **[3]**.

Human serum paraoxonase-1 (PON-1) that has lipophilic antioxidant characteristics. Participating in the removal and clearing of organo-phosphorus compounds. Also, by removal of carcinogenic lipid soluble radicals as byproduct of lipid peroxi-dation. PON-1 is one of the endogenous free-radical scavenging systems in the human body **[4,5]**. PON-1 is mani-factured in the liver and secreted into the blood, bound to less than 10% of the total high-density lipoprotein (HDL) particles **[5,9]**. Reduced PON-1 activity, with increased oxidative stress in diabetes mellitus, hypercholesterolemia, and cardiovascular disease **[6,20,28]**.

Hence, serum PON1 activity reflecting an index of liver function status **[9,10]**. Preliminary studies demonstrated decreased serum arylesterase activity significantly in Cirrhotic patients **[7,8]**. Previous investigators have measured PON1 activity were decreased significantly in serum of chronic liver disease patients such as; alcoholic liver disease, chronic hepatitis, and cirrhosis **[9-13]**. Kilic et al. have demonstrated that decreased serum arylesterase activity significantly in patients with chronic hepatitis **[13]**. Additionally, Xu et al. have shown that successful liver transplantation increased PON1 activity which was previously decreased PON1 activity in chronic liver diseases **[14]**. PON1 has been shown to protect liver damage by eliminating oxidative stress by CCl_4 **[15]**. Furthermore, Marsillach et al. have reported that PON1 protects hepatocytes against inflammation, fibrosis and thus liver diseases **[16]**. Kedage et al., demonstrated that liver disorders showing decreased Serum Paraoxonase 1 Activity Status **[17]**.

The present work aimed to investigate the usefulness of biomarkers as serum PON1 activity and concentration in cirrhotic and HCC patients as a sensitive and specific test by comparing it with an already established biomarker as AFP.

SUBJECTS AND METHODS

Subjects

This cohort prospective descriptive study was carried out in the Internal Medicine, Biochemistry Departments, Suez Canal University Hospital and Chemistry department of Faculty of Science, Suez Canal University, Ismailia, Egypt, during the Period from 19 March 2017 to 31 September 2017.

Eighty-eight subjects; 64 patients and 24 healthy controls were conducted in this study and classified into three groups:

Group 1. HCC group included 40 patients with HCC (26 males and 14 females, their mean age are 56±9.5 years old). Those were diagnosed according to clinical examination, laboratory and radiological investigations including abdominal Ultrasonography and Triphasic abdominal C.T. Scan. HCC patients clinical data were collected to determine tumor characteristics as tumor number, size, and micro/macrovascular involvement.

Group 2. Liver Cirrhotic (LC) group, including 24 patients (17 males and 7 females, their mean age is 56.3±10.3years old). The diagnosis of cirrhosis was based on clinical, biochemical, and ultrasonography.

Group 3. Control group, including 24 apparently healthy volunteers (18 males and 6 females, their mean age are 52.83 ±7.47years old). The control group had no evidence of liver disease by clinical or biochemical or imaging paramaters or known current medical illness at recruitment. All controls were not diabetics, negative viral serological markers. All patients and controls gave their informed consent which was ethically conducted in accordance with the Helsinki Declaration.

Blood sampling:

Six milliliters of venous blood samples were taken, from each participant and divided into, 2ml in tube containing sodium citrate for prothrombin time and the rest of blood was left to clot, then the serum was separated by 10 minutes centrifugation at 2300g and the serum samples then collected, divided into aliquots and stored at -20oC for further analysis.

Laboratory investigations:

Liver function tests in the form of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin and total bilirubin, total Protein, Albumin, prothrombin time (PT), and hepatitis viral markers including HCV antibody and HBsAg.

Tumor markers:

The serum concentration of AFP was determined by enzyme-linked immunosorbent assay (ELISA)-AFP Kit (Genway Company, USA) according to the method described in **[18]**. PON1 activity was measured using commercially available kits (Relassay®; Turkey) **[19]**. Enzyme-linked immunosorbent assay (ELISA) is used to determine PON1 concentration and activity **[20]**.

RESULTS

The Demographic data of the studied groups: according to the severity of liver disease using Child Classification in the Cirrhotic (LC) and HCC groups, classified as 17.5% of HCC group were child A, 35% were child B and 47.5% were child C compared to 33.3%, 54.2% and 12.5 % in the LC group respectively.

Table (1): Liver Function Tests of the study groups

Parameter	Group	N	Mean ± SD	Test-value	P For all groups	P Between groups comparison	
AST (U/L)	control	24	26±8.9	24.664	<0.001	P_1	<0. 01
	LC	24	49.5±25.1			P_2	<0.01
	HCC	40	63.6±43.8			P_3	<0.05
ALT (U/L)	control	24	18.8±8.6	30.757	<0.001	P_1	<0.01
	LC	24	33. 5±15.8			P_2	<0.01
	HCC	40	50.5±40.0			P_3	>0.05
ALP (U/L)	control	24	64.33±17.3	23.68	<0.001	P_1	<0.01
	LC	24	89.83±56.84			P_2	<0.01
	HCC	40	167.68±80.7			P_3	<0.01
Total bilirubin (mg/dl)	control	24	0.86±0.23	47.357	<0.001	P1	<0.01
	LC	24	4.3±4.7			P_2	<0.01
	HCC	40	6.1±4.9			P_3	>0.05
Direct bilirubin (mg/dl)	control	24	0.13±0.04	52.007	<0.001	P_1	<0.01
	LC	24	2.03±2.33			P_2	<0.01
	HCC	40	2.3±1.87			P_3	>0.05
Total protein (g/L)	control	24	7.93±0.55	55.138	<0.001	P_1	<0.01
	LC	24	5.61±1.10			P_2	<0.01
	HCC	40	5.74±0.92			P_3	<0.01
Albumin (g/L)	control	24	4.6±0.67	112.631	<0.001	P_1	<0.01
	LC	24	2.9±0.96			P_2	<0.01
	HCC	40	2.1±0.34			P_3	<0.01

t_1 & p_1 difference between LC group and the control group, t_2 & p_2 difference between HCC group and the control group and t3 & p3 difference between HCC group and the LC group, P<0.05 (significant), P>0.05 (insignificant)

Laboratory tests for liver function identified in Table 1. There was a significant increase in the mean level of serum AST, ALT, ALP and increase in serum total bilirubin and a significant decline in the mean level of serum albumin, total protein in both LC and HCC groups compared to the control group p=0.001. Although, total serum bilirubin, ALT and serum albumin showed no statistically significant difference was observed between cirrhotic and HCC. The mean level of AST and Alkaline phosphatase was decreased in the cirrhotic group than the HCC group (P<0.01).

Table (2): Comparison between the studied groups according to serum levels of AFP, PON1 concentration and activity.

Parameter	Group	N	Range	Mean± SD	Median	t-value		p-value	
AFP (ng/ml)	control	24	0.86- 7.24	3.0±1.6	3.06	t_1	2.11	p_1	<0.001
	LC	24	0.61- 31.00	6.38±7.66	2.7450	t_2	5.05	p_2	<0.001
	HCC	40	4.40 - 1337.00	323.30±401.03	130.95	t_3	4.99	p_3	<0.001
PON1 concentration (g/ml)	control	24	55.00-89	74.6±8.7	76.00	t_1	-8.04	p_1	<0.001
	LC	24	55.00- 151.00	108.3±28.1	110.50	t_2	-14.03	p_2	<0.001
	HCC	40	19.00- 88.00	47.9±15.3	46.50	t_3	-9.70	p_3	<0.001
PON1 activity (U/L)	control	24	111.00 -316.00	210.5±55.5	201.5000	t_1	14.39	p_1	<0.001
	LC	24	113.00 -211.00	149.3±23.9	110.50	t_2	5.90	p_2	<0.001
	HCC	40	49.00- 152.00	104.9±30.4	106.00	t_3	-6.48	p_3	<0.001

t_1 & p_1 difference between LC group and the control group, t_2 & p_2 difference between HCC group and the control group and t3 & p3 difference between HCC group and the LC group, P<0.05 (significant), P>0.05 (insignificant)

As illustrated in the table 2 and Figure 5, HCC patients had significantly higher serum AFP (ng/ml) compared to both the control group (323.30 ± 401.03 vs. 3.0±1.6) (p<0.001) and to LC patients (323.30±401.03 vs 6.38±7.66) (p<0.001), and still significantly higher in the LC patients compared to the control group (6.38 ± 7.66 vs. 2.79 ± 1.26) (P<0.001).

Regarding to the results of mean serum levels of PON1 concentration level (ng/ml), table 2, Figure 1 showed a significant reduced levels in the HCC group compared to the controls (47.9±15 vs 74.6 ± 8.7) (P<0.001) and to the LC group (47.9±15 vs 108.3±28.1) (P<0.001). Furthermore, an increase in serum PON1 concentration significantly was found in the LC group compared to the control group (108.3± 28.1 vs 74.6±8.7 (P<0.001). Regarding the PON1 activity, table 2 shows also a significant reduced serum PON1 activity (U/L) level in the HCC patients compared to the controls (104.9± 30.4 vs 210.5± 55.5) (P<0.001) and to the LC patients (104.9±30.4vs. 149.3± 23.9) (P<0.001). While in the LC group, a significant decrease in serum PON1 activity level was found in the LC group compared to the control group (149.3±23.9 vs 210.5±55.5) (P<0.001).

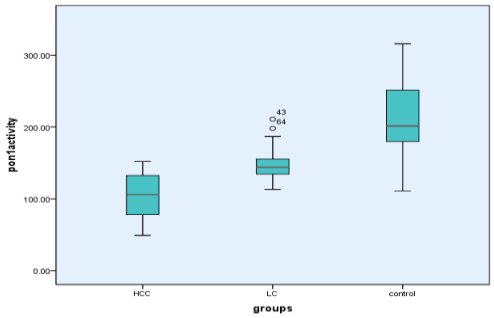
Serum PON1 concentration shows a significant difference in results among the different Child-Pugh classes (p_1<0.05. p_2<0.001, p_3<0.05) respectively. Serum PON1 activity is significantly higher in Child A than child C (p2<0.05), but there is no significant difference between the other groups: child B and child C, and Child A and child B (p_1>0.05), (p_3>0.05). Serum AFP concentration is significantly higher in Child A than Child C (p_2<0.05) and Child B than Child C (p_3<0.05), but there is no significant difference between Child A and Child B (Table 4).

Diagnostic Performance of studied markers

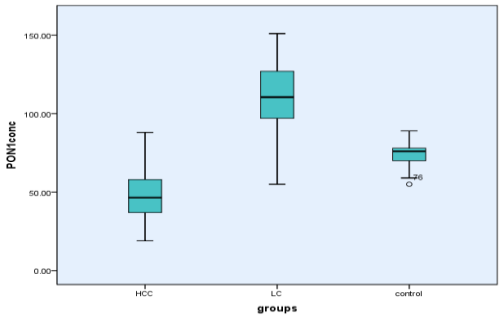
Analysis of the data in the ROC curve as in (Table 4, Figure 2), it showed the cut-off value (which had the highest Youden's index) for AFP to differentiate HCC from LC patients of 20.44 ng/ml. This gave 82.50% in sensitivity, 73.08 % in specificity, 82.50% in PPV, 79.17% in NPV. The area under the ROC curve (AUROC) was 0.92.

On the other hand, the cutoff is at a value 121.58 (U/L), serum PON1 activity had a sensitivity of 82.50%, a specificity of 70.83%. The PPV was 82.50% and the NPV was 70.83%. The AUROC was 0.86.

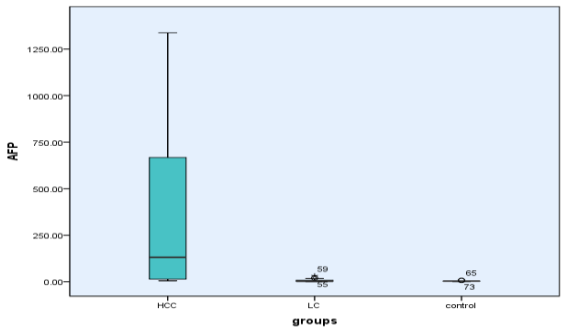
Furthermore, at 70.55 (U/L) optimal cut-off serum PON1 concentration had 88.37% sensitivity and 90.48% specificity. Positive Predictive Value PPV 95.00%, NPV 79.17% and AUROC was 0.96.



A: Comparison between PON1 activity levels in Patients with HCC, LC and control subjects



B: Comparison between PON1 concentration levels in Patients with HCC, LC and control subjects



C: Comparison between AFP levels in patients with HCC, LC and control subjects.

Figure 1: Box plots showing the level of studied markers in the HCC, LC and control groups

Table (3): Comparison of serum AFP, PON1 concentration and PON1 activity in HCC group according to Child-Pugh class

Parameter	Child-Pugh classes	NO. %	Range	Mean± SD	Median	t-value		P-value	
AFP (ng/ml)	Class A	7(17.5)	6-131.9	29.29 ±45.46	14.61	t_1	-0.79	p_1	>0.05
	Class B	14(35)	4.4-602	78.48± 160.55	303.5	t_2	-3.78	p_2	<0.05
	Class C	19(47)	128.18-1337	612.02±401.16	731.30	t_3	-5.23	p_3	<0.001
PON1 concentration (ng/ml)	Class A	7(17.5)	51-88	66.43± 12.55	66.0	t_1	2.84	p_1	<0.05
	Class B	14(35)	32-69	50.0±12.42	46.5	t_2	5.18	p_2	<0.001
	Class C	19(47)	19-58	39.578±12.42	43.00	t_3	2.50	p_3	<0.05
PON1 activity (U/L)	Class A	7(17.5)	111-145	125.86±13.16	123.00	t_1	1.64	p_1	>0.05
	Class B	14(35)	59-150	106.93±28.87	99.00	t_2	3.33	p_2	<0.05
	Class C	19(47)	49-152	95.74±32.94	86.00	t_3	1.04	p_3	>0.05

t_1 & p_1 difference between the A class and the B class ,t_2 & p_2 difference between A class and the C class and t_3 & p_3 difference between B class and C class, P<0.05 (significant) , P>0.05 (insignificant).

Serum PON1 concentration shows a significant difference in results among the different Child-Pugh classes (p_1<0.05. p_2<0.001, p_3<0.05) respectively. Child A had significantly higher Serum PON1 activity than Child C (p2<0.05), but there is no significant difference between Child B and Child C, and Child A and Child B (p_1>0.05), (p_3>0.05). Serum AFP concentration is significantly higher in Child C than Child A (p_2<0.05) and Child B (p_3<0.05), but there is no significant difference between Child A and child B (Table 4).

Table (4): Analysis showing Cut-off, Sensitivity, Specificity, AUC, PPV and NPV of the Studied Markers

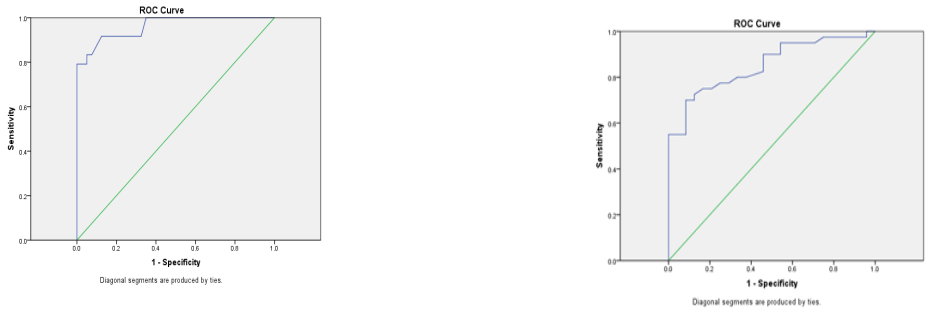
Parameter	AFP (ng/ml)	PON1 activity (U/L)	PON1 concentration (ng/ml)
Cut off value	20.44	121.58	70.55
Sensitivity (%)	86.84	82.50	88.37
Specificity (%)	73.08	70.83	90.48
Positive predictive value (%)	82.50	82.50	95.00
Negative predictive value (%)	79.17	70.83	79.17
Youden's index	0.60	0.53	0.79
Area Under the ROC	0.92	0.86	0.96
P- value	<0.001	<0.001	<0.001

PPV= Positive predictive value. NPV= Negative predictive value

Diagnostic Performance of studied markers

Analysis of the data in the ROC curve as in (Table 4, Figure 2), it showed the cut-off value (which had the highest Youden's index) for AFP to differentiate HCC from LC patients of 20.44ng/ml. This gave 82.50% in sensitivity, 73.08 % in specificity, 82.50% in PPV, 79.17% in NPV. The area under the ROC curve (AUROC) was 0.92.

On the other hand, the cutoff is at a value 121.58 (U/L), serum PON1 activity had a sensitivity of 82.50%, a specificity of 70.83%. The PPV was 82.50% and the NPV was 70.83%. The AUROC was 0.86. Furthermore, at 70.55 (U/L) optimal cut-off serum PON1 concentration had 88.37% sensitivity and 90.48% specificity. Positive Predictive Value PPV 95.00%, NPV 79.17% and AUROC was 0.



A: PON1 concentrations: ROC curve **B:** PON1 activity: ROC curve

Figure 2: The diagnostic performance of the studied markers in HCC patients vs. LC patients

DISCUSSION

The malignant transformation from Liver Cirrhosis to HCC is usually asymptomatic, being diagnosis late and thus increased mortality rate. The diagnosis of HCC in cirrhotic patients is often based on surveillance strategies every 6 months whose mainstay is the measurement of the levels of AFP **[21]** and ultrasound **[3,21]**. The performance characteristics of AFP as a screening diagnostic test apparently to be less satisfactory than its use as a prognostic test. Thus, there is a great need to establish another method for screening and diagnosis as early as possible for HCC **[21]**. PON 1 enzyme is a calcium-dependent glycoprotein produced in the liver and associated HDL4 **[4,27,28]**. PON1 prevents oxidative modification of low-density lipoprotein (LDL) particles and eliminating carcinogenic lipid-soluble radicals **[4]**.

In this study, HCC patients showed significantly reduced serum PON1 concentration compared with LC and healthy controls (P<0.001), with optimal cutoff 70.55 showed a higher performance than serum AFP with optimal cutoff 20.44ng/ml in diagnostic sensitivity (88.37% vs. 82.50%), specificity (90.48% vs. 73.08%), PPV (95.0% vs. 82.50), NPV (79.17% vs. 79.17) and AUROC was (0.96 vs. 0.92). Abdel Wahab et al. **[22],** studied the combination of potential circulating proteins in serum that are differentially expressed in HCC patients associated with HCV infection, in order to be used as a surrogate marker in early detection of HCC. They demonstrated that identified proteins including PON1 are linked to complement activation, coagulation cascades and lipids metabolism in HCV-related HCC patients.

In another study **[23]**, PON1 concentration was first reported as being a novel biomarker in HCC microvascular invasion (MVI). Furthermore, a study by Zhang et al. **[24]** reported the potential value of this elevated levels of PON1 protein for early detection of HCC. Additionally,the fucosylated PON1 may serve as a glycan biomarker for distinguishing early HCC from LC patients even with low AFP levels [24].

Additionally, we found that serum PON1 activity cutoff is at a value 121.58(U/L), showed lower performance than serum AFP with optimal cut-off 20.44 sensitivity of (82.50% vs 86.0%), a specificity of (70.83% vs 73.08%). The PPV was (82.50% vs. 80.15%) and the NPV was (70.83% vs. 79.17%) and AUROC was (0.86 vs. 0.92). A probable explanation of the decrease in serum PON1 may result from the suppression of PON1 attributed to the increased generation of reactive oxygen species in LC and HCC.

In the current study, we found an increased serum PON1 concentration despite decreased PON1 activity associated with the LC patients. This finding may initially appear contradictory, but it agrees with the previous observation of reduced PON1 activity and increased concentration in chronic liver patients **[25,26]**. The explanation of the increase was related to an inhibition of apoptosis in liver parenchymal cells **[27]**. Another evidence indicates that HDL inhibits cell apoptosis and that oxidized HDL loses this capability **[27]**. Also, the possibility for PON1, which is known to protect HDL from oxidation, would encourage the anti-apoptotic potential of HDL **[27]**. Serum PON-1 concentration was proved to be superior to Alpha-fetoprotein and PON-1 activity for early detection of HCC patients being highly sensitive and specific.

The results of this study suggest that serum enzyme concentration PON1 may be regarded as reliable biomarkers of the degree of hepatocellular damage and hepatocellular carcinoma in all studied patients groups, as it highly increased in the LC group while decreased in HCC group (P<0.001).

CONCLUSION

Hepatoma is the commonest primary liver cancer. Screening strategies every 6 months including AFP and ultrasound in cirrhotic patients. AFP, however, is a screening diagnostic marker with poor sensitivity and specificity and the ultrasound is highly dependent on the operator's experience.

The present work demonstrates the usefulness of biomarkers as serum PON1 activity and concentration in cirrhotics and HCC patients as a more sensitive and specific biomarker by comparing it with an established biomarker as AFP.

Ethical approval:
Consent for an interview was taken from each participant, who was assured about the confidentiality of his information. The faculty of medicine Suez Canal University research ethics committee approved the study

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Conflicts of interest: There are no conflicts of interest

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