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Assessment of Clinical Utility of Serum Paraoxonase 1 Activity in Chronic Liver Disease.

***Anand Pyati, **B.P. Preethi and *Dileep Rathi.**

*Department of Biochemistry, BLDEU's Shri B M Patil Medical College, Bangaramma Sajjan Campus, Sholapur Road, Bijapur – 586103, Karnataka, India.
**Department of Biochemistry, J.J.M. Medical College, Davangere – 577 004, Karnataka, India.

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Corresponding Author
Anand Pyati,
E-mail: anandpyati999@gmail.com
Cell: 07259678077

Abstract

Background and Objectives: Earlier studies have reported that serum basal paraoxonase 1(PON-1) activity measurement may add a significant contribution to the liver function tests. However its utility in clinical assessment of chronic liver disease patients is not fully established. This study was to find out whether the measurement of serum basal PON1 activity would be useful as an index of liver function status in chronic liver disease.
Materials and Methods: 100 patients (30 chronic hepatitis patients, 30 cirrhosis patients, and 40 healthy controls) were included in the study. Liver function tests were done and basal PON-1 activity was assayed.
Results: The serum basal PON1 activity was significantly decreased in both chronic hepatitis and cirrhosis cases when compared to controls. It was much lower in cirrhosis than in chronic hepatitis patients and was positively correlated with serum total protein and albumin, and negatively correlated with serum total bilirubin, ALT, and ALP ($p < 0.001$) in chronic hepatitis and cirrhosis cases but not in healthy controls. Diagnostic validity test showed, basal PON1 activity was a better discriminator of chronic liver disease than total protein, albumin and ALP.
Interpretation & Conclusion: The significant decrease in basal PON1 activity in chronic liver diseases is probably the consequence of liver dysfunction. Addition of serum PON1 activity measurement to the other conventional liver function tests could improve the evaluation of chronic liver diseases.
Key words: Alanine aminotransferase, Alkaline phosphatase, Bilirubin, Chronic hepatitis, Cirrhosis, Paraoxonase 1.

Introduction

The term "chronic liver disease" includes a large number of conditions having different etiologies, existing on a continuum between hepatitis infection and cirrhosis (1). Chronic liver disease and cirrhosis are the 10th leading cause of mortality. Among the various causes, hepatitis C virus (HCV) infection is the most frequent cause of chronic liver disease (2). Hepatitis B is another major cause of chronic liver disease. Chronic liver diseases are slow progressive diseases. The progression is subtle, and the liver function tests often remain within the normal limits until gross

disease becomes evident. To diagnose such a slow progressive liver disease before advancing to hepato-cellular necrosis and fibrosis, beside the conventional biochemical tests needs alternative parameters to evaluate liver damage. Also, currently it is widely accepted that the sensitivities of conventional liver function tests are low and insufficient for an accurate determination of the presence or absence of liver disease. Finding new non-invasive, reliable tests is an ongoing field of research that possesses clinical relevance (3).

Paraoxonase (PON, aryldialkylphosphatase) is a calcium-dependent ester hydrolase that catalyzes the hydrolysis of many xenobiotics (4), such as many OP compounds including paraoxon (from which it takes its name); the insecticides parathion and chlorpyriphos; nerve agents sarin and soman; arylesters like phenylacetate; as well as aliphatic lactones such as dihydrocoumarin, γ-butyrolactone and homocysteine thiolactone (5). The paraoxonase (PON) enzyme family comprises 3 members, PON1, PON2 and PON3, whose genes are located adjacent to each other on chromosome 7q21-22 (5). Paraoxonase 1 (PON1) is a glycoprotein, containing 355 amino acids, with a molecular mass of 43-45 kDa. PON1 is synthesized mainly by the liver. After synthesis, some of the PON remains inside the hepatocyte, and some of it is released into the blood where it binds to high density lipoprotein (HDL) by association with apolipoprotein A1 (4). Liver plays a key role in the synthesis of paraoxonase 1 (PON1). Also PON1 is a lactonase with the ability to degrade lipid peroxides in lipoproteins and in cells and that plays a protective role against oxidative stress and inflammation, which are key processes involved in the pathophysiology of chronic liver diseases. Alterations in circulating PON1 levels have been reported in a variety of diseases involving oxidative stress including chronic liver diseases. The role of PON1 activity may be particularly meaningful as an index of liver function status because previous studies suggest that PON1 activity had a high diagnostic accuracy when distinguishing patients with liver disease from control subjects and when added to a standard battery of liver function tests, increased the overall sensitivity without impairing the specificity. However, this measurement is still restricted to research and has not been extensively applied in routine clinical chemistry laboratories (6). The key objectives of the present study were: 1) To estimate and compare the standard liver function tests and serum basal PON1 activity in chronic liver disease patients and healthy controls; 2) To investigate the relationship between serum basal PON1 activity and the degree of liver damage in patients with chronic liver disease; 3) To find any correlation between serum basal PON1 activity and standard liver function tests; 4) To assess the efficacy of serum basal PON1 activity measurement, alone and in combination with the standard liver function tests, in the assessment of liver damage.

Materials and Methods

A cross sectional study of liver function tests and basal paraoxonase1 (PON1) activity in chronic liver disease patients was carried out for a period of one year. Approval from the institutional Ethical committee was obtained prior to the commencement of the study and also written voluntary consent was obtained for each subject separately, after explaining their participation in the study in their local language.

Subjects: A total number of 100 subjects comprising of 30 chronic hepatitis patients (21 men, 09 women; mean age of 42.0 ± 10.1 years), 30 liver cirrhosis patients (22 men, 08 women; mean age of 50.7 ± 9.7 years) and 40 age and sex matched healthy controls (21 men 19 women; mean age of 45.3 ± 11.6 years) were included in the present study. Chronic hepatitis was diagnosed by liver biopsy and cirrhosis was diagnosed on the basis of clinical features, echography (for splenomegaly or portal vein dilation) and endoscopy (for gastroesophageal varices) findings. Patients with diabetes, neoplasia, renal disease and cardiovascular disease were excluded from the study.

Methods: Liver function tests were done by standard methods using semi autoanalyser. Basal PON1 activity was measured by using p-nitrophenyl

acetate(7). The generated product, p-nitrophenol was calculated by using molar extinction coefficient of 17000/mol/cm at pH 8.0. Results were expressed as nmol/mL/min.

Statistical analysis: Descriptive data are presented as mean ± SD values. Differences between means of two groups were assessed with the Student *t* test. The Pearson's correlation coefficient was used to evaluate the degree of association between two variables. Diagnostic validity tests were performed for all the variables. Multiple logistic regression was used to estimate the ability of groups of variables to predict the presence or absence of liver disease. For all the tests, p-value of 0.05 or less was considered statistically significant.

Results

The levels of serum total bilirubin, ALT, ALP were significantly increased and the levels of serum total protein, albumin, and basal PON1 activity were significantly decreased in patients with chronic hepatitis and cirrhosis when compared to healthy controls and were statistically highly significant except for albumin in chronic hepatitis cases which was decreased, but was not statistically significant ($p = 0.35$). The mean levels of serum total bilirubin, ALT, ALP were much more increased and the mean levels of serum total protein, albumin, and basal PON1 activity were much more decreased in cirrhosis cases when compared to chronic hepatitis cases and the difference was statistically significant. The p value was highly significant for albumin, ALP and basal PON1 activity ($p < 0.001$) and it was significant for total bilirubin, total protein ($p < 0.01$) and ALT ($p < 0.05$).

Table 2 shows the Pearson's correlation between serum total bilirubin, total protein, albumin, ALT, ALP and serum basal PON1 activity in healthy controls and in cases of chronic hepatitis and cirrhosis. It is evident from the table that serum total protein and albumin were positively correlated with serum basal PON1 activity in both chronic hepatitis and cirrhosis cases and this correlation was statistically significant ($p < 0.001$). The serum total bilirubin, ALT, and ALP were negatively correlated with serum basal PON1 activity in both chronic hepatitis and cirrhosis cases. This correlation was also statistically significant ($p < 0.001$) except for the relation between ALP and PON1 activity in cirrhosis cases where serum basal PON1 activity decreases as the ALP level increases. There was no such correlation between standard liver function tests and basal PON1 activity in healthy controls.

Table 3 shows results of diagnostic validity test in predicting disease for a given standard liver function tests and basal PON1 activity level. Standard laboratory reference values were used as cut off values for standard liver function tests and median for basal PON1 activity for discriminating chronic liver disease cases from controls. It is evident from the table that basal PON1 activity was a better discriminator of chronic liver disease than total protein, albumin, ALP with sensitivity of 90%, specificity of 95%, positive predictive value of 96%, negative predictive value of 86% and diagnostic efficiency of 92%. But total bilirubin and ALT were better discriminators of the disease than basal PON1 activity. The usefulness of adding serum basal PON1 measurement to the standard panel of liver function tests was analyzed by multiple logistic regression analysis. We compared the ability of two different models to correctly differentiate between patients and controls. The coefficients of the equations of both models are shown in Table 4.

The equation for model 1 (standard liver function tests) was: $x = 0.252(\text{bilirubin}) - 0.554(\text{total protein}) + 1.044(\text{albumin}) + 0.020(\text{ALT}) + 0.005(\text{ALP}) - 3.26$. To predict the status of any one individual with respect to chronic liver disease, the biochemical terms in the equation should be substituted with their corresponding measured values. If the result (x) calculated from the equation is ≥ 0.04, this classifies the individual as a patient. Similarly, the equation for model 2 (basal PON1 + standard liver function tests) was: $x = 0.012(\text{bilirubin}) + 0.766(\text{total protein}) - 1.505(\text{albumin}) - 0.018(\text{ALT}) + 0.001(\text{ALP}) + 0.031(\text{PON1}) - 2.023$. Diagnostic

Results

Table.1 Serum total bilirubin, total protein, albumin, ALT, ALP and basal PON1 activity levels in controls, chronic hepatitis and cirrhosis cases.

Groups		Total bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	ALT (U/L)	ALP (U/L)	Basal PON1 activity (nmol/mL/min)
Controls		0.71 ± 0.11	6.78 ± 0.74	3.66 ± 0.53	20.7 ± 6.3	91.2 ± 38.4	177.5 ± 26.0
Chronic hepatitis cases		4.06 ± 1.77	6.21 ± 0.34	3.56 ± 0.34	135.3 ± 41.8	168.3 ± 48.0	101.5 ± 23.6
Cirrhosis cases		5.57 ± 2.34	5.91 ± 0.44	2.98 ± 0.58	111.7 ± 39.2	232.8 ± 73.0	50.6 ± 19.9
Controls vs Chronic hepatitis cases	p	< 0.001 (HS)	< 0.001 (HS)	0.35 (NS)	< 0.001 (HS)	< 0.001 (HS)	< 0.001 (HS)
Controls vs Cirrhosis cases	p	< 0.001 (HS)	< 0.001 (HS)	< 0.001 (HS)	< 0.001 (HS)	< 0.001 (HS)	< 0.001 (HS)
Chronic hepatitis vs Cirrhosis cases	p	< 0.01 (S)	< 0.01 (S)	< 0.001 (HS)	< 0.05 (S)	< 0.001 (HS)	< 0.001 (HS)

Values are Mean± SD; HS= Highly significant; S= Significant; NS= not significant

Table . 2 Relationship of serum total bilirubin, total protein, albumin, ALT, ALP levels with serum basal PON1 activity in healthy controls, chronic hepatitis and cirrhosis cases.

Variable	Basal PON1 activity					
	Controls		Chronic hepatitis		Cirrhosis	
	R	p	r	p	R	p
Total bilirubin	- 0.04	0.81	- 0.60	< 0.001	- 0.90	< 0.001
Total protein	- 0.03	0.85	0.59	< 0.01	0.65	< 0.001
Albumin	0.19	0.25	0.65	< 0.001	0.74	< 0.001
ALT	0.23	0.16	- 0.82	< 0.001	- 0.67	< 0.001
ALP	- 0.19	0.24	- 0.69	< 0.001	- 0.44	0.05

Table. 3 Diagnostic validity of serum total bilirubin, total protein, albumin, ALT, ALP, and basal PONI 1 activity

Cut-off value	Total bilirubin ≥ 3.7 mg/dL	Total protein ≤ 6.2 g/dL	Albumin ≤ 3.4 g/dL	ALT ≥ 93.0 U/L	ALP ≥ 167 U/L	Basal PON1 activity ≤ 113 (nmol/mL/min)
Sensitivity	90%	75%	68%	95%	80%	90%
Specificity	95%	68%	55%	95%	95%	95%
PPV	97%	78%	70%	96%	96%	96%
NPV	93%	64%	54%	95%	76%	86%
DE	95%	72%	63%	96%	86%	92%

PPV = Positive predictive value; NPV = Negative predictive value; DE = Diagnostic efficiency.

Table – 4: Coefficients of the two logistic regression analyses.

Model 1: Standard biochemical tests

Variable	Coefficient
Total bilirubin	0.252
Total protein	- 0.554
Albumin	1.044
ALT	0.020
ALP	0.005
Constant	- 3.26

Model 2: Basal PON1 and standard biochemical tests

Variable	Coefficient
Total bilirubin	0.012
Total protein	0.766
Albumin	- 1.505
ALT	- 0.018
ALP	0.001
Basal PON1	0.031
Constant	- 2.023

sensitivity of model 2 was superior to that of model 1 without any impairment of specificity. Thus, the traditional tests correctly classified 82% of the patients (i.e., sensitivity) and 100% of the controls (i.e., specificity). The addition of serum basal PON1 activity measurement to the group of standard tests increased the sensitivity of patient classification to 93%, whereas the specificity remained unchanged at 100%.

Discussion

PON1 activity has been demonstrated in different tissues such as liver (including microsomes), kidney, brain and lung and it has been studied extensively in relation to cardiovascular diseases, but there is less data available on the hepatic enzyme. Some of these enzymes are released in to the circulation and some portions are stored in the liver. Serum PON1 which is carried in the circulation bound to HDL particles, protects HDL from peroxidation. The putative function of PON1 in the liver is to provide hepatic protection against oxidative stress (8). In the present study, the serum basal PON1 activity was significantly lower in both chronic hepatitis and cirrhosis cases when compared to controls. This finding was in accordance with earlier studies (9, 10). Other salient observation in this study is that the decrease in PON1 activity in the serum of patients with chronic liver diseases was related to the degree of liver damage. PON1 activity was lower in patients with cirrhosis than in those with hepatitis. Two mechanisms explain the decreased PON1 activity in chronic liver disease patients. In one mechanism, a decrease in PON1 enzymatic activity or gene expression could be the consequence of the hepatic dysfunction. Supporting this hypothesis is the observation of an inhibition of microsomal PON1 activity in rats with chronically administered CCl_4. In the other mechanism, although hepatic PON1 concentrations may be normal, serum PON1 activity could be decreased as a consequence of an altered synthesis and/or secretion of HDL secondary, for example, to impaired lecithin:cholesterol acyl transferase (LCAT) activity. Alterations in HDL structure and concentration associated with decreases in hepatic LCAT activity are frequent in chronic liver diseases and a recent study described a decrease of serum PON1 activity in mice with LCAT deficiency resulting from LCAT gene targeted disruption (10). Our results also show that, Basal PON1 activity was positively correlated with serum total protein and albumin, and negatively correlated with serum total bilirubin, ALT, and ALP. This correlation was also statistically significant except for the relation between ALP and PON1 activity in cirrhosis cases, where serum basal PON1 activity had decreased as the ALP level increases, but was statistically not significant. There was no such correlation between standard liver function tests and basal PON1 activity in healthy controls. According to the diagnostic validity test, basal PON1 activity was a better discriminator of chronic liver disease than total protein, albumin, ALP with sensitivity of 90%, specificity of 95%, positive predictive value of 96%, negative predictive value of 86% and diagnostic efficiency of 92%. But total bilirubin and ALT were better discriminators of the disease than basal PON1 activity. We also analyzed the usefulness of adding basal serum PON1 measurement to the standard panel of liver function tests by multiple logistic regression analysis. The addition of serum PON1 activity measurement to the group of standard tests increased the sensitivity of patient classification to 93%, whereas the specificity remained unchanged at 100%. These results demonstrate that serum PON1 activity measurement may add a significant contribution to liver function tests.

Conclusion

The significant decrease of PON1 activity in chronic liver diseases is probably the consequence of liver dysfunction and is related to the severity of liver dysfunction. Addition of serum PON1 activity measurement to the other conventional liver function tests could improve the evaluation of chronic liver diseases. However further studies are needed to fully define this role and to establish the relationships between hepatic PON1 alterations and the pathophysiology of chronic liver disease.

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