

SERUM PARAOXONASE-1 ACTIVITY, OXIDATIVE STRESS & LIPID PROFILE IN PATIENTS WITH CHRONIC LIVER DISEASE

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ABSTRACT

Background/Aim: Chronic liver disease in the clinical context is a disease of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. Oxidative stress influences the pathophysiological changes leading to chronic liver disease. Paraoxonase-1 [PON1] is an esterase, exclusively synthesized by the liver exerts a protective effect against oxidative stress. The present study has two objectives: to estimate and compare the standard liver function tests, lipid profile, serum basal PON1 activity & malondialdehyde [MDA] in chronic liver disease patients and healthy controls & to find the correlation between serum basal PON1 activity, MDA and standard LFTs. **Materials and Methods:** In this study we included 40 diagnosed cases of chronic liver disease and 40 healthy age and sex matched subjects from whom blood was drawn to measure paraoxonase-1 activity manually using spectrophotometer, malondialdehyde by thiobarbitric acid method. Liver function tests- bilirubin, total protein, albumin, alanine transaminase, alkaline phosphatase and lipid profile were measured using clinical chemistry auto analyzer. **Results and observations:** Serum paraoxonase-1 activity, total protein, albumin levels, high-density lipoproteins are decreased and malondialdehyde, bilirubin, alanine transaminase and alkaline phosphatase are increased in patients with chronic liver disease. **Conclusion:** Serum PON1 activity has decreased significantly & MDA levels were increased significantly in chronic liver disease. Determination of PON1 activity may serve as a useful marker to assess severity of chronic liver disease.

KEYWORDS

Chronic liver disease, high-density lipoprotein, malondialdehyde, paraoxonase-1

INTRODUCTION

Chronic liver diseases are slow, progressive diseases characterized by advancing hepatocellular necrosis, inflammation and fibrosis. WHO estimates that about 3% of the world's population has been infected with Hepatitis C Virus and more than 170 million chronic carriers who are at risk of developing liver cirrhosis and hepatocellular carcinoma. Oxidative stress and inflammation plays a fundamental role in the onset and development of liver diseases. Oxygen free radicals cause lipid

peroxidation leading to destruction of PUFA producing toxic metabolites such as malondialdehyde (MDA) which is commonly used as a marker of lipid peroxidation. The ubiquitous presence of antioxidant enzymes may represent an important defence mechanism in diminishing the burden of the pro-oxidant stimuli. Paraoxonase-1 (PON1) is an enzyme synthesized in liver and has lactonase and esterase activities towards lipid peroxides and circulates in plasma bound to high-density lipoproteins (HDL)¹.

MATERIAL AND METHODS

The study was conducted in department of biochemistry, S.S Institute of Medical Sciences & Research Centre, Davangere between June 2010 to May 2011. Patients admitted to S.S Institute of Medical Sciences were enrolled in the study.

a. Subjects:

The study consisted of a total of 80 subjects, 40 patients with chronic liver disease 40 healthy controls. Based on the etiology of the liver disease patients were divided into two groups [Table 1]. CLD was diagnosed based on clinical evidence, radiography, laboratory investigations.

Table 1: Division of patients in to 2 groups based on etiology of liver disease

Groups	No of patients
Group I - Chronic viral hepatitis	15
Group II - Cirrhosis	25

Exclusion criteria: patients with diabetes, neoplasia, renal disease and cardiovascular disease were excluded from the study.

b. Collection of blood samples

After 12 hours of fasting about 5ml of blood was drawn under all aseptic precautions into plain vacutainers from the antecubital veins of healthy controls and patients. The blood was allowed to clot for about 30 minutes and then the serum was separated by centrifugation at 5000 rpm and stored at 4°C until the analysis.

c. Biochemical determinations

Paraoxonase assay

PON1 activity was estimated spectrophotometrically by the method using p-nitrophenylacetate as a substrate. The increase in the absorbance at 412 nm due to formation of p-nitrophenol was measured. Briefly, the assay mixture consists of 3ml of 20mM/L Tris-HCl buffer, pH 8.0 containing 1 m Mol CaCl₂, 15 mg of p-nitrophenylacetate dissolved in 0.5 ml of absolute ethanol & 50 µl of fresh serum. After mixing the contents kinetic measurements were taken immediately at every minute for 5 minutes at 412 nm at 25°C. First absorbance reading is taken as zero minute reading and subsequent absorbance readings were obtained by subtracting one minute reading with zero minute reading, likewise the latter minute reading was subtracted from previous minute readings. The

mean absorbance was calculated. Mean absorbance was used to determine PON1 activity. PON1 activity is expressed as Units/ml of serum i.e. 1 U= 1 nanomole of p-nitrophenol formed per minute².

Measurement of Serum MDA Concentration

Serum MDA levels were measured according to a method described elsewhere. The principle of the method was based on the spectrophotometric measurement of the color obtained during the reaction of thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of MDA-thiobarbituric acid complex and expressed in nmol/ml³.

Standard liver function and fasting lipid profile tests

Serum total and direct bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin, total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), triglycerides (TAG) levels were determined using clinical chemistry analyzer (Erba 360). LDL-cholesterol (LDL-C) values were derived from Friedewald formula⁴.

d. Statistical analysis

Statistical analysis was performed using statistical package for social sciences [SPSS-16]. The results were expressed as mean ± standard

deviation [SD]. The comparison between groups was done using Student's unpaired t test. Pearson's correlation was used to correlate between parameters. P - Value <0.05 was considered statistically significant.

RESULTS

A total of 40 cases and 40 controls were studied. As shown in **Table 2 & Table 3**: The mean age (years) in controls was [42.23±11.67] and cases [40.87±15.10] in chronic hepatitis & [47.56±11.03] years in cirrhosis. Total bilirubin in controls was [0.75±0.21] as compared to cases [5.24±0.60 in chronic hepatitis 5.11±0.234 in cirrhosis]. Total protein in controls was [6.81±0.57] as compared to cases [5.71±0.48] in chronic hepatitis & [5.56±0.42] in cirrhosis. Albumin in controls was [3.95±0.41] as compared to cases [3.20±0.55] in chronic hepatitis & [2.84±0.59] in cirrhosis. ALT in

controls was [24.88±11.27] as compared to cases [123.8±12.94] in chronic hepatitis & [117.24±48.3] in cirrhosis. ALP in controls was [89.40±26.8] as compared to cases [177.8±22.18] in chronic hepatitis & [195.60±87.7] in cirrhosis. LDL cholesterol in controls was [137±2.3] as compared to cases [86 ±16.4] in chronic hepatitis & [80±12.3] in cirrhosis. HDL cholesterol in controls was [46±1.8] as compared to cases [40±1.5] in chronic hepatitis & [39±2.3] in cirrhosis. Total cholesterol in controls was [186±23.1] as compared to cases [138±13.5] in chronic hepatitis & [132±12.6] in cirrhosis. **Table 4 & Figure 1**: Serum MDA in controls was [2.17±0.56] as compared to cases [7.52±0.41] in chronic hepatitis & [8.67±1.83] in cirrhosis. Serum PON1 activity in controls was [175.80±24.30] as compared to cases [69.67±10.86] in chronic hepatitis & [59.14±11.9] in cirrhosis.

Table 2: Results of Standard liver function tests & Lipid profile of healthy controls and patients with CLD.

Variables	Healthy controls	Chronic Hepatitis	Cirrhosis	p Value
Total bilirubin [mg/dl]	0.75±0.21	5.24±0.60	5.11±0.234	<0.001
Total protein [g/dl]	6.81±0.57	5.71±0.48	5.56±0.42	<0.001
Albumin [g/dl]	3.95±0.41	3.20±0.55	2.84±0.59	<0.001
ALT [U/L]	24.88±11.27	123.8±12.94	117.24±48.3	<0.001
ALP [U/L]	89.40±26.81	177.8±22.18	195.60±87.7	<0.001
LDL cholesterol	137 ± 2.3	86 ± 16.4	80 ± 12.3	0.025
HDL cholesterol	46 ± 1.8	40 ± 1.5	39 ± 2.3	0.042
Total cholesterol	186 ± 23.1	138 ± 13.5	132 ± 12.6	0.030
Triglycerides	183 ± 13.2	83 ± 5.6	79 ± 6.8	0.012

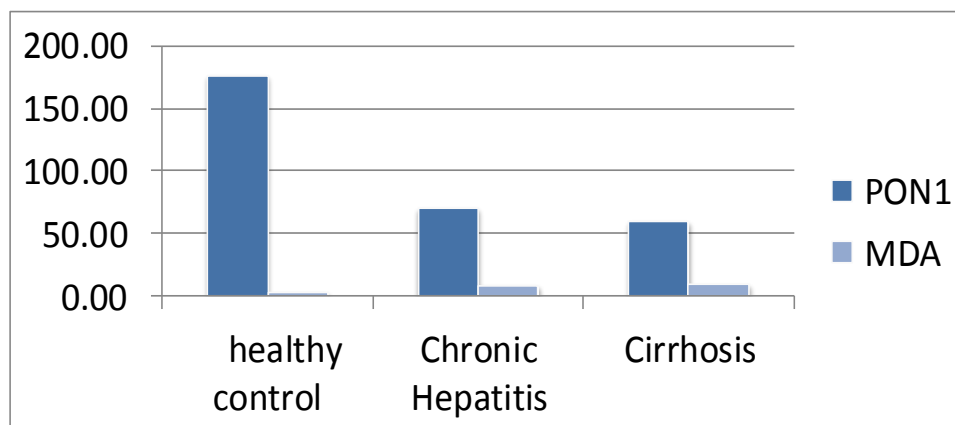
Table-3: Demographic, PON 1 status, MDA in healthy controls and patients with CLD

Variables	Healthy controls (n=40)	Chronic hepatitis (n=15)	Cirrhosis (n=35)	P value
Age(years)	42.23±11.67	40.87±15.10	47.56±11.03	
Sex (M/F)	28/12	11/4	22/3	
Total bilirubin(mg/dl)	0.75±0.21	5.24±0.60	5.11±0.234	<0.001
Total protein (g/dl)	6.81±0.57	5.71±0.48	5.56±0.42	<0.001
Albumin (g/dl)	3.95±0.41	3.20±0.55	2.84±0.59	<0.001
ALT (U/L)	24.88±11.27	123.8±12.94	117.24±48.3	<0.001
ALP (U/L)	89.40±26.81	77.8±22.18	195.60±87.7	<0.001
PON1(U/L)	175.80±24.30	69.67±10.86	59.14±11.9	<0.001
MDA(µmol/L)	2.17±0.56	7.52±0.41	8.67±1.83	<0.001

Table-4: PON1 status and MDA in chronic hepatitis and cirrhosis

Variables	Chronic hepatitis	Cirrhosis	P value
PON1(U/L)	69.67±10.86	59.14±11.98	<0.008
MDA (µmol/L)	7.52±0.41	8.67±1.83	<0.02

Figure 1: Graphical representation of PON1 activity and MDA levels in Healthy controls and patients with chronic liver disease.



DISCUSSION

Chronic liver diseases are slow, progressive diseases characterized by advancing hepatocellular necrosis, inflammation and fibrosis. WHO estimates that about 3% of the world's population has been infected with HCV and more than 170 million chronic carriers who are at risk of developing liver cirrhosis and Hepatocellular Carcinoma.

Oxidative stress and inflammation plays a fundamental role in the onset and development

of liver diseases. Oxygen free radicals cause lipid peroxidation leading to destruction of PUFA producing toxic metabolites such as malondialdehyde which is commonly used as a marker of lipid peroxidation. MDA can also initiate the formation of protein-aldehyde adducts. They are seen predominantly in the perivenous region and they coincide with signs of more advanced liver injury. It has been reported that patients with degenerative liver

disease had increased lipoperoxide levels in liver tissue and serum⁵.

Recently serum PON1 has been studied extensively in relation to cardiovascular diseases, whereas in contrast, there is a paucity of data on hepatic enzyme. PON1 activity has been observed in rat and human hepatic microsomes. Some of this enzyme is secreted into the circulation bound to HDL where as another portion is stored in the liver^{6, 7, 8}.

The physiologic role played by PON1 in the liver is unknown although preliminary observations suggest that this enzyme provides hepatic protection against oxidative stress. In the present study, the decrease in PON1 activity in serum of patients with chronic liver disease was related to the degree of liver damage. Previous authors have proposed two mechanisms to explain the decrease in activity in PON1 in liver disorder patients. First, as there is hepatic dysfunction, it is obvious that there is defective gene expression, which contributes to decreased PON1 in these patients. It has been reported that there was significant decrease in PON1 activity in Carbon tetra chloride induced liver cirrhosis secondary to increased free radicals⁹. Second, as a consequence of an altered synthesis and/or secretion of HDL-C, this may be due to impaired lecithin: cholesterol acyl transferase (LCAT) activity. We have observed positive correlation between PON1 activity and HDL-C levels in patients chronic liver disease.

Several workers have proposed earlier that viral hepatitis is associated with oxidative stress. Further, PON1 activity associated with HDL-C in plasma is thought to protect LDL-C oxidation¹⁰.

A previous study has stated that decrease in PON1 activity in patients with chronic liver diseases such as chronic hepatitis and cirrhosis, was related to degree of liver damage. Recently, Keskin *et al.* also have reported reduced baseline

and stimulated PON1 and arylesterase (ARE) activities in patients with CLD. Currently it is widely accepted that sensitivities of standard biochemical tests for liver function are low and insufficient for a reliable determination of the presence or absence of liver disease. Consequently a battery of tests to be performed to increase the sensitivity and specificity of the evaluation¹¹.

Results of our study demonstrate that serum PON1 activity measurement may add a significant contribution to liver function test. Its diagnostic accuracy is equivalent to that of ALT in patients with chronic hepatitis and far superior to that of other tests in patients with cirrhosis¹².

CONCLUSION

The results of our study suggests that there were higher oxygen free radicals production as evidenced by higher MDA and lower PON1 activity, supporting that there is increased oxidative stress in patients with CLD & decreased PON1 activity supports the decreased detoxification in CLD.

Determination of PON1 activity may serve as useful additional test in assessing degree and severity of CLD.

STUDY LIMITATIONS

Sample size in the present study was small. Large prospective studies in Indian population are needed to support the results of present study.

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