Serum paraoxonase – Marker of alcoholic liver cirrhosis

B. Jyotchna Devi[1,*], V. Uma Lakshmi, Naazia Arifuddin[3], J. Rama Rao[4], Ch. Kiranmai[5]

[1]Assistant Professor, [2]Retired as Professor, [3]Consultant, [4]Professor and HOD, [5]Tutor, Dept. of Biochemistry, [1,5]Great Eastern Medical School and Hospital, Ragolu, Andhra Pradesh, [2]Osmania Medical College, Hyderabad, Telangana, [3]Prime Hospital, [4]Malla Reddy Institute of Medical Sciences, Hyderabad, Telangana, India

*Corresponding Author: B. Jyotchna Devi
Email: jyotchna.appaji@gmail.com

Abstract

Serum Paraoxonase is an esterase, along with Apolipoprotein A1 and Clusterin (Apolipoprotein J), PON 1 is associated with HDL. This study was done to assess the significance of serum Paraoxonase as an biomarker along with standard liver function tests in evaluating alcoholic cirrhosis. Serum Paraoxonase levels was estimated in 30 patients diagnosed with alcoholic cirrhosis and 30 healthy blood donors. Paraoxonase levels were significantly lower in cases as compared with controls (P<0.0001). The ROC showed 100% sensitivity of PON 1 and specificity equivalent to that of bilirubin, ALT, AST in alcoholic cirrhosis.
Conclusion : Serum Paraoxonase is sensitive marker for patients with alcoholic liver disease and oxidative stress and should be included as an additional parameter along with the other parameters in liver function tests.

Keywords: Alcoholic cirrhosis, Antioxidant, Paraoxonase, Liver function tests.

Introduction

Liver plays an important role in metabolism, detoxification and excretion of many xenobiotic compounds. Although viral infection is one of the main causes of liver injury, xenobiotics, excessive drug therapy, environmental pollutants and chronic alcohol ingestion can also cause hepatic injury.[1] Most of these have been reported to generate free radicals and reactive oxygen species which are the major etiologic factors in liver pathogenesis.[2]

The major primary diseases of the liver are viral hepatitis, alcoholic liver diseases, non- alcoholic fatty liver diseases and hepatocellular carcinoma.[3] Iron overload, copper overload, chronic ethanol consumption, non-alcoholic steatohepatitis and viral hepatitis are all associated with cellular oxidative constituent damage.[4]

Paraoxonase is an enzyme, has 3 isoenzymes named PON 1, PON 2 and PON 3 located on chromosome 7q21.3-22.1.[5] It was first recognized by its hydrolytic activity on organophoshorous compounds such as Paraoxon.[6] It is considered that it might have a role in antioxidant system of an organism, due to its ability to hydrolyse lipid peroxides and homocysteine-thiolactone.[7]

Liver plays an integral part in the synthesis and metabolism of plasma lipoproteins.[8] It plays a key role in the synthesis of serum PON1 and the gene expression has been confined only to the liver.[9] Also, properties shared by hepatic and serum PON1 are identical, as elucidated by in vitro biochemical tests.[10] Hence these observations raise a question on the utility of the measurement of serum PON1 activity as an index of liver function status.

Aims and Objectives

1. Whether Liver diseases produce any changes in levels of serum Paraoxonase as liver is the major site of production.
2. To assess the activity of serum Paraoxonase in alcoholic cirrhosis.

Materials and Methods

Setting: A case control study was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad. Ethical issues involved in the project were reviewed and approved by Ethics Scientific Committee of Osmania Medical College. Consent to withdraw blood from patients was taken after explaining the nature of the study being undertaken.

Sources of Sample and Data:
1. Department of Biochemistry, Osmania General Hospital.
2. Department of Biochemistry, MNJ Cancer Institute.
3. Department of Medicine, Osmania General Hospital.
4. Department of Liver Care Unit, Osmania General Hospital.

Cases: Patients diagnosed as cirrhosis of liver, with or without any associated co-morbidities were taken as cases.

Controls: Healthy voluntary blood donors were taken as controls.

Sample Collection: Blood samples were collected by venipuncture with strict aseptic precaution. 5ml of blood was taken in plain vacutainer. All the blood samples were centrifuged at 3000 rpm for 10 minutes and serum separated. One part of the serum sample was

taken for analysis of Liver parameters which includes Total Bilirubin, Direct Bilirubin, AST, ALT, ALP, Total protein and Albumin and another part was stored at -20ºC for PON 1 analysis later. Grossly hemolyzed and lipemic samples were excluded.

Biochemical Parameters: The test parameters included in liver function test are total bilirubin (Diazo method of Pearlman and Lee), Direct bilirubin (Diazo method of Pearlman and Lee), Aspartate transaminase (Modified IFCC), Alanine transaminase (Modified IFCC), Alkaline Phosphatase (Kinetic method recommended by IFCC), total proteins (Biuret method introduced by Kingsley and modified by Henry) and Albumin (Doumas et al using BCG). All the parameters were performed on Transasia chem 5 semi-autoanalyzer using Erba path reagents.

Serum PON was estimated using 4-nitrophenylacetate as substrate. It was acquired from Sigma Aldrich Chemicals. 250μl of 1 in 20 prediluted serum and 2ml Tris HCl (25mM pH 7.4 25ºC) containing 1mM $CaCl_2$, 5% methanol and 0.625mM 4-nitrophenylacetate. Rate of generation of 4-nitrophenol was determined at 402nm.[11] (ε_{402}= 14000 $M^{-1}cm^{-1}$ at pH 7.4). Kinetic curves were linear for 1 min.

Statistical Analysis

The data was analyzed using Graph Pad Prism Demo and software version 6 and the results were expressed as Mean and Standard deviation of various parameter. A P value <0.05 was considered to be statistically significant. ROC curve analysis was done to assess maximum sensitivity and maximum specificity and diagnostic efficiency.

Results

Table 1

Parameters	Controls MEAN ± S.D	Cases MEAN ± S.D
Total Bilirubin	0.82 ± 0.51	7.2 ± 8.0
Direct Bilirubin	0.36 ± 0.31	2.93 ± 2.93
AST	27.7 ± 13.44	112.7 ± 118
ALT	24.31 ± 14.36	55.65 ± 38.23
ALP	70.63 ± 15.01	93.53 ± 31.06
Total proteins	7.21 ± 0.96	6.13 ± 2.2
Albumin	4.3 ± 0.72	2.43 ± 1.04
PON	988 ± 238	306.9 ± 192.5

Table 2: Sensitivity, specificity and best cutoff value in discriminating cases and controls

	Total Bilirubin	Direct Bilirubin	AST	ALT	ALP	Total protein	Albumin	PON 1
Sensitivity	87.10	96.67	70.97	58.06	76.67	58.06	77.42	93.55
Specificity	93.33	83.33	96.67	96.55	66.67	93.33	100	100
BCV	> 1.455	> 0.45	> 52.5	> 45.5	> 76.5	< 6.100	< 3.35	< 581.9

Table 3: Area under curve (AUC), Diagnostic efficiency (DE) and significance (P value) in discriminating analyzed parameters in cases and controls

	Total Bilirubin	Direct Bilirubin	AST	ALT	ALP	Total protein	Albumin	PON 1
AUC	0.9495	0.9452	0.8828	0.7547	0.7628	0.7032	0.9172	0.9796
DE	91.6	86.6	84.9	76.6	71.6	76.6	89.9	98.3
P Value	< 0.0001	< 0.0001	< 0.0001	0.0007	0.0005	0.0064	<0.0001	<0.0001

Discussion

The most important causes of liver diseases are alcohol abuse, obesity and viral infections.[12] Alcoholic liver disease (ALD) encompasses a broad spectrum of hepatic alterations ranging from steatosis and minimal injury to advanced fibrosis and cirrhosis.[13] The involvement of oxidative injury in ethanol toxicity has emerged from reports showing that alcohol-fed animals and patients with ALD present with high content of lipid peroxidation products in their livers and in their circulation.[14] The possible role of oxidative stress in

promoting an inflammatory reaction in ALD has emerged from the observation that lipid peroxidation end-products are able to develop an immune response[15,16] and that liver-associated lymphocytes isolated from ethanol-fed rats have an increased capacity to secrete pro-inflammatory cytokines.[17] Ethanol-induced lipid peroxidation also increases the production of the pro-fibrogenetic tissue growth factor β-1 by Kupffer cells[18] and the expression of the collagen α2(1) gene by activated stellate cells.[19]

The enzyme paraoxonase (PON) has esterase and lactonase activity[20] and is involved in protection against xenobiotic toxicity.[21] PONs reduce LDL oxidation,[22] but PON2 decreases cellular oxidative stress and even prevents apoptosis in vascular endothelial cells[23] PON 1is extensively studied enzyme and altered values of it has been reported in several diseases with oxidative stress.

The present study was done to support the fact that PON1 is a good sensitive indicator of Liver damage due to oxidative stress incurred during alcohol abuse.

From Table 1 it is evident that PON 1 levels alter along with other standard Liver parameters when a pathological event occurs

ROC analysis as presented in Table 2 shows that it exhibits the highest sensitivity (93.55%) when compared with standard liver parameters and specificity is equivalent to Albumin (100%) and superior to other parameters.

Ferré et al. (2002),[24] found similar results in a prospective cohort study of 175 patients with chronic liver disease, in which Serum ALT, AST, Bilirubin and ALP were elevated in patients with chronic hepatitis and cirrhosis (p<0.001). P-value with respect to total proteins was non-significant in chronic hepatitis patients but significant in cirrhosis patients (<0.01). Albumin was decreased in both the groups, more so in cirrhotic patients.

PON 1 shows better diagnostic efficiency (98.3) than the other parameters in discriminating between cases and controls and a significant P- value which is equivalent to Bilirubin and AST.

In the same study Ferré et al. (2002) ROC analysis supported the proposition that the measurement of baseline serum PON1 activity is a good candidate for inclusion among those used to evaluate liver damage. Its diagnostic accuracy is equivalent to that of ALT in patients with chronic hepatitis and far superior to that of the other tests in patients with cirrhosis.

Marsillach et al. 2007[25] demonstrated that serum PON1 activity was decreased in alcoholic patients, and that the magnitude of the alteration was related to the degree of liver damage.

In the present study several mechanisms may be proposed to explain the decrease of serum PON 1 activity in chronic liver diseases.

First, these patients present with an increased free radical production, and it has been previously reported that PON 1 is inactivated after hydrolyzing lipid peroxides (Aviram et al. 1999).[26]

Second, alterations in HDL structure and composition can affect PON1 activity (Deakin et al. 2002; James and Deakin, 2004).[27,28]

It is widely accepted that the present non – invasive tests for liver function are of low sensitivities to know the presence or absence of liver disease with oxidative stress and finding a reliable one of clinical value is an ongoing research. Most of the studies suggest that

measurement of serum PON1 activity may add additional information without altering the specificity and at the same time increasing the sensitivity when amongst the other tests included in Liver function. (Ferré et al. 2002; Marsillach et al. 2007; Camps et al. 2007).[24,25,29]

The main drawback is that the use of toxic and unstable substrates to measure PON1 activity hampers the implementation of this assay in routine practice. However, the recent development of new assays for serum PON1 measurement by using non-toxic substrates, such as the lactonase assay employing 5-thiobutyl butyrolactone (TBBL), makes this proposal closer to a practical development. (Marsillach et al. 2008).[30]

However a practical result of the present study is the demonstration that the relatively simple PON 1 activity measurement could significantly improve the current efficacy of the laboratory's evaluation of patients with suspected liver disease.

References

1. King PD and Perry MC. Hepatotoxicity of chemotherapy. *The Oncologist.* 2001;6:162-176.
2. DeBethizy, J. D., and Hayes, J. R. (2001). Metabolism: a determinant of toxicity. In A.W. Hayes (Ed) Principles and Methods of Toxicology (pp-77–136). Philadelphia: Taylor and Francis.
3. Lim YS, Kim WR: The global impact of hepatic fibrosis and end-stage liver disease. *Clin Live Dis.* 2008;12:733.
4. Cahill A, Cunningham CC, Adachi M, et al. Effects of alcohol and oxidative stress on liver pathology: the role of the mitochondrion. *Alcohol Clin Exp Res.* 2002;26(6):907–15.
5. La Du BN. Structural and functional diversity of Paraoxonases. *Nat Med.* 1996;2:1186-7.
6. Ferré N, Camps J, Prats E, Vilella E, Paul A, Figuera L, et al. Serum paraoxonase activity: A new additional test for the improved evaluation of chronic liver damage. Clin. *Chem.* 2002;48:261–8.
7. Aviram M, Rosenblat M. Paraoxonases 1, 2 and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. *Free Radical Biol Med.* 2004;37:1304–16.
8. Murray, Bender, Kennelly. Harper's Illustrated Biochemistry. McGraw Hill; 2012; 29th edn; p244.
9. Kelso GJ, Stuart WD, Richter RJ, Furlong CE. Jordan-Starck TC, Harmony JA. Apolipoprotein J is associated with paraoxonase in human plasma. *Biochemistry.* 1994;33:832–9.
10. Gonzalvo MC, Gil F, Herna′ndez AF, Rodrigo L, Villanueva E, Pla A. Human liver paraoxonase (PON1): Subcellular distribution and characterization. *J Biochem Mol Toxicol.* 1998;12:61–9.
11. Haagen L, Brock A: A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of $-nitrophenyl acetate by phenylacetate. *Eur J Clin Chem Clin Biochem.* 1997;25:293-297.
12. Jordi Camps, Judit Marsillach, Anna Rull. Interrelationships Between Paraoxonase-1 and Monocyte Chemoattractant Protein-1 in the Regulation of Hepatic Inflammation.

13. Day CP. Genes or environment to determine alcoholic liver disease and non-alcoholic fatty liver disease. *Liver Int.* 2006;26:1021–1028.

14. Albano E. Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc.* 2006;65(03):278–290.

15. Mottaran E, Stewart SF, Rolla R. Lipid peroxidation contributes to immune reactions associated with alcoholic liver disease. *Free Rad Biol Med.* 2002;32:38–48.

16. Thiele GM, Freeman TK, Klassen LW. Immunological mechanisms of alcoholic liver disease. *Semin Liver Dis.* 2004;l24:273–287.

17. Batey RG, Cao Q, Gould B (2002) Lymphocyte-mediated liver injury in alcohol-related hepatitis. *Alcohol.* 27:37–41.

18. Tsukamoto H, Lu SC. Current concepts in the pathogenesis of alcoholic liver injury. *FASEB J.* 2001;15:1335–1349.

19. Nieto N (2007) Ethanol and fish oil induce NFkB transactivation of the collagen a2(1) promoter through lipid peroxidation-driven activation of PKC-PI3K-Akt pathway. *Hepatology.* 2007;45:1433–1445.

20. Billecke S, Draganov D, Counsell R, Stetson P, Watson C, Hsu C, La Du BN. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. *Drug Metab Dispos.* 2000;28:1335-1342.

21. Costa LG, Cole TB, Vitalone A, Furlong CE. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. *Clin Chim Acta.* 2005;352:37-47.

22. Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. *Free Radic Biol Med.* 2004;37:1304-1316

23. Horke S, Witte I, Wilgenbus P, Kruger M, Strand D, Forstermann U. Paraoxonase-2 reduces oxidative stress in vascular cells and decreases endoplasmic reticulum stress induced caspase activation. *Circulation.* 2007;115:2055-2064

24. Ferré N, Camps J, Prats E. Serum paraoxonase activity: a new additional test for the improved evaluation of chronic liver damage. *Clin Chem.* 2002;48:261–268.

25. Marsillach J, Ferré N, Vila MC. Serum paraoxonase-1 in chronic alcoholics: Relationship with liver disease. *Clin Biochem.* 2007;40:645–650.

26. Aviram M, Rosenblat M, Billecke S. Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radical Biol Med.* 1999;26:892–904.

27. Deakin S, Leviev I, Gomaraschi M, Calabrese L, Franceschini G, James RW. Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high affinity, saturable, desorption mechanism. *J Biol Chem.* 2002;277:4301–8.

28. James RW, Deakin SP (2004) The importance of high-density lipoproteins for paraoxonase-1 secretion, stability, and activity. *Free Rad Biol Med.* 2004;37:1986–1994.

29. Camps J, Marsillach J, Joven J. Measurement of serum paraoxonase-1 activity as a potential biomarker for chronic liver impairment. *Clin Chim Acta.* 2007;386:114–115.

30. Marsillach J, Aragonès G, Beltrán R. (2008) The measurement of the lactonase activity of paraoxonase-1 in the clinical evaluation of patients with chronic liver impairment. *Clin Biochem.* doi: 10.1016/j.clinbiochem.2008.09.120

How to cite this article: Devi B. J, Lakshmi V. U, Arifuddin N, Rao J. R, Ch. Kiranmai. Serum paraoxonase – Marker of alcoholic liver cirrhosis. Int J Clin Biochem Res. 2018;5(4):513-516.