Indian J Physiol Pharmacol 2003; 47 (3): 343–346

STATUS OF BLOOD ANTIOXIDANT ENZYMES IN ALCOHOLIC CIRRHOSIS

SURESH CHARI* AND MADHUR GUPTA

Department of Biochemistry,
Indira Gandhi Medical College,
Nagpur – 440 018

(Received on September 25, 2002)

Abstract : Chronic alcohol consumption is associated with increased incidence of variety of illnesses including cirrhosis. Studies have shown that ethanol consumption may result in increased oxidative stress with increased formation of lipid peroxides and free radicals. However, very few reports are available on their involvement in the toxicity of alcoholic cirrhosis. The present study was undertaken in 44 male subjects to evaluate the role of oxidative stress in liver injury with special reference to alcoholic or non alcoholic cirrhosis. It was observed that the parameters of liver function like total bilirubin, Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), γ Glutamyl transfarase (γ GT) were increased in cirrhotic (alcoholic or non alcoholic) patients as compared to normal controls. However antioxidant enzymes like Superoxide dismutase (SOD) and Glutathine peroxidase (GPx) lipid peroxidation marker, Malondialdehyde (MDA) showed significant changes only in alcoholic cirrhosis and not in non alcoholic cirrhosis when compared with normal controls. The possibility of assessment of antioxidant enzymes to differentiate between alcoholic or non alcoholic or non alcoholic cirrhosis is postulated.

Key words: alcohol cirrhosis antioxidant enzymes lipid peroxidation

INTRODUCTION

Free radicals are implicated in the pathogenesis of liver damage due to viral hepatitis, Wilson's disease, haemochromatosis (1) and ishaemic reperfusion injury eg: following transplantation (2). Cellular damage arises from oxidative stress ie: an imbalance between reactive oxygen species generating and scavenging systems (antioxidant enzymes). Liver injury due to acute or chronic alcohol abuse has been attributed to its oxidative metabolism at the cytoplasmic and microsomal/peroximal level (3). With this view in mind, it was thought pertinent to evaluate the role of oxidative stress in liver injury, specifically cirrhosis due to alcoholism or any other cause.

METHODS

Forty four male patients of cirrhosis (27– alcoholic, established on accepted clinical

*Corresponding Author and address : 53/C/1, Gokulpeth, Nagpur – 440 010

biochemical criteria (4) admitted in medicine wards of India Gandhi Medical College, Nagpur over one year period were considered for this study. A control group of 25 non alcoholic healthy individuals of similar age group without liver disease, obesity and any other inflammatory disease were selected for the study.

Blood was taken from individuals in heparinized bulbs and centrifuged at 1500xg for 30 minutes. Analysis was performed within 24 hours of sample collection. All chemicals used were of reagent grade. The plasma separated was analyzed for liver function tests using fully automated chemistry analyzer. Malondialdehyde (MDA) an indicator of lipid peroxidation was estimated by the method of Yagi (5). The haemolysate prepared from the red cells was used for the estimation of antioxidant enzymes; glutathione perosidase (GPx) (6) and superoxide dismutase (SOD) (7).

RESULTS

Table I indicates that certain parameters of liver function test (LFT) like total bilirubin and enzymes were increased in cirrhotic patients as compared to normal control irrespective of whether the patients were alcoholic or non alcoholic. These altered biochemical findings are in addition to histopathological and clinical confirmation of cirrhosis.

Table I also indicates that Changes in all parameters were non significant when alcoholic cirrhosis was compared with non alcoholic cirrhosis except for γ Glutamyl Transfarase (γ GT) which showed significant difference (P<0.001).

TABLE I: Liver function tests in normal control; alcoholic and non-alcoholic cirrhosis.

Liver function tests in serum	*Normal controls (n = 25)*	*Alcoholic cirrohsis (n = 27)*	*Non-alcoholic cirrohsis (n = 17)*
Total Bilirubin (mg/dl)	0.85±0.52	4.54± 3.79***	2.92±1.21***
AST (U/L)	30.40±15.81	62.24± 34.86*	54.89±29.26*
ALT (U/L)	23.61±19.62	41.12± 20.14*	40.86±17.98*
ALP (U/L)	102.04±78.81	183.22± 98.26*	177.62±92.20*
Total protein (g/dl)	5.52±1.24	5.94± 0.96	6.18±0.92
Albumin (g/dl)	2.16±0.17	2.21± 0.62	2.39±0.74
Globulin (g/dl)	3.36±0.52	3.72± 0.35	3.79±0.19
γ GT (U/L)	19.82±9.86	90.45± 42.12***	41.71±24.86**

– Values are mean + SD, P values when cirrhotic (alcoholic or non alcoholic) were compared with normal controls *P<0.05; **P<0.01; ***P<0.001.

– Changes in all parameters were non-significant when alcoholic cirrhosis was compared with non alcoholic cirrhosis except for γ GT (P<0.001).

TABLE II: MDA and antioxidants in normal; control and cirrhotic patients.

Enzyme	*Normal controls (n = 25)*	*Alcoholic cirrohsis (n = 27)*	*Non-alcoholic cirrohsis (n = 17)*
Plasma MDA (nmol/ml)	1.03±0.08	1.67±0.51***	1.16±0.21
Erythrocytic SOD (U/mgHb)	6.17±1.29	5.28±0.77**	5.92±0.16
Erythrocytic GPx (U/gHb)	13.11±4.02	10.25±3.21*	11.25±2.83

Values are mean + SD, *P<0.05; **P<0.01; ***P<0.001.
Changes in non alcoholic cirrhotic as compared to normal controls were non significant.

Table II shows a significant change in the value of MDA (P<0.001), SOD (P<0.01) and GPx (P<0.05) in patients of alcoholic cirrhosis when compared with normal control. No significant change was observed when non–alcoholic cirrhotic was compared with normal control for these parameters.

DISCUSSION

It has been suggested earlier that alcohol damage to liver can, among other factors, be mediated through the action of toxic oxygen radicals generated by ethanol (8, 9). Alterations in the pro oxidant and anti oxidant levels have also been demonstrated when alcoholic cirrhotic were compared with nonalcoholic cirrhotic patients (10) and with non–cirrhotic controls as well (11). Moreover, significant decrease in the activity of erythrocyte SOD and GPx was observed in alcoholics with no significant change in the activity of these enzymes in plasma (12).

Our study was restricted to assess the status of the antioxidant enzymes in patients with alcoholic and nonalcoholic cirrhosis and we have demonstrated an altered erythrocyte pro–oxidant and anti oxidant status in alcoholic cirrhotic patients which was not evident in the non–alcoholic cirrhosis. This could therefore be used as a good discriminator between patients with alcoholic and non–alcoholic cirrhosis. The biochemical reasons to explain these changes are however not clear at present.

Earlier studies have demonstrated a close association between oxidative damage and alcohol abuse. It has been suggested that free radical intermediates produced during ethanol metabolism might be responsible for causing oxidative damage (13). It has been also demonstrated that ethanol impairs the hepatic antioxidant potential (14).

Lipoperoxidation, a degradative process of membranous polyunsaturated fatty acids, had been suggested to represent an important mechanism of ethanol toxicity on the liver. An increase in lipid peroxidation as concluded by increase in MDA in alcoholic cirrhotic observed in this study in difficult to explain at this juncture. It is suggested that the disturbances of iron metabolism reported earlier in human alcoholics (15) may contribute to an enhanced steady state concentration of reactive free radicals leading to lipoperoxidative damage and cellular injury.

The susceptibility of a given tissue to peroxidation is however, a function of the overall balance between Pro–oxidant and antioxidant defense systems. The latter involves both intracellular and extra cellular protective factors where nutrients play an important role. Reduced levels of vitamins in general and Vitamin E in particular (16) have been found in serum of alcoholics. This may reduce antioxidant capacity and promote generation of free radicals and lipid peroxides resulting in tissue damage and disease. Thus the role of nutrition in favor of an anti oxidant defense mechanism cannot be ruled out.

REFERENCES

1. Von Herbay A, Stahl W, Neideran C et al. Diminished plasma levels of vitamin E in patients of severe viral hepatitis. *Free Radic Res* 1996; 25: 461–466.
2. Nordstrom G, Seeman T, Hasselgren PO. Beneficial effect of allopurinol in liver ischaemia. *I Surgery* 1985; 97: 679–684.
3. Lieber CS. Biochemical and molecular basis of alcohol induced injury to liver and other tissues *N Engl J Med* 1988; 319: 1639–1650.
4. Sherlocks S, Dooley SJ. In: Diseases of the liver and biliary system 10th ed London: Blackwell Science Ltd 1977; 371–384.
5. Yagi K. Toxicity of lipid peroxides in processed food. *Biochem Rev* 1980; 4: 42–46.
6. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab & Clin Med* 1967; V70: 158–169.
7. Crapo JD, McCord JM, Fridovich I. Preparation and assay of superoxide dismutase. *Methods in Enzymol* 1978; L111: 382–393.
8. Zhao M, Matter K, Laissure JA et al. Copper/ zinc and manganese superoxide dismutases in alcoholic liver disease: immunohistochemical quantitation. *Histol Histopathol* 1996; 11: 899–907.
9. de la Maza MP, Petermann M, Bunout D et al. Effect of long term vitamin E supplementation in alcoholic cirrhotics. *J Am Coll Nurt* 1995; 14: 192–196.
10. Nalini G, Hariprasad C, Narayanan VA. Oxidative stress in alcoholic liver disease. *Indian J Med Res* 1999; 110: 200–203.
11. Hadi Yasa M, Kacmaz M, Serda Ozturk H et al. Antioxidant status of erythrocytes from patients with cirrhosis. *Hepatogastroenterology* 1999; 46: 2460–2463.
12. Guemouri L, Lecomte E, Herbeth B et al. Blood activities of antioxidant enzymes in alcoholic before and after withdrawal. *J Stud Alcohol* 1993; 53(5): 626–629.
13. Clot P, Tabone M, Arico S et al. Monitoring oxidative damage in patients with liver cirrhosis and different daily alcohol intake. *Gut* 1994; 35: 1637–1643.
14. Rouach H, Fataccioli V, Gentil M et al. Effects of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. *Hepatology* 1997; 25: 351–355.
15. Nordmann R, Ribiere C, Rouach H. Involvement of iron and iron-catalyzed free radical production in ethanol metabolism and toxicity. *Enzyme* 1987; 37: 57–69.
16. Dupont I, Bodenez P, Berthou F, Simon B, Bardon LG, Lucas D. Cytochrome P-450 2E1 activity and oxidative stress in alcoholic patients. *Alcohol Alcohol* 2000, 35(1): 98–103.