



Original Research Article

Oxidative Stress and Antioxidant Vitamin Levels in Alcoholic Liver Disease Patients

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ABSTRACT

Alcoholism is a socio-behavioral problem with an increasingly detrimental impact on society. Alcoholic liver disease is a major cause of morbidity & mortality in lower socioeconomic strata. Ethanol causes liver damage through generation of lipid peroxidation by free radicals. The present case control study was designed which included 90 study subjects with clinically diagnosed alcoholic liver disease and 90 normal healthy controls. The study and control group were matched with age, sex & socioeconomic status. Serum analysis was done to estimate malondialdehyde (MDA), as a measure of oxidative stress, serum bilirubin and antioxidant vitamins E & C. Serum bilirubin and MDA levels were significantly ($p < 0.001$) increased in the study group as compared to the normal healthy controls. The levels of vitamin E & vitamin C were found to be significantly ($P < 0.001$) decreased in the study group as compared to that of controls. Poor nutrition or malabsorption leads to deficiency of these vitamins. This may impair the anti-oxidative defense leading to ethanol induced oxidative stress and then to liver damage

Key words: Alcoholic liver disease, Antioxidants, oxidative stress, Vitamin E, Vitamin C

INTRODUCTION

Alcohol consumption is the common cause of liver disease in both the urban & rural population. Three decade research in ethanol metabolism has established that ethanol is hepatotoxic. Alcohol can affect wide range of organ system. Some of its effects are directly due to the action of either alcohol or its metabolites. Whereas others are related to nutritional deficiencies associated with alcohol intake.^[1]

Rather than type of alcohol beverage or ingestion pattern, the amount and duration of alcohol becomes the important determinant of the liver injury. Alcoholic fatty liver occurs in most heavy drinkers but is reversible on cessation of alcohol consumptions. An inflammatory lesion characterized by infiltration of the liver with leukocytes and liver cell necrosis is thought, to be the major precursor of cirrhosis.^[2]

Free radicals are thought to be the basic factor for oxidative damage in many pathological conditions including alcoholic liver disease (ALD). Many cellular components like DNA, proteins, lipids etc. are damaged by free radicals.^[3] One of the characteristic features of oxidative stress is enhancement of lipid peroxidation. Previous studies have reported an increased oxidative stress after alcohol intake.^[4,5]

Among the dietary antioxidant, vitamin E and vitamin C are the major ones. Acting as a chain reaction terminator along with vitamin C, Vitamin E by donating a hydrogen atom, has the ability to inhibit generation of free radicals and thereby protect polyunsaturated fatty acids from peroxidation in the membrane and prevent further oxidative damages including lipid peroxidation.^[6, 7, 8, 9] The risk of developing severe alcoholic liver disease was a cumulative one determined both by the amount of alcohol consumed and the duration of alcohol.^[10, 11]

MATERIAL AND METHODS

The present case control study was designed to study the levels of MDA and antioxidant vitamins E & C. The study was carried out in the department of biochemistry, Government Medical College, Miraj. The study group consists of 90 patients with clinically diagnosed as alcoholic liver disease and 90, age, sex matched normal healthy controls. The patients were recruited from department of medicine, Government Medical College, Miraj, between the age group 25-50 years. The patients were diagnosed by department of medicine on the basis of USG findings with altered echogenesity, echotexture, increase in size of hepatic portal vein, splenomegaly etc. and the severity was confirmed by measuring serum bilirubin level. And the patients having other causes of liver damage like viral hepatitis (past or present) or drug induced liver damage were excluded from the study.

The patients were divided as mild (group I), moderate (group II) and severe disease (group III) on the basis of serum bilirubin concentration and prothrombin time. Group I includes patients having serum bilirubin concentration less than or equal to 5 mg/dl with normal Prothrombin time (<20 seconds). Group II included patients with serum bilirubin concentration more than 5 mg/dl and with normal Prothrombin time (<20 seconds). Group III, includes the patients with the serum bilirubin concentration more than 5 mg/dl along with prothrombin time more than 20 seconds.

The blood samples were collected in plain bulb and allowed to clot. After 1 hr the serum was separated by centrifugation at 2500 rpm for 10 minutes. Serum was free from hemolysis and turbidity. All the analyses of the biochemical parameters were

performed on the same day. Serum bilirubin concentration was measured by Jendrassik & Groff^[12] method. The oxidative stress was measured in terms of MDA; the analysis of MDA in serum was performed using Kei Satho^[13] method. Vitamin E was assayed by Baker and Frank^[14] method and vitamin C was measured by using method of Ayekyaw et al.^[15] The results were statistically evaluated by using 'z' test.

RESULTS

In the present study the levels of serum bilirubin, MDA and antioxidant vitamins were determined, and expressed as mean \pm SD. The mean age of the study group was 39.27 ± 8.71 years and that of control group was 33.59 ± 6.94 years. There was no significant difference found between mean age of the study and control group. The serum bilirubin level in patients of ALD (4.7 ± 0.81 mg/dl) was found to be

significantly ($p < 0.001$) higher than the normal healthy controls (0.92 ± 0.07 mg/dl). The determinant of oxidative stress MDA level was also found to be significantly ($p < 0.001$) increased in the study group (9.5 ± 0.62 nmol/ml) as compared to the normal healthy controls (6.9 ± 0.39 nmol/ml).

Where as, the levels of antioxidant vitamins E & C were significantly ($p < 0.001$) reduced in patients (0.35 ± 0.05 mg/dl and 0.42 ± 0.06 mg/dl respectively) than the normal healthy controls (0.69 ± 0.06 mg/dl and 0.85 ± 0.09 mg/dl respectively). The difference between means of the control group and each group on the basis of severity was also found to be significantly increased for bilirubin and MDA as the severity of the liver injury increases. The difference between means of the control group and each group was found to be significantly decreased for vitamin E & C as the severity of the liver injury increases. The results are depicted in table no. 1 and 2.

Table 1- Levels of Serum bilirubin (mg/dl) and MDA (nmol/ml) in study group and normal healthy controls.

Groups	No. of cases	Bilirubin (mg/dl)	MDA (nmol/ml)
Controls	90	0.92 ± 0.07	6.9 ± 0.39
Group I	35	$4.5 \pm 0.50^*$	$9.4 \pm 0.52^*$
Group II	30	$5.51 \pm 0.24^*$	$10.4 \pm 0.56^*$
Group III	25	$6.3 \pm 0.22^*$	$14.1 \pm 0.8^*$
Total study	90	$4.7 \pm 0.81^*$	$9.5 \pm 0.62^*$

* $P < 0.001$ highly significant

Table 2- Levels of vitamin E and vitamin c in study group and normal healthy controls.

Groups	No. of cases	Vitmain E (mg/dl)	Vitamin C (mg/dl)
Controls	90	0.69 ± 0.06	0.85 ± 0.09
Group I	35	$0.38 \pm 0.01^*$	$0.7 \pm 0.04^*$
Group II	30	$0.27 \pm 0.08^*$	$0.46 \pm 0.07^*$
Group III	25	$0.22 \pm 0.01^*$	$0.31 \pm 0.03^*$
Total study	90	$0.35 \pm 0.05^*$	$0.42 \pm 0.06^*$

* $P < 0.001$ highly significant

DISSCUSION

Alcoholic liver diseases are very common in lower socio-economic strata due to heavy drinking habits and multiple nutritional deficiencies. Alcoholic liver disease encompasses a spectrum of injury, ranging from simple steatosis to frank cirrhosis. It may well represent the oldest form of liver injury known to humankind. Alcohol remains a major cause of liver disease worldwide.^[16]

ALD spans a clinical and histological spectrum, from fatty liver to alcoholic hepatitis to alcoholic cirrhosis. Fatty liver develops in most people who abuse alcohol for a long period. However, this condition is generally asymptomatic and entirely reversible with abstinence. Although the majority of people who abuse alcohol for an extended duration do not develop advanced lesions of alcoholic liver disease, approximately 15% to 20% develop alcoholic hepatitis and/or cirrhosis, which may develop in succession or exist concomitantly. Specific laboratory abnormalities reflect the severity of alcohol-induced liver injury and have prognostic utility.^[17]

There are several mechanisms reported for ethanol induced liver damage. One of the mechanisms for ethanol induced hepatotoxicity is generation of lipid peroxidation by free radicals but its role has been controversial and the mechanism by which it occurs is unclear.^[4] After metabolism, alcohol is converted to acetaldehyde. Acetaldehyde is a reactive molecule that can oxidize and covalently bind to a variety of functional groups is thought to be a major cause of alcoholic liver injury. Acetaldehyde causes depletion of mitochondrial glutathione, impairs mitochondrial β oxidation of fatty acids, and promotes formation of oxygen-free radicals that cause peroxidation of membrane lipids.

Along with ethanol, acetaldehyde may damage liver cell membranes, especially those of mitochondria, by altering membrane fluidity and modifying membrane-bound enzymes and transport proteins.^[18]

Antioxidants play a critical role in the defense against oxidative stress. Many antioxidants are present in the diet e.g. vitamin E & C, β carotene etc. However poor nutrition or malabsorption leads to deficiency of antioxidant vitamins. This may impair anti-oxidative defense leading to ethanol induced oxidative stress and then to liver damage.

In the present study we have investigated serum bilirubin which was found to be significantly ($p < 0.001$) increased in patients with ALD than the controls. Lipid peroxidation was determined in terms of MDA, which was significantly ($p < 0.001$) higher in the study group than the normal healthy controls, these results are in accordance with Matsumuru T.^[11] Our results also showed a significant ($p < 0.001$) rise in the MDA level with the increase in severity of liver disease in each group as compared to controls, Masalkar et al^[19] reported same results.

We also estimated serum vitamin E & C levels, which was found significantly decreased in the study subjects than the normal healthy controls. It is also observed that the level of decreases significantly with increase in the severity of the liver disease, which may be seen due to increased demands of these antioxidant vitamins to protect against enhanced oxidative stress. The similar results were found by E Lecomte et al^[9] who studied the effect of alcohol consumption on antioxidants and MDA in alcoholics.

CONCLUSION

From this study we can conclude that, serum MDA can be used to detect severity of alcoholic liver disease. As we have observed a significant decrease in vitamin E & vitamin C and the increase in oxidative stress with severity of the disease, further studies with chain breaking antioxidant supplementation have to be performed to examine the possible beneficial effect of this supplementation in alcoholics.

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