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ORIGINAL ARTICLE

Antioxidant Vitamins And Enzymes Status In Patients With Alcoholic Liver Disease

JANANI A V* and SURAPANENI K M**

ABSTRACT

OBJECTIVES: The exact pro-oxidant and antioxidant status in alcoholic liver disease among the chronic alcoholics is still not clear. Consumption of excess amounts of ethanol causes liver damage by several mechanisms. 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. **METHODS:** To add a new insight to the question, changes in the levels of antioxidant vitamins ascorbic acid and plasma vitamin E (non enzymatic antioxidant parameters) and activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase in erythrocytes were measured in 30 patients with alcoholic liver disease (study subjects) and compared to 30 age and sex matched healthy subjects (controls). Statistical analysis between controls and patients was performed by the unpaired *t*-test using the SPSS package. **RESULTS:** It was observed that there was a significant increase in the activities of SOD and GPx and a significant decrease in erythrocyte ascorbic acid, plasma vitamin E levels and catalase activity in patients with alcoholic liver disease, among chronic alcoholics when compared to controls. **CONCLUSIONS:** The results of our study have shown higher oxygen free radical production, evidenced by the decreased levels of ascorbic acid, vitamin E and catalase activity, supporting the hypothesis that there is increased oxidative stress in patients with alcoholic liver disease. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The decreased concentrations of the antioxidant vitamin status support the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of alcoholic liver disease among chronic alcoholics. These data reveal that antioxidant defense mechanisms might be impaired in these patients. These findings also provide a theoretical basis for the development of novel therapeutic strategies, such as antioxidant supplementation.

KEY WORDS: Alcoholic liver disease, ascorbic acid, vitamin E, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, alcoholic liver disease.

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INTRODUCTION

Alcoholic Liver Disease (ALD) is the disease considered to be a major cause of morbidity and mortality, with increasing incidence day by day especially in the developing countries including India (1). This disease is induced / caused due to the consumption of excess alcohol. Chronic consumption of alcohol causes accumulation

of the fatty acids in hepatocytes thereby decreasing the functional capacity of the liver (2). The ingested alcohol in chronic alcoholics also alters various metabolic pathways inside the liver (3, 4) which ultimately leads to the production of the reactive oxygen species (ROS) (5). Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage (6). Free radicals are formed in both physiological and pathological conditions in mammalian tissues (7). The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies (8). Influence of free radicals and presence of oxidative damage that is alteration in the oxidant -antioxidant profile is known to occur in chronic alcoholism (9, 10). Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (11) and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (12) They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes.

In our previous study we showed that lipid peroxidation was significantly increased along with the significant decrease in glutathione levels in chronic alcoholics with alcoholic liver disease (13). However the antioxidant vitamins and antioxidant enzyme status was not assessed. Therefore in this study, concentrations of antioxidant vitamins along with the activities of antioxidant enzymes were estimated in patients with alcoholic liver disease and compared to controls.

In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant–antioxidant status in chronic alcoholics with alcoholic liver disease. Erythrocyte ascorbic acid and plasma vitamin E levels were estimated along with the activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) in erythrocytes as an index of antioxidant status. The activity of the enzyme catalase was also measured. These parameters were estimated in RBCs to assess the disturbances in oxidant–antioxidant status and their effect on erythrocytes. Alterations in antioxidant enzymes have been reported in various studies (14).

MATERIALS & METHODS:

The study was conducted in the Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha University, Chennai, T.N, India. Thirty male patients of alcoholic liver disease established on accepted clinical biochemical criteria (15) were chosen for the study. An equal number of age matched healthy subjects were also investigated. The control and patient groups had the same socioeconomic background. Therefore, changes in analytes due to nutritional factors are minimal. Written consents were also taken from the patients prior to the study. The controls chosen for the study were non alcoholic healthy individuals of similar age group without liver disease, obesity and any other inflammatory disease. Patients suffering from disease of any origin other than alcohol intake were excluded from the study. Patients were subjected to detailed clinical examination and laboratory investigations. The Controls and patients were divided into 2 groups.

Group 1: Thirty healthy age matched controls.

Group 2: Thirty patients with alcoholic liver disease.

The venous blood samples obtained from these subjects were used for the estimation of ascorbic acid, SOD, GPx, catalase and MDA in erythrocytes and vitamin E in plasma. The venous blood samples obtained under asceptic conditions, from these subjects in fasting state were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the measurement of the activity of vitamin E. Ascorbic acid levels were estimated in plasma by the method of Tietz (16). Plasma vitamin E levels were estimated by the method of Baker H et al (17). SOD (EC 1.15.1.1) activity was determined in the hemolysate by the method of Marklund & Marklund (18). Catalase (EC

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1.11.1.6) activity was measured by the method of Beers and Sizer (19). The activity of Glutathione Peroxidase (GPx, EC 1.11.1.9) was measured as described by Paglia and Valentine (20) in erythrocytes All reagents used were of analytical reagent grade and were obtained form Sigma Chemicals, St. Louis, MO.

STATISTICAL ANALYSIS:

Statistical analysis between group 1 (controls) and group 2 (study subjects) was performed by the student t-test using the SPSS package for windows. The data were expressed as mean ± SD. $p < 0.05$ was considered as significant.

RESULTS:

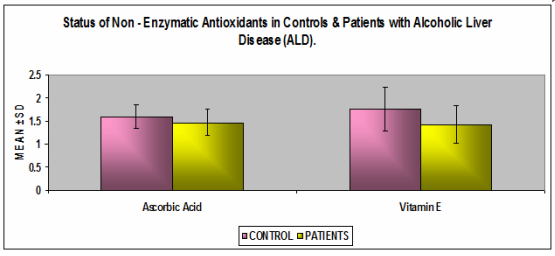
The mean ± SD of antioxidant vitamins –ascorbic acid and vitamin E in controls and patients with Alcoholic Liver Disease are indicated in the Table1 & Figure 1. Table 1 shows that these antioxidant vitamins were significantly decreased in patients with alcoholic liver disease as compared to controls.

The mean + SD of erythrocyte SOD, GPx and Catalase are indicated in Table 2 and Figure 2. There was a statistically significant increase in the erythrocyte antioxidant enzymes – SOD, GPx activity in patients with alcoholic liver disease as compared to controls. The level of erythrocyte catalase activity was significantly decreased in group 2 (study subjects) compared to group1 (controls).

Table/Fig 1: shows the mean ± SD values of non enzymatic antioxidants in controls & patients with alcoholic liver disease.

Parameter	Group1 (Controls) (mean ± SD) n=30	Group2 (Study Subjects) (mean ± SD) n=30
Ascorbic Acid (mg / dl)	1.59 ± 0.26	1.47 ± 0.29 *
Vitamin E (mg / dl)	1.76 ± 0.49	1.42 ± 0.41 **

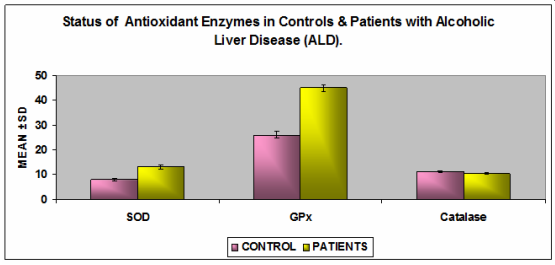
* P < 0.05 compared to controls ** P < 0.01 compared to controls



Table/Fig 2 shows the mean ± SD values of antioxidant enzymes in controls & patients with alcoholic liver disease.

Parameter	Group1 (Controls) (mean ± SD) n=30	Group2 (Study Subjects) (mean ± SD) n=30
SOD (U / mg of protein)	8.02 ± 0.61	13.11 ± 0.85 ***
GPx (U / gm of Hb)	26.06 ± 1.47	44.98 ± 1.36 ***
Catalase (nmole H_2O_2 decomposed / mg protein / 1 min)	11.28 ± 0.42	10.45 ± 0.37 ***

*** P < 0.001 compared to controls



DISCUSSION:

In our study we have observed that, there was a significantly lower level of antioxidant vitamins and increased activity of antioxidant enzymes in patients with alcoholic liver disease compared to controls.

We observed a significant decrease in the levels of erythrocyte ascorbic acid, and plasma vitamin E (non enzymatic antioxidant defense system) in patients with alcoholic liver disease when compared to controls. Free radical generation can induce oxidative stress. The decrease in the levels of these non enzymatic antioxidant vitamin parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in alcoholic liver disease. Similar reports of decreased antioxidant vitamins levels in chronic alcoholics with alcoholic liver disease reported by various studies (2, 21). These findings were supported by our earlier study (13), in which a significant reduction in the levels of glutathione and significant decrease in glutathione – S - transferase activity were observed in chronic alcoholics with alcoholic liver disease compared to controls. A marked increase in lipid peroxidation in these patients was also noted. These results indicate that in ALD patients the efficiency of the antioxidant vitamins in counteracting the damaging effects of free radicals is significantly reduced. Thus the results of our present study suggest that a

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cumulative functional insufficiency of the antioxidant vitamin system may play an essential role in the development of oxidative stress in patients with alcoholic liver disease.

The levels of erythrocyte MDA were significantly higher in chronic alcoholics with alcoholic liver disease as compared to controls as reported by our earlier study (13). In chronic alcoholics, the chronic consumption of alcohol causes the excessive accumulation of fatty acids in hepatocytes there by causing damage to the liver by decreasing its functional capacity (2). It has been postulated that alcohol damage to liver can be mediated through the action of toxic oxygen radicals generated by ethanol, one among the other factors (22, 23). It is also postulated that ethanol induces cytochrome P450 2E1 there by causing generation of excess ROS leading to the production of oxidative stress (24). On the other hand acetaldehyde the metabolic end product of the ethanol oxidation by alcohol dehydrogenase or by cytochromes causes the consumption of antioxidants and inactivation of antioxidants and responsible for the increased generation of free radicals (25).

In our study the erythrocyte Antioxidant enzyme, i.e. Super Oxide Dismutase (SOD) & Glutathione Peroxidase (GP_x) activities have been increased significantly in patients with alcoholic liver disease. Similar reports of raised SOD & GP_X activities have been reported in patients with alcoholic liver disease (26). SOD is the important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GP_X, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes (27). The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. GPx provides an effective protective mechanism against cytosolic injury, because it eliminates H2O2 and lipid peroxides by reduction, utilising GSH (28).

In the present study, we have observed a significant decrease in the catalase activity in patients with alcoholic liver disease compared to controls. Catalase is the enzyme which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (29). Similar reports of decreased catalase activity were observed in alcolic liver disease patients by Das et al (30).

CONCLUSION

In Conclusion, Oxidative stress may be involved in chronic alcoholics. The results of our study have shown higher oxygen free radical production, evidenced by increased levels of MDA and decreased levels of ascorbic acid, vitamin E and catalase activity, supporting the hypothesis that there is increased oxidative stress in patients with alcoholic liver disease. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The decreased concentrations of the antioxidant vitamin status support the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of alcoholic liver disease among chronic alcoholics. These data reveal that antioxidant defense mechanisms might be impaired in these patients. These findings also provide a theoretical basis for the development of novel therapeutic strategies, such as antioxidant vitamin supplementation.

Our results suggest the necessity for therapeutic co-administration of antioxidant vitamins along with conventional drugs to such patients with alcoholic liver disease in the initial stages to prevent the oxidative damage and deterioration of the tissues. The findings implicate oxidative stress in the disease and cite the biochemical rationale for clinical trials of antioxidants to prevent and treat alcoholic liver disease. However due to the limited number of cases included in this study, more studies may be required to substantiate the results and arrive at a definite conclusion in terms of safety and efficacy of adding antioxidant therapy as secondary therapy for the treatment of alcoholic liver disease.

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