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Research Article

ERYTHROCYTE LIPID PEROXIDATION AND ANTIOXIDANTS IN CHRONIC ALCOHOLICS WITH ALCOHOLIC LIVER DISEASE

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ABSTRACT

Objectives: The exact pro-oxidant and antioxidant status in chronic alcoholics is still not clear. Consumption 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, erythrocyte antioxidant glutathione (GSH) and Malondialdehyde (MDA) levels in erythrocytes and plasma glutathione – S – transferase (GST) activity were estimated in Thirty male chronic alcoholics (patients) with alcoholic liver disease and compared to Thirty age and sex matched healthy subjects (controls). Patients were subjected to detailed clinical examination and laboratory investigations. 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 significantly lower erythrocyte GSH levels and plasma GST activity in patients with alcoholic liver disease when compared to controls. There was a significantly higher erythrocyte MDA levels in patients with alcoholic liver disease when compared to controls. **Conclusions:** The results of our study suggests that there was higher oxygen free radical production, as evidenced by higher MDA and lower GSH, supporting the hypothesis that there is increased oxidative stress in patients with alcoholic liver disease and decreased GST activity supports the decreased detoxification capacity in chronic alcoholics with alcoholic liver disease.

Key words: Chronic Alcoholics, Glutathione (GSH), Malondialdehyde (MDA), Glutathione – S – Transferase (GST), Alcoholic liver disease.

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 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 glutathione (GSH) levels were estimated as an index of antioxidant status. Malondialdehyde (MDA) levels were measured as thiobarbituric acid-reacting substances (TBARS), which serve as an index of extent of lipid peroxidation. These parameters were estimated in RBCs to assess the disturbances in oxidant–antioxidant status and their effect on erythrocytes. Glutathione–S– transferase (GST) levels were estimated in plasma. GST is an enzyme involved in antioxidant defense and also involved in detoxication. Alterations in antioxidant enzymes have been reported in various studies [13].

MATERIALS AND 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 [14] 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 GSH and MDA in erythrocytes and GST 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 GST. GSH was estimated by the method of Beutler et al., using dithio-bis-nitrobenzoic acid (DTNB) [15]. MDA was determined as a measure of TBARS (16) and GST was measured by using 1-chloro-2,4-dinitrobenzene (CDNB) (17). All reagents used were of analytical reagent grade. DTNB, CDNB and thiobarbituric acid 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 $\pm$ SD. $p < 0.05$ was considered as significant.

Table 1: Table shows the mean ± SD values of various liver function test parameters in controls and patients with alcoholic liver disease among the chronic alcoholics

LFT Parameter	Group 1 (Controls) (mean ± SD) n=30	Group 2 (Study Subjects) (mean ± SD) n=30
AST (U/L)	31.64 ± 2.22	45.54 ± 2.52 **
ALT (U/L)	37.62 ± 0.57	53.72 ± 0.76 ***
ALP (U/L)	164.74 ± 7.02	231.04 ± 18.12 **
Total Protein (g/dl)	7.88 ± 0.49	8.87 ± 0.74 NS
Albumin (g/dl)	2.65 ± 0.25	1.89 ± 0.21 ***
Total Bilirubin (mg/dl)	0.73 ± 0.45	2.67 ± 0.64 ***
GGT (U/L)	21.98 ± 1.40	93.78 ± 4.90 ***

** P < 0.01 compared to controls, *** P < 0.001 compared to controls, NS – Not Significant as Compared to Controls

Table 2: Table shows the mean ± SD values of erythrocyte GSH, MDA and plasma GST activity in controls and patients with alcoholic liver disease among the chronic alcoholics

Parameter	Group1 (Controls) (mean ± SD) n=30	Group2 (Study Subjects) (mean ± SD) n=30
Glutathione (GSH) (mg / gm of Hb)	12.94 ± 1.88	11.63 ± 1.37 ***
MDA (n mol / gm of Hb)	5.18 ± 1.22	10.81 ± 1.64 ***
GST (μmoles / dl of Plasma)	12.86 ± 1.29	11.08 ± 1.25 ***

*** P < 0.001 compared to controls

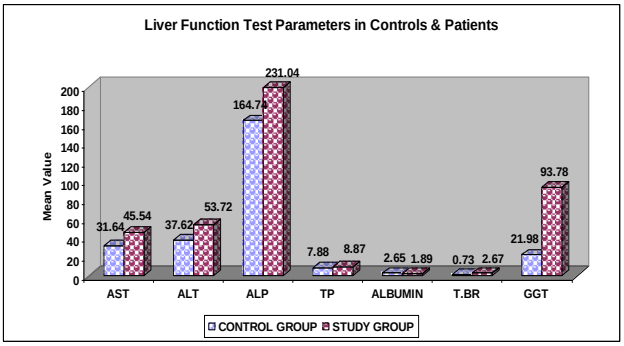

Figure 1: It shows the mean ± SD values of various liver function test parameters in controls and patients with alcoholic liver disease among the chronic alcoholics

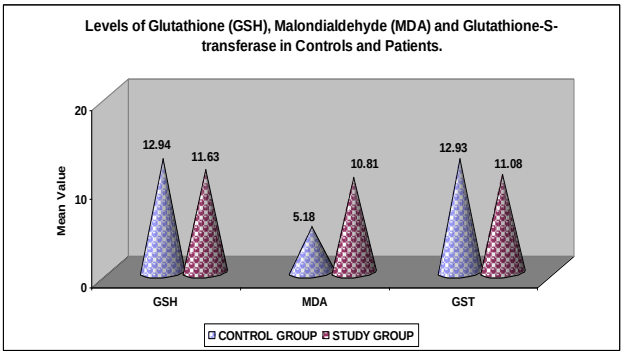

Figure 2: It shows the mean ± SD values of erythrocyte GSH, MDA and plasma GST activity in controls and patients with alcoholic liver disease among the chronic alcoholics

RESULTS

The mean ± SD of various Liver Function Test parameters – AST, ALT, ALP, Total Proteins, Albumin, Total Bilirubin and GGT levels in controls and patients with Alcoholic Liver Disease are indicated in the Table1 and Figure 1. Table 1 shows that these parameters of liver function test (LFT) were significantly increased along with a significant decrease in albumin in patients with alcoholic liver disease as compared to controls.

The mean + SD of erythrocyte GSH and MDA and plasma GST are indicated in Table 2 and Figure 2. There was a statistically significant increase in the erythrocyte MDA levels in patients with alcoholic liver disease as compared to controls. The levels of erythrocyte GSH and plasma GST activity were significantly decreased in group 2 (study subjects) compared to group1 (controls).

DISCUSSION

In our study we have observed that, there was a significantly lower level of erythrocyte GSH and plasma GST levels in patients compared to controls. The levels of erythrocyte MDA were significantly higher in chronic alcoholics with alcoholic liver disease as compared to controls. 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 [18, 19]. It is also postulated that ethanol induces cytochrome P450 2E1 there by causing generation of excess ROS leading to the production of oxidative stress [20]. 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 [21].

In the present study the lipid peroxidation product i.e. MDA levels have been increased significantly in erythrocytes of the patients with alcoholic liver disease compared to controls. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. The free radical intermediates produced during ethanol metabolism might be responsible for causing oxidative damage .These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. An elevated MDA level has been reported in patients with Alcoholic liver disease among the chronic alcoholics [22].

We observed a significant decrease in the levels of serum glutathione (GSH) (non enzymatic antioxidant defense system) in patients with alcoholic liver disease when compared to controls. The decrease in the levels of these non enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in these patients. Similar reports of decreased erythrocyte GSH, Ascorbic acid and Vitamin E levels in chronic alcoholics with alcoholic liver disease reported by various studies [2].

The GST is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood [23]. In this study, a significant decrease is observed in the levels of plasma GST levels in chronic alcoholics with alcoholic liver disease, as compared to normal healthy individuals. Similar results in the status of antioxidant enzymes were observed by suresh chari et al [22]. Decrease in GST activity in chronic alcoholics might indicate decreased detoxification or free radical scavenging capacity in alcoholic liver disease caused due to the excessive consumption of alcohol. This decrease in GST activity may result from decreased enzyme production or enzyme inactivation. In contrast to our study

seema gupta et al [1] reported increased activity of this enzyme in patients with ALD.

CONCLUSION

In Conclusion, Oxidative stress may be involved in chronic alcoholics. The results of our study have shown higher oxygen free radical production & decreased glutathione, support to oxidative stress in chronic alcoholics with alcoholic liver disease. These facts suggest that oxidative stress may be one of the contributing factors in the pathogenesis of ALD. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage. The results also suggest the necessity for therapeutic co-administration of antioxidants along with conventional drugs to such patients.

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