ABSTRACT
Alcoholism can lead to various medical complications, like perturbed alcohol metabolism, liver cirrhosis and hormonal changes associated with pancreatitis, osteoporosis, immune impairment and impaired fertility. Most of the toxic and metabolic disorders that results from long-term ethanol abuse can be explained on the basis of the metabolism of ethanol by alcohol dehydrogenase (ADH) and the associated generation of reduced NADH or by the Microsomal ethanol-oxidizing system (MEOS), with the induction of Cytochrome P-4502E1 and other Microsomal enzymes, as well as by the toxic effects of acetaldehyde produced by both pathways, primarily in the liver. 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 conducted to evaluate some of the components of antioxidant defense system and oxidative damage in 80 male patients of alcoholic cirrhosis. The results were compared with 70 healthy non alcoholic male volunteers. Patients were subjected to detailed clinical examination and laboratory investigations. Blood samples were collected for estimating Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH), Glutathione peroxidase (GPx) and Glutathione Reductase (GR). However, the core utility of MDA was found to be significantly (p<0.001) increased, and the blood antioxidants was found to be significantly decreased (p<0.001) in alcoholic cirrhosis patients compared to controls. There was a significant negative correlation of MDA with SOD, CAT, GSH, GPx and Glutathione. 
Reductase (GR) (p<0.05), and GGT and GSH (P<0.05), these results give enough evidence of increased oxidative stress and compromised antioxidant defense system in patients with alcoholic cirrhosis.

KEY WORDS: Alcohol cirrhosis, Antioxidant enzymes, Lipid peroxidation.

1. INTRODUCTION

Earlier the alcohol dependence syndrome was recognized as a clinical syndrome. In 1992 International Classification of Disease-10th edition (ICD-10).\(^1\) has identified alcohol as psychoactive substance, which leads to mental and behavioral disorders. Over the past few decades, the disease concept of substance use disorders, particularly alcoholism, has become well accepted. Epidemiological studies in India showed that the cultural tradition and permissiveness of alcohol use is associated with higher rates of alcohol use disorder.\(^2\)

Most of the toxic and metabolic disorders that results from long-term ethanol abuse can be explained on the basis of the metabolism of ethanol by alcohol dehydrogenase (ADH) and the associated generation of reduced NADH or by the Microsomal ethanol-oxidizing system (MEOS), with the induction of Cytochrome P-4502E1 and other Microsomal enzymes, as well as by the toxic effects of acetaldehyde produced by both pathways, primarily in the liver.\(^3\) The acetaldehyde produced by the oxidation of ethanol also has toxic effects, inhibiting the repair of alkylated nucleoproteins,\(^4\) decreasing the activity of key enzymes, and markedly reducing oxygen utilization in mitochondrial damaged by long-term ethanol consumption.\(^3\) Moreover, acetaldehyde promotes cell death by depleting the level of GSH, inducing lipid peroxidation, and increasing the toxic effect of free radicals. By binding to the tubulin of microtubules, acetaldehyde blocks the secretion of proteins.\(^5\) Long-term consumption of ethanol induces the microsomal ethanol-oxidizing system.\(^6\) Liver-biopsy specimens from subjects who have recently drunk alcohol have a 5-to 10-fold increase in levels of Cytochrome P-4502E1, the key enzyme in the oxidation of ethanol,\(^7\) and an increase in the corresponding messenger RNA (mRNA).\(^8\)

Alcohol abuse is associated with cancers of the alimentary and respiratory tracts and possibly, with breast cancer.\(^9\) One contributory factor is the activation of carcinogens through the induction of Cytochrome P-4502E1. Alcohol also enhances the mutagenicity of tobacco-derived products. A third contributing factor is vitamin A deficiency,\(^10\) an long-term ethanol consumption induces in part by accelerating hepatic Microsomal degradation of retinol.\(^11\)
The incidence of ALD is increasing day by day specially in the developing countries including India. Hence the present study was planned with the objectives to investigate the oxidative damage and the efficiency of endogenous antioxidant defense system in patients of alcoholic cirrhosis.

2. MATERIALS AND METHODS
This study was carried out after getting clearance from Institutional Ethical Review Committee, Grant Medical College & Sir J J. Groups of Hospitals, Byculla, Mumbai. In the present investigation, attempts were made to design a discrimination procedure to separate alcoholics from controls and patients with non-alcoholic hepatic diseases using a combination of the most promising test. The most powerful discrimination model was constructed with the batteries of screening instruments for detecting alcohol problems. CAGE,[12, 13, 14] Michigan Alcohol Screening Test (MAST).[15] Alcohol Use Disorder Identification Test (AUDIT).[16, 17] and Severity of Alcohol Use Disorder Data (SADD).[18] Eighty male patients of alcoholic cirrhosis (established on accepted clinical biochemical criteria,[19] admitted in medicine wards of Grant Medical College and Sir J.J.Group of Hospital Byculla, Mumbai, was considered for this study. A control group of 70 non alcoholic healthy males of similar age group without liver disease, and any other inflammatory disease were selected for the study.

Within 24 hours of admission & overnight fasting conditions total 10ml of venous blood samples were collected. From blood samples collected in plain tubes serum was separated by centrifuging at 2500 rpm for 7 minutes at room temperature and were used for estimation of Serum γ-Glutamyl Transferase (GGT), Serum glutamic-oxalacetic transaminases (SGOT), Serum glutamic-pyruvic transaminases (SGPT), and Malondialdehyde (MDA). Hemolytic or turbid samples were discarded. Blood sample collected in EDTA tube were used for estimating MCV. Hemolysate prepared from heparinised blood specimens were used for estimation of activities of CAT, SOD, GPx, GR. Citrated blood collected was utilized for estimation of blood GSH.

Serum Malondialdehyde (MDA) was estimated by the method of Buege and Aust,[20] Erythrocyte CAT activity was estimated by Hugo Aebi.[21] method. Method proposed by Marklund and Marklund,[22] was used to estimate erythrocyte SOD activity, GPx was determined by following the method described by Paglia and Valentine [23], GR assay was performed by the method of Carlberg and Mannervik,[24] blood Glutathione (GSH) was done
by the method of Beutler et al.\textsuperscript{[25]} All spectrophotometric reading was taken on Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.

**Statistical analysis**

All the samples were run in duplicates, differences were statistically assessed using student t-Test,\textsuperscript{[26]} by using statistical software Minitab, were one-way ANOVA is being applied. The results obtained were expressed as Mean ± Standard deviation (SD), and correlations between variables were studied by using Pearson’s correlation coefficient test.

**3. RESULTS**

Table 1: indicates that certain parameters of liver function test (LFT) like total bilirubin and enzymes were increased in cirrhotic patients as compared to normal control these altered biochemical findings are in addition to histopathological and clinical confirmation of cirrhosis.

Table 2: shows a significant change in the value of MDA (P<0.001), SOD (P<0.001), CAT (P<0.001), GSH (P<0.001), GPx (P<0.001) and GR (P<0.001) in patients of alcoholic cirrhosis when compared with normal control.

Table 3: indicates significantly negative correlation between MDA against SOD, CAT, GSH, GSH-Px, GR and GGT against GSH in alcoholic cirrhosis patients (P<0.05).

**TABLE 1: Liver function tests in normal control and alcoholic cirrhosis.**

<table>
<thead>
<tr>
<th>LIVER FUNCTION TEST</th>
<th>NORMAL CONTROLS (n=70)</th>
<th>ALCOHOLIC CIRRHOSIS (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.85± 0.52</td>
<td>4.54± 3.79*</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.32± 1.30</td>
<td>8.34± 0.72#</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.20± 0.8</td>
<td>4.29± 0.44#</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>5.56± 0.66</td>
<td>6.32± 0.29#</td>
</tr>
<tr>
<td>GGT(11-50 U/I for men)</td>
<td>19.70± 8.9</td>
<td>150.20± 8.7*</td>
</tr>
<tr>
<td>SGOT(0-40 IU/L)</td>
<td>28.40± 3.5</td>
<td>80.70± 4.3*</td>
</tr>
<tr>
<td>SGPT(0-40 IU/L)</td>
<td>19.33± 6.8</td>
<td>69.72± 8.1*</td>
</tr>
<tr>
<td>MCV(82-98 FL)</td>
<td>88.45± 2.8</td>
<td>144.55± 2.6*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation.

- Differences between Controls and Alcoholic Patients were significant for all biochemical and hormonal parameters (p<0.001).
- Non significant difference of parameter.
### TABLE 2: MDA and antioxidants in normal control and alcoholic cirrhosis.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL CONTROLS (n=70)</th>
<th>ALCOHOLIC CIRRHOSIS (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td>3.088 ± 2.07</td>
<td>8.22 ± 4.8 *</td>
</tr>
<tr>
<td>Superoxide dismutase (u/gHb)</td>
<td>1750.8 ± 37.7</td>
<td>1287.8 ± 5.7 *</td>
</tr>
<tr>
<td>Catalase (u/gHb)</td>
<td>18.55 ± 7.4</td>
<td>13.44 ± 3.05*</td>
</tr>
<tr>
<td>Glutathione (mg/dl of RBCs)</td>
<td>82.63 ± 5.4</td>
<td>43.21 ± 0.83*</td>
</tr>
<tr>
<td>Glutathione peroxidase (u/gHb)</td>
<td>40.33 ± 2.9</td>
<td>19.68 ± 3.08*</td>
</tr>
<tr>
<td>Glutathione Reductase (u/gHb)</td>
<td>0.89 ± 0.2</td>
<td>0.32 ± 0.31*</td>
</tr>
</tbody>
</table>

- Results are expressed as mean ± standard deviation.
- Differences between Controls and Alcoholic Patients were significant for all biochemical and hormonal parameters (p<0.001).

**Table 3: Correlation analysis between MDA concentration and antioxidants in alcoholic cirrhosis patients and serum GGT activity with blood Glutathione levels (r values)**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>ALCOHOLIC CIRRHOSIS (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA/SOD</td>
<td>-0.64**</td>
</tr>
<tr>
<td>MDA/CAT</td>
<td>-0.72**</td>
</tr>
<tr>
<td>MDA/GSH</td>
<td>-0.62**</td>
</tr>
<tr>
<td>MDA/GSH-Px</td>
<td>-0.69**</td>
</tr>
<tr>
<td>MDA/GSH-R</td>
<td>-0.76**</td>
</tr>
<tr>
<td>GGT/GSH</td>
<td>-0.59**</td>
</tr>
</tbody>
</table>

- ** Significant (P<0.05)
- r = correlation coefficient.

### 4. DISCUSSION

Earlier studies suggests that alcohol damage to liver can be due to the other factors, and mediated through the action of toxic oxygen radicals generated by ethanol [27, 28]. Alterations in the pro oxidant and anti oxidant levels have also been demonstrated when alcoholic cirrhotic were compared with nonalcoholic cirrhotic patients and with non cirrhotic controls [29, 30].

Our results are in good agreement and run consistently well with the previous reports by Matsurma, et al., Suematsu, et al., Naveau, et al., Dupont, et al., Paramahamsa, et al., Ucar, et
al. and Das, et al., which states that both serum and erythrocyte TBARS content was increased significantly in alcoholic patients as compared to their controls. Paramahamsa, et al. found erythrocyte lipid peroxidation was 4-fold and 6-fold higher in alcoholic diabetics, compared to the control group, similarly Mottaran, et al., states that the fighters of anti-MDA antibodies were significantly higher in heavy drinkers than in controls. El-Sokkary, et al. reported that MDA and (4HDA) 4-hydroxy alkenals levels were significantly increased in brain, heart, lungs and testes of alcohol treated rats compared to their controls. At the same time Schlorff, et al, found plasma Malondialdehyde (MDA) levels were significantly elevated with respect to an increase in time after ethanol ingestion in rats indicating time dependent augmentation of lipid peroxidation. Further data given by Atamer, et al were MDA levels in liver, heart, kidney and brain were increased in short and long term ethanol treated rats as compared to normal controls. Further chronic ingestion of ethanol significantly increased MDA content in rat liver reported by Husain, et al. Therefore, present data on increase of serum MDA concentration in alcohol cirrhosis subjects compared to their non alcoholic healthy controls, shown in (Table.no.2), run parallel with the reports documented by various investigators in their works.

The present study has illustrated similar findings with reference to the activity of SOD in study group. (Table.no.2), Dismutation reaction involving two \( O_2^- \) radicals and catalyzed by SOD results in the generation of \( H_2O_2 \). The present investigation, where erythrocyte SOD activities in alcoholic cirrhosis patients displayed low statistically significant activities than their respective controls, showed resemblance with the result obtained by Guemouri, et al. were they measured erythrocyte (E) and plasma (P) activities of (SOD) Superoxide dismutase in 58 male alcoholics without evidence of severe liver disease and in control (C) group of 78 healthy men. They found that E-SOD mean activities were lower in alcoholics than controls. Veerappan, et al. have encountered similar kind of observation, where they found the activities of erythrocyte antioxidant (SOD) was decreased significantly in alcoholic hypertensive patients with/ without diabetes, when compared to normal control subjects. The low statistically significant value of SOD in alcoholic cirrhosis group probably signifies that, this enzyme may be involved in depleting free radical induced oxidative stress in alcoholic cirrhosis groups. Moreover, it also gives us a probable hint that free radicals generated might be involved in this enzyme inactivation. All of this data is evident by elevated levels of lipid peroxidation in alcoholic cirrhosis subjects compared to their respective non alcoholic controls. Thus the precipitous fall in SOD activity in alcoholic
patients (Table no.2) indicates that there may be marked decrease in the ability of these alcoholic cirrhosis patients tissues to detoxify $O_2^-$ radicals and protect the cell from oxidative damage.

Reactive oxygen species like H$_2$O$_2$ which are formed during metabolic pathways and also during alcohol metabolism is been removed by CAT. So CAT becomes more important when the concentration of H$_2$O$_2$ far exceeds the physiological level. Our findings demonstrated statistically significant decrease in the CAT activities in alcoholic cirrhosis patients compared to their controls. Our findings showed resemblance with the previous results, obtained by Zhou, et al. and later in Zhou, et al. where they observed CAT activity of RBC in male alcoholics was significantly decreased with the increasing time and quantity of alcohol abuse. Further Veerappan, et al., reported that erythrocyte CAT activity was significantly decreased in alcoholic hypertensive patients with/without diabetes as compared to normal subjects. Bogdanska, et al., have also reported similar observation, when they measured erythrocyte CAT activity in patients with alcohol dependence syndrome, where CAT showed a significant decrease in the activity in alcohol abuser group, compared to the normal healthy control group.

Reduced Glutathione (GSH), a tripeptide is a major non-protein thiol in living organisms that play a central role in coordinating the body’s antioxidant defense process. Present studies shows that red cell GSH content in alcoholic cirrhosis group (Table 2) was found to be significantly decreased compared to their respective non alcoholic controls, indicating that GSH may be escaping through red blood cells. Measurement of GSH concentration also provides a direct means of determining the degree of oxidant stress in erythrocytes for lipid peroxidation, which suggests oxidant stress in alcoholic cirrhosis patients. Experimental evidences of our study run consistently well in accordance with these findings, where the work has revealed a significant decrease in GSH levels. Large number of concluding evidences shows that GSH concentration gets significantly decreased in alcoholic subjects. Altomare, et al reported in their study, alcoholic patients exhibited significant decrease of hepatic GSH compared to control subjects. Total GSH content in blood of alcohol liver disease patients was significantly lower than healthy control subjects as documented by Naveau et al., report by Cardin, et al stated that serum GSH levels were significantly lower in chronic alcoholic liver damage patients than control subject. A year latter Czuczejko et al in their study on patients of alcoholic liver injury found that, the red cell GSH
concentration was significantly lowered compared to healthy controls. Veerappan et al also reported similar observation where they found the erythrocyte and plasma GSH level was significantly decreased in the alcoholics compared to normal subjects. [44]

Our biochemical investigation suggest that higher GGT values in alcoholic cirrhosis patients (Table-1) offer’s direct evidence, that this enzyme might be involved to hydrolyze GSH, so as to provide cells with amino acid’s necessary for replenishment of which is indicative of low-values of GSH. At the same time these low values can also lead to increased oxidative stress which is noticeable by increased lipid peroxidation (Table.no.2) among alcoholic cirrhosis patients as compared to their respective controls.

Further investigations on erythrocyte GPx and GR were also carried out in alcoholic cirrhosis patients. Our study displayed significantly reduced activity of GPx in alcoholic cirrhosis subjects (Table.no.2) which clearly indicates diminished levels of its substrate (i.e.) GSH and inability to dispose H₂O₂. The decrease in activities of Gpx could also be due to deficiency of selenium in their diet. Experimental evidences of the present studies are consistent in accordance with these previous findings, where the work has revealed a significant decrease in Gpx activity of erythrocyte in alcoholics. According to the report of Guemouri, et al erythrocyte Gpx activity was found to be reduced in alcoholics. [43] In several papers few investigators like Zhou et al and Zima, et al reported that Gpx activities in RBC of male alcoholics were significantly decreased as compared to healthy control Volunteers. [52] Veerappan, et al have also reported similar, observations, [44] where the erythrocyte enzymatic antioxidant Gpx was significantly decreased in alcoholic hypertensive patients with/without diabetes as compared to normal healthy nonalcoholic controls. One year later Das, et al also found significant decline in erythrocyte Gpx activity in alcoholics with moderate and high alcohol intake in comparision to normal healthy individuals. [37] Report by Bogdanska et al showed that activity of erythrocyte Gpx was decreased in alcoholics, this decrease was not statistically significant as compared to the control group. [47]

Further studies on erythrocyte GR revealed gradual decrease of its activities in alcoholic cirrhosis patients compared to their respective controls. (Table.no.2), which explains the incapability of this enzyme to provide a continuous flow of GSH as a substrate for Gpx enzyme. A decreased erythrocyte GR activity in alcoholic dependent population showed resemblance with the report of Das et al, where they obtained significant decline in GR activity in alcoholic patients with moderate and high alcohol intake to the control group. [37].
In 2007 Das et al also found statistically significant decrease activity of GR in rat brain, who was fed chronically with ethanol\textsuperscript{[53]}. Previous reports based on animal trials by Husain et al reported significant decreased GR activity in rat heart after chronic ethanol consumption\textsuperscript{[54]}. Further Schlorff et al reports stated that plasma GR activity was found to be significantly decreased in ethanol treated rats in dose dependent manner\textsuperscript{[55]}

The significant increase in MDA levels in alcoholic cirrhosis patients compared to healthy controls suggest that alcoholics are subjected to oxidative stress. To highlight the antioxidant defense system as well as oxidative damage, it is reasonable to evaluate the status of ratios i.e. MDA/SOD, MDA/CAT, MDA/GSH, MDA/GSH-Px and MDA/GSH-R, a decreased ratio observed in the present study suggests that with increase in oxidative stress, and corresponding proportionate decrease in antioxidant defense system. This fact was substantiated by negative correlation observed between MDA and all the endogenous antioxidant enzyme Parameters which reflects that antioxidant defense system is compromised with increased free radical generation during alcohol metabolism.

The measurement of serum GGT levels is known as a sensitive marker of hepatobiliary disorders\textsuperscript{[56]} and it has been reported to be induced by drugs including alcohol\textsuperscript{[57]}. As GGT is a membrane bound enzyme, oxidative stress induced damage to the membranes of hepatocytes seems to contribute to the increased activity of GGT as observed in the present study. This is substantiated by the observation where GGT showed negative correlation with GSH indicating that with a decrease in GSH concentration there is increase in the activity of GGT. The present study clearly demonstrates the compromise in the endogenous antioxidant system in the patients of alcoholic cirrhosis, which in turn is due to alcohol, induced oxidative stress. Antioxidants treatment of patients in the process of achieving sobriety and at risk for progression of ALD with orally administered agents with antioxidant properties such as (SAMe) S-Adenosyl –L-Methionine and betaine as both possess a lipotropic property that enhances fat export and play an intermediary role in the metabolic pathway that enhances intracellular glutathione and betaine which decreases homocysteine, a candidate pro-oxidant and profibrotic amino acid could be beneficial. Large, controlled clinical trials are needed for proving the beneficial role of these agents in the treatment of alcoholic cirrhosis with increased oxidative stress and derranged antioxidant profiles.

**CONFLICT OF INTEREST**

No competing financial interests exist.
ACKNOWLEDGEMENTS

We would like to acknowledge all the member's from our laboratory.

5. REFERENCES


LIST OF ABBRIVATIONS

ADH- alcohol dehydrogenase.
MEOS- Microsomal ethanol- oxidizing system.
MDA- Malondialdehyde.
GGT- γ-Glutamyl Transferase.
ALD- Alcohol liver disease.
MAST- Michigan Alcohol Screening Test.
AUDIT- Alcohol Use Disorder Identification Test.
SADD- Severity of Alcohol Use Disorder Data.
SGOT- Serum glutamic-oxalacetic transaminases.
SGPT- Serum glutamic-pyruvic transaminases.
EDTA- Ethylene Diamine Tetra Acetic acid.
MCV- Mean Corpuscular Volume.
CAT- Catalase.
SOD- Superoxide dismutase.
GSH-Px- Glutathione Peroxidase.
GR- Glutathione Reductase.
GSH- Glutathione.
4HDA- 4-hydroxy alkenals.
H2O2- Hydrogen peroxide.
E-SOD- erythrocyte Superoxide dismutase.
SAMe- S- Adenosyl –L-Methionine.