SERUM MALONDIALDEHYDE, VITAMIN-C STATUS IN ALCOHOLIC LIVER DISEASES

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ABSTRACT
Alcoholic liver disease (ALD) is an alcohol induced disease with multiple factors influencing its development and manifestations. It is proposed that alcohol ingestion produces metabolic imbalances in liver leading to increased formation of reactive oxygen species and decreased levels of antioxidants. The study intends to understand the mechanism of the disease by evaluating the role of lipid peroxidation and antioxidant status in the patients of ALD. The present study involved 40 participants of which 20 were patients admitted with diagnosis of ALD and other 20 were age and sex matched healthy controls who fulfilled inclusion criteria. In ALD patients compared to controls significant increase in following parameters was observed. Malondialdehyde and uric acid are significantly increased compared to controls (P<0.001), total bilirubin and the direct bilirubin also increased significantly (P<0.001). The levels of AST, ALT and AST/ALT ratio, ALP and GGT were significantly increased (P<0.001). The levels of vitamin C, total protein and albumin were significantly reduced in patients with alcoholic liver disease compared to controls with P<0.05 and P<0.001 respectively. ALD is due to increased oxidative stress and decrease of antioxidants. Malondialdehyde a lipid peroxidation product is a sensitive marker of ALD and its severity.

Key words: Alcoholic liver disease; Malondialdehyde, lipid peroxidation, Vitamin C, Reactive Oxygen Species (ROS).
INTRODUCTION
Alcoholic liver disease (ALD) is an alcohol-induced disease with genetic, psychosocial and environmental factors influencing its development and manifestations. The disease is often progressive and is major source of morbidity and mortality[1]. ALD is one of the major medical complications of alcohol abuse and alcohol is the major cause of liver cirrhosis [2]. Serum albumin level is reduced in chronic liver disease as well as in the inflammatory states, and become one of the severity markers of liver cirrhosis [3, 4]. Free oxygen radicals have important toxic effects; chiefly the hydroxyl radical and to a lesser extent the superoxide anion lead to peroxidation of membrane lipids thereby causing production of MDA and 4HNE. These substances directly induce hepatocytic damage with generation of proinflammatory cytokines, activation of spindle cells, and fibrogenesis[5-7].

In the liver GSH plays a key role against lipid peroxidation through its influence both on enzyme catalyzing systems and other antioxidants such as vitamin E, vitamin C and selenium, which is crucial components of the non-enzymatic pathway [8]. Chronic use of alcohol is suggested to cause depletion in hepatic GSH, particularly in mitochondrial GSH. GSH depletion is attributed to a defect in translocation of cytosolic reduced GSH to mitochondria and/or a decrease in GSH resynthesis capacity. This ethanol-induced depletion in the mitochondrial antioxidant defense mechanism is proposed to increase hepatic damage by rendering cells more sensitive to the oxidative stress from inflammatory cytokines such as tumor necrosis factor (TNF)-α and making them targets for free oxygen radicals [7].

Role of Vitamin C as antioxidants is indicated by its known free radical scavenging action. Vitamin C offers the most effective protection against plasma lipid peroxidation. Ascorbic acid serves as both an antioxidant and a pro-oxidant. As an antioxidant vitamin C exerts a sparing effect on the antioxidant actions of vitamin E and selenium on the other hand, excess amounts (~1nM) may act as a pro oxidant in the presence of the transition metals Fe³⁺ or Cu²⁺ generating cofactors of activated oxygen radicals during the promotion of lipid peroxidation [9].

Ascorbic acid (vitamin C) is an important antioxidant both within cells and in the plasma [10]. It has been shown to regenerate α -tocopherol from thotocopheroyxl radical in vitro [11] but this ‘sparing’ action of vitamin C for vitamin E has yet to be convincingly demonstrated in vivo [12].
MATERIALS AND METHODS

In the present study, twenty ALD patients, between the age group of 31-50 years, who attending the Rajah Muthiah Medical College Hospital, Chidambaram were included after taking the inform consent from the patients and the study was approved by the ethical and research committee of RMMC&H to use human subjects in the research study. Twenty age, and sex matched subjects were selected as controls. Experiments were done in accordance with the Helsinki Declaration of 1975.

Selected subject’s blood samples were collected with all aseptic precautions. 6 ml of blood was collected from a large peripheral vein. It was allowed to clot for 30 minutes in a clean dry test tube and was subjected to centrifugation at 2000×g for 10 min. The separated serum samples were analyzed for liver function test were done using conventional standardized methods and estimate serum malondialdehyde (MDA) by TBA method, ascorbic acid (2,4 – Dinitrophenyl hydrazine Method), uric acid levels (Uricase/PAP method) and CRP are analyzed by ELISA method in both groups in the Biochemistry lab. The chemicals and reagents used for the procedures were of analytical grade. These tests are carried out as part of routine laboratory services at the centre.

A total number of 40 subjects participated in present study. Twenty clinically diagnosed cases of alcoholic liver disease patients were selected. Detailed medical history and relevant clinical examinations data of these patients were collected. Twenty age and sex matched control subjects, who were healthy non-smokers at the time of study were selected. Alcoholic liver disease patients with infectious diseases, smokers were excluded from this study. Patients suffering from non-alcoholic liver disease, diabetes mellitus, hypertension, chronic inflammatory liver disease and smokers were excluded from the study. Patients suffering from psychiatric disease, pregnant women and children below 18 years were not included in the study.

Analytical methods

For malondialdehyde

The MDA was estimated by the standard method where the Serum lipid peroxide was mixed with Methyl Phenyl indole in acidified medium and after incubation for 48°C for 60 minutes, the resultant chromophore intensity was recorded at 586 nm [13].
For serum ascorbic acid: Ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4, DNPH to form the derivative bis-2, 4 dinitrophenylhydrazone. This compound, in strong sulfuric acid, undergoes rearrangement to form a product with an absorption that is measured at 520nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen [14].

Statistical analysis
The statistical analysis was carried out by using the SPSS (Statistical Package for Social Sciences) software. The Student't'- test was applied for the statistical analysis and the results were expressed in mean ± SD, P values (p <0.001) were considered as highly significant.

RESULTS & DISCUSSION
Among twenty alcoholic liver disease patients, the total bilirubin level in patients with alcoholic liver disease was 3.15± 0.48mg/dl while in the controls it was 0.642 ± 0.13 mg/dl. The direct bilirubin level in patients with alcoholic liver disease was 1.02±0.19 mg/dl. While in the controls it was 0.12±0.03 mg/dl. The levels of total bilirubin and the direct bilirubin were significantly increased in patients with alcoholic liver disease compared to controls (P<0.001). Hyperbilirubinemia is common and is accompanied by modest increases in the alkaline phosphatase level. Derangement in hepatocytes synthetic function indicates more serious disease.

Conjugated hyperbilirubinemia implied liver or biliary tract disease. Since the rate limiting step in bilirubin metabolism is not conjugation of bilirubin, rather transport of bilirubin into bile canaliculi. Thus elevation of conjugated fraction was seen in cirrhosis patients in comparison to controls. The total protein concentration in patients with alcoholic liver disease were 6.618 ± 0.11g/dl, while in the controls it was 7.9 ± 0.51 g/dl. The level of albumin in patients with Alcoholic liver disease was 3.75 ± 0.12 g/dl, while in the controls it were 4.83 ± 0.21 g/dl. The levels of total protein and albumin were significantly reduced in patients with Alcoholic liver disease compared to controls (P<0.001). Hypoalbuminemia and coagulopathy are common in advanced liver injury. An increase in the circulating polymorphonuclear cell number >5500/µL parallels the occurrence of the lobular infiltration of neutrophils observed in the florid lesion of alcoholic hepatitis. The results are shown in table I.
The observed hypoalbuminemia in the study group reflects severe liver damage and decreased albumin synthesis. Hypoalbuminemia was observed in cirrhotic & Alcoholic patients in comparison to controls.

Table I: Biochemical characteristics of alcoholic liver disease patients and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALD (n=20)</th>
<th>Control (n=20)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±S.D</td>
<td>Mean ±S.D</td>
<td></td>
</tr>
<tr>
<td>Total proteins (gm/dl)</td>
<td>6.74±0.15</td>
<td>7.5±0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>3.95±0.13</td>
<td>4.63±0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total serum bilirubin (mg/dl)</td>
<td>3.5 ± 0.38</td>
<td>0.65±0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>1.05± 0.21</td>
<td>0.13±0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>92.35±20.17</td>
<td>16.48±1.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>66.82±13.66</td>
<td>18.38±1.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST / ALT</td>
<td>1.39±0.31</td>
<td>0.89±0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>165.68±10.45</td>
<td>62.06±8.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>131.1±18.16</td>
<td>31.84±5.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vit C (mg/dl)</td>
<td>0.79± 0.05</td>
<td>1.37± 0.89</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>9.89± 0.892</td>
<td>2.63±0.739</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum uric acid (mg/dl)</td>
<td>6.12 ± 0.544</td>
<td>4.78 ± 1.337</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.2 ± 0.47</td>
<td>3.93 ± 0.588</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

The AST concentration in patients with alcoholic liver disease was 92.35±20.17IU/L, while in the controls it was 16.48±1.91IU/L. The level of ALT in patients with alcoholic liver disease was 66.82 ± 13.66 IU/L, while in the controls it was 18.38 ± 1.10 IU/L. The AST/ALT ratio in patients with alcoholic liver disease was 1.39 ± 0.31, while in the controls it was 0.89 ± 0.10. The levels of AST, ALT and AST / ALT ratio were significantly increased in patients with alcoholic liver disease compared to controls. (P < 0.001). The results are shown in table I.

The ALP level in patients with alcoholic liver disease was 165.68±10.45IU/L, while in the controls it was 62.06±8.04IU/L. The level of ALP was significantly increased in patients with alcoholic liver disease compared to controls (P<0.001). The GGT level in patients with alcoholic liver disease was 131.1 ± 18.16 IU/L, while in the controls it was 31.84±5.06 IU/L. The level of GGT was significantly increased in patients with alcoholic liver disease compared to controls (P<0.001). The results are shown in table I.

The activity of GGT is increased after chronic alcohol consumption. The determination of gamma-glutamyltransferase (GGT) activity in the serum is commonly used as a screening test for alcoholism, since striking elevations of serum GGT activities can
be observed in patients with a high alcohol intake over a prolonged period [15,16]. Enhanced serum enzyme activities are also found in patients with various stages of alcoholic liver disease including alcoholic fatty liver, alcoholic hepatitis, alcoholic liver fibrosis and cirrhosis. Since enzyme alterations in the serum are commonly observed even during the early stage of alcoholic liver disease, such as alcoholic fatty liver, the determination of GGT activity in the serum is a useful test for early recognition of alcoholism. Moreover, the assessment of the adult and fetal form of GGT in the serum facilitates a clear dissociation between early stages of alcoholic liver diseases, such as alcoholic fatty liver, and late stages such as alcoholic liver cirrhosis. Recent studies have suggested that the activity enhancement in the serum is primarily due to hepatic enzyme induction, rather than to liver cell injury, and can be ascribed to the action of ethanol itself but not to dietary imbalance with respect to carbohydrates.

The vitamin C & Serum uric acid levels in patients with alcoholic liver disease was 0.79 ±0.05 mg/dl & 6.12 ±0.544, while in controls it was 1.37±0.89 mg/dl & 4.78 ± 1.337. The levels of Vitamin C & uric acid were significantly reduced in patients with alcoholic liver disease compared to controls (P<0.05 & < 0.001).

The malondialdehyde levels in patients with alcoholic liver disease were 9.89± 0.892 nmol/ml, while in the controls it was 2.63±0.73nmol/ml. The level of malondialdehyde was significantly increased in patients with alcoholic liver disease compared to controls (P<0.001).

This study results have indicated that there was accumulation of oxidative stress markers with increasing alcohol intake resulting in further damage to liver as revealed by abnormal liver function tests. This study results were similar to previous research reports which suggested that there was an increased risk of liver injury with chronic alcohol intake [17,18]. Studies in the past have also revealed that there was an increase in chronic ethanol exposure [19,20]. It has been experimentally demonstrated in chronic ethanol fed rats that ethanol tends to increase lipid peroxidation and is responsible for elevated malondialdehyde (MDA) production [21].

Our study revealed that there was a rise in MDA levels associated with high alcohol consumption as observed by a previous studies [22]. Previous studies have demonstrated that
there was an association between reduced antioxidant levels and increased oxidative stress markers with increasing alcoholic consumption resulting in liver injury [23,24].

The current study results have also indicated that there was an added risk of liver injury with increased alcohol consumption as evident from the abnormal liver function tests. There was as a negative correlation of oxidative stress markers with levels of alcohol consumption and markers of liver injury.

In conclusion, it is evident from the results of this study and the existing literature that there was a compromise of antioxidant defense system with rise in oxidative stress markers and subsequent decrease in antioxidants levels between the two groups with alcohol consumption when compared to normal controls.

Elevated serum uric acid (UA) levels strongly reflect and may even cause oxidative stress, insulin resistance, and metabolic syndrome, which are risk factors for the progression of liver disease [25]. Urac acid (UA), the final oxidation product of purine metabolism involved in gouty arthritis and kidney stones genesis, has been found to be also associated with different cardiometabolic diseases, like hypertension, kidney disease, metabolic syndrome and cardiovascular disease[26,27]. In these settings, available data prompted to speculate that hyperuricemia is not only an epiphenomenon of metabolic alterations, but also a factor directly involved in the pathogenesis of the above cited disorders[28-33].

REFERENCES


