IMBALANCE OF OXIDANTS AND ANTIOXIDANT SYSTEMS IN SUBJECTS WITH CIRRHOSIS

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ABSTRACT: Oxidative stress has been increasingly implicated in the pathogenesis of cirrhosis. Ethanol and various viral infections will increase the production of reactive oxygen species (ROS) in the liver, resulting in an imbalance between oxidants and antioxidants. Thus determination of oxidants along with antioxidants, stated the role of oxidative stress more accurately in the pathogenesis of liver cirrhosis. In the present study we measured the markers of prooxidants, erythrocyte malondialdehyde (MDA), antioxidants that included erythrocyte catalase, reduced glutathione (GSH), glutathione peroxidase (GPx). 30 subjects with age 25-60 years, who were diagnosed as having liver cirrhosis by the department of Gastroenterology, Narayana Medical Hospital were included, 30 normal healthy individuals of the same age were selected as control. The results clearly indicated that the levels of pro oxidants, MDA were high in cirrhotic subjects than in the controls with p value of 0.0001. The levels of antioxidant enzymes GSH, Catalase were low in cirrhosis with p value of 0.0001 (GSH) and 0.067(Catalase). But the mean value of glutathione peroxidase was high in cirrhosis than in controls. This may be due to conterregulation with oxidative stress. Hence this study indicates the role of oxidative stress in liver cirrhosis and it clearly defines the imbalance between oxidants & antioxidants.

Key words: Antioxidant enzymes, Cirrhosis, lipid peroxidation.

Abbreviations: ROS (Reactive Oxygen Species) , GSH(Reduced Glutathione), GPx (Glutathione Peroxidase) , MDA (Malondialdehyde).

INTRODUCTION

Cirrhosis is a world wide health problem affecting 15-29% of the total population. It is the end stage of liver fibrosis characterized by nodule formation (Benyon, et.al;2001). Cirrhosis is commonly associated with abnormalities in the systemic circulation and impaired primary hemostasis (Violi F, et.al; 1994). Gastrointestinal & oesophageal variceal bleeding are the major causes of death in patients with cirrhosis. The Red Blood Cells(RBC) were found to be lysed at 20-30% level in the haemorrhagic blood of liver cirrhosis patients (Kelly DA, et.al; 1987). In general the oxidative stress and the red cell membrane integrity play important role in cell lysis. Metabolism of various endo and exogenous compounds and viruses generate reactive oxygen species (ROS), which are involved in the pathogenesis of liver diseases. ROS rapidly react with a variety of molecules and there by interfere with cellular function. Inadequate removal of ROS may cause cell damage by attacking membrane lipids, proteins and inactivate enzymes thus mediating several forms of tissue damage (Cengiz, et.al; 2005).
Oxidative stress has been implicated in pathophysiology of a large number of diseases or disorders which are initiated and exacerbated by prooxidants such as various drugs including alcohol & food additives. Ingested alcohol produces striking metabolic imbalances in the liver. It leads to the formation of reactive oxygen species which are capable of initiating lipid peroxidation & subsequently hepatocyte apoptosis and necrosis (Szuster, et.al; 2002). The role of erythrocyte as an oxygen carrying device repeatedly exposes it to the risk of oxidative injury. In addition, intermediate products of oxidative denaturation of haemoglobin(Hb), the hemichromes interact with superoxide & hydrogen peroxide to generate hydroxyl radicals and thus create another source of potential damage to the cell.

Increased amounts of met-haemoglobin, hemichromes & denatured Hb(Heinz bodies), decreased membrane free sulfhydryl groups, all have been reported as evidence of oxidative damage in RBC of cirrhotic patients (Chiu, et.al; 1989, Poli G, 2000). Many studies have proven the role of the oxidative stress in pathogenesis of liver disorders. Thus the main idea of this study is to study the role of imbalance between the prooxidants and antioxidants, which are responsible for tissue damage in patients with cirrhosis.

Materials and Methods:
The study was conducted over a period of one year, in association with the department of Gastroenterology, Narayana Medical College Hospital, Nellore, AP. Subjects admitted with liver cirrhosis in the Department of Gastroenterology were included as cases. 30 cirrhotic patients were selected for the study. We excluded the cases with malignancy, biliarytract disease, primary biliary cirrhois, autoimmune, metabolic and drug induced liver cirrhosis. In all the study cases liver cirrhosis was diagnosed according to laproscopy, with (or) without liver biopsy, all of them had evidence of abnormal liver function tests lasting for at least six months. 30 normal healthy individuals in the age group of 25-60 yrs working in Narayana Medical college were included as controls.

Exclusion criteria were concurrent use of antioxidant drugs; co-existing disease like Diabetes Mellites, Chronic Kidney Disease, gastrointestinal bleed (or) blood transfusion with in previous 2 weeks. Through a standard questionnaire, data on alcohol consumption, smoking, history of diabetes, Hypertension & past history of any hepatitis infection & family history of hepatitis were enquired. Informed written consent was taken from both the patients & controls and this study was approved by institutional ethics committee.

Blood samples were collected in EDTA tubes. Plasma was separated by centrifugation at 2500 rpm. Plasma was removed and erythrocytes were carefully sampled from the bottom of the tubes to minimize the contamination with leukocytes, washed 3 times with isotonic saline solution.RBC were used for estimation of Reduced Glutathione, Glutathione peroxidase, Catalase & MDA. Antioxidant enzymes were measured on the same day of collecting the sample.

Estimation of Reduced Glutathione
The content of glutathione in red blood cells was determined spectrophotometrically at 412 nm by the reaction with DTNB (Di Thio NitroBenzoic acid.) according to Owens and Belcher (Owen ,et.al; 1965). The GSH activity was measured in units μ mol /gHb.

The Hemoglobin content of each haemolysate was measured by Cyanohemoglobin method.
Estimation of Catalase in RBC
Sediments of erythrocytes were rinsed 3 times using 0.9% NaCl and were lysed in 3 volumes of cold, redistilled water and left in ice for 30 minutes. The samples were not centrifuged before Catalase was measured. The haemolysate was diluted 500 times with phosphate buffer (60nM), PH 7.4. Kinetic reaction was carried out in a cuvette mixed with 10 ml of Phosphate buffer, 20ul haemolysate and 1ml of H2O2 and the initial absorbance was measured immediately at wavelength of 240nm. The kinetic changes of absorbance were measured every 5 seconds,(0, 5, 10,15 secs) on spectrophotometer. The unit of catalase activity was expressed as K/ gm Hb (Abi H 1984).

MDA determination
By the method of Draper and Hadley, based on the reaction of MDA with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form pink colored pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2 – 3 at 95°C for 15min. After cooling the absorbance was read at 532nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxy propane). Results were expressed as nanomoles / gm of Hb (Draper H, et.al; 1990).

Estimation of Glutathione Peroxidase (GPx)
GPx was measured by paglia et al method ( Paglia, et.al ;1967). Results were expressed as U/gHb.

RESULTS
Data analysis was done using SPSS programme. Results were expressed as mean ± standard deviation. p value was used to compare the groups. p value < 0.05 was considered significant. Table 1 shows that mean values of erythrocyte antioxidant enzymes GSH, Catalase were decreased in patients with cirrhosis than in normal subjects. The mean values of GPx and MDA were high in cirrhosis. p value was significant for GSH (0.0001), GPx (0.023), &MDA (0.0001).

Table-1: Comparison of study variables in subjects with cirrhosis and Controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=30) Mean ± S.D</th>
<th>Controls (n=30) Mean ± S.D</th>
<th>‘t’ test</th>
<th>‘p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Glutathione μmol/gHb</td>
<td>1.87 ± 0.45</td>
<td>3.36 ± 0.72</td>
<td>9.61</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Glutathione peroxidase U/gHb</td>
<td>65.4 ± 5.31</td>
<td>61.2 ± 8.37</td>
<td>2.32</td>
<td>0.023’</td>
</tr>
<tr>
<td>Catalase K/gHb</td>
<td>37.1 ± 4.57</td>
<td>39.1 ± 3.71</td>
<td>1.86</td>
<td>0.067</td>
</tr>
<tr>
<td>Malondialdehyde nmol/gHb</td>
<td>360.3 ± 86.2</td>
<td>267.6 ± 43.4</td>
<td>5.2</td>
<td>0.0001’</td>
</tr>
</tbody>
</table>

n = number of subjects, p value < 0.05 significant,
DISCUSSION

Reactive oxygen species are oxygen containing molecules that are produced during normal metabolism. The organism has enzymatic & non enzymatic antioxidant systems neutralizing the harmful effects of the endogenous ROS products. Under certain conditions, the oxidative (or) antioxidative balance shifts towards the oxidative status as a result of increase in ROS and / impairment in antioxidant mechanism (Halliwell B 1994).

Oxidative stress is well recognized to be a key step in the pathogenesis of ethanol associated liver injury. Toxic substances generated during the metabolism of alcohol in the liver may contribute to the development of alcoholic liver disease (Nordmann R, et.al; 1992, Situnayake RD, et.al; 1990). Chronic hepatitis B virus infection also causes the hepato cellular damage by increasing oxidative stress, which are considered to be the important risk factors for the development of cirrhosis (Carmela, et.al; 2003 ). According to our study there is a large difference in the mean values of antioxidants GSH & catalase in erythrocytes of patients & controls. GPx & MDA were highly elevated in cirrhotic patients. Decreased antioxidants GSH & catalase clearly indicates that there is role of oxidative stress in the pathophysiology of liver diseases.

Malondialdehyde is a highly reactive compound that is not typically observed in pure form. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form advanced glycation end products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Moore, et.al; 1998 , Del Rio, et.al; 2005). (Subir Kumar Dase, et. al ; 2005), found patients suffering from liver disease either due to non alcohol (or ) excessive alcohol intake showed depletion of GSH levels. In consistent with this our study also proved the reduced levels of GSH in cirrhotic patients. The reduced levels of GSH may be explained on the basis of its utilization in scavenging the free radicals, Oxidation of glutathione to its oxidized form by GPx in detoxification of hydrogen peroxide, suppression of glutathione synthesis by ethanol (Varsha Desai, et.al; 1991).

The two antioxidant enzymes GPx & catalase, protect the RBC against peroxides that are generated intracellularly (or) exogenously. According to Geetha et. al; enzymatic antioxidants were low in cirrhotic subjects except GPx. The results of our study co-incide with these findings. The high GPx in the cirrhotic subjects is may be due to counter regulation with oxidative stress (Geetha A etal 2007). Catalase is a common enzyme found in nearly all living organisms, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. The decrease in erythrocyte catalase activity may be due to excessive generation of Oxygen (superoxide) leading to inactivation of the enzyme (Gaetani G, et.al; 1996).

Thus ours results indicated that patients with cirrhosis had lower levels of GSH, Catalase in RBC, MDA is high in cirrhotics. GPx though an antioxidant enzyme is slightly elevated due to its counter regulation with oxidative stress.

In Conclusion, the present study clearly demonstrates the elevated levels of GPx due to counter regulation with oxidative stress. Elevated MDA levels support increased lipid peroxidation. The decreased concentration of GSH & catalase support the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of cirrhosis.
REFERENCES