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Original Research Article

STUDY OF BIOCHEMICAL CHANGES, ANTIOXIDANT STATUS AND OXIDATIVE STRESS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

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ABSTRACT

Introduction: Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide**.** ALD is caused by drinking too much alcohol. A large proportion of drinkers developed fatty liver, alcoholic hepatitis and cirrhosis, the more serious types of alcoholic liver diseases due to drinking more than the recommended limits of alcohol over many years. The aim of this study was to find out the biochemical changes related to liver function, alteration in antioxidant status and oxidative stress in patient with alcoholic liver disease.

Materials and Methods: In the present study, total (n = 249) subjects were recruited from the Department of Medicine, SAIMS, Indore. 124 diagnosed cases of alcoholic liver disease, and 125 healthy control subjects. To observe the biochemical changes related to liver function measured by semi-auto analyzer. The levels of antioxidants such as SOD, CAT and oxidative stress marker; malondialdehyde (MDA) were estimated by spectrophotometric methods.

Results: The present study shown that the plasma MDA were significantly increased ($p<0.001$) and blood levels of SOD, CAT activity, and total protein were significantly decreased ($p<0.001$) in ALD as compared with group healthy controls, and also significant of biochemical parameters in study.

Conclusions: The results of this study suggested that drinking more than the recommended maximum amounts enhanced the development of ALD. Significant alteration in antioxidant levels and enhanced oxidative stress increase the severity of alcoholic liver disease.

KEY WORDS: SOD (Superoxide dismutase), CAT (Catalase), AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline phosphatase), GGT(Gamma Glutamyl transfererase), Malondialdehyde (MDA), Alcoholic liver disease (ALD), oxidative stress (OS).

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BACKGROUND

Alcoholism is a serious public health problem and major medical complications of alcohol abuse in worldwide. Excessive amount of alcohol results, liver damage, release of inflammatory cytokines, impaired oxidative stress, lipid peroxidation reaction, and also acetaldehyde toxicity and these can be causes inflammation of liver, apoptosis and finally fibrosis of liver cells [1]. Oxidative stress (OS) is the imbalance between generating free radicals and scavenging radical system i.e., increased production of free radical or reduced antioxidant defense activity. Oxidative stress is also the cause of development of liver disease [2].

Malondialdehyde (MDA**)** is produced by lipid peroxidation and also it is the key marker of oxidative stress [3]. Lipid peroxidation followed

by response of inflammatory, hepatic stellate cells activation can be leading to liver fibrosis, and finally the liver cirrhosis. Increased plasma MDA levels related to the excessive consumption of alcohol and also associated with pathogenesis and progression of liver disease. However, very few studies are measured the role of oxidative stress in pathogenesis of liver disease. Antioxidants are the compounds that prevent oxidation of lipids, proteins molecules and insufficient concentrations of antioxidants in the body cause damage of the cells [4]. High intake of alcohol in the study group has lower activity of blood SOD, CAT, and causes damage mitochondrial and ATP reduction, leading to alcoholic liver disease [5].

PATIENTS AND METHODS

The present study was carried out in the Department of Biochemistry, Sri Aurobindo Institute of Medical sciences (SAIMS) and P.G. Institute, Indore, M.P., India during March, 2016 to February, 2017. The patients includes in the study were consuming alcohol for 10-25 years & suffering from alcoholic liver disease Total 249 subjects were enrolled for the study, 124 ALD pateints (83 males and 41 females) diagnosed on the basis of history clinical symptoms were selected from study and age was between 35 to 70 years for both genders. According to the Paton, high alcohol intake group; those had been drinking > 80g alcohol per day for at least 5 year [6]. 125 normal healthy controls (79 males and 46 females) without any habit of alcohol drinking. The pateints suffering from diabetic, obesity, essential hypertension, thyroid disease, nephritic disease, pregnant women, cardiovascular associated liver disease, malignancy, asthma, gout and other infectious diseases were excluded from the study. The study was approved by the Institutional Ethical Committee and patients were recruited for the study after taking their written informed consent. A detailed physical examination was done which included measuring of height, weight and blood pressure.

Sample collection and analysis of biochemical parameters: Blood was collected after an overnight fasting in anticoagulation tubes. Plasma, serum and hemolysate were prepared and stored at -4°C. Blood samples transferred in EDTA tubes were centrifuged at 3000 rpm for 15 min and their plasma fractions were stored at -20°C to measure MDA levels. Activity of blood SOD, CAT, plasma glucose, and blood samples collected in plain tubes were used for the analysis of serum liver function tests and serum lipid profile. Estimation of blood Catalase activity done by Aebi (1984) method [7]. Determination of Superoxide Dismutase (SOD) by Marklund and Marklund (1974) method [8]. Plasma MDA was measured by spectrophotometric method at 531nm [9], and biochemical parameters done by semi- auto analyzer (Erba).

Statistical analysis: The statistical analysis was carried out by the SPSS statistics version 20.0. Values are presented as means ± standard deviation (Means ±SD). $p<0.05$ was considered as significant level.

RESULTS

Age distribution in study group and control group: The age distribution in alcoholic liver disease and healthy control subjects are listed in below table 1. Out of total (n = 249) subjects, were 124 of ALD patients, and were 125 of control. According to age distribution, 45 to 60 years age group subjects were in higher numbers as compare to other age groups.

Table 1: Age distribution of ALD cases and healthy controls.

Age (year)	ALD (n = 124)	Healthy controls (n = 125)
30 – 45	17	27
46- 60	73	61
61- 75	34	37
Total	**124**	**125**

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI) in ALD patients: The SBP, DBP BMI, in ALD patients and healthy control are shown in table 2. The BMI SBP and DBP were found markedly statistically significantly in ALD pateints as compared to healthy controls.

Table 2: SBP, DBP and MBI in ALD pateints compared with controls.

Parameters	ALD (n =124)	Healthy controls (n =125)	p value
SBP (mm/Hg)	122.70±9.86	112.68±6.88	p<0.001
DBP (mm/Hg)	78.75±6.07	73.49±6.76	p>0.001
BMI (kg/m^2)	20.57 ± 2.2	19.72 ± 1.38	p< 0.02

Data are presented as mean ± SD, $p<0.05$ was considered as significant level

Liver function test in ALD pateints compared with controls: The biochemical parameters-AST, ALT, ALP, GGT, total protein and total bilirubin, in ALD patients and healthy control are shown in table 3. The AST, ALT, ALP, GGT, and total bilirubin were found significantly increased and no significant difference of total protein in ALD pateints as compared to healthy controls.

Table 3: Liver function test in ALD pateints compared with controls.

Liver profile test	ALD (n =124)	Healthy controls (n = 125)	p value
SGPT (IU/L)	125.57±39.23	29.57±7.78	Significant
SGOT(IU/L)	118.74±47.74	28.76±7.13	< 0.001
GGT(IU/L)	105.67±32.66	22.08±10.37	< 0.001
ALP(IU/L)	193.67±54.24	93.52±26.32	< 0.001
T .P.(g/dl)	4.87±0.67	7.08±0.73	< 0.001
T.B. (mg/dl)	0.96±0.33	0.62±0.15	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

Lipid profile in ALD pateints compared with controls: Serum total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C in ALD pateints and healthy control are shown in Table 4. The serum total cholesterol, triglyceride, LDL-C and VLDL-C levels were found significantly increased and no significant difference of HDL-C ALD pateints as compared to healthy controls.

Table 4: Lipid profile in ALD with compared to controls.

Lipid Profile	ALD (n =124)	Healthy controls (n = 126)	p value
T.Chole (mg/dl)	258.52± 36.27	179.2 ±21.10	< 0.001
TG (mg/dl)	191.16 ±40.64	121.21±13.71	< 0.001
LDL-C (mg/dl)	178.53±35.97	114.31±17.94	< 0.001
HDL-C (mg/dl)	41.12±9.98	40.71±7.02	0.47
VLDL-C (mg/dl)	37.94 ±7.66	24.22 ±2.79	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

Table 5: Blood Activity of SOD, CAT and plasma MDA levels in ALD with pateints compared with controls.

Oxidative and antioxidant	ALD (n =124)	Healthy controls (n = 125)	p value
SOD (U/g of Hb)	2.80 ±1.04	5.6 ±1.12	< 0.001
Catalase (U/g of Hb)	3.56 ±0.87	6.88 ±1.08	< 0.001
MDA (µmol/L)	8.57 ±2.31	2.81±0.53	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

Activity of SOD, CAT and plasma MDA levels in ALD with pateints compared with controls: Blood activity of SOD, CAT and plasma MDA levels in ALD pateints and healthy control are shown in table 5. The plasma MDA levels significantly increased and blood activity of SOD and catalase significantly decreased in ALD patients as compare to controls.

DISCUSSION

The incident of ALD is increasing day by day in the developing countries specially, India. Chronic heavy drinking induces liver injury results ALD, even alcoholic liver cirrhosis. [10] BMI was markedly significantly increased in ALD pateints. Body mass index (BMI) is an independent predictor of fat infiltration of the liver, and it is used to screen for weight that may lead to problem of health [11].We have observed in the present study the significant increased level of plasma MDA (p<0.001) and significantly decrease antioxidant SOD and CAT (p<0.001) in alcoholic liver disease, our study documented by Gupta S et al., (2005) [12] found to significantly increased (p<0.001) level of plasma MDA in ALD in comparison to controls. Role of oxidative stress in the pathogenesis of chronic liver disease has been proposed by many authors using animal and human studies [13]. Nalini G et al., (1999) [14] have noted that a significantly increase in the level of plasma MDA in cirrhotic pateints. Excessive consumption of alcohol is associated with changes in cell function and the oxidant-antioxidant system. Capacity of reduced antioxidant has been found in liver disease and may promote the fee radical generation, lipid peroxide, and lipid peroxidation mediated by free radical is reasoned to damage of cell.

According to some previous studies; Chen YL et al (2011); Pujar S et al., (2011) [15, 16] have reported that the significantly decreased erythrocyte SOD (p<0.05), and catalase activity (p<0.05) in ALD pateints. The decreased antioxidant enzyme could be due increase in MDA which can cross link with the amino group of enzyme protein The elevated free radical and their metabolites decrease the plasma antioxidant status in ALD. Chari S et al (2003); Janani AV et al., (2010) [17, 18] demonstrated that the blood SOD activity were significantly lower (p<0.001) in alcoholic liver disease as compared to the controls.

We observed, ALD with heavy drinker had significantly low body weight, low BMI, compared

to control groups. Addolorato et al., [19] observed that alcoholics, as compared with social drinkers showed a lower body weight due to reduction of fat mass and other study, World et al., [20] shows that reduce body weight was the best clinical indicator of apparently of the alcohol abuse.

We observed, the increase levels of AST, ALT, ALP, GGT, and total bilirubin in alcoholic liver disease, our study compression of Das SK et al., (2005) [21] have shows that the significant levels of serum alanine aminotransfrase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and ã-glutamyltransferase (GGT) activities were observed in alcoholic liver in comparison to healthy controls. Levels of alkaline phosphatase are also helpful in identifying the cause and severity of liver damage. These findings indicate that, with the heavy dose of alcohol, subjects are facing oxidative stress.

In the present results, we observed, the lipid profile (TC, TG, and LDL, VLDL and HDL) were found to be statistically significant ($p<0.001$) and level of HDL was found to be not significant ($p<0.005$) in ALD pateints in comparison to the control group in both genders. Similar result documented by Boemeke L et al., (2015) [22] have found that markedly significantly increased ($p<0.001$) levels of total cholesterol (TC), triglyceride (TG), LDL-C and HDL-C in ALD. The role of liver in lipid and lipoprotein metabolism, therefore hypertriglyceridemia has been correlated with hepatocyte fat accumulation.

CONCLUSIONS

Consumption of alcohol is associated with a biochemical changes in liver and the oxidant-antioxidant system. Early monitoring of GGT, ALP, AST and ALT in detecting severity of alcohol induced liver damage. Increased lipid peroxidation and depletion of antioxidants could occur as a consequence of free radical generation due to alcohol consumption. There is need of some future studies which will focus on whether these effects will be reversed by antioxidant supplementation from abstinence alcohol or not.

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