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Biochemistry

Study of Correlation between Stress Marker Enzymes and Other Biochemical Parameters in Alcoholic Liver Disease

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Original Research Article

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Abstract: Alcohol related liver disease is a major cause of morbidity and mortality worldwide. Chronic alcohol consumption leads to hepatocellular injury, fat accumulation and liver inflammation. Progression of ALD is well characterized and is actually a spectrum of liver diseases, which ranges initially from simple steatosis, to inflammation and necrosis (steatohepatitis), to fibrosis and cirrhosis. The study was designed to determine effects of stress marker enzymes and other biochemical parameters in alcoholic liver disease patients with reference to the normal healthy individuals. 175 alcoholic liver disease patients were enrolled for the study & were compared to 150 normal healthy individuals of the same age. Those fulfilling inclusion & exclusion criteria were enrolled for the study & the blood samples were analysed for lipid profile, LFT, plasma MDA and SOD**.** Significant higher concentrations of MDA (P<0.001), GGT (P<0.001) and lower concentration of SOD (P<0.001) & Protein (p<0.001) was demonstrated in patients with alcoholic liver disease when compared with normal healthy individuals controls. The risk of alcoholic liver disease (ALD) increases in a dose & time dependent manner with consumption of alcohol.
Keywords: MDA, SOD, GGT, TG, HDL, ALD.

INTRODUCTION

According to the latest WHO data issued in May 2014 Liver disease related deaths in India reached 2.44% of overall deaths. Liver disease is responsible for more than 55% of deaths from alcohol abuse and the prevalence of Alcoholic liver disease remains correlated with the per capita alcohol consumption [1**]**. Liver disease includes a vast range of conditions that disturbs the normal functioning of the liver for example alcoholic liver disease, non-alcoholic fatty liver disease, liver cirrhosis, etc. Excessive alcohol consumption can lead to numerous forms of alcoholic liver diseases. Alcohol toxicity is ranked as the third most common cause of moribidity and mortality and accounts for 3.8% of deaths worldwide and 4.6% of disability adjusted life years cases (DALYs) [2]. Chronic alcohol consumption causes hepatocellular injury, fat accumulation, and liver inflammation and sometimes leads to liver cirrhosis or hepatocellular carcinoma. The pathogenesis of alcoholic liver disease (ALD) is a consequence of chronic alcohol consumption.

Progression of alcoholic liver disease (ALD) is well characterized and is actually a spectrum of liver diseases, which ranges initially from simple steatosis (fatty liver), to inflammation and necrosis (alcoholic steatohepatitis), fibrosis, and finally cirrhosis, and hepatocellular carcinoma (HCC) [3, 4**]**. The mechanisms involved in the development and progression of Alcoholic liver disease include alcohol and alcohol metabolism, dysregulated inflammation, oxidative stress and altered extracellular matrix (ECM) metabolism.

Ethanol is the most psychoactive substance. Chronic ethanol administration is able to induce Oxidative Stress (OS) [5**]** in liver and in the extra hepatic tissue that in turn is related with the imbalance between the pro oxidants and the antioxidant system [6**]** Oxidative stress is caused by excess ROS production, which leads to apoptosis and necrosis. ROS can also lead to a free radical chain reaction with unsaturated fatty acids generating toxic lipid intermediates. Alcoholics substitute up to 50% of their total daily

calories with alcohol, leading to nutritional deficiencies that can be further complicated by nutrient malabsorption [7**]**. The net effect is that alcoholics often have lower levels of key dietary antioxidant molecules [8**]** and an overall decreased antioxidant status. Therefore, oxidative stress in alcoholic liver disease is most likely triggered both by an increase in pro-oxidant production, as well as by a decline in antioxidant defences. Clearly, the most obvious pathologic changes to liver during alcohol exposure occur in the hepatocytes. Moreover, the accumulation of indices of oxidative stress (e.g., lipid peroxides) is primarily a hepatocellular event during alcohol consumption. There are also many potential sources of pro-oxidants; two major suspected sites in hepatocytes are the alcohol-inducible CYP2E1 and mitochondria. It is now clear that the reduction of O_2 to H_2O by the mitochondria is not complete and that 1%–2% of O_2 consumption by mitochondria leads to the formation of O_2^-. Alcohol exposure increases the yield of O_2^- from this cellular component in the liver. Therefore, it is likely that pro-oxidant production from this cellular compartment is key for the development of severe alcoholic liver disease. A key component in the growth of alcohol-induced liver injury is inflammation, involving both resident (e.g., Kupffer cells) and recruited (e.g., neutrophils and lymphocytes) inflammatory cells.

METHODOLGY

The present study was undertaken in the Department of Medical Biochemistry, MGM Medical College Indore (M.P.). The study group comprise of 175 histologically & ultrasound scan proven Alcoholic Liver Disease patients and 150 Healthy Individuals matched for age and dietary habits were treated as controls. The study subjects were randomly selected irrespective of age or occupational status. The age of subjects ranged between 20-70 years and the educational status ranged from illiteracy to post-graduation. Alcoholic liver disease patients selected for the study were, further confirmed by questionnaire, laboratory investigations and clinical findings. The patients suffering from renal disorders, diabetes mellitus, obesity, hypertension, thyroid disease, cardiovascular disease, viral hepatitis, hepatitis A & B, Asthma patients, Patients of lung cancer, any other malignancy, Gout, TB, HIV, Malnutrition, Malabsorption and or suffering from any other infectious diseases were excluded from the study.

Prior to taking out blood samples a detailed and accurate history, including Anthropometric measurements of the subjects was done. Anthropometric evaluation included measurement of BMI. Blood sampling was, performed in the morning, following a not less than 12 hr. fasting period. Biochemical parameters analysed were Plasma MDA, serum SOD, AST, ALT, GGT, Protein, Albumin, Bilirubin, Cholesterol, Triglyceride, HDL, LDL and VLDL. The Institutional Ethics Committee granted ethical approval. All the data was computed and analysed by using statistical packages for social science (SPSS) software version 20. Values are presented as Mean ± SD. $p<0.05$ is considered as Significant and $p<0.001$ is considered as highly significant.

RESULTS

The basic demographic details of the study subjects is shown in Table 1. Table 2 shows the lipid profile level between cases & controls. Table 3 shows Liver Function Test between cases and controls. Table 4 shows oxidative stress & antioxidant level between cases and controls.

Table-1: Baseline characteristics of controls and cases

S.no.	variables	Controls	Cases
1	Age (Years)	41.2±7.9	46.2±7.85
2	BMI (kg/m^2)	21.11±1.54	25.61±3.01

Table-2: Lipid Profile level between controls and cases

S.no.	Variables	Controls	Cases	p-Value
1	Total Cholesterol (mg/dL)	160.93±24.74	204.50±37.34	<0.001
2	Triglyceride (mg/dL)	98.14±26.06	132.03±45.81	<0.001
3	HDL (mg/dL)	52.5±10	43.93±9.46	<0.001
4	LDL (mg/dL)	99.92±20.51	137.52±33.04	<0.001
5	VLDL (mg/dL)	19.63±5.21	26.46±9.19	<0.001

Table-3: Liver Function Test between controls and cases

S.no.	Variables	Controls	Cases	p-Value
1	AST (IU/L)	25.57±6.27	97.28±57.20	<0.001
2	ALT (IU/L)	25.28±5.52	79.36±37.14	<0.001
3	GGT (IU/L)	26.54±8.23	180.25±37.49	<0.001
4	Bilirubin (mg/dl)	0.62±0.27	3.91±3.02	<0.001
5	Protein (mg/dl)	7.46±0.45	5.99±0.93	<0.001

Table-4: Oxidative Stress & Antioxidant level between controls and cases

S.no.	Variables	Controls	Cases	p-Value
1	MDA (nmol/ml)	1.8±0.75	5.71±2.95	<0.001
2	SOD (U/g of Hb)	6.83±1.26	4.06±1.38	<0.001

DISCUSSION

Alcohol-related liver disease is a major cause of morbidity and mortality worldwide. Chronic alcohol consumption leads to hepatocellular injury, fat accumulation, and liver inflammation and sometimes leads to liver cirrhosis or hepatocellular carcinoma. The pathogenesis of alcoholic liver disease (ALD) is a consequence of chronic alcohol consumption. The clinical syndrome of ALD carries a poor prognosis, such as liver cirrhosis [9**]** or hepatocellular carcinoma. The pathogenesis of ALD is uncertain, but the relevant factors include metabolism of alcohol to toxic products, oxidative stress, acetaldehyde adducts, abnormal methionine metabolism, malnutrition, the activation of endotoxin, and impaired hepatic regeneration [3**]**.

Results of present study indicates that there is an increase in plasma MDA level & decrease in SOD activity in alcoholic liver disease patients, the present finding could be in relation to increased exposure to oxidant environment that can destabilize RBC membrane by lipid peroxidation and cause significant leakage of these intracellular enzymes. There are evidences for plasma membrane becoming leaky owing to extensive damage by peroxidative attack that allows leakage of cytosolic enzymes from whole cells [11, 12. 13, 14]. Additionally increased alcohol consumption leads to poor dietary intake of antioxidant minerals adversely affecting the activity of these metalloenzymes.

In our present study significantly increased values of gamma glutamyltransferase, aspartate aminotransferase and alanine aminotransferase activities was observed in alcoholics in comparison to healthy controls (Table 3). Alanine amino transferase (ALT) and aspartate amino transferase (AST) are present in high concentration in hepatocytes. These enzymes leak into the circulation when hepatocytes or their cell membranes are damaged. In the present study, we also found hyperbilirubinemia and hypoproteinemia, which is in accordance with the study conducted by Das S.K. *et al*.

We have found that serum total cholesterol values were higher in alcoholic patients compared with the normal, healthy individuals. The serum LDL cholesterol and VLDL cholesterol & triglycerides levels are also significantly increased in alcoholics when compared with the control group. However, the level of HDL Cholesterol decreases in alcoholics as compared to the control groups. Heavy drinking puts more fat into the circulation in body, raising triglycerides level. Our results were similar with other studies like Varghese *et al*. [13] and Singh b *et al*.[14].

CONCLUSION

Alcohol consumption leads to liver diseases that may present with clinical and biochemical features, mainly impaired serum lipid profile. Increased serum lipids have been implicated in liver diseases. Monitoring GGT, AST and ALT in combination is a sensitive means of detecting severity of alcohol induced liver damage. Increased lipid peroxidation and depletion of antioxidant enzymes could occur as a consequence of free radical generation due to alcohol consumption. All these parameters in combinations may be useful indicator for identification and determination of severity of alcoholic liver diseases. We conclude that there is a need to educate people regarding the ill effects of alcohol on the liver. Hence, Government should take the necessary initiatives to ban such harmful practices in order to develop a healthier and prosperous society.

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