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RESEARCH ARTICLE

THE ROLE OF ANTIOXIDANT ENZYME GLUTATHIONE PEROXIDASE IN THE ETIOPATHOGENESIS OF ALCOHOLIC LIVER DISEASE

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ABSTRACT

Background & Objectives: Chronic alcohol consumption has long been associated with progressive liver disease from steatosis to inflammation, development of hepatic cirrhosis, and the subsequent increased risk of hepatocellular carcinoma. The present study focusses on alcohol induced oxidative stress associated with imbalance between oxidants and anti-oxidant defense system leading to molecular damage. **Materials & Methods:** In this case-control study, clinically diagnosed patients of Alcoholic liver disease (n=50) and age matched healthy controls (n=50) were enrolled.The levels of AST, ALT, AST/ALT ratio, ALP, serum protein, Malondialdehyde, Superoxide dismutase and Glutathione Peroxidase were estimated in both the groups and compared using Independent student's t-test. **Results:** The levels of AST, ALT, AST/ALT ratio, ALP, Malondialdehyde, Superoxide dismutase, Glutathione Peroxidase were significantly higher in cases as compared to controls whereas the levels of serum total proteins were significantly lower in cases as compared to controls. **Interpretation & Conclusion:** The study suggests that oxidative stress may be one of the contributing factor in the pathogenesis of ALD as indicated by increase in level of oxidants and decrease in level of antioxidant enzymes. Therefore, antioxidant supplement should be a part of the management of ALD as it would help in lowering the oxidative stress and the resulting peroxidation.

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INTRODUCTION

The pathogenesis of alcoholic liver disease (ALD) is a consequence of chronic alcohol abuse and approximately 44% of the 26,000 deaths from cirrhosis are due to ALD in the United States (Amini, 2010). Alcoholic hepatitis, the clinical presentation of ALD, remains to be a common life threatening cause of liver failure, especially when it is severe. Chronic alcohol consumption has long been associated with progressive liver disease from steatosis to inflammation, development of hepatic cirrhosis, and the subsequent increased risk of hepatocellular carcinoma. The spectrum of the ALD ranges from a simple steatosis to frank cirrhosis. The exact amount of alcohol consumption which places, a person at risk of developing ALD is not known but most of patients of ALD give history of drinking more than 100 gm per day of alcohol (Mandayam, 2004) which corresponds to 6-7 drinks per day. Alcohol consumption and consequently ALD is less prevalent in India in comparison to other countries, but in the last few years, the ALD was shown downward trend in the developed countries but the prevalence is increased in the developing countries including India (Basra, 2011).

ALD has four stages

- Alcoholic steatosis (this is reversible after the stopping of alcohol consumption)
- Acute alcoholic hepatitis
- Alcoholic cirrhosis
- End stage ALD (Ray, 2007)

The ingested alcohol in chronic alcoholics also alters various metabolic pathways inside the liver, which ultimately leads to the production of reactive oxygen species (ROS) (Liber, 1988). Inadequate removal of ROS may cause cell damage by attacking membrane lipids, proteins and inactivating enzymes thus mediating several forms of tissue damage. Liver is a major organ attacked by ROS (Sanchez-Valle, 2012). Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The mitochondrion, microsomes and peroxisomes in parenchymal cells can produce ROS. Moreover, Kupffer cells, hepatic stellate cells and endothelial cells are potentially more exposed or sensitive to oxidative stress-related molecules.

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A variety of cytokines like TNF-α can be produced in Kupffer cells induced by oxidative stress, which might increase inflammation and apoptosis. The oxidative stress not only triggers hepatic damage by inducing irreversible alteration of lipids, proteins and DNA contents but more importantly, also modulates pathways that control normal biological functions. Since these pathways regulate genes transcription, protein expression, cell apoptosis, and hepatic stellate cell activation; oxidative stress is regarded as one of the pathological mechanisms that results in initiation and progression of various liver diseases, such as chronic viral hepatitis, alcoholic liver diseases and non-alcoholic steatohepatitis (Feng, 2011; Singal, 2011). The protection of cells against damage from oxygen and its metabolites can be accomplished through enzymatic and nonenzymatic means. In our study, we estimated the levels of Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and catalase (CAT) and as they are considered to be the primary antioxidant enzymes, since they are involved in the direct elimination of reactive oxygen species.

Glutathione peroxidase is a cytoplasmic and mitochondrial enzyme important for detoxifying H_2O_2 in almost all the cells. Glutathione-s-transferase (GST) is glutathione dependent antioxidant enzymes, which catalyzes the reaction between the -SH group and potential alkylating agents. Glutathione is an effective antioxidant and important component of antioxidant network that protects our body against the effect of free radicals. Glutathione is small protein which is built with three amino acids cystine, glutamic acid, glycine and generally exist in reduced GSH and oxidized status (Balkrishnan, 2008). Superoxide dismutase (SOD) is a very important enzyme that functions as a cellular anti-oxidant. It has isoenzymes in different organelles as like copper-zinc SOD in cytoplasm, manganese SOD in mitochondria these help in maintaining a low concentration of superoxide anion(O_2^-). SOD catalyzes the dismutation of superoxide anion and the absence of this enzyme is lethal. The amount of superoxide dismutase is controlled by specific redox sensitive genes in cells (Stewart et al., 2002). ROS are highly reactive molecules that are naturally generated in small amount during the body metabolic reactions and can react with the cellular molecules such as lipid, protein or DNA (Balkrishnan, 2008). Malondialdehyde MDA is a reactive dialdehyde originating from the non-enzymatic lipid peroxidation of a variety of unsaturated fatty acids during phagocytosis by monocytes and from arachidonic acid catabolism in thrombocytes. MDA adducts have been found in the liver of alcohol consumers.

MATERIALS ANDMETHODS

The study was Hospital based cross-sectional study carried out in the Department of Biochemistry, SGT Medical College, Hospital & Research Institute, Gurugram, Haryana during 2015-2016. Patients aged >20years and <60years who reported to the outpatient department (OPD) of Medicine Department, SGT Hospital, Gurugram, were evaluated for Alcoholic liver disease. Fifty (50) patients diagnosed with alcohol liver disease fulfilling the inclusion criteria were enrolled for the study after obtaining written informed consent. Fifty (50) healthy age and socioeconomic matched controls were taken as controls.

Inclusion criteria: The cases were selected based on established and accepted clinical and biochemical criteria. All the cases were subjected to detailed history, clinical examination and laboratory investigations

Criteria for diagnosis of Alcoholic Liver Disease: Quantity and duration of alcohol intake are the most important risk factors involved in the development of alcoholic liver disease.

- History, physical examination and lab tests.
- Amount of alcohol intake > 100gm/day[2].
- Duration of alcohol intake more than 10 years.
- USG of whole abdomen.
- AST level is raised as compare to ALT (2-6 times).

Exclusion criteria

- Patients suffering from concomitant systemic disorders such as kidney disease, heart disease or endocrinal disorders.
- Patients on hormonal replacement therapy, oral pills or steroids.

METHODOLOGY

Group I: 50 patients with alcoholic liver disease
Group II: 50 healthy age matched controls

The Institutional Ethics Committee approved the study. Before their participation, the volunteers were fully informed about the nature and purpose of the study and written informed consent was obtained from each. The patients were subjected to detailed clinical examination and laboratory investigations. 6ml of venous blood after fasting for 12-14 hours was collected aseptically by venipuncture from the antecubital vein of the patients and the controls into plain vacutainers and the serum was separated by centrifuging the blood at 3000 rpm for 10 minutes and the serum samples were preserved at –20°C for subsequent analysis.

The following parameters were analyzed in serum by fully auto-analyzer EM-200(erba).

- AST (SGOT)
- ALT (SGPT)
- ALP
- Total protein

The following parameters were done by ELISA method by commercially provided kits by BIOVENDER R &D

- Glutathione peroxidase
- Superoxide dismutase (SOD)
- Malondialdehyde (MDA)

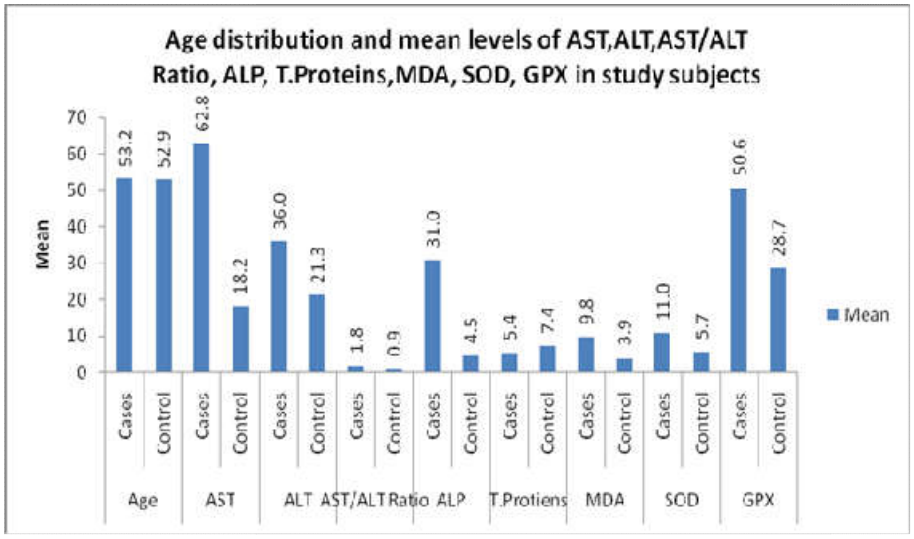
Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 24.0, for Windows (SPSS, Inc., Chicago).The quantitative data were expressed as Mean ± Standard Deviation (SD). Unpaired Student's t–test was used to compare the values (PCOS *vs* Controls) and Pearson correlation coefficient was used to elucidate the relationship between the variables. At a confidence interval of 95%, *p*-values<0.05 were considered statistically significant.

RESULTS

Demographic features: Total study subjects enrolled for the study were one hundred (50 cases and 50 controls).

Table 1: Age distribution and levels of AST, ALT,AST/ALT Ratio, ALP, Total Plasma Proteins, Malondialdehyde, Superoxide dismutase, Glutathione Peroxidase in study subjects

Study subjects		N	Mean	Std. Deviation	Std. Error Mean	p value
Age	Cases	50	53.2000	3.81725	.53984	
	Control	50	52.9200	2.73182	.38634	
AST(IU/L)	Cases	50	62.8400	6.90271	.97619	0.000
	Control	50	18.2400	3.88382	.54926	
ALT(IU/L)	Cases	50	35.9800	6.01186	.85021	0.000
	Control	50	21.3000	4.72186	.66777	
AST/ALT Ratio	Cases	50	1.7870	.31593	.04468	0.000
	Control	50	.8688	.15038	.02127	
ALP(IU/L)	Cases	50	30.9800	4.37288	.61842	0.000
	Control	50	4.5232	1.38840	.19635	
Total Proteins (gm/dl)	Cases	50	5.3608	.15529	.02196	0.000
	Control	50	7.3666	.15228	.02154	
Glutathione Peroxidase(U/gmHb)	Cases	50	50.6364	5.40398	.76424	0.000
	Control	50	28.6594	6.34343	.89710	
Malondialdehyde(mmol/L)	Cases	50	9.7970	.36982	.05230	0.000
	Control	50	3.8954	.45861	.06486	
Superoxide dismutase(U/gmHb)	Cases	50	11.0140	.58554	.08281	0.000
	Control	50	5.7048	.33309	.04711	



The mean age ± standard deviation of the cases was found to be 53.2 ± 3.81 and of the controls was 52.92 ± 2.73.The levels of AST,ALT,AST/ALT ratio, ALP, Malondialdehyde, Superoxide dismutase, Glutathione Peroxidase were significantly higher in cases as compared to controls whereas the levels of serum total proteins were significantly lower in cases as compared to controls.

DISCUSSION

Liver injury due to acute or chronic abuse has been proved dependent on metabolic products of ethanol viz. acetaldehyde and ROS account for the various functional derangements[11] accompanying alcohol abuse. These facts suggest that oxidative stress may be one of the contributing factor in the pathogenesis of ALD. Raised levels of serum transaminases observed in the present study may be due to increased permeability of cell membrane following the oxidative damage. Moreover, the ratio of ALT/AST was found to be reversed in ALD. Similar findings were reported by Ramesh Pradhan et al in 2014, who observed that serum aspartate amino transferase (AST) & alanine amino transferase (ALT) and their ratio was significantly ($p<0.01$) increased in ALD patients as compared to the controls. The reversal of ratio may be because of release of mitochondrial AST by alcohol itself or through its toxicity by its metabolites and/or oxidative stress.

In the present study there was significant increase in the levels of ALP in patients with ALD whereas the levels of proteins in ALD patients were decreased. In the present study, the erythrocyte antioxidant enzymes i.e. Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Malondialdehyde (MDA) activities have been increased significantly in ALD patients. Evidence is accumulating that intermediates of oxygen reduction may in fact be associated with the development of alcoholic liver disease. GPx is an oxidative stress inducible enzyme, which plays a significant role in the peroxyl scavenging mechanism & in maintaining functional integrity of cell membranes (Chandra, 2000). In 2016, Ashok Shinde et al. (2012) also reported that there was a significant increase in MDA (Malondialdehyde) in patients of ALD as compared to controls. The rise in the activity of GPx could be due to its induction to counter the effect of increased oxidative stress. GPx provides an effective protective mechanism against cellular injury, because it eliminates hydrogen peroxide and lipid peroxidation by reduction, utilizing reduced glutathione (GSH). Both increased and decreased SOD activities in the blood of alcoholics have been reported (Chen, 2011; Chari, 2003; Chari, 2003; Janani, 2010). The increase in erythrocyte SOD activity may probable be an adaptive response towards oxidative stress. Our finding regarding SOD activity is supported by the work of DM Vasudevan and Subirkumar Das. (2005). They found an increase in erythrocyte SOD activity.

The probable reason of increase in SOD activity in our study may be that, in alcohol induced oxidative stress; there may be up regulation of enzyme activity towards oxidative stress. Over expression of SOD activity may result in increased dismutation of superoxide to H_2O_2.The significant increase in MDA levels in alcoholics compared to controls suggests that alcoholics are subjected to more oxidative stress. In 2016, Ashok Shindeet al[15] also studied the oxidative stress and antioxidative status in patients with alcoholic liver disease and observed that there were a significant increase in MDA (Malondialdehyde) in cases as compared to controls. In 2010, Maithreyi et al. (2010) investigated that the erythrocyte lipid peroxidation and antioxidants in chronic alcoholics with alcoholic liver diseases. They included 30 male patients of alcoholic liver disease and Thirty healthy age matched controls in the study. They concluded that there was a statistically significant increase in the erythrocytes MDA levels in patients with alcoholic liver disease as compared to controls. The present study clearly demonstrates that due to alcohol induced oxidative stress the anti-oxidant defense system is compromised. It is reasonable to suggest that apart from the standard medical care for these patients, antioxidant supplement should be a part of the management. This will help in lowering the oxidative stress and the resulting per oxidation. It is expected that in future a more rational treatment plan for the poor victims of alcohol can be devised.

Conclusion

The present study supports the hypothesis that excessive alcohol intake increases the hepatic oxidative stress. Hence, it is not surprising that the antioxidants, as well as the GSH-precursors, are being explored as a supportive treatment or to prevent further deterioration in ALD. The use of the biological markers of antioxidant depletion (GSH) and hepatic damage (MDA), may be of help for the risk assessment in ALD. A better understanding of the pathophysiological mechanism and the correlation between the alcohol intake, the biological markers, and oxidative stress, is an important issue for the better management of ALD. However due to limited number of cases in our study, future study should be planned with more number of cases to substantiate the results, arrive at a definite conclusion and to throw more light on oxidative stress &antioxidative status in patients with ALD.

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