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LIPID PEROXIDE LEVELS AND ANTIOXIDANT STATUS IN ALCOHOLIC LIVER DISEASE

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ABSTRACT

The present study was conducted to evaluate some of the components of antioxidant defense system and oxidative damage in 20 male patients of alcoholic liver disease (ALD). The results were compared with 20 healthy male smokers and 20 healthy male non-smokers volunteers. Patients were subjected to detailed clinical examination and laboratory investigations. Blood samples were collected for estimating reduced glutathione (GSH), total thiols (T-SH) malondialdehyde (MDA), transaminases (AST, ALT), glutathione-S-transferease (GST) and gamma-glutamyl transferase (GGT). Serum aspartate amino transferase (AST)/alanine amino transferase (ALT) ratio was significantly ($p<0.01$) reduced in ALD patients as compared to the controls. However, the core of utility of MDA and GST was found to be significantly ($p<0.01$) increased in ALD patients compared to controls. There was a significant negative correlation of MDA with both GSH and T-SH. Plasma GGT levels were significantly ($p< 0.01$) increased in alcoholics and the enzyme showed a significant positive correlation with MDA. These results give enough evidence of increased oxidative stress and compromised antioxidant defense system in patients with ALD.

KEY WORDS

alcoholic liver disease; oxidative stress; antioxidant defense system.

INTRODUCTION

Alcoholic liver disease (ALD) is an alcohol induced disease with genetic, psycho-social and environmental factors influencing its development and manifestations (1). The disease is often progressive and is considered to be a major cause of morbidity and mortality (2). In recent years, oxidative stress has been implicated in the path physiology of a large number of disease or disorders which are initiated and /or exacerbated by pro-oxidants such as various drugs including alcohol and food additives (3). Besides, ingested alcohol produces striking metabolic imbalances in the liver (4, 5) it leads to the formation of reactive oxygen species (ROS) (6). Inadequate removal of ROS may cause cell damage by attacking membrane lipids, proteins and inactivating enzymes thus mediating several forms of tissue damage (7).

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At present, except for the abstinence of alcohol abuse, there is no effective modality of either prevention or treatment. The incidence of ALD is increasing day by day specially in the developing countries including India. The present study was planned with the objectives to investigate the oxidative damage and the efficiency of antioxidant defense system in patients of alcoholic liver disease in the socioeconomic belt of Rohtak, Haryana.

MATERIAL AND METHODS

The present study included patients residing in an around Rohtak city of Haryana state. The study group comprised of 20 male patients of alcoholic liver disease, having history of alcohol intake for more than five years with daily intake of 80-160 gm continuously. Twenty healthy male smoker (Bidis) volunteers, as well as 20 healthy non-smoker volunteers served as controls. They were all age-matched and had same socio-economic status. Patients suffering from disease of any origin other than alcohol intake were excluded from the study. Patients were subjected to detailed clinical examination and laboratory investigations. A score of 1 was given for, feeling the need to cut down intake, annoyed at the suggestion of

drinking, guilt of excessive drinking and a drink in the morning and total score of 2 or more suggested alcohol related problems.

Heparinised whole blood samples were collected for estimating biochemical parameters. Reduced Glutathione (GSH) was done by the method of Beutler et al (8) and total thiols were estimated according to the method of Ellman (9). Plasma malondialdehyde (MDA) was estimated by the method of Buege and Aust (10) and glutathione-S – transferase by the method of Habig et al (11). Enzyme gamma- glutamyl-transferase was estimated according to the method of Szasz (12).

RESULTS

Group 1 comprised 20 healthy non-smokers and Group II comprised 20 healthy smokers which were compared with the group III (study group) comprising of 20 alcoholics. The age distribution was found to be non significant ($p>0.05$) between all groups, the mean values being 45.15+4.74 (group I), 43.15+4.87 (group II) and 48.00+6.27 (group III). Tobaacco exposure was found to be same in groups II and III($p>0.05$). total leukocyte count, differential leukocyte count, erythrocyte sedimentation rate and hemoglobin (Hb) were carried out in alcoholics. The serum AST/ALT ration was significantly ($p<0.001$) increased in group III compared to group II (Table 1). Glutathione (GSH) levels were significantly ($p<0.001$) reduced in group II compared to group I and in group III when compared to group II. Total thiols and non-protein bound-thiols were found to be decreased at the same significance levels and had same pattern as GSH(T a b l e 1) . P l a s m a malondialdehyde (MDA) levels were significantly ($p<0.001$) decreased in group II when compared with group I and in group III when compared to group II (Table 1). Ration of GSH/MDA and NP-GSH/MDA showed significant ($p<0.001$) decreasing trend in group, starting from group I through group II and group III (Table 1). Glutathione-S-transferase levels were significantly ($p<0.001$)reduced in group III compared to group I and II (Table 1). Plasma-gamma- glutamy transferase (GGT) were found to be increased in group III significantly ($p<0.001$) compared to group I and II.

Ethical approval from the Post Graduate Institute of Medical Education. Rohtak ethical committee was attained before starting the present study.

STATISTICAL ANALYSIS

The data was analyzed using students unpaired't' test and the values were expressed as mean ± S.D. P-value less than 0.05 was considered as the significant value.

DISCUSSION

Free radical mediated damage to macromolecule plays

TABLE 1. BIOCHEMICAL ALTERATIONS IN HEALTHY NON-SMOKERS, HEALTHY SMOKERS AND ALCOHOLICS.

GROUPS	I Healthy non smokers (N=20)	II Healthy smokers (N=20)	III Alcoholics (N=20)
Serum AST (IU/L)	18.75±7.14	23±7.14	116.65±44.8*
Serum ALT (IU/L)	22.25±7.50	26.90±6.19	48.55±18.80*
AST/ALT	0.72±0.09	0.75±0.85	2.33±0.95*
GSH (m mol/L)	1.59±0.12	1.23±0.13	0.95±0.14*
T-SH (m mol/L)	6.01±0.72	4.83±0.41	3.39±2.4*
NON GSH (m mol/L)	4.64±0.71	3.62±0.38	2.43±0.17*
MDA (m mol/L)	3.48±0.63	5.22±0.62**	7.97±1.40*
GSH/MDA	0.40±0.09	0.23±0.05	0.12±0.14*
T-SH/MDA	1.67±0.48	0.93±0.02	0.42±0.13*
GST(IU/L)	3.70±2.0	4.95±1.02	34.47±11.8*1
GGT (IU/L)	11.2±4.40	15.3±4.57	81.8±12.4*

p Value : < 0.001
* Group II v/s Group III, **Group I v/s II
Values are expressed as ± S.D

a crucial role in the pathophsiology of atherosclerosis, inflammation, carcinogenesis, ageing, drug reaction and toxicity (13). Liver injury due to acute or chronic abuse has been proved to be dependent on its oxidative metabolism at the cytosolic, peroxisomal and /or microsomal level. It is not ethanol itself but rather its metabolic products such as acetaldehyde and ROS that accounts for the various functional derangements accompanying alcohol abuse(4). The induction of cytochrome P450 2E1' (CYP450 2E1) by ethanol leads to increasedgeneration of reactive oxygen species leading to the development of oxidative stress (15) which is also potentiated by redox shift associated with ethanol oxidation by alocohol dehydrogenase(16). Acetaldehyde, a major metabolic product of ethanol by either alcohol dehydrogenase (ADH) or CYP450 2E1 catalyzed oxidation, promotes oxidative stress not only via consumption and inactivation of antioxidants but also by increased generation of free radicals (17). These facts suggest that oxidative stress may be one of the contributing factor in the pathogenesis of ALD. The smoking pattern in alcoholic patients was similar to the pattern observed in healthy smokers. Thus tobacco smoke acts as an additional risk factor in development of ALD(18).

Raised levels of serum transaminases observed in the present study may be due to increased cell membrane permeability because of oxidative damage. Moreover, the ratio of AST/ALT used in discriminating alcoholic liver disease from other liver disorders(19), was found to be reversed in ALD. 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. The present study was conducted to test the oxidant/antioxidant that oxidative stress may be one of the contributing factor in the pathogenesis of ALD.

The smoking pattern in alcoholic patients was similar to the pattern observed in healthy smokers. Thus tobacco smoke acts as an additional risk factor in development of ALD (18). Raised levels of serum transaminases observed in the present study may be due to increased cell membrane permeability oxidative damage. Moreover, the ratio of AST/ALT used in discriminating alcoholic liver disease from other liver disorders (19), was found to be reversed. It was the reversal of ratio may be because of release of mitochondrial AST by alcohol itself or through its metabolites and/or oxidative stress.

The present study was conducted to test the oxidant/ antioxidant hypothesis in the pathogenesis of ALD. Reduced glutathione (GSH), a major intracellular non-enzymatic antioxidant and chief constituent of thiol pool was significantly reduced in alcoholics, which was in consistent with other reports (20-22). This observation may be explained on the basis of (i) its utilization in scavenging the free radicals, (ii) its involvement in maintaining non-GSH critical protein sulfhydryls in reduced state, (iii) acting as co-factor for GST during detoxification of xenobiotics including alcohol, (iv) oxidation of glutathione to its oxidized form by glutathione peroxides in detoxification of hydrogen peroxide and /or lipid peroxides (v) suppression of glutathione synthesis by ethanol (22,23). Greater degree of reduction in GSH in alcoholics may be because of synergistic action of smoking and alcoholism as smoke is reservoir of variety of oxidants (18). The levels of total thiols are deranged whenever there is oxidative stress. A strong significant positive correlation of GSH with total-SH was observed in the present study, which suggests that as the concentration of GSH goes on decreasing there is simultaneous decrease in total thiols in alcoholics. It suggests that it is not only GSH which is being used for detoxification of free radicals but protein thiols (Non-GSH) may also be taking part in maintaining the critical sulfhydryls proteins in the reduced form.

The significant increase in MDA levels in healthy smokers and alcoholics compared to healthy non smokers suggest that alcoholics and healthy smokers are subjected to more oxidative stress. Alcoholics seem to have still greater degree of oxidative stress which may be due to compounding effect of smoking. To highlight the antioxidant defense system as well as oxidative damage, it is reasonable to evaluate the status of ratios i.e. GSH/MDA and T-SH/MDA, a decreased ratio observed in the present study suggests that with increase in oxidative stress, there is corresponding proportionate decrease in antioxidant defense system. This fact was substantiated by negative correlation observed between MDA and T-SH as well as GSH. This reflects that antioxidant defense system is compromised with increased free radical generation during alcohol metabolism.

Hepatic damage due to alcohol resulted in release of glutathione-S-transferase from hepatocytes, hence increased levels were observed in the present study. The enzyme also showed a negative correlation with GSH and total thiols. This may by because of increase in the activity of GST due to either alcohol or alcohol induced oxidative stress. The GST showed a positive MDA levels which is because the enzyme is an oxidative stress inducible enzymes or alcohol itself may be responsible for its induction (21).The measurement of serum GGT levels is known as a sensitive marker of hepatobiliary disorders(25) and it has been reported to be induced by drugs including alcohol(26). As GGT is a membrane bound enzyme, oxidative stress induced damage to the membranes of hepatocytes seems to contribute to the increased activity of GGT as observed in the present study. This is substantiated by the observation of positive significant correlation of MDA

with the enzyme in alcoholics. Moreover, GGT showed negative correlation with GSH indicating that with a decrease in GSH concentration there is increase in the activity of GGT.

The present study clearly demonstrates the compromise in the AODS in the patients of ALD, which in turn is due to alcohol induced oxidative stress. It is reasonable to suggest that apart from the standard suggestive care for these patients, antioxidant supplement especially the thiol precursors (e.g. N-acetyl cysteine) should form part and parcel of the physician's prescription. However, it would be useful to evaluate other aspects of the AODS which were not explored in the present study, such as the antioxidant vitamins. It is expected that the future would witness a more rational treatment plan for the poor victims of alcohol.

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