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Serum Total Thiol Status in Alcohol Abusers

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Abstract: Thiol (-SH) groups are the major intracellular and extracellular reducing agents. This study estimates such thiol groups in alcohol abusers. Serum total thiols and liver function test parameters were estimated by spectrophotometric methods in alcohol abusers on admission (group I) and thirty days after alcohol abstinence along with life style modification (group II) and in non-alcoholic healthy controls. Serum amino transaminases, gamma glutamyl transpeptidase levels were increased and total thiols, total proteins and albumin levels were decreased in group I cases compared to group II cases and controls. Total thiols status improved significantly along with tranasaminases and transapeptidase with thirty days of alcohol abstinence and life style modification. In conclusion, total thiol status is decreased in alcohol abusers and abstinence of alcohol along with life style modification improves thiol antioxidant status and liver function.

Key words: Alcohol abusers, antioxidants, total thiols, liver function tests

INTRODUCTION

Hepatocytes synthesize albumin, which is a major plasma protein. The SH groups present on protein are considered as major antioxidants *in vivo* and most of them are present over albumin (Himmelfarb *et al*., 2000). The thiol groups are the major reducing groups present in our body fluids (Himmelfarb *et al*., 2001). Contribution of glutathione to total thiol status is minor; hence majority of total thiol status is contributed from albumin bound thiol groups (Hu, 1994). The levels of protein SH in the body indicate antioxidant status and low levels of protein SH correlated with increased levels of lipid hydroperoxides (Prakash *et al*., 2004) and Advanced Oxidation Protein Products (AOPP) (Himmelfarb *et al*., 2000).

Chronic consumption of alcohol causes fatty acids accumulation in hepatocytes and decreases its functional capacity (Lieber, 2000). Presence of free radicals and oxidative damage in alcoholism has been proved by several authors by measuring various oxidants and antioxidants in the body fluids (Albano, 2006; Lieber, 1997). The involvement of free radical mechanisms in the pathogenesis of alcoholic liver disease is demonstrated by the detection of lipid peroxidation markers in the liver and in the serum of patients with alcoholism, as well as by experiments in alcohol-feed rodents that showed a relationship between alcohol-induced oxidative stress and the development of liver pathology (Albano, 2006). Recent study by Lash indicated that there is depletion or oxidation of mitochondrial glutathione pool in alcoholic liver disease (Lash, 2006), but very few studies reported serum total thiol status in alcohol abusers associated with alcoholic liver disease. To our knowledge none of the previous studies reported the effect of alcohol abstinence on thiols status.

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In light of these data, the current study was designed to investigate, a) the levels of serum total thiols in alcohol abusers, b) the effect of alcohol abstinence on total thiol status and c) the relationship between total thiols, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Gamma Glutamyl Transferase (GGT) levels in alcohol abusers at the time of admission and thirtieth day after abstinence and in non-alcoholic healthy volunteers.

MATERIALS AND METHODS

Subject

The study was carried out on 50 alcohol abusers and 55 non-alcoholic healthy volunteers in the department of Biochemistry, Kasturba Medical College, Manipal, India in October 2006. Informed consent was taken from all subjects involved in the study and was approved by institutional review board. Alcohol abusers were recruited from AV Baliga Memorial Hospital, Udupi, India, who voluntarily attended the alcohol de-addiction camp conducted in the same hospital. They were consuming 70-90 g day^{-1} ethanol since 18±10 years before attending the camp. All alcohol abusers were admitted in the same hospital and were on oral benzodiazepines for seven days followed by 800 mg of Disulfiram on the first day then the dosage was gradually decreased to maintenance dose of 200 mg day^{-1}. They were given life style modification training including daily yoga, pranayama, meditation, prayers, moderate diet less in cholesterol and salt, energy 2400 kcal day^{-1}, individual counseling, group therapy, family counseling. They were trained for ten days in the hospital and were discharge on advice to continue it for themselves and report after thirty days. On reporting, history was taken and few of them were continued taking alcohol after discharge from the hospital and they were excluded from this study. The alcohol abusers were classified into two groups, group I-cases at the time of admission, group II-thirtieth day after admission. Healthy volunteers were non-alcoholic, non-smokers and free from any chronic inflammatory diseases and were not on any kind of medications. The other demographic and clinical characteristics are reported in Table 1.

Samples and Reagents

Under aseptic conditions blood samples (5 mL) were drawn into plain vacutainers, free of iron contamination, from antecubital veins of cases at the first medical examination and thirtieth day after abstinence and healthy volunteers. The collected blood was allowed to clot for 30 min and then centrifuged at 2000 g for 15 min for clear separation of serum. All assays were performed immediately after serum was separated. Special chemicals were obtained from Sigma chemicals, St Louis, MO, USA. All other reagents obtained were of analytical grade.

Methods

Total Thiols

Serum total thiols were measured by a spectrophotometric method using 5′ 5′ dithio-bis (2-nitrobenzoic acid) (DTNB) (Motchnik, 1994). Nine hundred microliter of 0.2 M Na_2HPO_4

Table 1: Demographic and clinical characteristics of alcohol abusers and non-alcoholic healthy controls (expressed in mean±SD)

Characteristics	Healthy controls	Alcohol abusers
Age (years)	30±6	39±8.7
Sex (Male)	55	50
Duration of ethanol intake (years)	-	18±7
Amount of ethanol (g day^{-1})	-	80±10
Disease	-	Alcoholic liver disease
Hypertension	-	1
Bipolar disorder	-	1
Alcohol related psychosis	-	1

containing 2 mM Na_2EDTA, 100 µL serum and 20 µL of 10 mM DTNB in 0.2 M Na_2HPO_4 were taken in an Eppendorf tube and warmed to 37°C. The solution was mixed in a vortex mixer and transferred to a cuvette and the absorbance was measured at the end of 5 min at 412 nm. Both sample and reagent blanks were prepared and absorbances were noted at 412 nm. The absorbance of sample and reagent blank were subtracted from serum absorbance values to obtain the corrected values. The calibration curve was produced using glutathione dissolved in Phosphate Buffered Saline (PBS). The total thiol concentration in serum was determined from the standard curve using the corrected absorbance values for serum.

Liver Function Tests

Serum transaminase [aspartate amino transferase (AST) and alanine amino transferase (ALT)] activities, γ-glutamyl transpeptidase (γ-GGT) activity, total protein and albumin levels were estimated by using automated analyser (Hitachi 911).

Statistical Analysis

The results were expressed as mean±standard deviation (SD). A $p<0.05$ was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-10, Chicago, USA). Analysis of variance (ANOVA) was used to calculate the mean values followed by multiple comparisons by post-hoc test to compare mean values in three groups. Pearson correlation was applied to correlate between the parameters.

RESULTS AND DISCUSSION

Serum total thiol levels were significantly decreased and transaminases and gamma glutamyl transpeptidase levels were significantly increased in group I cases compared to group II and healthy controls. There was significant increase in total thiols and significant decrease in transaminases and gamma glutamyl transpeptidase activities on alcohol abstinence for 30 days (group II cases) compared to alcohol abusers (group I cases) (Table 2). Protein thiols correlated positively with and albumin ($r = 0.430$, $p<0.01$). However, transaminases and gamma glutamyl transpeptidase did not correlate with total thiols.

Previous studies demonstrated the role of metallothionein and zinc in protecting liver against alcohol induced oxidative liver damage (Zhou *et al*., 2002b). The availability of thiol groups either as glutathione or protein thiols will reduce the powerful oxidants to protect biomolecules. Present study demonstrates decrease in total thiols (both glutathione and protein bound thiols) in alcohol abusers and such decrease in thiols in alcohol abusers may increase the oxidative injury to biomolecules. Presence of oxidative stress in alcohol abusers reported by other authors may be due to decrease in thiol antioxidant (Zhou *et al*., 2002a). Since albumin carries majority of thiol groups available over

Table 2: Total thiols and liver function test parameters in alcohol abusers before and after abstinence and in non-alcoholic healthy controls (expressed in mean±SD)

Parameters	Healthy controls (n = 55)	Alcohol abusers before abstinence (n = 50)	Alcohol abusers 30 days after abstinence (n = 50)
AST ($U\ L^{-1}$)	17±4	110±20*	25±6
ALT ($U\ L^{-1}$)	14±2	178±32*	21±3
GGT ($U\ L^{-1}$)	15±3	148±20*	24±6
Total thiols (µmoles L^{-1})	342±34	183±12*	310±20
Total protein (g dL^{-1})	7.5±0.8	6.0±0.6**	7.0±0.9
Albumin (g dL^{-1})	4.8±0.4	3.2±0.5**	3.7±0.8

*: $p<0.01$ compared to healthy controls and alcohol abusers after abstinence, **: $p<0.05$ compared to healthy controls

proteins, decrease in total proteins particularly albumin levels may decrease available thiol groups to fight against oxidants (Himmelfarb *et al*., 2000; Prakash *et al*., 2004). Thirty days of alcohol abstinence along with life style modification that included regular yoga and meditation significantly decreased serum levels of amino transferases, GGT and improved the levels of protein thiols.

Positive correlation of protein thiols with albumin indicates majority of thiols that contribute to antioxidant status resides on proteins, particularly albumin. Hence levels of albumin determine the total thiol status in the body. Baraona *et al*. (1977) reported alcohol induced protein accumulation in the liver. They reported that alcohol causes decreased export of synthesized proteins from hepatocytes which lead to decrease in circulating albumin. This may lead to decreased availability of thiols present over albumin for it antioxidant function. Fiorelli *et al*. (2002) reported increased consumption of reduced glutathione with increasing severity of alcoholic non-alcoholic liver cirrhosis. In this scenario decreased availability of thiols may increase the oxidative damage in alcohol abusers associated with alcohol induced liver disease. This study reports decreased total thiol status, which is a major antioxidant available in the body fluids, in alcohol abusers and supports the previously published studies reporting decreased antioxidants level in alcohol abusers. However, lack of correlation between total thiols and transaminases and gamma glutamyl transpeptidase requires further properly designed studies on large number of homogenous study group to know the role of thiol status in various liver diseases.

In conclusion, total thiols are decreased in alcohol abusers and abstinence from alcohol along with life style modification improves thiol antioxidants and liver function.

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