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Original Article

Biochemistry Section

Significance of the Glutathione-S-Transferase Activity and the Total Thiols Status in Chronic Alcoholics

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ABSTRACT

Objectives: The present study was conducted to assess the activity of glutathione -S-transferase (GST) and its relationship with total thiols (T-SH) in patients with alcohol liver disease (ALD).

Methods: Twenty male chronic alcoholics who had a history of alcohol abuse for more than five years were included in the present study and twenty healthy male volunteers who matched in age and socio-economic status, served as the controls. The plasma GST activity and the T-SH concentration were assayed.

Results: The activity of plasma GST was significantly higher in the chronic alcoholics as compared to that in the healthy controls. There was a significant decrease in the T-SH concentration and an increase in the aminotransferase (AST and ALT) and alkaline phosphatase (ALP) activities. Moreover, there was a significant negative correlation of the GST activity with the T-SH concentration ($r = 0.45$; $p< 0.001$).

Conclusions: The findings of the present study indicated that GST was a sensitive marker in the diagnosis of ALD. A significantly low concentration of T-SH was observed in the alcoholics as compared to that in the controls. Both these parameters (plasma GST activity and total thiols) showed a significantly negative correlation, indicating that there was an increase in the activity of GST with a decrease in the concentration of T-SH.

Key Words: Alcohol Liver Disease, Glutathione-S-transferase (GST), Thiols, Biochemistry

INTRODUCTION

The liver is the primary organ for alcohol metabolism and thus, it is an especially vulnerable organ to alcohol-related injury. Now- a-days the alcohol liver disease is a major cause of morbidity and mortality in India [1]. The amount and the type of alcohol which is ingested is the most important risk factor for the development of alcohol liver disease (ALD). The chronic consumption of alcohol causes fatty acid accumulation in the hepatocytes and it decreases their functional capacity. The alcohol induced formation of free radicals and the oxidative damage in alcoholism have been proved by several authors by measuring various oxidants and anti-oxidants in the body fluids [2, 3]. The involvement of free radical mechanism in the pathophysiology of ALD is demonstrated by lipid peroxidation markers in the liver, in the serum of patients with alcoholism and in alcohol fed rodents [2]. Hepatocytes synthesize albumin, which is a major plasma protein. The total thiol (T-SH) groups which are present on the plasma proteins, particularly on albumin, are considered to be major anti-oxidants in vivo [4]. The levels of protein -SH in the body indicate the antioxidant status and low levels of protein -SH have been shown to correlate with lipid hydroperoxides [5] and advanced oxidation protein productions (AOPP) [4].

Glutathione-S-transferases (GSH-s-trans; EC 2.5.1.18) are a family of enzymes which are present in the cytosol of most of the cells which play an important role in the process of the detoxification of a number of potentially harmful electrophilic compounds by conjugating them to glutathione. In addition to this, GST also shows glutathione peroxidase activity [6] and it is known for the detoxification of hydrogen peroxide (H_2O_2).The activity of GST can be induced by several chemicals/ drugs, alcohol being one of them. GST-alpha is found specifically at high concentrations in the human liver. It is released quickly in large quantities into the blood during hepatocellular damage. Because of the half-life of GST alpha in plasma is ~1hr, its concentration will follow the changes in the hepatocellular damage more rapidlythan aspartate amino transferase (AST) or alanine amino transferase (ALT) which have plasma half lives of ~17 hours and ~47 hours respectively [7].

Keeping the above facts in view, the current study was designed to investigate a) the levels of T-SH, b) the activity of GST in plasma and c) the relationship between T-SH and GST in alcohol abusers at the time of admission and in non-alcoholic healthy volunteers.

MATERIALS AND METHODS

The study was conducted on twenty chronic alcohol abusers and twenty non-alcoholic healthy volunteers in the Department of Biochemistry, in collaboration with the Department of Medicine, PGIMS, Rohtak (Haryana). The study group comprised of male patients of alcoholic liver disease who had a history of alcohol intake for more than five years, with a continued daily intake of 80-160 gm of alcohol. Patients who were suffering from jaundice, cirrhosis and chronic liver disease due to alcohol intake were only included in the study and others were excluded from it. The study was approved by the local ethical committee and before their participation, the patients and volunteers were fully informed about the nature and the purpose of the study. A written consent was obtained from each of them. The patients were subjected to a detailed clinical examination and laboratory investigations. Liver function tests such as total and conjugated bilirubin, total protein and albumin levels, along with the enzymes (AST and ALT) were evaluated by using standard methods.

Under aseptic conditions, blood samples were drawn into heparinized vacutainers which were free of iron contamination, from the antecubital veins of the patients and the healthy volunteers. The

plasma was immediately separated by centrifuging the samples at 1000 rpm for 15 minutes. The activity of plasma GST was estimated by the method of Habig et al (1974) [8]. The plasma total thiols (T-SH) were estimated according to the method which was described by Ellman (1959) [9]. All the reagents and the chemicals which were used in this study were obtained from Sigma Chemicals Co. USA.

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM. A P value of <0.05 was considered to be statistically significant. The Student's independent 't'-test was used to test whether the differences between the subject groups of the patients and the healthy individuals were significant. Linear regression analysis was used to obtain the correlation coefficient between GST and T-SH.

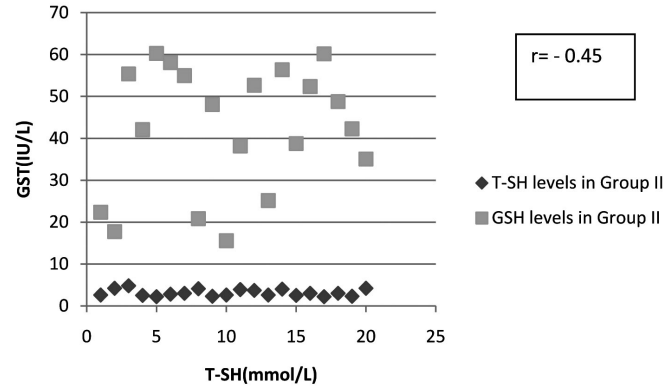
RESULTS

The [Table/Fig-1] shows the results of the present study. There was a significant increase in the activities of plasma AST, ALT, ALP and GST in the chronic alcoholics as compared to those in the healthy controls (p<0.001). The plasma total thiol levels were found to be significantly lower in the alcoholics as compared to those in the healthy controls (p<0.001). We observed that plasma GST correlated positively with serum AST and serum ALT (p<0.001) and negatively with serum total thiols (p<0.001). Moreover, the AST/ALT ratio was significantly increased in chronic alcoholics as compared to that in the controls (p<0.001).The levels of the total plasma proteins and albumin were significantly lower in patients with ALD as compared to those in the healthy controls (p<0.001).

Parameters	Healthy Controls (n=20)	Chronic Alcoholics (n=20)
Age (yrs.)	41.20 ± 5.24	45.75 ± 7.55*
Total Protein (g/dl)	7.14 ± 0.13	4.84 ± 0.12**
Albumin (g/dl)	4.46 ± 0.53	3.05 ± 0.32**
Total Bilirubin (mg/dl)	0.54 ± 0.31	2.40 ± 0.58**
Conjugated Bilirubin (mg/dl)	0.11 ± 0.13	0.90 ± 0.49**
Unconjugated Bilirubin (mg/dl)	0.42 ± 0.20	1.49 ± 0.21**
AST (IU/L)	20.5 ± 5.54	123.5 ± 53.82**
ALT (IU/L)	25.0 ± 5.64	49.05 ± 15.52**
AST/ALT	0.8 ± 0.20	2.65± 1.31**
ALP (IU/L)	54.45 ± 7.96	122.0 ±21.13**
GST (IU/L)	1.47± 0.53	42.24±14.96**
T-SH (mmol/L)	6.15± 0.69	3.13± 0.58**

[Table/Fig-1]: GST activity, Total thiols and liver function tests in chronic alcoholics and in healthy controls (expressed in mean ± SEM)

* p>0.05 (non-significant) compared to healthy controls and chronic alcoholics.
** p< 0.001 (highly significant) compared to healthy controls and chronic alcoholics.



[Table/Fig-2]: Scattergram showing correlation between glutathione-s-transferase (GST) and Total thiols (TSH) in group II.

DISCUSSION

Free radical mediated damage to macro-molecules plays a crucial role in the patho-physiology of a wide variety of diseases including atherosclerosis, inflammation, carcinogenesis, ageing, drug reaction and toxicity [10]. Liver injuries due to acute or chronic alcohol abuse have been proved to be dependent on its oxidative metabolism at the cytosolic, peroxisomal and /or the microsomal levels. It is not ethanol itself, but rather its metabolic products such as acetaldehyde and the reactive oxygen species (ROS), that account for the various functional derangements which accompany alcohol abuse [11]. The induction of cytochrome P450 2E1(CYP450 2E1) by ethanol leads to an increased generation of ROS, leading to the development of oxidative stress [12]. This is also potentiated by the redox shift which is associated with ethanol oxidation by alcohol dehydrogenase [13]. Acetaldehyde dehydrogenase is enhanced due to the inactivation of the anti-oxidants [14].These facts suggest that oxidative stress may be one of the contributing factors in the pathogenesis of ALD.

Elevated levels of alanine transaminase (ALT) and aspartate transaminase (AST) are regarded as indicators of liver damage and the isolated elevation of AST than ALT (with the AST/ALT ratio being >2) strongly suggests alcoholic liver disease [15]. Thus, the plasma levels of these enzymes have been routinely checked to assess the liver function. In our study, there was a significant increase (p<0.001) in the AST, ALT and the ALP levels in chronic alcoholics as compared to those in the healthy controls. This indicated the presence of alcohol-induced hepatocyte damage in chronic alcoholics. The ratio of AST/ALT in alcoholics as compared to the normal healthy controls was found to be significantly elevated. Moreover, the increase in unconjugated bilirubin in alcoholics also supported the fact that their liver functions are deranged. The derangement of the liver function tests suggested the damage of the hepatocytes.

Glutathione-S-transferase (GST), is involved in the binding, transport and detoxification of a wide variety of endogenous and exogenous toxic compounds. Moreover, it is an important ancillary enzyme of the anti-oxidant defence system. Ethanol is known as an inducer of the GST activity in the hepatocytes and the determination of this enzyme in humans has been suggested as a useful monitor of cellular induction [16]. We also have found significantly raised levels of GST in the chronic alcoholic patients. This could be due to the release of GST from the hepatocytes into the circulation following hepatic damage by oxidative stress, which was produced due to the generation of ROS during alcohol metabolism. It may indicate the active consumption of reduced thiols from the total thiol pool due to the increased GST activity in chronic alcoholics.

It has been well established that GST is primarily involved in the cellular detoxification processes and that the elevated, circulating GST activity is considered to be an early index of the increased load on the hepatocytes in detoxifying toxins. It is related to indicate increased presence of oxidative stress. Thiol groups are found in all the body cells and they are indispensable for life [17,18]. Thiols are extra-ordinarily efficient anti-oxidants which protect the cells against the consequences of the damage which is induced by free radicals, due to their ability to react with the latter [17,19]. Both the intra-cellular and the extra cellular redox states of thiols play a critical

role in the determination of the protein structure and function, the regulation of the enzymatic activity of the transcription factors and antioxidant protection [20]. In a recent study, it was calculated that the SH protein groups contributed a 52.9% load to the measured serum total antioxidant capacity in healthy subjects [21].

The plasma total thiols are considered to be the major components of anti-oxidant defence system [2] and they are reported to be decreased in general alcoholics [22-24]. In the present study, we have also demonstrated the decreased levels of T-SH in chronic alcoholics. Muttigi et al (2009) [25] have reported the increased GST activity and the decreased total thiols status in general alcoholics. The low levels of protein thiols which correlate negatively with the levels of AOPPs, a condition which is termed as 'thiol stress' may be contribute in to the pathogenesis of ALD. The activity of plasma GST correlated negatively with the concentration of T-SH. In alcoholics, thiols stress may be quantitatively important due to its reversible and treatable nature. Moreover, the reduced total thiols pool may have been caused due to the decreased concentration of albumin, as was observed in the present study. Albumin is known to be synthesized in the liver and damage to the hepatocytes might be responsible for the decrease in the albumin concentration. Albumin is the main contributing factor to the total T-SH pool of plasma.

CONCLUSIONS

The strong negative correlation of GST with the T-SH levels indicated that as the concentration of T-SH decreased, there was a corresponding increase in the activity of GST. This may be because of the increased in the alcohol induced oxidative stress and the increased utilization of T-SH from the thiols. Our study confirmed the observations of earlier studies, but with a different patient population in a different geographical location.

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REFERENCES

[1] Gupta S, Panday R, Katyal R, Aggarwa HKI, Aggarwal RP, Aggarwal SK. The lipid peroxide and antioxidant status in alcoholic liver diseases. *Indian J Clin Biochem*. 2005; 20: 67-71.
[2] Albano E. Alcohol, oxidative stress and free radical damage. *Proc. Nutr. Soc.*, 2006; 65: 278-90.
[3] Lieber CS. Role of oxidative stress and antioxidant therapy in alcoholic and non-alcoholic liver diseases. *Adv. Pharmacol.*, 1997; 38: 601-28.
[4] Himmelfarb J, McMonagle E, McManamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney Int*. 2000; 58: 2571-78.
[5] Prakash M, Upadhya S, Prabhu R. Protein thiols oxidation and lipid peroxidation in patients with uremia. *Scand J Clin Lab Invest*. 2004; 64:599– 604.
[6] Beckett GJ, Hayes JD. Glutathione S-transferases: biomedical applications. *Adv Clin Chem*. 1993; 30: 282-380.
[7] Adachi Y, Horii K, Takahashi Y, Tonihata M. Serum glutathione S-transferase activity in liver disease. *Clin Chem Acta*. 1980; 106: 243-55.
[8] Habig WH, Pubst MJ, Jakoby WB. Glutathione S-transferase, the first enzymatic step in mecrapturic acid formation. *J Biochem*. 1974; 249: 7130-9.
[9] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959; 82: 70-7.
[10] Jose MM, Javer FP, Florence C, Susana C, Antonia, C. S-adenosyl methionine in alcoholic liver cirrhosis: A randomized, placebo controlled, double- blind, multi-centre clinical trial. *J Hepatol*.1999; 30:1081-89.
[11] Hirnwich HE, Nahum LH, Pakieten N, Fazekas JF, Bots H DU. Effects of alcohol on the metabolism. *Am J Physiol*. 1982; 101: 57- 68.
[12] Morimotto M, Hagbjork Al, Nariji AA. Role of cytochrome P450 2EI in the pathogenesis of alcoholic liver disease. Alcohol.1993;10: 459-64.
[13] Nordmann R, Ribiere C, Rouach H. Implication of free radical mechanisms in ethanol included cellular injury. *Free Radical Biol. Med*.1992; 12: 219-40.
[14] Peters TJ, Ward RJ. Role of acetaldehyde in the pathogenesis of alcohol liver disease. *Mol. Aspects Med*.1998; 10: 179-90.
[15] Sorbi D, Boynton J, LIndor KD. The ratio of aspartae aminotransferse to alaine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol.* 1999; 94, 1018-22.
[16] Loguercio V, Degirolamo A, Cuomo F, Argenzio C, Iannotta D, Disalvo A, et al. Determination of Plasma α-Glutathione-S- Transferase in chronic alcohol abusers: Relationship with alcohol intake and liver involvement. *Alcohol Alcoholism*.1998; 33:366 -72.
[17] Amacat G. Anti-oxidant effects of sulfur-containing amino acids. *Yonsei Med J*.2004; 45: 776-88.
[18] Moriarty Craige SE, Jones D. Extracellular thiols and the thiol/ disulfide redox in metabolism. *Annu Rev Nutr*. 2004; 24: 481-509.
[19] Sen CK. Redox signaling and the emerging therapeutic potential of the thiol anti-oxidants. *Biochem Pharmacol*. 1998; 55: 1747-58.
[20] Wlodek L. Beneficial and harmful effects of thiols. *Pol J Pharmacol*. 2002; 54: 215 - 23.
[21] Erel O. A novel automated direct measurement method for the total antioxidant capacity with the use of a new generation, more stable, ABTS radical cation. *Clin Biochem*. 2004; 37: 277-8.
[22] Prakash M, Shetty JK, Tripathy S, Verma M, Saddinamane V, Bhandary PV. Serum total thiol status in alcohol abusers. *Asian J Biochem*. 2008; 3: 48-51.
[23] Prakash M, Shetty JK, Tripathy S, Verma M, Saddinamane V, Bhandary PV. Serum paraoxonase in alcohol abusers which was associated with alcoholic liver disease. *Clin Chim Acta*. 2000 ; 378: 232- 34.
[24] Lash LH. Mitochondrial glutathione transport: Physiological, pathological and toxicological implications. *Chem Biol Interact*. 2006 ; 163: 54- 67.
[25] Muttigi MS, Prabhu LS, Kedage V, Prakash M, Shetty JK, Devaramane V, et al. Glutathione-S-Transferase activity and total thiol status in chronic alcohol abusers before and 30 days after alcohol abstinence. *Online J Health Allied Scs*. 2009; 8: 1-5.

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