

**Original Article**

**Glutathione S-Transferase activity and total thiol status in chronic alcohol abusers before and 30 days after alcohol abstinence**

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## Abstract:

**Background:** Glutathione S Transferase (GST) has been involved in detoxification process in the liver and its activity has been shown to be increased in alcohol abusers. In the current work we measured the GST activity, total thiol status, AST, ALT, and direct bilirubin in chronic alcohol abusers before and 30 days after alcohol abstinence and lifestyle modification.

**Methods:** Serum and urine GST activity and total thiol status were determined using spectrophotometric methods and serum transaminases were determined using clinical chemistry analyzer. **Results:** We found, significant increase in serum and urine GST ( $p < 0.001$ ), AST ( $p < 0.001$ ), ALT ( $p < 0.001$ ), and decrease in total thiol status ( $p < 0.001$ ) in chronic alcohol abusers. GST activity significantly decreased ( $p < 0.001$ ) and total thiol status were improved significantly ( $p < 0.001$ ) 30 days after alcohol abstinence and lifestyle modification. **Conclusion:** This study provides preliminary data to suggest the role of GST as prognostic indicator of alcohol abstinence with possible trend towards an improvement in liver function.

**Key Words:** Glutathione S Transferase; Total thiols; Alcoholic liver disease; Alcohol abstinence

## Introduction:

Liver plays a key role in many metabolic processes and one of the important functions being detoxification of drugs, chemicals and toxic substances. Detoxification process occurs in two phases. Phase 1 involves the use of cytochrome P-450 system. Phase 2 reactions generally limit further biotransformation by enhancing elimination, which often involves addition of a large polar molecule like reduced glutathione, to primary metabolites, thereby leading to detoxification. Thus phase 2 enzymes are believed to have antioxidant and anticarcinogenic potential.(1) Reactive oxygen species (ROS) are generated during alcohol metabolism as a result of the generation of both NADH from the dehydrogenase and NADPH from the metabolism by cytochrome P-450 2E1. In addition, ROS are generated by alcohol related cell damage, which suggests that ROS are central to alcohol related liver damage.(2) There is considerable evidence in implicating ROS and their products in the pathogenesis of alcoholic liver disease (ALD).(3,4) Increased presence of ROS and oxidative damage to cells in alcoholism has been proved by several authors by measuring various oxidants and antioxidants in the body fluids.(4,5)

Mammalian cells express a number of enzyme systems to detoxify ROS and their by-products, including superoxide dismutase, glutathione peroxidase, glutathione S-transferase (GST) and catalase. GST is a cytoplasmic class of large family of enzymes with their maximal activity seen in the hepatocytes. GST's are believed to exert a critical role in cellular protection against ROS. Within the hepatocytes, GST's are involved in conjugating reduced glutathione to electrophiles, hydroperoxides and xenobiotics derived from the metabolism of ethanol, drugs and other toxins.(6) The total thiol status in the body, especially thiol (-SH) groups present on protein, are considered as major plasma antioxidants *in vivo* and most of them are present over albumin and are major reducing groups present in our body fluids.(7,8)

The objectives of the current study were to 1) determine the levels of GST both in serum and urine along with the total thiols in chronic alcohol abusers, 2) establish relationship between GST and total thiols in this patient population, 3) know the effect of thirty days of alcohol

abstinence on the levels of GST and total thiols, both in serum and urine sample compared to healthy controls.

## Material and Methods:

### Subjects and samples

The study was carried out in the department of biochemistry, Kasturba Medical College, Manipal, India. Alcohol abusers were recruited from Dr AV Baliga Memorial Hospital, Psychiatry and alcohol de-addiction unit, Udupi, India, who voluntarily attended the alcohol de-addiction camp conducted in the same hospital. Informed consent was taken from all subjects involved in the study and study was approved by the institutional ethics committee. Fifty two alcohol abusers were admitted on the first day of de-addiction camp. They were consuming 50-100 g ethanol per day since 15±8 years before attending the camp. All alcohol abusers were put on oral benzodiazepines and 250 mg of disulfiram twice daily for five days followed by maintenance dose of 250 mg per day.

All the subjects involved in the study were given lifestyle modification training including daily yoga, pranayama, meditation, prayers, moderate diet less in cholesterol and salt, energy of 2400 kcal per day, individual counseling, group therapy and family counseling. They were trained for ten days in the hospital and were discharged on advice to continue it for themselves and report after thirty days. On reporting, history was taken, and the cases not adhering to the study criteria during their home stay were excluded from the study (21 cases). The alcohol abusers were classified into 2 groups: pre abstinence (group I)—cases at the time of admission, post abstinence (group II) — cases 30 days after alcohol abstinence and life style modifications. Healthy volunteers were non-alcoholic, non-smokers and free from any chronic inflammatory diseases and were not on any kind of medications. Under aseptic conditions blood was drawn into plain vacutainers from controls and alcohol abusers, allowed to clot for 30 min, and then centrifuged at 2000×g for 15 min for separation of serum. All assays were performed immediately after serum was separated.

### Reagents

Special chemicals like GSH, 1-chloro 2,4-dinitrobenzene (CDNB), 5' 5' dithio-bis (2-nitrobenzoic acid) (DTNB)

were obtained from Sigma chemicals, St Louis, MO, USA. All other chemicals were of analytical grade.

### Biochemical determinations

#### Serum and urine GST assay

One mL reaction mixture containing 850  $\mu$ L of 0.1 M Phosphate buffer pH 6.5, 50  $\mu$ L CDNB 20 mM, 50  $\mu$ L 20 mM GSH, was preincubated at 37<sup>o</sup> C for 10 min. Reaction was started by adding 50  $\mu$ L serum or urine. GST activities were assayed kinetically by noting changes in absorbance at every 1 min interval for 5 min at 340 nm. Serum and urine GST activity was determined by using molar extinction coefficient 9.6 mM<sup>-1</sup> cm<sup>-1</sup> (9-11) and was expressed in IU.

#### Serum and urine total thiol assay

100  $\mu$ L serum or urine was added to reaction mixture containing 900  $\mu$ L 2 mM Na<sub>2</sub>EDTA in 0.2 M Na<sub>2</sub>HPO<sub>4</sub>, 20  $\mu$ L 10 mM DTNB in 0.2 M Na<sub>2</sub>HPO<sub>4</sub>, incubated at room temperature for 5 min and absorbance was read at 412 nm. Similarly absorbance of sample blank and reagent blank was subtracted from serum and urine absorbance values to obtain corrected values. The calibration curve was produced using GSH dissolved in phosphate buffered saline (PBS). Total thiol levels were determined using molar extinction coefficient 1600 M<sup>-1</sup> Cm<sup>-1</sup>. (12)

#### Liver Function Tests

Serum aspartate transaminase (AST) and alanine transaminase (ALT) (13,14) and direct bilirubin (15) were

determined using clinical chemistry analyzer (Hitachi 912).

#### Statistical Analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS-16, Chicago, USA). The results were expressed as mean $\pm$ standard deviation (SD). A p-value <0.05 was considered statistically significant. One-way analysis of variance (ANOVA) was used to compare mean values in three groups, followed by multiple comparison *post hoc* tests. Pearson's correlation was applied to correlate between the parameters.

#### Results:

As depicted in Table 1, serum GST, AST, ALT and urine GST levels were significantly increased in pre abstinence cases compared to post abstinence and healthy controls (p<0.001). Serum total thiols (p<0.001), and urine total thiols significantly decreased in pre abstinence cases when compared to post abstinence (p<0.001), and healthy controls (p<0.05). There was no significant difference in levels of serum GST and thiols, and urine GST and thiols in post abstinence cases compared to healthy controls, but there was a significant difference in the activities of AST (p<0.05) and ALT (p<0.001) compared to healthy controls. On applying Pearson correlation, serum GST activity correlated positively with urine GST (r=0.856, p<0.001) (Fig 1), AST (r=0.922, p<0.001), and ALT (r=0.855, p<0.001), and negatively with serum total thiols (r= -0.611, p<0.001) and urine total thiols (r= -0.452, p<0.001). Further, urine GST correlated positively with serum AST (r=0.904, p<0.001) and ALT (r=0.855, p<0.001), and negatively with serum total thiols (r= -0.612, p<0.001) and urine total thiols (r= -0.437, p<0.001).

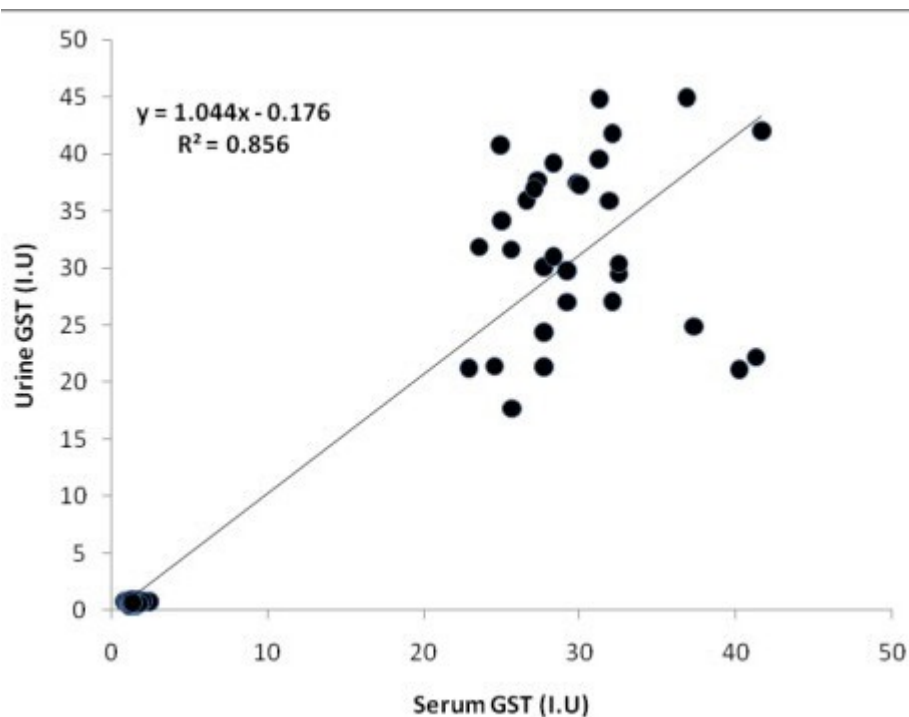
**Table 1: Demographic and biochemical parameters in chronic alcohol abusers pre-abstinence (group I) and 30 days after alcohol abstinence (group II), and in healthy controls (expressed in mean  $\pm$  SD).**

	Healthy controls (n=30)	Chronic alcohol abusers	
		Pre-abstinence (Group I) Cases (n=31)	Post- abstinence (Group II) Cases (n=31)
Age (years)	42 $\pm$ 14	49 $\pm$ 15	49 $\pm$ 15
Sex (M/F)	27/3	30/1	30/1
AST (U/L)	18.21 $\pm$ 3.89	71.98 $\pm$ 12.47*	24.85 $\pm$ 4.03 <sup>#</sup>
ALT (U/L)	15.09 $\pm$ 3.94	52.98 $\pm$ 10.83*	22.79 $\pm$ 3.06 <sup>#</sup>
Direct bilirubin ( $\mu$ M)	5.98 $\pm$ 2.9	2.22 $\pm$ 1.53*	5.13 $\pm$ 1.53 <sup>#</sup>
Serum GST (IU)	1.18 $\pm$ 0.54	30.05 $\pm$ 5.01*	1.54 $\pm$ 0.32 <sup>#</sup>
Urine GST (IU)	1.22 $\pm$ 0.24	31.98 $\pm$ 7.76*	0.65 $\pm$ 0.13 <sup>#</sup>
Serum total thiols ( $\mu$ M)	371.66 $\pm$ 58.20	282.05 $\pm$ 15.99*	386 $\pm$ 77.48 <sup>#</sup>
Urine total thiols( $\mu$ M)	20.2 $\pm$ 11.68	12.61 $\pm$ 2.92**	26.09 $\pm$ 11.72 <sup>#</sup>

\*p< 0.001 compared to healthy controls.

\*\*p< 0.05 compared to healthy controls.

<sup>#</sup>p< 0.001 compared to group I.



**Figure 1: Correlation between serum GST and urine GST in alcohol abusers before and after abstinence**

### Discussion

Ethanol is capable of inducing GST activity in the hepatocytes and the determination of these enzymes in humans has been suggested as a useful monitor of cellular induction.(6) We have measured serum and urinary GST activity in chronic alcohol abusers before and 30 days after alcohol abstinence. In our study, Serum GST activity was significantly increased in alcohol abusers associated with ALD. Serum GST activity correlated positively with elevated liver enzymes, indicating induction of GST synthesis by chronic ingestion of alcohol and its release to blood due to the damage to hepatocytes. Levels of GST were decreased significantly to normal range just within 30 days of abstinence from alcohol, whereas the levels of other liver enzymes like AST and ALT were still slightly higher than normal. Significant negative correlation of serum GST with serum thiols may indicate the active consumption of reduced thiols from total thiol pool with increased GST activity in chronic alcohol abusers before abstinence.

Interestingly, urine GST levels were found to be increased in alcohol abusers before abstinence, possibly due to increased presence of GST in serum and its excretion in urine, and urine GST levels decreased following 30 days of alcohol abstinence along with decrease in the levels of serum GST. It has been shown by previous authors that serum GST activities will increase after an acute ethanol load in approximately 40% of chronic alcohol abusers, and it has been

suggested that individuals who display this response may go on to develop cirrhosis.(16) Early recovery of serum and urine GST on alcohol abstinence denotes its prognostic indication in patients with alcohol abstinence.

In our study, we have observed that the levels of serum and urine total thiols in alcohol abusers were significantly decreased when compared to healthy controls, indicating enhanced oxidative stress. We speculate that the decrease in total thiols in the urine of chronic alcohol abusers when compared to healthy controls could be because of increased oxidation of thiol groups in the serum due to the existing oxidative stress. Further, we have found significant improvement in the levels of serum and urine total thiols after alcohol abstinence and lifestyle modification. Improvement in total thiol levels correlated negatively with improvement in the levels of serum and urine GST activity.

In conclusion, chronic alcohol intake possibly induces GST which detoxifies ROS generated by consuming available total thiol pool in the body. Abstinence from alcohol for just 30 days along with lifestyle modifications will improve the levels of available total thiol pool and decreases GST activity. Further more, this study may provide the preliminary data to suggest the role of GST as prognostic indicator of alcohol abstinence with possible trend towards an improvement in liver function.

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