

Circulatory Glutathione s transferases estimation in chronic alcoholics vising Urban and Rural health center.

ABSTRACT:

Introduction: Excessive alcohol consumption is a global healthcare problem with enormous social, economic, and clinical consequences, accounting for 3.3 million deaths in 2012. Glutathione (GSH) is tri-peptide thiol with chemical name γ glutamyl-cysteinyl-glycine the properties of glutathione are conferred to it by highly reactive thiol present in one of its constituent amino acids- cysteine, hence they referred as GSH. Xenobiotics form thioether (-S) linkage with GSH. The reaction is catalysed by enzyme known as glutathione S Transferases (GSTs). The cytoplasmic GSTs are important in the xenobiotic metabolism and are present in higher concentration in liver.

Material and Method: The present study was conducted in the Dept. of Biochemistry in collaboration with Dept. of General Medicine at Datta Meghe Medical College, Nagpur. In present study includes 40 diagnosed alcoholic liver disease patients and 40 non-alcoholic healthy subjects as control group who are permanent nt of study area.

Result: The level of GST was raised in chronic alcoholic patients i.e. study group (43.25 ± 15.94) as compare to control group (1.57 ± 0.55). At the other hand the level of total thiol were decreased in study group (3.12 ± 0.55) as compare to control group.

Conclusions: The strong negative association between glutathione-s-transferase (GST) and total thiol (T-SH) levels suggested that as the concentration of total thiol (T-SH) decreased, glutathione-s-transferase activity increased (GST). . This may be attributed to an increase in alcohol-induced oxidative stress and increased T-SH utilization from thiols.

Key words: Glutathione-S- transferase, alcoholic liver disease, total thiols, UHTC & RHTC

INTRODUCTION:

Alcoholism is a major public health issue with far-reaching consequences for not only individual health and social status, but also for society. Long-term alcohol intake is detrimental to almost every organ in the body, with metabolic pathway dysfunction, reactive oxygen species production and oxidative stress, and impairment/induction of detoxifying enzymes all contributing to these effects.¹

The key molecular mechanism involved in the pathogenesis of chronic alcoholism problems is considered to be oxidative stress.^{1,2}

In recent days, the alcoholic liver disease is a significant cause of morbidity and mortality; it is highly susceptible to alcohol-related injury.³

The most important risk factor for the development of **alcoholic liver disease** is the type and brand of alcohol consumed (ALD). Chronic alcoholism contributes to accumulation of fatty acid in hepatocytes, which reduces functional ability. Several researchers have demonstrated that the production of free radicals and oxidative damage in alcoholism by testing different type of oxidants and antioxidants.^{4,5}

Albumin, a major plasma protein, is formed by hepatocytes. *In vivo*, the *T-SH* present on plasma proteins, especially albumin is important antioxidants⁶ osmotic, transport and nutritive functions.

Protein -SH levels in the body suggest antioxidant status, and low protein -SH levels have been linked to lipid hydroperoxides and advanced oxidation protein output (AOPP).^{7,6}

Several chemicals/drugs can trigger GST activity, with alcohol being one of them. In the human liver, GST-alpha is present in extremely high concentrations. During hepatocellular injury, it is rapidly released in large amounts into the bloodstream. GST alpha can respond to changes in hepatocellular damage faster than aspartate amino transferase (AST) or alanine amino transferase (ALT), which have plasma half-lives of 17 hours and 47 hours, respectively. ^{5,8}

Considering the above, the current research was designed to look into

- a) T-SH levels in alcoholics
- b) GST activity in plasma in alcoholics

MATERIAL AND METHOD

The Present study was conducted at Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur in the Dept. of Biochemistry. Study includes 40 alcohol abusers and 40 non-alcoholic age sex matched healthy subjects.

This study involved chronic alcoholic patients who had a long term history of alcohol abuse and a daily alcohol intake of 80-160 gm.

The research study approved by the institutional ethics committee, and the purpose of study informed to patients and volunteers prior to participating. Each of them signed a written consent form. The subjects showed a rigorous clinical evaluation as well as laboratory testing.

Inclusion Criteria:

- Alcohol abusers aged between 30 to 55 years
- Alcohol intake for at least 3 years
- Severe drinker (alcohol addicts)

Exclusion Criteria:

- Aged Less than 30 year and More than 55 years
- Occasional Drinkers
- Acute or Chronic liver patients
- Patients suffering from HIV, HBsAg, MTB, DM and other chronic diseases.

SAMPLE COLLECTION:

5 mL overnight fasting blood samples were drawn under aseptic conditions into heparinized and simple vacutainers free of iron contamination. The plasma was separated right away by centrifuging the samples for 10 minutes at 3000 rpm. The separated plasma were labeled and kept in refrigerator till analysis.

BIOCHEMICAL ANALYSIS:

- Total protein was estimated by biuret method.
- Serum albumin was estimated by bromocresol green (BCG) binding method.
- AST/SGOT and ALT/SGPT was estimated by international federation of chemistry (IFCC) Kinetic method.
- Glutathione-S- transferase and Total Thiol (T-SH) was estimated by colorimetric method.

RESULT:

Table no: `1 Concentration of Biochemical parameters in study group and normal subjects

Parameter	Healthy subject (40) MEAN±SD	alcoholics subject (40) MEAN±SD	P-Value
Age (Yrs)	40.19±5.20	46.15±7.50	P = 0.0001
Serum protein (gm/dl)	7.16±0.14	4.50±0.10	P < 0.0001
Serum Albumin (gm/dl)	4.10±0.45	3.00±0.30	P < 0.0001
Total Bilirubin (mg/dl)	0.53±0.30	2.50±0.55	P < 0.0001
Conjugated Bilirubin (mg/dl)	0.10±0.12	0.92±0.48	P < 0.0001
Unconjugated Bilirubin (mg/dl)	0.43±0.19	1.50±0.20	P < 0.0001
AST/ SGOT (IU/L)	21.6±5.56	126±56.85	P < 0.0001

ALT/ SGPT (IU/L)	26.0±6.64	50.03±16.53	P < 0.0001
GST (IU/L)	1.57±0.55	43.25±15.94	P < 0.0001
Total thiol (T-SH) mmol/l	6.16±0.68	3.12±0.55	P < 0.0001

The [Table1] shows the activities of serum AST/SGOT, ALT/SGPT, and GST were found significantly high in chronic alcoholics ($p < 0.001$). The plasma total thiol levels in alcoholics were found to be significantly lower than those in healthy controls ($p < 0.001$). The levels of total plasma proteins and albumin in patients with alcoholic liver disease (ALD) were significantly lower than in healthy controls ($p < 0.001$).

DISCUSSION:

It has been discovered that oxidative metabolism at the cytosolic, peroxisomal, and/or microsomal levels is needed for liver injuries caused by acute or chronic alcohol abuse. The multiple functional derangements associated with alcohol consumption are caused by metabolic products such as acetaldehyde and reactive oxygen species (ROS), not by ethanol itself.^{5,9}

Free radical-mediated macromolecular damage is significant in the pathophysiology of many diseases, including atherosclerosis, inflammation, carcinogenesis, ageing, drug reaction, and toxicity.^{5, 10}

Ethanol induces the enzyme cytochrome P450 2E1 (CYP450 2E1), which contributes to an increase in the production of reactive oxygen species (ROS) and the creation of oxidative stress.¹¹ Elevated levels of ALT and AST are considered markers of liver damage, and a higher AST than ALT (AST/ALT ratio > 2) strongly predict alcoholic abusers.^{5, 12}

The present study highlight that chronic alcoholics have lower levels of total protein, albumin, and thiols (T-SH) i.e. 4.50 ± 0.10 , 3.00 ± 0.30 and 3.12 ± 0.55 respectively as compared to control group 7.16 ± 0.14 , 4.10 ± 0.45 and 6.16 ± 0.68 respectively.

Muttigi et al.¹³ In general alcoholics, increased GST activity and decreased total thiols status have been reported in their study. Low levels of protein thiols, which are negatively associated with Advanced oxidation protein products (AOPPs) levels, a condition known as 'thiol stress,' may play a role in the pathogenesis of ALD.

In general long-term alcoholics, increased GST activity and decreased total thiols status have been documented. Low levels of protein thiols, which are negatively associated with AOPP levels, a condition known as 'thiol stress,' may play a role in the pathogenesis

of ALD. In chronic alcoholics, this suggested the existence of alcohol-induced hepatocyte damage. The abnormal liver function tests indicated that the hepatocytes had been impaired.

Glutathione-S-transferase (GST) is involved in the binding, transport, and detoxification of a few toxic compounds, both endogenous and exogenous. Furthermore, it is an effective antioxidant defense system ancillary enzyme. Ethanol is a recognized inducer of GST activity in hepatocytes, and testing this enzyme in humans has been proposed as a useful measure of cellular induction.^{5,14}

We also discovered that chronic alcoholic patients had significantly higher levels of GST than healthy control subjects. This may be attributed to the release of GST from hepatocytes into the bloodstream after hepatic damage caused by oxidative stress, which was caused by the development of reactive oxygen species (ROS) throughout alcohol metabolism. It may mean that chronic alcoholics are deliberately consuming reduced thiols from the total thiol pool as a result increased GST activity.

Mamta Singh et al In addition, total protein albumin and total thiols (T-SH) were found to be lower in chronic alcoholic patients relative to healthy controls, while serum AST/SGOT, ALT/SGPT, and glutathione-s-transferase (GST) were found to be higher in chronic alcoholic patients ($p < 0.001$).¹⁵

Thiols' intracellular and extracellular redox states are essential in the determination of protein structure and function, as well as the regulation of transcription factor enzymatic activity and antioxidant defence.¹⁶

CONCLUSION:

The strong negative association between glutathione-s-transferase (GST) and total thiol (T-SH) levels suggested that as the concentration of total thiol (T-SH) decreased, glutathione-s-transferase activity increased (GST). This may be attributed to an increase in alcohol-induced oxidative stress and increased T-SH utilization from thiols. Our results corroborated those of previous research, but with a different patient population in a different area.

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