

Serum GST Activity and Total Thiols Status in Patients with Liver Disease Secondary to Various Disorders

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Abstract: *Introduction:* There have been several reports on levels of GST and thiol status in hepatocellular damage. Very few comparative studies are available to know the levels of GST and thiols in various disorders causing hepatocellular damage. Current study was undertaken to know serum GST activity and total thiols status in patients with various disorders causing liver damage.

Subjects and methods: Study consisted of 95 patients with various disorders affecting liver leading to hepatocellular damage and 53 healthy controls not on any kind of medication or any therapeutic regimen. Serum GST activity and total thiols were measured using spectrophotometric methods, and standard liver function test was done using clinical chemistry analyzer Hitachi 912.

Results: The serum GST activity in patients with acute viral hepatitis, cirrhosis with portal hypertension, leptospirosis, left ventricular failure and falciparum malaria did not differ markedly, however, there was significant increase in serum GST activity ($p < 0.001$) in patients with sepsis and multi organ failure. Total thiols were significantly decreased ($p < 0.001$) in all these patients.

Discussion: With exception of sepsis with multi-organ failure, no statistically significant increase in serum GST activity was found in hepatocellular damage caused secondarily to various diseases. Low levels of total thiols in these patients indirectly indicate increased presence of oxidative stress.

Keywords: Liver disease, acute viral hepatitis, sepsis, GST, total thiols, cirrhosis with portal hypertension.

INTRODUCTION

There have been several reports on levels of Glutathione S-transferase (GST) and thiol status in hepatocellular damage [1]. Very few comparative studies are available to know the levels of serum GST and total thiols in various disorders causing hepatocellular damage. Hepatic disease is characterized by hyperbilirubinemia, is regarded as one of the most important cellular events accounting for the adverse effects of inflammation and toxins [2]. Etiologically hepatocellular damage is complex and multifactorial in origin [3, 4]. A series of interconnected biochemical changes initiated by inflammation and toxins have been documented to directly affect the cellular function, including an aberration in the processing of bilirubin by hepatocytes leading to hyperbilirubinemia [2].

The main risk factors associated with hepatocellular damage are infection with viruses such as hepatitis B and C viruses [4], parasites such as plasmodium falciparum [5], and leptospira [6], and toxin such as alcohol [7]. The hepatocellular damage caused by all these risk factors involves an element of oxidative damage to hepatocytes by free radical generation [7, 8]. Free radical mediated damage to biological membrane increases the permeability permeability of the cell

membrane resulting in release of low molecular weight reduced glutathione (GSH), GST and other cytosolic constituents into the blood stream [7].

GST (EC. 2.5.1.18) is a family of phase II xenobiotic detoxifying enzymes, catalyses the conjugation of GSH thiolate anion to electrophiles, including the secondary products of lipid peroxidation [9]. Apart from detoxification of xenobiotics, GST also binds to unconjugated bilirubin in cytosol and transports to endoplasmic reticulum for conjugation with glucuronic acid thereby prevent its efflux from the cell [10]. Several authors suggested that, GST is a sensitive and specific biomarker of cell permeability in various etiologies leading to inflammation of hepatocytes [11, 12]. An alpha-GST is found to be at high concentrations in the human liver and is released in response to hepatocellular damage. GST has been found to be unaffected by muscle damage, extra-hepatic inflammation, and hemolysis, and is therefore presumed to be more specific than transaminases [13].

Reduced thiols (-SH) are the major antioxidants in the cell and the source for such reduced thiols are GSH and protein bound thiols. Both GSH and protein bound thiols contributes maximum to the total thiols pool in the cell [14]. Low levels of total thiols pool have been shown to be associated with various disorders with increased generation of free radicals [1, 15]. Decreased levels of protein thiols were found to be associated with alcoholic liver disease [16, 17]. The current study was primarily undertaken to know the levels of GST and total thiols status in various disorders

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associated with hepatocellular damage. The secondary objective of this study was to know the extent of GST and total thiols pool variation in these disorders.

SUBJECTS AND METHODS

The study group consisted of 95 subjects with various disorders affecting liver and 53 healthy controls. The patients were recruited from medical out patient department and emergency wards before starting specific treatment for the disease condition. The various liver disorders that were affecting liver was acute viral hepatitis (n = 14), cirrhosis with portal hypertension (n = 33), leptospirosis (n = 14), sepsis and multi organ failure (n = 15), left ventricular failure (n = 9), and falciparum malaria (n = 10). Liver disease was diagnosed based on clinical evidence, radiography, and laboratory investigations. The healthy controls were not on any kind of prescribed medication or dietary restrictions. The demographic and other biochemical data are depicted in Table 1 and 2. Informed consent was taken from all subjects involved in the study. The current study was approved by institutional ethical committee for human research.

Blood samples (5 mL) were drawn into plain vacutainers from the antecubital veins of healthy controls and patients.

The blood was allowed to clot for 30 min and centrifuged at 2000g for 15 min for clear separation of serum. All the assays were performed immediately after the clear separation of serum.

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 GST Assay

One mL reaction mixture containing 850 μ L of 0.1 M Phosphate buffer pH 6.5, 50 μ L of CDNB 20 mM, 50 μ L of 20 mM GSH were preincubated at 37 °C for 10 min. Reaction was started by adding 50 μ L of serum. GST activities were assayed kinetically by noting changes in absorbance at every 1 minute interval for 5 minute at 340 nm. Serum GST activity was determined by using molar extinction coefficient 9.6 mM⁻¹ cm⁻¹ [18] and was expressed in IU.

Table 1. Results of Standard Liver Function Tests of Healthy Controls and Patients with Liver Diseases (Expressed as Mean \pm SD)

	Healthy Controls (n=53)	Acute Viral Hepatitis (n=14)	Cirrhosis with Portal Hypertension (n=33)	Leptospirosis (n=14)	Sepsis (n=15)	Left Ventricular Failure (n=9)	Falciparum Malaria (n=10)
Total bilirubin (mg/dL)	0.80 \pm 0.28	8.22 \pm 6.01**	4.64 \pm 4.19**	3.62 \pm 3.2	2.10 \pm 0.69	1.57 \pm 1.18	3.88 \pm 1.8
Direct bilirubin (mg/dL)	0.34 \pm 0.16	4.9 \pm 4.06**	2.8 \pm 2.5**	3.12 \pm 2.32	0.88 \pm 0.69	0.6 \pm 0.5	2.5 \pm 1.21
AST (U/L)	18.2 \pm 4.01	244 \pm 105.7**	104.82 \pm 74.92*	219.4 \pm 209.1**	227.5 \pm 184.3**	52 \pm 15.21	85.20 \pm 15.8
ALT (U/L)	15.02 \pm 3.9	219.6 \pm 88.2**	65.82 \pm 54.92*	116.3 \pm 84.4**	151.5 \pm 106.2**	48 \pm 31.01	87.4 \pm 46.6*
ALP (U/L)	75.7 \pm 2.41	167 \pm 62.75*	155.5 \pm 62*	279 \pm 186.9**	223.4 \pm 158**	175 \pm 125.3	209 \pm 33.53**
Total protein (g/dL)	7.14 \pm 0.29	7.45 \pm 0.65	7.61 \pm 0.9	7.12 \pm 1.05	5.5 \pm 0.17**	6.30 \pm 0.8	5.88 \pm 0.72**
Albumin (g/dL)	3.64 \pm 0.23	3.7 \pm 0.77	3.26 \pm 0.86	3.25 \pm 1.12	2.24 \pm 0.37**	2.76 \pm 0.45*	2.66 \pm 0.32**
Globulin (g/dL)	3.5 \pm 0.17	3.52 \pm 0.08	4.38 \pm 1.09**	3.87 \pm 0.75	3.26 \pm 0.44	3.6 \pm 0.49	3.22 \pm 0.94

**p < 0.001 compared to normal controls *p < 0.05 compared to normal controls.

Table 2. Demographic and Antioxidant Status of Healthy Controls and Patients with Liver Diseases (Expressed as Mean \pm SD)

	Healthy Controls (n=53)	Acute Viral Hepatitis (n=14)	Cirrhosis with Portal Hypertension (n=33)	Leptospirosis (n=14)	Sepsis and Multiorgan Failure (n=15)	Left Ventricular Failure (n=9)	Falciparum Malaria (n=10)
Age (years)	34 \pm 12	47 \pm 11	39 \pm 9	39 \pm 6	35 \pm 8	36 \pm 11	34 \pm 7
Sex (M/F)	31/22	9/5	19/14	6/8	11/4	6/3	6/4
GST (IU)	1.2 \pm 0.55	1.06 \pm 0.27	0.97 \pm 0.46	0.95 \pm 0.21	4.74 \pm 0.45**	1.62 \pm 1.21	1.08 \pm 0.28
Total thiols (μ M)	375.1 \pm 57.9	119.7 \pm 53.9**	117.3 \pm 67.7**	119.3 \pm 42**	138.3 \pm 115.6**	138.3 \pm 115.6**	107.1 \pm 81.7**

**p < 0.001 compared to normal controls.

Serum Total Thiols Assay

Hundred μL of serum was added to reaction mixture containing 900 μL of 2 mM Na_2EDTA in 0.2 M Na_2HPO_4 and 20 μL of 10 mM DTNB in 0.2 M Na_2HPO_4 , 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 absorbance value to obtain corrected value. The calibration curve was produced using GSH dissolved in phosphate buffered saline (PBS). Total thiols level was determined using molar extinction coefficient $1600 \text{ M}^{-1}\text{Cm}^{-1}$ [19] and expressed as $\mu\text{moles/L}$.

Standard Liver Function Tests

Serum total and direct bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, albumin and globulin levels 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 all groups, followed by multiple comparison *post hoc* tests. Pearson's correlation was applied to correlate between the parameters.

RESULTS

As depicted in Table 1, AST and ALT were increased markedly in patients with acute viral hepatitis ($p<0.001$), leptospirosis ($p<0.001$), and sepsis and multi organ failure ($p<0.001$), and moderately elevated in patients with cirrhosis with portal hypertension ($p<0.05$), left ventricular failure ($p<0.05$) and falciparum malaria ($p<0.05$). ALP was markedly increased in patients with leptospirosis ($p<0.001$), falciparum malaria ($p<0.001$), and sepsis and multiorgan failure ($p<0.001$), and moderated elevated in acute viral hepatitis ($p<0.05$), cirrhosis with portal hypertension ($p<0.05$). Total protein and albumin markedly decreased in patients with falciparum malaria ($p<0.001$) and sepsis and multi organ failure ($p<0.001$). Globulin increased only in patients with cirrhosis with portal hypertension ($p<0.001$).

As depicted in Table 2, the serum GST activity in patients with acute viral hepatitis, cirrhosis with portal hypertension, leptospirosis, left ventricular failure and falciparum malaria did not differ markedly when compared to healthy controls. Significant increase in serum GST activity ($p<0.001$) was found only in patients with sepsis and multi organ failure when compared to healthy controls. However, there was no correlation between increased GST with any of the standard liver function test parameters. Total thiols were significantly decreased ($p<0.001$) in all these patients when compared to healthy controls.

DISCUSSION

Several authors have reported the role of serum GST activity as a marker of hepatocellular damage [11, 12]. In the

present study, we have found no significant difference in the serum GST activity and decreased total thiols in patients with acute viral hepatitis, leptospirosis, and falciparum malaria when compared to healthy controls. Decreased total thiols indicate that presence of oxidative stress in these patients. Presence of oxidative stress in patients with liver disease has been reported by other authors [7, 8, 17]. Further Sohail M *et al.*, suggested that decrease in expression of endogenous antioxidant enzymes including GST, and up-regulated oxidative defense mechanisms against inflammation plays an important role in host defense mechanism [20]. This decrease in antioxidant defense to survival against inflammation may play a key role in hepatocellular damage in these patients. However, in contrast to findings reported by Adachi Y *et al.* [21], in our study there was no significant rise in serum GST activity in patients with acute viral hepatitis.

Vukasovic *et al.* have shown the presence of oxidative stress in left ventricular failure [22], however, we have found no significant increase in serum GST activity but there was significant decrease in total thiols in patients with left ventricular failure. We have found no significant increase in serum GST activity but significantly decreased total thiols level in cirrhosis patients with portal hypertension. However, in animal models, Czetczot H *et al.* have shown decreased glutathione and increased expression and activity of glutathione dependent antioxidant enzymes including GST in cirrhotic liver tissue [3]. Similarly, Erh-Hao L *et al.* have shown increase in α -GST in animals during recovery from cirrhosis [23].

In patients with sepsis and multiorgan failure, we have found significant increase in serum GST activity and significant decrease in total thiols. These findings are in consistent with previous reports [2, 24], however, at present it is difficult to speculate the possible mechanism for increased presence of serum GST activity only in patients with sepsis and multiorgan failure. The studies on animal model shown increase in serum GST activity 5 hour after the onset of sepsis and they have reported GST is more sensitive indicator of hepatocellular damage than aminotransferases and lactate [24]. The difference in findings between human subjects and animal models and increased presence of serum GST activity in sepsis needs to be addressed by future studies.

The absence of significant difference in serum GST activity in patients with acute viral hepatitis, leptospirosis, falciparum malaria, left ventricular failure, and cirrhosis with portal hypertension patients in our study needs future prospective follow up studies with large sample size in each group to understand the possible mechanism and clinical outcome. Absence of correlation between serum GST activity and standard liver function tests in patients with sepsis and multi-organ failure may possibly indicate serum GST may be contributed from cells other than hepatic cells. Previous studies have reported the presence of oxidative stress in various liver disorders [7, 8, 17]. Presence oxidative stress in patients with liver disease secondary to various disorders could have consumed considerable amount of total thiols in our patient group.

In conclusion, with exception of sepsis with multi-organ failure, no statistically significant increase in serum GST activity was found in hepatocellular damage caused secon-

daily to various diseases. A low level of total thiols in these patients indicates increased presence of oxidative stress.

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