



Original Article:

A Comparative Study Between Alcoholics of Koraga Community, Alcoholics of General Population and Healthy Controls for Antioxidant Markers and Liver Function Parameters

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Abstract:

Objectives: It is well established that long-term alcohol consumption leads to liver cirrhosis and other related disorders. Sufficient work has been done on biochemical markers of liver damage and antioxidant status of chronic alcoholics in general population. In the current study chronic alcoholics from a community called Koraga are analysed for the same parameters in a view to assess the extent of liver damage as compared to healthy controls and other alcoholics. **Methods:** Serum and urine samples from Koraga alcoholics (n=28), general alcoholics (n=30) and healthy controls (n=31) were analysed for liver function parameters and antioxidant markers. Liver function parameters were determined by automated analyzer. Markers of antioxidant status were estimated spectrophotometrically. The data was analysed using SPSS version 16.0. **Results:** There was significant increase in serum AST, serum ALT, serum GST and urine GST in both general and Koraga alcoholics when compared to healthy controls (p<0.0001). Serum ALT, serum GST and urine GST activity was significantly higher in general alcoholics when compared to Koraga alcoholics (p<0.001). Serum and urine total thiol levels were significantly lower in general alcoholics when compared to healthy controls and Koraga alcoholics (p<0.0001). We have observed no difference in total thiols level between healthy controls and Koraga alcoholics, in fact, there was significant increase in urine total thiols level in Koraga alcoholics compared to healthy controls (p<0.001). On Pearson's correlation serum AST, serum ALT correlated positively with serum and urine GST (p<0.0001) and negatively with serum total thiols (p<0.0001). Serum GST correlated negatively with serum total thiols (p<0.0001). **Conclusion:** Results of our study possibly indicate that the extent of alcohol induced liver damage in Koraga subjects is comparatively lower than general alcoholics, even though the alcohol consumption is found to be higher in them. There may be some mechanism that is rendering them resistant to alcoholic liver damage which needs to be explored through further studies at molecular level.

Key Words: Koraga, alcoholics, total thiols, GST, cirrhosis, antioxidant status

Introduction:

Liver plays a major role in the detoxification of toxic compounds such as alcohol that generate free radicals which aid in the alcohol-mediated oxidative stress.¹ Acute and chronic ethanol consumption has been shown to increase the production of reactive oxygen species, lower cellular antioxidant levels, and enhance oxidative stress in many tissues, especially the liver.² Ethanol-induced oxidative stress plays a major role in the mechanism of ethanol induced liver injury.³ The metabolic effects of alcohol are due both to its direct action and to that of its first metabolite acetaldehyde, and can also be connected with the changes in redox state. Differences in ethanol distribution, bioavailability and hepatic metabolism can provide insight into the protective and predisposing factors in alcoholism.⁴

Elevated serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) are regarded as an indication of liver damage and isolated elevation of AST than ALT (with AST/ALT ratio >2) strongly suggests alcoholic liver disease.⁵ Thus, serum levels of these enzymes have been routinely checked to assess the liver function.

Glutathione-S-transferases (GSTs), a cytoplasmic class of enzymes with their maximal activity seen in the hepatocytes are believed to exert a critical role in cellular protection against damage caused by ROS.⁶ Within the hepatocytes, GSTs are involved in conjugating reduced glutathione to electrophiles, hydroperoxides and xenobiotics derived from the metabolism of ethanol, drugs and other toxins.⁷ 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.⁸

Chronic consumption of alcohol causes accumulation of fatty acids in hepatocytes and decreases their functional capacity.⁹ Alcoholic liver diseases may be caused by oxygen radicals such as superoxide and hydroxyl radicals, generated during the metabolism of ethanol by the microsomal ethanol oxidising system (MEOS).¹⁰ The involvement of free radical mech-

anisms in the pathogenesis of alcoholic liver disease have been demonstrated by the detection of lipid peroxidation markers in the liver and in the serum of patients with alcoholism.¹¹ Experiments in alcohol-fed rodents have also shown a relationship between alcohol-induced oxidative stress and the development of liver pathology.¹² 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.^{12,13} Depletion or oxidation of mitochondrial glutathione pool in alcoholic liver disease has also been reported.^{14,15}

From the available literature it appears that sufficient work has been done on the biochemical markers of liver damage and antioxidant status in general chronic alcoholics. In the current study, chronic alcoholics from a community called Koraga, found inhabiting in regions of Kasaragod district in the Karnataka-Kerala state border, India, are analysed for the liver function test parameters and antioxidant status to assess the extent of liver damage and antioxidant status in them as compared to general alcoholics and healthy controls.

Materials and Methods:

Subjects

The study was carried out on 28 chronic alcoholics of Koraga community, 30 general chronic alcohol abusers and 31 non-alcoholic healthy volunteers. Both general and Koraga community alcoholics were recruited from who voluntarily attended the alcohol de-addiction camp conducted in the hospital. Consumption of alcohol in Koraga community is considered common social practice within the community and they routinely consume alcohol along with other family members irrespective of age and sex. Food habits and the type of alcohol consumption in Koraga population were almost similar to that of general population and they were consuming 150-170 grams of ethanol per day for 15±8 years. General alcohol abusers were consuming 70-90 grams of alcohol per day for 7±3 years. Blood and urine samples from chronic alcohol abusers were taken at the time of first visit to the hospital before starting any kind of treatment. On history, alcohol abusers were found not to be on any type of medication. Healthy volunteers were non-alcoholics, non-smokers and free from any chronic inflammatory diseases and were not on any kind of medications. Informed consent was taken from all the subjects involved in the study. This study was approved by the institutional ethical review board for human research.

Venous blood from healthy controls, general alcoholics and Koraga community alcoholics was drawn into sterile, plain vacutainers. The blood was allowed to clot for 30 minutes and then centrifuged at 2000×g for 15 min for separation of serum. The serum is then assayed for liver function markers such as ALT, AST, total bilirubin (TB), direct bilirubin (DB) and antioxidant markers such as GST enzyme activity and total thiols

status. All assays were performed immediately after the separation of serum. Random urine samples were collected in sterile containers with toluene as preservative and analysed within 30 minutes of collection.

Reagents

Special chemicals such as 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), 1-chloro 2, 4-dinitrobenzene (CDNB) and reduced glutathione (GSH) were obtained from Sigma Chemicals Co. (St Louis, MO). All other reagents used were of analytical grade.

Biochemical determinations

Determination of liver function test parameters

Serum AST, ALT, TB and DB levels were estimated using a clinical chemistry automated analyzer Hitachi 912.

Determination of serum and urine total thiols status

Serum and urine total thiols were measured by a spectrophotometric method using DTNB.¹⁶ In brief, 900 µl of 0.2 M Na₂HPO₄ containing 2 mM Na₂ EDTA, 100µl of serum or urine and 20µl of 10 mM DTNB in 0.2 M Na₂HPO₄ were taken in an Eppendorf tube and warmed to 37°C. The solution was mixed with a vortex mixer and transferred to a cuvette and the absorbance was measured at the end of 5 minutes at 412nm. Simultaneously sample and reagent blanks were also prepared and their absorbance values were ascertained at 412nm. The absorbance of the sample and the reagent blanks were subtracted from the serum absorbance values. Corrected absorbance values were used to calculate the total thiols status from the calibration curve produced using GSH dissolved in phosphate buffer saline (PBS). The molar extinction coefficient 1600 M⁻¹cm⁻¹ was derived from the calibration curve to calculate the total thiols status in individual samples and the total thiols level was expressed as µmoles/L of serum or urine sample.

Determination of serum and urine GST activity

Serum and urine GST activity was measured by the method described by Habig et al.¹⁷ Briefly, 850µl of phosphate buffer of pH 6.5, 50µl of CDNB, 50 µl GSH were added and incubated at 37°C for 10 minutes. This was followed by the addition of 50 µl of serum or urine sample. The absorbance was read at 340nm at 1 minute interval for 5 minutes. The mean difference in absorbance values between each minute interval was taken to calculate the GST activity using molar extinction coefficient 9.6 mM⁻¹ cm⁻¹ and GST activity was expressed in IU.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences, version 16.0 (SPSS Inc. Chicago, USA). One-way analysis of variance followed by Post-Hoc test was used to compare the mean values between the three groups. Pearson's correlation was applied to correlate between the parameters. The results were expressed as mean±SD in a tabular form. A p-value <0.05 was considered statistically significant.

Results:

Table-1: Liver function parameters and antioxidant markers in chronic Koraga alcoholics and general alcoholics as compared to healthy controls (values expressed as Mean±SD).

	Healthy controls (n=31)	General alcoholics (n=30)	Koraga alcoholics (n=28)
TB(mg/dl)	0.80±0.28	1.05±0.44 ^a	0.94±0.39
DB(mg/dl)	0.26±0.11	0.53±0.21 ^a	0.25±0.18
AST(IU)	18.22±3.89	51.27±23.50 ^b	43.07±16.75 ^b
ALT(IU)	15.09±3.94	49.26±24.78 ^{bc}	36.96±15.95 ^b
Serum GST (IU)	0.92±0.15	28.82±17.73 ^{bd}	7.99±1.62 ^e
Serum total thiols (µM)	362.00±59.03	151.90±29.62 ^b	332.26±78.76 ^f
Urine GST (IU)	0.66±0.13	48.75±20.42 ^{bd}	0.99±0.24
Urine total thiols (µM)	27.43±5.65	19.29±5.17 ^b	33.34±10.77 ^{ef}

p- values: a<0.05, b<0.0001, e<0.01 compared to healthy controls; c<0.01, d<0.0001 compared to Koraga alcoholics.; f<0.0001 compared to general alcoholics.

As depicted in the Table 1, serum AST and serum ALT levels were found to be significantly increased in both general ($p < 0.0001$, $p < 0.0001$) and Koraga ($p < 0.0001$, $p < 0.0001$) alcohol abusers compared to healthy controls, respectively. It was also observed that serum ALT activity was significantly higher in general alcoholics when compared with that of Koraga alcoholics ($p < 0.01$). A significant increase in serum GST activity was observed in both general ($p < 0.0001$) and Koraga ($p < 0.01$) alcoholics compared to healthy controls. We have observed a significant elevation of serum GST in general alcoholics when compared to Koraga alcoholics ($p < 0.0001$). Urine GST activity of general alcoholics showed significant increase when compared with healthy controls ($p < 0.0001$) and Koraga alcoholics ($p < 0.0001$). However, urine GST activity in Koraga alcoholics did not differ significantly from that of healthy controls. Serum and urine total thiols were significantly decreased in general alcoholics as compared to healthy controls ($p < 0.0001$, $p < 0.0001$) and Koraga alcoholics ($p < 0.0001$, $p < 0.0001$). However, serum total thiols level of Koraga alcoholics showed no significant difference as compared to control group, whereas urine total thiols were significantly higher in Koraga alcoholics compared to healthy controls ($p < 0.001$).

On Pearson's correlation, serum ALT and serum AST correlated positively with serum GST ($r = 0.529$, $p < 0.0001$ [Figure 1], $r = 0.505$, $p < 0.0001$) and urine GST ($r = 0.424$, $p < 0.0001$, $r = 0.430$, $p < 0.0001$), and negatively with serum total thiols ($r = -0.515$, $p < 0.0001$ [Figure 2], $r = -0.427$, $p < 0.0001$), respectively. Also, serum GST correlated negatively with serum total thiols ($r = -0.656$, $p < 0.0001$) [Figure 3] and urine total thiols ($r = -0.356$, $p < 0.001$), and positively with urine GST ($r = 0.588$, $p < 0.0001$) [Figure 4]. Serum total thiols correlated positively with urine total thiols ($r = 0.381$, $p < 0.0001$) [Figure 5], and negatively with urine GST ($r = -0.723$, $p < 0.0001$). Urine GST correlated negatively with urine total thiols ($r = -0.554$, $p < 0.0001$) [Figure 6].

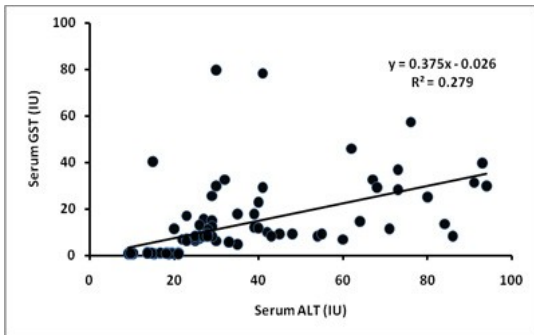


Figure 1. Correlation between serum ALT and serum GST

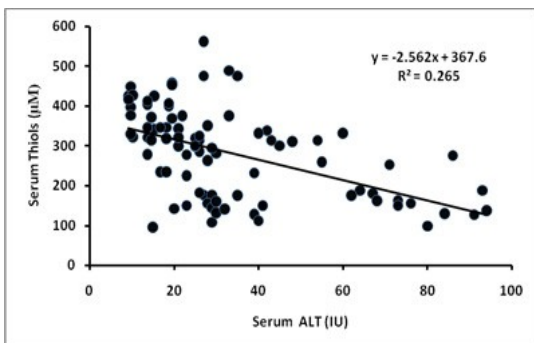


Figure 2. Correlation between serum ALT and serum thiols

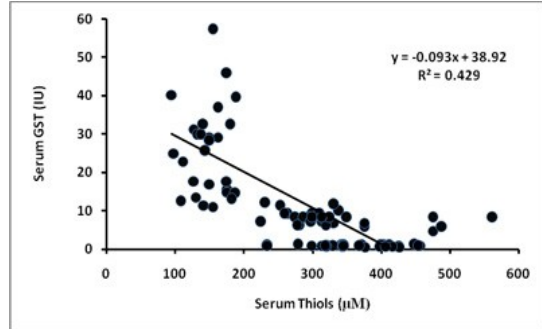


Figure 3. Correlation between serum thiols and serum GST

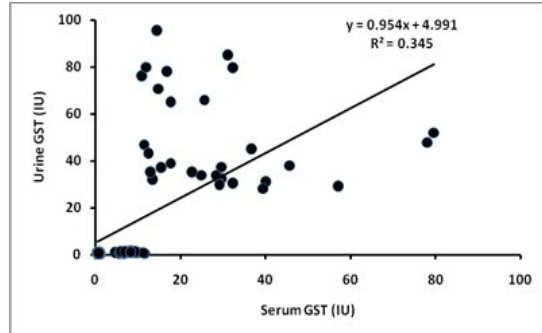


Figure 4. Correlation between serum GST and urine GST

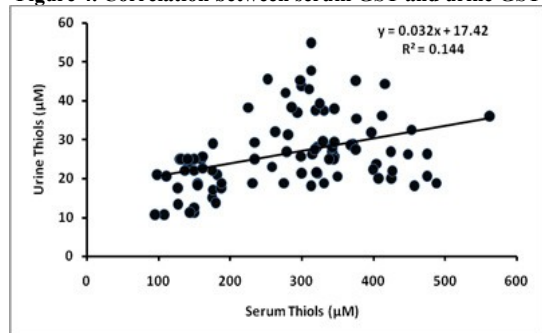


Figure 5. Correlation between serum thiols and urine thiols

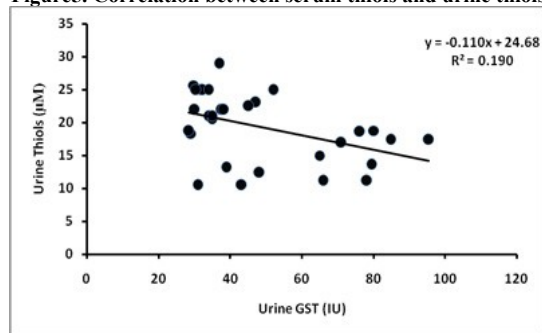


Figure 6. Correlation between urine GST and urine thiols

Discussion:

We have observed significant increase in serum AST and ALT activity in both the general alcoholics (AST/ALT ratio 1.04) and Koraga alcoholics (AST/ALT ratio 1.16) indicating the presence of alcohol-induced hepatocyte damage in these alcoholics. However, it has also been observed that ALT activity in general alcoholics was significantly higher when compared with that of Koraga alcoholics ($p < 0.01$) and there was significant increase in TB and DB in general alcoholics and

such increase was not observed in Koraga alcoholics. These findings probably may indicate that the extent of damage to hepatocytes caused by ethanol in Koraga community appears to be less as compared with that of general alcoholics.

It has been well established that GSTs are primarily involved in the cellular detoxification processes and elevated circulating GST activity is considered to be an early index increased load on hepatocytes in detoxifying toxins and is related to indicate increased presence of oxidative stress.⁷ In our study, serum AST and serum ALT correlated positively with serum and urine GST and negatively with serum and urine total thiols, and serum GST correlated negatively with serum total thiols (figures 1-4) indicating increased generation of alcohol induced free radicals and consumption of available antioxidant pool in them. Although serum GST activity was found to be significantly increased in both the general alcoholics and Koraga alcoholics, the extent of increase in GST activity in Koraga alcoholics (mean 7.99 IU) was significantly lower ($p < 0.0001$) as compared to that found in general alcoholics (mean 28.82 IU). It has also been observed that there was no significant increase in urine GST activity in Koraga alcoholics which was almost comparable to that of healthy controls. These findings also indicate that there is lesser extent of damage to hepatocytes in Koraga alcoholics when compared to the extent of damage induced by alcohol in general alcoholics although the amount of alcohol consumption in Koraga alcoholics was two times higher than that of general alcoholics.

Recently Muttigi et al¹⁸ have reported increased GST activity and decreased total thiol status in general alcoholics. We have observed significant increase in urine GST activity in general alcoholics, and this increase in GST activity in urine may be due to increased glomerular filtration of circulating low molecular weight GST present in higher concentration in general alcoholics facilitated by the impairment of renal tubular function caused by chronic alcohol consumption.¹⁹ On the other hand, Koraga alcoholics with significantly lesser increase in serum GST activity compared to general alcoholics showed no significant increase in urine GST activity. This probably indicates a lesser degree of alcohol-induced hepatocyte and renal tubular damage in them.

Serum total thiols are considered to be the major body antioxidants²⁰ and are reported to be decreased in general alcoholics.^{15, 21} Earlier studies have also reported the depletion of cellular total thiols pool in patients with alcoholic liver disease.¹⁴ In our study we have observed similar findings in general alcoholics but interestingly total thiols status of Koraga alcoholics were similar to that of healthy controls in spite of consuming such an increased amount of alcohol for more than 15 years on daily basis. Urinary total thiols status in Koraga alcoholics was even higher than that of healthy controls. We were surprised to observe these findings in Koraga alcoholics and there is no literature available to explain these findings in them. Although speculative, we hypothesize that there may be some mechanism at molecular and genetic level which is protecting them from alcohol toxicity. There may be some unknown mechanism that is increasing the total thiols pool in them thereby protecting them from alcohol induced free radical generation and subsequent tissue damage.

It has been reported that both alcoholism and susceptibility to develop cirrhosis appear to be largely genetically determined and the rate of cirrhosis is much higher if a patient has a parent with alcoholic cirrhosis.²² Severity of liver damage is often correlated with the amount of alcohol consumption in patients with a history of heavy alcohol abuse.²³ Contrary to these reports, even with a rich family history of alcoholism, the severity of liver cirrhosis and other alcohol-induced impairments seen in the Koraga alcoholics is comparatively lesser than general ones. Thus the findings in our study suggest the possibil-

ity that chronic alcohol abusers of the Koraga community appear to be less susceptible to the alcohol-induced liver damage by unknown mechanism. Whether this is due to some adaptive changes at the molecular level occurred since generations needs to be explored through further studies. The studies at the molecular level may decipher the possibility of the existence of resistance against alcohol-induced liver damage and the molecular mechanisms responsible for such resistance in these Koraga alcoholics.

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