International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

*Original Research Article*

# A Comparative Study between Yakshagana Artists and Healthy Controls for Antioxidant Markers and Liver Function Parameters

Virupaksha Devaramane[1], Panambur V Bhandary[1], Harini Ramanan[2], Ann T Thomas[2], Amrita Rishi, Harisha Kasturi[2], Mukund P Srinivasan[2], Chethan A Jaiprakash[2], Safiulla B Syed[2], Puneeth H Somashekar[2], Mungli Prakash[2], Veena Nayak[3*]

[1] Department of Psychiatry, Dr A V Baliga Memorial Hospital, Doddanagudde, Udupi, India.
[2]Department of Biochemistry, Kasturba Medical College, Manipal, India-576104
[3] Department of Pharmacology, Kasturba Medical College, Manipal, India-576104

*Correspondence Email: drveenayak@gmail.com

*Received: 10/02//2012* *Revised: 14/02/2012* *Accepted: 5/03/2012*

## ABSTRACT

**Objectives:** The lifestyle of yakshagana artists is comparatively different from other people which predispose them to physical and mental stress. In the current study yakshagana artists are analysed for antioxidant markers and liver function parameters to assess the extent of stress and liver damage as compared to healthy controls.
**Methods**: Serum samples from yakshagana artists (n=78) and healthy controls (n=80) were analysed for liver function parameters and antioxidant markers. Liver function parameters were determined by automated analyzer. Markers of antioxidant status were estimated spectrophotometrically.
**Results**: There was significant increase in serum AST, serum ALT, serum total bilirubin, serum GST and serum MDA, and significant decrease in serum total thiols in yakshagana artists when compared to healthy controls ($p<0.0001$). On Pearson's correlation serum AST, serum ALT correlated positively with serum GST ($p<0.0001$) and negatively with serum total thiols ($p<0.0001$). Serum MDA correlated negatively with serum total thiols ($p<0.0001$).
**Conclusion**: Results of our study indicates there is presence of stress in yakshagana artists which may be because of their lifestyle and consumption of alcohol in them may compound this stress and can cause impairment in liver function.
**Key Words:** yakshagana artists, total thiols, GST, MDA, antioxidant status.

## INTRODUCTION

Yakshagana is a folk melodrama popular in coastal Karnataka. It is way of telling stories (usually from epics or puranas) by means of song and dance. This folk melodrama is usually carried out in twilight hours. The yakshagana artists are dressed up in bright colored dressings added with various painting applied to the face. This melodrama is usually played in all seasons except on rainy days since it is played out door. Most of the yakashgana artists are at risk of alcohol and nicotine abuse because of peer pressure and nature of the play.

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]

Gluthathione-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. [4] 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.[5] The involvement of free radical mechanisms 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. [6]

In the current study, yakshagana artists are analysed for the liver function test parameters and, oxidant and antioxidant status to assess the extent of liver damage and antioxidant status in them as compared to healthy controls.

## MATERIALS AND METHODS

### Subjects

The study was carried out on 78 yakshagana artists and 80 non-alcoholic healthy volunteers. Yakshagana artists were recruited from health camp conducted exclusively for yakashagana artists residing in and around Udupi district. All the subjects participated in the health camp were professional yakshagana artists practicing it for around 10±3 years, approximately 6 months in a year. Most of them were smokers (average 24 beedies or 12 cigarettes per day) and alcoholics consuming 70-90 grams of alcohol per day for 7±3 years. Blood samples from yakshagana artists were taken at the time of the camp visit before starting any kind of medication. In history, yakshagana artists 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.

### Samples

Venous blood from yakshagana artists and healthy controls were collected in centrifuge tubes. 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), oxidant marker malondialdehyde (MDA), and antioxidant markers such as GST enzyme activity and

total thiols status. All assays were performed immediately after the separation of serum.

**Reagents**

Special chemicals such as 5 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), 1-cholro 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 aspartate transaminase (AST), alanine transaminase (ALTa0, total bilirubin (TB) and direct bilirubin (DB) levels were estimated using a clinical chemistry automated analyzer Hitachi 912.

**Determination of serum total thiols status**

Serum total thiols were measured by a spectrophotometric method using DTNB. [7] In brief, 900 µl of 0.2 M $Na_2HPO_4$ containing 2 mM $Na_2$ EDTA, 100µl of serum and 20µl of 10 mM DTNB in 0.2 M $Na_2HPO_4$ were taken in an Eppendorf tube and warmed to 37ºC. 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 $M^{-1}cm^{-1}$ 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 sample.

**Determination of serum GST activity**

Serum GST activity was measured by the method described by Habig et al. [8] Briefly, 850µl of phosphate buffer of pH 6.5, 50µl of CDNB, 50 µl GSH were added and incubated at 37ºC for 10 minutes. This was followed by the addition of 50 µl of serum 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.

**Determination of serum MDA**

In a test tube, 500µl each of serum sample, 1% TBA and glacial acetic acid was taken, the resultant mixture was vortexed and heated at 100°C for 30-minute. After cooling to room temp the absorption maxima of the chromogen formed was recorded and the absorbance readings were noted in that wavelength. The TBA-adduct formed is expressed in MDA units using extinction co-efficient $1.56 \times 10^{-5}$ . [9,10]

## STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for Social Sciences, version 16.0 (SPSS Inc. Chicago, USA). Independent sample t test was used to compare the mean values between the two 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.

## RESULT

As depicted in the table 1, serum AST ($p<0.0001$), serum ALT ($p<0.0001$), serum total bilirubin ($p<0.001$) levels were found to be significantly increased yakshagana artists compared to healthy controls. A significant increase in serum GST activity ($p<0.0001$) and MDA ($p<0.0001$) was observed in yakshagana artists compared to healthy controls. Serum total thiols were significantly decreased in yakshagana artists as compared to healthy controls ($p<0.0001$) On Pearson's correlation, serum ALT and serum AST correlated positively with serum GST ($r = 0.467$, $p<0.0001$, $r = 0.465$ $p<0.0001$), respectively. Serum total thiols correlated negatively with serum MDA ($r = -0.412$, $p<0.001$) (figure 1).

**Table 1: Liver function parameters and antioxidant markers in yakshagana artists and healthy controls (values expressed as mean±SD).**

| | Healthy controls (n=80) | Yakshagana Artists (n=78) |
|---|---|---|
| TB(mg/dl) | 0.69±0.26 | 0.87±0.36♣ |
| DB(mg/dl) | 0.10±0.10 | 0.11±0.13 |
| AST(IU) | 18.45±4.87 | 46.15±16.45* |
| ALT(IU) | 15.25±4.93 | 37.82±20.37* |
| Serum GST (IU) | 0.92±0.16 | 8.32±7.31* |
| Serum total thiols (μM) | 364.00±60.12 | 246.34±85.46* |
| Serum MDA (nmoles/L) | 223.70±49.81 | 326.79±121.29* |

p- values: *<0.0001, ♣<0.001 compared to healthy controls.

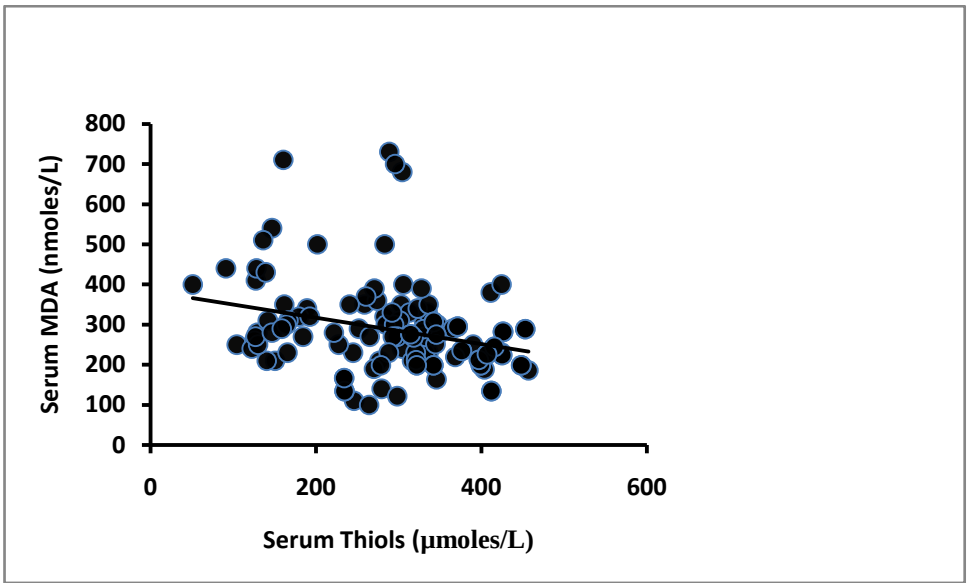


**Figure 1. Correlation between serum thiols and serum MDA**

## DISCUSSION

We have observed significant increase in serum AST, ALT and TB in yakshagana artists indicating the presence of alcohol-induced hepatocyte damage in these artists. 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. [11] In our study, serum AST and serum ALT correlated positively with serum indicating increased hepatocytes damage in these subjects and the increased presence of GST in serum possibly indicates increased oxidative damage to hepatocyte membrane leading to leakage of intracellular GST into blood stream. Presence of increased generation of free radicals in these subjects is evident from the significant increase in MDA levels in yakshagana artists.

Serum total thiols are considered to be the major body antioxidants [12] and are reported to be decreased in alcoholics. [13, 14] Earlier studies have also reported the depletion of cellular total thiols pool in patients with alcoholic liver disease. [13, 15] In our study we have observed similar findings in yakshagana artists in whom there was significant decrease in total thiol pool and this decrease in thiol pool was accompanied by significant increase in serum GST and MDA. This may possibly explain that as the toxins in the form of alcohol or the lead based paints they apply over the face for the drama may increase the free radical generation and oxidative membrane damage leading to increased leakage of detoxifying enzymes like GST and increased presence of lipid peroxidation marker like MDA in the blood stream.

## CONCLUSION

In conclusion, yakashagana artists are at increased stress due to their lifestyle

and habits like smoking and alcohol abuse and they need lifestyle modification.

## ACKNOWLEDGEMENT

We sincerely thank Dr Sripathi Rao,Dean, Kasturba Medical college and Dr Sudhakar Nayak, Head of the department of Biochemistry for research facilities provided. We are also thankful to the management of Dr AV Baliga Memorial Hospital for providing clinical data and other information related to subjects involved in the study

## REFERENCES

1. Nordmann R, Ribiere C, Rouach H. Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radic Biol Med 1992;12: 219-40.
2. Cederbaum AI. Introduction-serial review: alcohol, oxidative stress and cell injury. Free Radic Biol Med 2001;31: 1524-26.
3. Aparajita Dey, Cederbaum AI. Alcohol and oxidative liver injury. Hepatalogy 2006; 43:S63-S74.
4. Hayes PC, Bouchier IAD, Beckett GJ. Glutathione-S-transferase in humans, in health and disease. Gut 1991; 32: 813-18.
5. Himmelfarb J, McMongale E, McManamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. Kidney Int 2000; 58: 2571 –78.
6. Tsukamoto H, Lu SC. Current concepts in the pathogenesis of alcoholic liver injury. FASEB J 2001;15: 1335-49.
7. Motchnik AP, Frei B, Ames NB. Measurement of antioxidants in human blood plasma: Protein Thiols. In, Packer L (ed). Oxygen radicals in biological systems; Methods in Enzymology, Packer L. (Ed.) Vol-234. California, Academic Publishers, 1998; 243-75.
8. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-Transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-9.
9. Gutteridge JMC. Thiobarbituricacid-reactivity following iron-dependent free-radical damage to amino acids and carbohydrates. FEBBS Lett 1981; 128:343-346.
10. Kei Satoh. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978; 90: 37-43.
11. 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 an liver involvement. alcohol and alcoholism 1998; 33: 366-72.
12. 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.
13. 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.
14. Prakash M, Shetty JK, Tripathy S, Verma M, Saddinamane V, Bhandary PV. Serum paraoxonase in alcohol abusers associated with alcoholic liver disease. Clin Chim Acta 2007; 378: 232-34.
15. Muttigi MS, Prabhu LS, Kedage V, Prakash M, Shetty JK, Devaramane V, Bhandary PV. 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: 5

**********