PUB: 4/2014

International Journal of Biochemistry. Photon 109 (2014) 289-295
https://sites.google.com/site/photonfoundationorganization/home/international-journal-of-biochemistry
Original Research Article. ISJN: 4438-5728

International Journal of Biochemistry **Photon**

A Comparative and Correlative Study of Sialic Acid, Malondialdehyde and Antioxidant Status in Blood and Saliva of Male Chronic Alcoholics

Susanna T.Y.[a*], Shivashankara A.R.[b], Malathi M.[b]

[a] Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India
[b] Father Muller Medical College, Mangalore, Karnataka, India

Article history:
Received: 09 December, 2013
Accepted: 16 December, 2013
Available online: 11 January, 2014

Abbreviations:
DNA: Deoxyribonucleic acid, FSA: Free Sialic Acid, MDA: Malondialdehyde, PBSA: Protein Bound Sialic Acid

Keywords:
Alcoholism, Antioxidants, Malondialdehyde, Saliva, Sialic acid, Thiols

Corresponding Author:
Susanna T.Y.*
Assistant Professor of Biochemistry
Email: drsusannty@gmail.com
Phone: +919538925391

Shivashankara A.R.
Associate Professor of Biochemistry
Email: arshiva72@gmail.com
Phone: +919880146133

Malathi M.
Professor of Biochemistry
Email: malathi.mala@hotmail.com
Phone: +919480229866

Abstract
The most commonly used laboratory diagnostic procedures employ analyses of blood constituents. Saliva as a diagnostic tool offers distinctive advantages. Whole saliva can be collected non-invasively, without the help of skilled technician and special equipments. The present study aimed to evaluate the effect of chronic alcoholism on Sialic acid and oxidant-antioxidant status in blood and saliva, and to correlate the changes in biochemical parameters of saliva with the changes in blood, hence making a prompt attempt to develop saliva as a tool in detection, diagnosis and management of alcoholism. Blood and saliva samples from fifty chronic alcoholics and fifty normal healthy controls, were analyzed for Sialic acid, malondialdehyde (MDA) and antioxidants. In blood and saliva of chronic alcoholics, the levels of free Sialic acid (FSA), protein bound Sialic acid (PBSA), MDA and uric acidwere significantly higher while protein thiols were lower in comparison to controls. A significant correlation was observed between the levels in blood and saliva, with respect to PBSA, MDA and uric acid in alcoholics, no such correlation was seen with regard to FSA and protein thiols. Monitoring oxidative stress may be useful for prognosis, treatment follow up and prevention of complications, in alcohol-dependent individuals. Saliva analysis provides an additional laboratory tool supportive to blood in the biochemical assessment of alcoholism.

Citation:
Susanna T.Y., Shivashankara A.R., Malathi M., 2014. A Comparative and Correlative Study of Sialic Acid, Malondialdehyde and Antioxidant Status in Blood and Saliva of Male Chronic Alcoholics. International Journal of Biochemistry. Photon 109, 289-295.

1. Introduction

1.1 Alcoholism and its Mechanism

Alcohol is detrimental to human health and wellbeing. Alcoholism is a serious health issue with socioeconomic consequences. According to the World Health Organization, in 2000 alcohol-related death and disability accounted for 4.0% of the global burden of disease, ranking as the fifth most detrimental risk factor of 26 examined (NIAA, 1995).

Chronic alcohol consumption causes toxic effects on the body with involvement of multiple molecular phenomena and metabolic pathways. Alcohol is known to induce generation of free radicals and cause impairment of antioxidant defense systems (Zakhari et al., 2006; Das et al., 2007). Previous studies have reported increased levels of oxidation products of lipids, proteins and DNA, and decreased levels of antioxidants in experimental animals subjected to chronic consumption of alcohol (Das et al., 2007; Maneesh et al., 2007; Yeh et al., 2007). Researchers have also found increased lipid peroxidation and decreased antioxidants in blood, in chronic alcoholics.

Identification of alcoholics especially in early stages of alcohol abuse is crucial in preventing adverse health effects and social consequences. Many biochemical parameters in blood and urine have been proposed as biomarkers of alcoholism. Common biomarkers include, carbohydrate deficient transferring, gamma glutamyl transferase and amino transferases (Das et al., 2005; Maithreyi et al., 2010; Das et al., 2008; Sharpe et al., 2001).

1.2 Saliva as a Diagnostic Tool

Saliva as a diagnostic tool offers distinctive advantages. Whole saliva can be collected non-invasively, and without the help of skilled technician and special equipments. Analysis of saliva may provide a cost-effective approach for the screening of large populations. Methods have been developed to assay alcohol in saliva (smalle et al., 1999).Studies have reported decreased levels of proteins, amylase, electrolytes, and increased levels of Sialic acid and acetaldehyde in saliva in chronic alcoholics (Dutta et al., 1992; Ponnio et al.,2006). A recent study has reported an increased malondialdehyde level and decreased levels of GSH and SOD in the saliva in chronic alcoholics; and on alcohol deaddiction for one month malondialdehyde decreased while GSH increased to near control values (Chiramel et al., 2010).

There is paucity of studies on salivary markers of diseases in general, salivary biomarkers of alcoholism in particular. Hence, the present study has made an attempt to analyze salivary biochemical parameters in alcoholics and to find the correlation between blood and saliva with respect to changes in biochemical parameters.

2. Experimental

2.1 Objectives of the Study

This hospital–based study aimed to estimate Sialic acid, MDA , protein thiols and uric acid in blood and saliva of chronic alcoholics in comparison to healthy non alcoholic controls. This study also aimed to assess the correlation between blood and saliva with respect Sialic acid, MDA, protein thiols and uric acid in chronic alcoholics.

2.2 Source of Data and Study Design

This study was done at Father Muller Medical College and Hospital, Mangalore. The study was conducted over a period of one year from May 2010 to April 2011. The study protocol was approved by Ethics Committee of the institution. Voluntary informed consent was taken from the subjects of the study. The study subjects aged from 20-60 years, and comprised of Alcoholics (group-1) and Controls (group-2).

Group-1: Fifty, male, chronic alcoholics admitted to the Deaddiction Center for alcohol withdrawal treatment comprised this group. Diagnosis of alcohol dependence syndrome was made by the treating psychiatrist. These subjects consumed moderate to heavy doses of alcohol for five years and more, and were devoid of obvious clinical manifestations. Detailed history of alcohol intake, clinical complications if any, habits in particular smoking and tobacco chewing, were collected by giving them a questionnaire. Chronic alcoholics (heavy/moderate) with alcohol abuse for five years or more, consuming any type of alcoholic beverage were included.

Group-2: Fifty, age-matched, clinically apparently healthy male volunteers were included as controls.

Individuals with any systemic illness (Diabetes, Hypertension, Cardiovascular disease, Viral/Bacterial Hepatitis, Alcoholic hepatitis, tumors, meningitis), history of current use of hepatotoxic and nephrotoxic drugs, occasional and problem drinkers, tobacco chewers, and smokers were excluded from the study groups.

2.3 Collection of Samples

Unstimulated whole saliva sample was collected according to the method of Navazesh (1993). The collected samples were centrifuged at 3000 rpm for 15 minutes and supernatants were collected. Five ml. Blood was collected in EDTA vaccutainer taking aseptic precautions, centrifuged to separate plasma and cells. From the packed red blood cells, hemolysate was prepared.

2.4 Assays

Level of MDA, the marker of lipid peroxidation, was estimated in the hemolysate and saliva as thiobarbituric acid-reactive substances (TBARS), by the method of Ohkawa et al. (1979). Levels of PBSA and FSA in plasma and saliva were assayed by the method of Yao et al. (1989), which is based on reaction of Sialic acid with acid-ninhydrin reagent.Initially, proteins were precipitated using ethanol; Sialic acid level in the precipitate was taken as PBSA, and Sialic acid level in supernatants was taken as FSA. Protein thiols were measured by a spectrophotometric method

using 5, 5/ dithio, bis-2- nitrobenzoic acid (DTNB)(Prakash et al.,2009); and uric acid by uricase method using reagent kit procured from Olympus diagnostics. All the assays were standard spectrophotometric methods; Systronics 118 UV visible spectrophotometer was used for all assays.

2.5 Statistical Analysis

The Data were expressed as mean with standard deviation. Significance of the difference between alcoholics and controls was evaluated by Student's "t" test and Mann Whitney "U" test. Using Logistic regression analysis, correlation of MDA level with other biochemical parameters, and correlation between values of each parameter in blood and saliva in alcoholics, was analyzed.

3. Results

The results of present study are presented in tables 1 and 2 , figures 1 to 3 .The present study observed significantly increased levels of uric acid ,FSA and PBSA in plasma and saliva of chronic alcoholics when compared to controls ($p< 0.001$). Level of MDA in red blood cell hemolysates and saliva was significantly higher in alcoholics in comparison to controls ($p< 0.001$). The levels of protein thiols in plasma and saliva of alcoholics were significantly lower, when compared to controls ($p < 0.001$).

The plasma level of uric acid in alcoholics was significantly higher by 85% when compared to controls; salivary uric acid was higher by 96% in alcoholics. The results were highly significant ($p< 0.001$).

The correlation analysis was done with respect to the level of each biochemical parameter in blood and saliva of chronic alcoholics. There was significant correlation between blood and salivary levels with respect to PBSA, MDA, and uric acid; no such correlation was seen with regard to FSA and protein thiols (Fig 1-3).

4. Discussion

Saliva as a laboratory tool in clinical medicine has generated interest in recent years. Saliva analysis was promoted mainly considering the advantages in its collection. Saliva collection

Table 1: Levels of Free Sialic acid (FSA) and Protein Bound Sialic acid in Plasma and Saliva of Chronic Alcoholics and Controls (Values are mean ± SD of number samples indicated; Range of the values is indicated in parentheses)

	Group-1 (Alcoholics) N = 50	Group-2 (Controls) N = 50	% Difference wrt Controls
FSA, Plasma (mg/dl)	11.44 ± 0.4 * (11.33-11.54)	9.3 ± 0.6 (9.15-9.49)	+ 23 %
FSA, saliva (mg/dl)	4.64 ± 0.3 * (4.56-4.72)	2.4 3 ± 0.44 (2.3-2.55)	+ 91%
PBSA, plasma (mg/dl)	5.94 ± 0.5 * (5.8-6.07)	3.46 ± 0.7 (3.27-3.64)	+ 72 %
PBSA, saliva (mg/dl)	1.4 ± 0.2* (1.4 – 1.5)	0.93 ± 0.2 (0.9-1.0)	+51%

*P value < 0.001; highly significant
(Significance of difference between alcoholics and controls).

Table 2: Levels of MDA, Protein thiols and Uric acid in Blood and Saliva of Chronic Alcoholics and Controls (Values are mean ± SD of number samples indicated; Range of the values is indicated in parentheses)

	Group-1 (Alcoholics) N = 50	Group-2 (Controls) N = 50	% Difference wrt Controls
MDA , hemolysate (nmol/dl)	1236 ± 157* (1191.29-1280.69)	455 ± 130 (418.14-491.72)	+ 172
MDA, saliva (nmol/dl)	61.8 ± 13* (58.18-65.50)	22.88 ± 8 (20.60-25.17)	+ 170
Protein thiols, plasma (µmol/l)	356 ± 48.2* (342.23-369.69)	713 ± 131 (676.22-750.49)	- 50
Protein thiols, saliva (µmol/l)	74.7 ± 36* (64.61-84.91)	204.9 ± 37 (197.34-218.40)	- 63
Uric acid, plasma (mg/dl)	8.23 ± 0.9* (7.97-8.48)	4.46 ± 1 (4.17-4.75)	+ 85
Uric acid, saliva (mg/dl)	4.34 ± 0.4* (4.22-4.46)	2.21 ± 0.7 (2.0-2.42)	+ 96

* P value < 0.001; highly significant
(Significance of difference between alcoholics and controls).

Figure 1: Correlation between PBSA in plasma and PBSA in saliva in chronic alcoholics

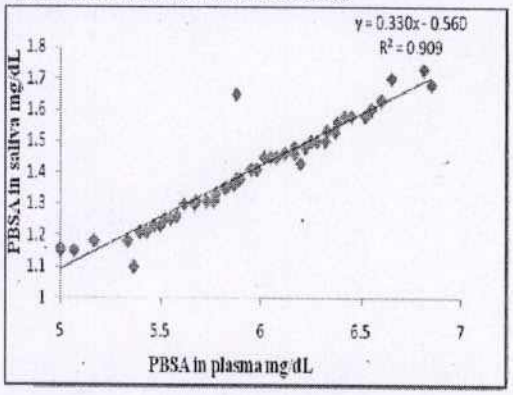


Figure 2: Correlation between MDA in Hemolysate and MDA in saliva in chronic alcoholics

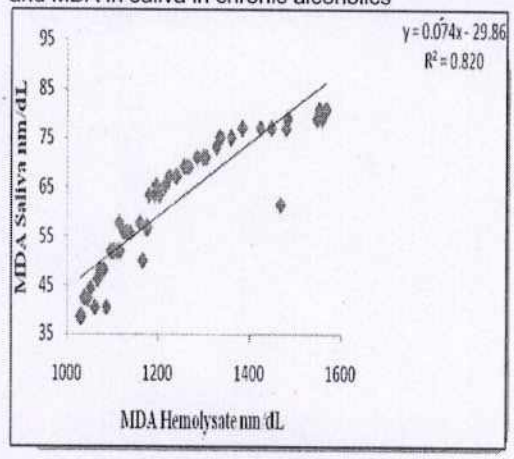
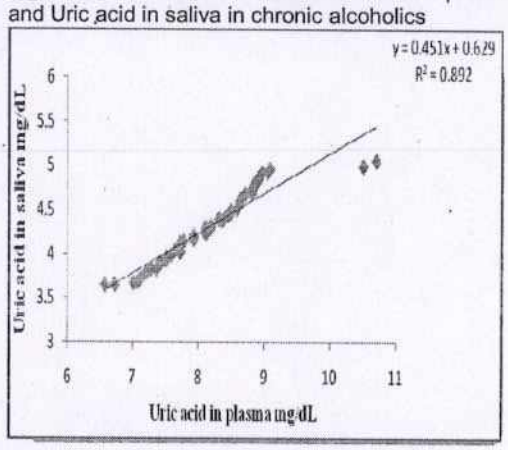


Figure 3: Correlation between Uric acid in plasma and Uric acid in saliva in chronic alcoholics



has least compliance problems and it could be the biological fluid of choice when repeated sampling is required. Whole saliva is considered suitable for evaluation of systemic diseases while gland-specific saliva is considered ideal for evaluation of gland-specific pathology (Kaufman et al., 2002; Ziang et al., 2009; Streckfus et al., 2002). Saliva is equipped with enzymatic and non-enzymatic antioxidants (Nagler et al., 2007).

Sialic acid is an essential moiety of salivary glycoproteins such as mucins (Tenonvo et al., 1989) .Sialic acids are acetylated derivatives of neuraminic acid, they are important constituents of both glycoproteins and glycolipids particularly located at their termini, and thus are important in a variety of cellular functions (Schauer et al., 1982; Schauer et al., 2000). The present study observed elevation of FSA by 1.2 fold in plasma and 1.9 fold in saliva in chronic alcoholics. Increase in PBSA was by 1.7 fold and 1.5 fold in plasma and saliva respectively. Earlier, Guvendik et al (Guvendik et al., 2006) observed that total Sialic acid (TSA) in serum elevated in chronic alcoholics with and without hepatosteatosis, and recommended Sialic acid assay as a part of clinical tests for identifying alcohol abuse regardless of hepatosteatosis, as well as its value to demonstrate the amount of alcohol consumed. Earlier, Ponnio et al (Ponnio et al.,2006) and Kurtul et al (Kurtul et al.,2004) have demonstrated increased TSA in the saliva of chronic alcoholics, and suggested TSA to be a non-invasive marker of alcoholism. An altered level of Sialic acid in alcoholics has been suggested to be due to changes in the expressions and levels of sialylated glycoproteins such as mucins, alpha-1 acid glycoprotein, transferrin and alpha-1 antitrypsin (Chrostek et al., 2007). The present study has revealed positive correlation of Sialic acid levels with MDA and uric acid, and negative correlation with protein thiols, both in plasma and saliva of chronic alcoholics. Findings of this study suggest possible role of Sialic acid in alcohol-induced oxidative stress. Elevated FSA and PBSA could be the response of the body tissues against the altered oxidant-antioxidant status .Increased Sialic acid level could also suggest increased cell turnover and aberrant glycations due to chronic ethanol intoxication.

Malondialdehyde is the sensitive and convenient indicator of degree of lipid peroxidation. Increased MDA level in blood or saliva suggests increased lipid peroxidation due to alcohol induced generation of free radicals. The present study has observed 2.7 fold elevation of MDA both in saliva and red blood cell lysates of alcoholics. Findings of this study are in accordance with the observations of earlier studies with respect to MDA in blood in chronic alcoholics (Subham et al., 2009; Seema et al., 2005). Oxidative stress is proposed as the key mechanism in the

pathogenesis of alcoholic complications. This study observed a significant correlation of MDA levels in hemolysate with the plasma levels of FSA, PBSA, protein thiols and uric acidic chronic alcoholics. With regard to the saliva, MDA correlated significantly with PBSA and uric acid, in chronic alcoholics. Overall, MDA showed a positive correlation with FSA, PBSA and uric acid, and negative correlation with protein thiols, in alcoholics.

Uric acid is the end product of purine catabolism, and important extracellular free radical scavenger. Uric acid is the major nonenzymatic antioxidant of plasma and saliva (Halliwell et al., 2007; Nagler et al., 2002; Herschkovich et al., 2007). The present study observed significantly higher levels of uric acid in plasma and saliva of chronic alcoholics when compared to controls. The primary cause for the occurrence of hyperuricemia in alcoholic patients is increased generation of NADH as a result of the oxidation of ethanol to acetaldehyde which increases the conversion of pyruvate to lactate, the later metabolite alters the transport of uric acid with the production of hyperuricemia and a concomitant decrease in urinary uric acid excretion (Zakhari et al., 2006; Liberopoulos et al., 2004).

Thiols constitute the major portion of the total body antioxidants and they play a significant role in defense against reactive oxygen species. Total thiols are composed of both intracellular and extracellular thiols either in the free form as glutathione, or thiols bound to proteins. In this study, levels of protein thiols in plasma and saliva were significantly lower in chronic alcoholics in comparison to controls which could be due to impaired antioxidant defense. Previous studies have reported decreased blood levels of total thiols and glutathione in patients with alcoholic liver disease (Seema et al., 2005). With regard to the salivary thiols, recent studies have shown lower GSH levels in alcoholics (Chiramel et al., 2010; Shivashankara et al., 2011), and restoration of salivary GSH after alcohol withdrawal (Chiramel et al., 2010).

We attempted correlating salivary values with those of blood with respect to PBSA, FSA, uric acid, MDA and protein thiols in chronic alcoholics. We observed significant correlation of salivary MDA, PBSA and uric acid with the respective values in blood where as other parameters did not show such correlation.

Research Highlights

Alcohol induces lipid peroxidation as evident from increase in MDA level in blood and saliva. The oxidant antioxidant status is tilted in favor of oxidants which could be the key, molecular phenomenon in the development of alcoholic complications. Monitoring oxidative stress markers and uric acid in alcoholics could be of diagnostic and prognostic value.

Limitations of the Present Study

This study had a smaller sample size and male alcoholics. We did not monitor the effect of alcohol withdrawal regimen on the biochemical parameters. The study did not correlate Sialic acid, MDA, FSA, PBSA and uric acid with conventional biomarkers of alcoholism such as gamma glutamyl transferase and aminotransferases. Our study did not consider duration of alcohol abuse and alcohol dose for correlation analysis.

Recommendations

Saliva analysis in alcoholics has to be given due thrust. Future studies employing large sample size, correlating biochemical changes in saliva with those of blood, are required. The future studies need to take into account the type of alcoholic beverage consumed, and correlate the biochemical findings with dose and duration of alcohol consumption. Changes in salivary flow rate, which may influence the concentrations of biochemical parameters in saliva, needs consideration in the future studies.

Funding and Policy Aspects

Priority should be given for alcohol research as there are reports of increasing number of young individuals becoming alcohol dependent, and there is a need for detecting alcoholism and its complications at early stages.

Justification of Research

This study was taken considering the need for alcohol biomarkers which could detect alcoholism in early stages, and in monitoring alcoholic complications. Saliva is an underused diagnostic tool. There is paucity of studies on salivary biomarkers of alcoholism especially in the Indian scenario.

Conclusions

Significant changes were evident in blood and saliva samples of chronic alcoholics with respect to Sialic acid, lipid peroxidation and antioxidants. Monitoring oxidative stress may be useful for prognosis, follow up of treatment and prevention of complications, in alcohol-dependent individuals. Saliva analysis provides an additional, non-invasive diagnostic and prognostic tool if not an alternative to blood, time being. However, salivary diagnostics has the potential to develop and establish as a laboratory tool supportive and alternative to laboratory tests done with blood samples, in clinical medicine.

Author's Contribution and Competing Interests

Dr. Susanna TY was involved in concept design, literature search, sample collection, experimental work and drafting the manuscript. Dr. Shivashankara AR designed the study protocol, literature search, drafting the manuscript and oversaw the experimental work. Dr. Malathi M. involved drafting the manuscript and overseeing the work.

Competing interests

None declared

Acknowledgement

The authors wish to acknowledge the support and encouragement by the management of Father Muller Medical College. We are grateful to Rev. Fr. Patrick Rodrigues, Director; Rev. Fr. Denis D'Sa, Administrator; Dr. JP Alva, Dean.

References

Chrostek L., Cylwik B., Krawiec A., Korcz W., Szmitkowski M., 2007. Relationship between serum sialicacid and sialylated glycoproteins in alcoholics. Alcohol and Alcoholism, 42,588-592.

Chiramel K.J., Shivashankara A.R., 2010. A study of salivary malondialdehyde and glutathione in chronic alcoholics before and after alcohol withdrawal. Conference abstracts Medicon 2010, Australasian Medical Journal. 3,532.

Das S.K., Vasudevan D.M., 2005. Monitoring oxidative stress in patients with non-alcoholic and alcoholic liver disease. Indian Journal of Clinical Biochemistry. 20, 24-28.

Das S.K., Vasudevan D.M., 2007. Alcohol-induced oxidative stress. Life Sciences. 81, 177-187.

Das S.K., Hiran K.R., Mukherjee S., Vasudevan D. M., 2007. Oxidative stress is the Primary event: effects of ethanol consumption in brain. Indian Journal of Clinical Biochemistry. 22, 99-104.

Das S.K., Dhanya L., Vasudevan D.M., 2008. Biomarkers of alcoholism: an update Ed review Scandinavian Journal of Clinical Laboratory Investigations. 68, 81-92.

Dutta S.K., Orestes M., Vengulekur S., Kwo P., 1992. Ethanol and human saliva: effect of chronic alcoholism on flow rate, composition and epidermal growth factor. American Journal of Gastroenterology. 87, 350-354.

Guvendik G., Idiz N., Bosgelmez I., Soylemezoglu T., Dogan Y.B., Ilhan I.O., 2006. Comparative assessment of diagnostic accuracy of serum Sialic acid and several conventional biomarkers in alcohol-dependent individuals. Turkish Journal of Pharmaceutical Sciences. 3, 19-30.

Halliwell B., Gutteridge J.M.C., 2007. The chemistry of free radicals and related reactive species.Free radicals in biology and medicine, 4th edition. Oxford Press: oxford. 30-78.

Herschkovich O., Shafat I., Nagler R.M., 2007. Age-related changes in salivary antioxidant profile: possible implications for oral cancer. J Gerontol A BiolSci Med Sci. 62, 361-366.

Indian alcohol policy alliance., 2008. Alcohol Related Harm in India - a fact sheet.Indian alcohol policy alliance http://www.indianalcoholpolicy.org/alcohol_atlas. New Delhi.

Kaufman E., Lamster I.B., 2002. The diagnostic applications of saliva-a review.Crit Rev Oral Biol Med. 13,197-212.

Kurtul N., Cil M.Y., Bakan E., 2004. The effects of alcohol and smoking on serum, saliva, and urine Sialic acid levels. Saudi. 25,1839-44.

Liberopoulos E.N., Miltiadous G.A., Elisaf MS., 2004. Alcohol intake, serum uric acid concentrations, and risk of gout. Lancet. 364, 246-247.

Maneesh M., Dutta S., Chakrabarti A., Vasudevan D.M., 2007. Experimental Therapeutic intervention with alpha tocopherol in ethanol induced testicular Injuries in rats. Indian J Clin Biochem. 22, 138-142.

Maithreyi R., Janani A.V., Krishna R., Sweta A., Edwin R.R., Mohan S.K., 2010. Erythrocyte lipid peroxidation and antioxidants in chronic alcoholics with alcoholic liver disease. Asian J Pharmaceut Clin Res. 3,183-185.

National Institute on Alcohol Abuse and Alcoholism, 1995.

Diagnostic Criteria for Alcohol Abuse and Dependence-Alcohol Alert No. 30 http://pubs.niaaa.nih.gov/publications/aa30.htm. Retrieved 25th 2011.No. 30 PH 359.

Nagler R.M., Klein I., Zarzhevsky N., Drigues N., Reznick A.Z., 2002. Characterization of the differentiated antioxidant profile of human saliva. Free Radic Biol Med. 32, 268-277.

Navazesh M., 1993. Methods for collecting saliva. Ann N Y Acad Sci. 20, 72-73.

Ohkawa H., Ohishi N., Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95, 351-358.

Ponnio M., Alho H., Heinala P., Nikkari S.T., Sillanaukee P., 2006. Serum and saliva levels of Sialic acid are elevated in alcoholics; Alcohol Clin Exp Res. 23, 1060-1064.

Prakash M., Shetty S., Tilak P., Anwar N., 2009. Total thiols; Biomedical importance of and their alteration in various disorders. Online J Health Allied Scs. 8(2), 2.

Schauer R., 1982. Chemistry, metabolism, and biological functions of Sialicacids. Adv Carbohydr Chem Biochem. 40, 131-234.

Schauer R., 2000. Achievements and challenges of sialicacid research. GlycoconjJ 17, 485-499.

Seema G., Rajesh P., Ranjan K., Aggarwal H.K., Aggarwal R.P., Aggarwal S.K., 2005. Lipid peroxide levels and Antioxidant status in Alcoholic liver disease, Indian Journal of Clinical Biochemistry. 20, 67-71.

Sharpe P.C., 2001. Biochemical detection and monitoring of alcohol abuse and Abstinence. Ann clin Biochem. 38, 652-664.

Shivashankara A.R., Susanna T.Y., Chiramel K.J., Kuriakose S., Malathi M., 2011. Blood and Salivary gamma glutamyl transferase and oxidant-antioxidant status in chronic alcoholics: a comparative and correlative study, National J Med Sci. 1,137-141.

Smalle K.H., Hofmann G., Kaufmann P., Lueger A., Brunner G., 1999. Q.E.D. alcohol test: a simple and quick method to detect ethanol in saliva of patients in emergency departments; comparison with the conventional determination in blood. Intensive Care Med. 25, 492-495.

Streckfus C.F., Bigler L.R., 2002. Saliva as a diagnostic fluid. Oral Diseases 8, 69-76.

Subhani T.F., Nasar M.A., Jarrari A., D'Souza V., Naseer M.A., Shakeel F., 2009. 5'-nucleotidase, oxidative stress and antioxidant status in alcohol consumers and cirrhotic patient. Biochemia Medica. 19,277-86.

Tenouvo J.O., 1989. Human saliva-Clinical Chemistry and Microbiology. Tenovo text book .vol 1. CRC Press: Florida.

Yao K., Ubuka T., Masuoka N., Kinuta M., Ikeda T., 1989. Direct determination of bound Sialic acids in sialoglycoproteins by acidic ninhydrin. Anal Biochem 179, 332-335.

Yeh M.Y., Burnham E.L., Moss M., Brown L.A.S., 2007. Chronic alcoholism alters systemic and pulmonary glutathione redox status. Am J Respir Crit Care Med 176,270-276.

Zakhari S., 2006. Overview: how is alcohol metabolized in the body?. Alcohol Res Health 29, 245-255.

Ziang L., Xiao H., Wong D.T., 2009. Salivary biomarkers for clinical application. Mol Diagn Ther 13, 245-259.