

BLOOD AND SALIVARY GAMMA GLUTAMYL TRANSFERASE AND OXIDANT-ANTIOXIDANT STATUS IN CHRONIC ALCOHOLICS : A COMPARATIVE AND CORRELATIVE STUDY

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ABSTRACT

Background :

Saliva is an unused or underused diagnostic tool. There is paucity of studies on salivary biochemical changes in alcoholism. There is a need for studies which correlate the biochemical changes in saliva with those of blood.

Aims and Objectives :

The study aimed to correlate the changes in gamma-glutamyl transferase (GGT) and oxidant-antioxidant status in blood and saliva of chronic alcoholics.

Materials and Methods :

Blood and saliva samples of chronic alcoholics (n=50) and healthy controls (n=50) were analyzed for GGT, malondialdehyde, GSH, SOD and GST. Statistical significance of the results was evaluated by Student's t test and Karl Pearson Correlation analysis.

Results:

Activities of GGT in plasma and saliva of alcoholics were higher when compared to controls. Level of GSH and activities of SOD and GST were lower in red blood cells and saliva of chronic alcoholics in comparison to controls. All the results were statistically significant. The direction of change (increase / decrease), was similar in blood and saliva in each biomarker of alcoholism. However, there was no significant correlation between saliva and blood with respect to any of these parameters.

Conclusions:

Chronic consumption of alcohol led to increased activity of gamma-glutamyl transferase, and oxidative stress indicated by increased lipid peroxidation and decreased antioxidants in blood and saliva. Further studies with large sample size, correlation of alcohol biomarkers of blood and saliva, consideration of all the relevant socio demographic variables and salivary flow rate measurement, are needed.

INTRODUCTION:

Alcoholism is a serious health issue with socioeconomic consequences. Chronic consumption of alcohol causes multiple structural and functional derangements^[1,2]. 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. Major biomarkers include, transaminases, gamma-glutamyl transferase (GGT) and carbohydrate-deficient transferrin^[2]. GGT is induced by alcohol, and serum levels rise in response to acute hepatocellular damage. Levels are especially high in patients with severe alcoholic liver disease^[2].

Alcohol is known to induce generation of free radicals and cause impairment of antioxidant defense systems^[3]. 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. Researchers have also found increased lipid peroxidation and decreased antioxidants in blood, in chronic alcoholics^[4-7].

Saliva as a laboratory tool in clinical medicine, has generated interest in recent years. It is an underused diagnostic tool. Whole saliva can be collected non-invasively without the need for skilled persons and special equipments. Saliva collection has least compliance problems^[8,9]. Saliva has been analyzed in systemic diseases, infectious conditions and psychiatric disorders^[8-13]. Previous studies have reported decreased levels of proteins, amylase, electrolytes, and increased levels of sialic acid and acetaldehyde in saliva in chronic alcoholics^[14,15]. There is paucity of studies on salivary GGT and oxidative stress markers in alcoholism, and studies which correlate the biochemical changes in saliva with those of blood in alcoholics.

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Materials and Methods:

This study was carried out at Father Muller Medical College and Hospital, Mangalore. The study protocol was approved by the Ethics Committee of the institution. The study subjects were divided into two groups :

Group-I : Controls – apparently healthy individuals (males) in the age group of twenty to seventy years; n = 50

Group-II : Alcoholics – chronic alcoholics (alcohol abuse for five years or more, all males) admitted to the Deaddiction center and in-patient wards. The diagnosis of alcohol-dependence syndrome was done by the treating psychiatrist. Detailed history of alcohol intake, clinical complications, use of tobacco, were collected from the subjects; n=50.

Occasional drinkers, problem drinkers and patients with systemic illness, chronic and acute infections including hepatitis, smokers and tobacco chewers, were excluded from the study. Written informed consent was obtained from each subject.

Five ml. of blood was collected taking aseptic precautions, immediately centrifuged to separate cells and plasma. From the packed cells, red blood cell hemolysates were prepared. Unstimulated whole saliva sample was collected according to the method of Navazesh^[16]. The sample was collected between 9 am – 12 noon. The subjects were asked to rinse the mouth thoroughly to remove any food debris and then after ten minutes, were asked to spit into sterile plastic containers, avoiding forcible spitting. The collected samples were immediately centrifuged at 3000 rpm for 15 minutes and supernatants were collected. The plasma, hemolysates and saliva were stored at – 20 C till the analyses were done. All the analyses were performed on the same day of collection. The methods for analyses were standardized taking blood and saliva samples from normal, healthy individuals of different age groups, and reproducibility of results was ensured.

In the plasma, red blood cell hemolysate and saliva of alcoholics and controls, the assays were carried out. Glutamyl transferase (GGT) activity in saliva and plasma was assayed by the kinetic spectrophotometric method using L-gamma glutamyl 3-carboxy 4-nitroanilide as the substrate. GGT catalyzes the transfer of the glutamyl group from L- γ - glutamyl- 3- carboxy - 4 - anilide to glycylglycine with formation of L- γ -glutamylglycylglycine and 5-amino-2-nitrobenzoate. Increased absorbance at 405 nm was measured^[17]. Malondialdehyde in red blood cell hemolysates and saliva was estimated as thiobarbituric acid-reactive substances (TBARS), by the

method of Ohkawa et al^[18]. Malondialdehyde, the end product of lipid peroxidation, forms a complex with thiobarbituric acid, the absorbance of which is measured at 535 nm. Glutathione (GSH) in red blood cell hemolysates and saliva was estimated based on reduction of 5,5' dithiobis-2 nitrobenzoic acid (DTNB) by GSH, and measurement of absorbance at 412 nm^[19]. Activity of superoxide dismutase in red blood cell hemolysates and saliva was assayed by the method of Nandi and Chatterjee^[20]. Superoxide-mediated oxidation of pyrogallol was inhibited by SOD, which was measured as an increase in absorbance at 420 nm. One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autooxidation. Glutathione S-transferase (GST) activity in saliva and red blood cell hemolysates was assayed by the method of Habig et al^[21]. This assay was based on conjugation of glutathione (GSH) with 1-chloro, 2,4-dinitrobenzene (CDNB) to form a thioether. Increased absorbance at 340 nm was measured. A unit of GST activity was defined as the amount of enzyme that catalyzes the formation of one micromole of product per minute under the conditions of the assay. In the red blood cell hemolysates, hemoglobin levels were estimated and the levels / activities of biochemical parameters were expressed in terms of value per gram Hb.

The significance of differences in the values of parameters among controls and alcoholics, was evaluated by Student's 't' test. Karl Pearson correlation analysis was employed to find out correlations between blood and salivary levels of each parameter.

Results:

All the study subjects were males as the Deaddiction Center of Father Muller Medical College Hospital admits only male alcoholics as per its provisions. GGT activities in plasma and saliva of alcoholics were significantly higher when compared to controls. GSH level, and SOD and GST activities were significantly lower in red blood cell hemolysates and saliva of chronic alcoholics, in comparison to controls. All the results were statistically significant (P = 0.001). (Table 1.)

The change in each biochemical parameter in saliva, was compared correlated with that of blood, in chronic alcoholics. All the parameters showed similar direction of change (increase / decrease) in saliva and blood. However, there was no significant correlation between saliva and blood with respect to any of these parameters.

DISCUSSION

The present study observed increased activities of gamma-glutamyl transferase in blood and saliva of chronic alcoholics. Elevated activities of GGT are found in the blood of patients with alcoholic liver disease and also in heavy drinkers. Blood GGT as an alcohol biomarker, is well established^[2]. But, studies on salivary GGT in alcoholics are scarce. Salivary activity of GGT showed significant elevation in liver cirrhosis, cholecystitis, hepatic tumors, acute pancreatitis and diabetic ketoacidosis. Salivary GGT activities were unmodified in fatty liver, infectious hepatitis, cholelithiasis, and mumps^[22,23]. Elevated GGT in saliva as well as blood indicates potential clinical application of salivary GGT assay as an alcohol biomarker. However, the present study showed varying degrees of increase in blood and salivary GGT. Blood GGT increased by 158 % while salivary GGT increased by 22%, and there was no correlation between blood and salivary activities of GGT in chronic alcoholics.

Increased generation of free radicals and decreased antioxidant capacity of the body are implicated in the etiopathogenesis of many diseases, and in the toxic manifestations of many compounds. Alcohol consumption is associated with a number of changes in cell functions and the oxidant-antioxidant status^[1,2]. Present study revealed increased malondialdehyde levels in red blood cells and saliva of chronic alcoholics. Activities of superoxide dismutase and GST, and level of glutathione were decreased in red blood cells and saliva of chronic alcoholics. Malondialdehyde is a convenient and sensitive marker of lipid peroxidation. Increased malondialdehyde levels in red blood cells and saliva suggest increased lipid peroxidation due to generation of free radicals, on long-term exposure to alcohol. SOD is a first line defense against free radicals as it scavenges the superoxide radical produced in the initial stages of free radical-mediated oxidative damage. Decreased SOD in red blood cells and saliva suggests depletion of this enzyme due to increased oxidative stress and increased generation of superoxide radicals.

GSH is the major thiol and an efficient reducing agent in our body. It is involved in free radical scavenging reaction catalyzed by glutathione peroxidase, and in detoxification of xenobiotics catalyzed by glutathione S-transferase^[24]. GST is an enzyme involved in phase-II of detoxification reactions. It uses glutathione as the co-substrate. By virtue of its detoxification of products of lipid peroxidation, GST is also an antioxidant enzyme^[24,25].

Decreased GST activity observed in this study indicates depletion of antioxidant capacity in blood and saliva, and utilization of GST-mediated reaction for the detoxification of metabolites of alcohol. Savolainen et al found a strong association between occurrence of GST M1 "null" genotype and alcoholic liver disease^[26].

Previous studies have reported altered oxidant-antioxidant status in the blood and tissues of experimental animals subjected to chronic alcohol consumption,^[2,5,6,27-29] and in the blood of chronic alcoholics^[2-4, 7,30]. However, there is paucity of studies on salivary parameters of oxidative stress in alcoholism. Zima et al suggested that pathways of ethanol metabolism could produce free radicals which affect the antioxidant system^[3]. Ethanol itself, and the resultant hyperlactacidemia and elevated NADH, increase xanthine oxidase activity, which results in the production of superoxide radicals. Microsomal ethanol oxidation aggravates the oxidative stress by impairing the antioxidant systems^[1,3].

Saliva is equipped with an antioxidant system comprising mainly of peroxidase, superoxide dismutase, catalase, glutathione and uric acid^[31]. Increased oxidative stress and altered oxidant-antioxidant status in saliva, has been observed in oral cancer, diabetes mellitus and renal failure^[32-34]. Significant change in salivary malondialdehyde and antioxidants was seen in alcoholics. In our study, each of the oxidative stress markers (malondialdehyde, GSH, SOD and GST) showed the changes on their levels / activities in the same direction (increase / decrease) in blood and saliva though there was no significant correlation between blood and salivary values in chronic alcoholics.

CONCLUSIONS

Chronic alcohol consumption is associated with changes in blood and salivary levels or activities of GGT, malondialdehyde, GSH, SOD and GST. The direction of change (increase / decrease), was similar in blood and saliva in each biomarker of alcoholism. However, there was no significant correlation between saliva and blood with respect to any of these parameters. Saliva analysis as laboratory tool in assessment of alcoholism has advantages over that of blood. Whole saliva can be collected non-invasively without the need for skilled persons and special equipments, and saliva collection has least compliance problems and it could be the biological fluid of choice when repeated sampling is required. We did not correlate the biochemical markers with the type,

Table 1. GGT, Malondialdehyde, GSH, SOD and GST in Blood and Saliva of Chronic Alcoholics and Controls

Parameters	CONTROLS (N =30)	ALCOHOLICS (N=30)	% difference	P value
GGT, plasma (U/L)	20.90± 1.213	53.83 ± 4.878	+ 157%	P<0.001
GGT , Saliva (U/L)	4.363 ± 0.14	5.317 ± 0.311	+ 22%	P=0.005
Malondialdehyde, red blood cell (nmol/g Hb)	220.2 ± 8.7	292 ± 8.8	+ 33%	P = 0.002
Malondialdehyde, saliva(nmol/dL)	21.66 ± 4.21	40.87 ± 4.33	+ 89%	P < 0.001
GSH, red blood cell (µmol/ g Hb)	13.23 ± 2.3	9.23 ± 1.9	- 30%	P = 0.002
GSH, saliva (µmol / dL)	9.75 ± 0.33	8.01 ± 0.59	- 18%	P=0.005
SOD, red blood cell (U/g Hb)	13 ± 1.7	8.5 ± 0.98	- 35%	P= 0.001
SOD , saliva(U/g Hb)	3.9 ± 0.19	2.9 ± 0.17	- 26%	P=0.004
GST, red blood cell (U/g Hb)	0.345 ± 0.014	0.286 ± 0.025	- 17%	P<0.001
GST, saliva (U/L)	1.370 ± 0.166	0.967±.158	- 29%	P=0.005

Values are mean ± S.D.

Units : 1) One unit of enzyme activity is defined as the amount of enzyme required to convert one micromole of the substrate to product in one minute (for GGT and GST); 2) One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation.

quantity, frequency and duration of alcohol intake. Hence, further studies with large sample size, correlation of alcohol biomarkers of blood and saliva, consideration of all the relevant sociodemographic variables and salivary flow rate measurement, are needed before establishing application of saliva as a fluid alternative to blood in clinical medicine.

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Key Words : Alcoholism, Antioxidants, Gamma-glutamyl transferase, Glutathione, Lipid peroxidation, Oxidative Stress, Saliva, Superoxide Dismutase

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