Original Article

A Correlative Study on the Aminotransferases and Gamma Glutamyl Transferase in the Saliva and Serum of Chronic Alcoholics Before and After Alcohol Deaddiction

SHIVASHANKARA A.R., CLAUDIA JOHNNY, MALATHI M, ARUN KUMAR K., AVINASH SS, TONI THOMAS

ABSTRACT

Objective: The present study was taken up to evaluate the effects of chronic alcoholism on the activities of γ-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum and saliva and to assess the effects of alcohol abstinence on these enzyme-activities.

Methods: This was a case-control study. Fifty chronic male alcoholics (with a history of alcohol abuse for five years or more) aged between 30-70 years, who were admitted to the Deaddiction Center for Alcohol Withdrawal Treatment, were the subjects. Age-matched, normal, healthy controls were also included in the study. GGT, ALT and AST were assayed in the saliva and serum of fifty chronic alcoholics before and after the deaddiction of one month.

Results: The activities of the enzymes in the saliva as well as in the serum were significantly higher in the alcoholics as compared to that in the healthy controls. The serum GGT, ALT and AST levels were higher by 4.4 fold, 2 fold and 1.8 fold respectively while the salivary activities of these enzymes were higher by 2.1, 3.2 and 2.6 fold respectively in the alcoholics. The activities of these enzymes in the serum and saliva decreased significantly after one month of the alcohol withdrawal regimen. There was a significant correlation between the serum and the salivary activities in the alcoholics with respect to each enzyme.

Conclusions: The enzymes ALT, AST and GGT in blood and saliva served as the markers of alcoholism and alcohol deaddiction. The strong correlation between the salivary and serum activities of the enzymes in alcoholics indicates the potential use of saliva as an alternate to blood in the future.

Key Words: Alanine aminotransferase, Alcoholism, Aspartate aminotransferase, Gamma glutamyl transferase

INTRODUCTION

Alcoholism is a serious health issue with socioeconomic consequences. The chronic consumption of alcohol causes multiple structural and functional derangements [1], [2]. The identification of alcoholics, especially in the 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 the biomarkers of alcoholism. The major biomarkers include alanine amino transferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), and carbohydrate-deficient transferrin [2], [3].

GGT is induced by alcohol, and the serum levels rise in response to the acute hepatocellular damage. The levels are especially high in patients with severe alcoholic liver disease. These are more likely to be elevated in the regular rather than the episodic drinkers [2], [3]. The ALT and AST levels are elevated in patients with viral hepatitis, toxic hepatitis, cholestasis, cirrhosis, liver carcinoma, and alcoholic liver disease. The serum AST and ALT levels are often raised in patients who are alcoholics although generally not to more than 2-4 times the upper limits of the normal range [2-4]. An AST/ALT ratio which is > 1.5, strongly suggests an alcohol- induced damage to the liver, and a ratio which is > 2.0 confirms this diagnosis [2], [3].

The use of saliva as a diagnostic fluid has gained importance in recent years. The whole saliva collection procedure is non-invasive and it does not need skilled technicians and special equipments. The salivary analysis in diseases could become a cost-effective approach. An altered salivary chemical composition has been reported in oral diseases, cancer, diabetes mellitus, cystic fibrosis, autoimmune disorders, psychiatric disorders and hormonal disorders [5], [6]. Previous studies have reported decreased levels of proteins, amylase and electrolytes and increased levels of sialic acid and acetaldehyde in the saliva of chronic alcoholics [7], [8].

There is a paucity of studies on the analysis of the salivary chemical constituents in general, and the evaluation of alcoholism by saliva analysis in particular, from India. Hence, the present study was taken up to evaluate the effects of chronic alcoholism on the activities of GGT, ALT and AST in serum and saliva and to assess the effects of alcohol abstinence on these enzyme-activities. We also aimed to compare and correlate the changes in the activities of GGT, ALT and AST in saliva with the changes in the enzyme-activities in serum, in alcoholics.

MATERIALS AND METHODS

This study was carried out at the Father Muller Medical College and Hospital, Mangalore. The study protocol was approved by the institutional ethics committee. The study subjects were divided into two groups: Group-1 (alcoholics) and Group-2 (controls). Group-I.A: Alcoholics – fifty chronic alcoholics (with a history of alcohol abuse for five years or more) who were admitted to the Deaddiction

Center for Alcohol Withdrawal Treatment. They were in the age group of 30-70 years. The diagnosis of the alcohol-dependence syndrome was done by the treating psychiatrist. A detailed history of alcohol intake, clinical complications and the use of tobacco were collected from the subjects; n=50. The same subjects were followed up after the treatment period of one month and they comprised group 1B. Group-2: Age- and sex-matched, apparently healthy volunteers (n=30) were included as the controls in this study. Occasional drinkers, patients with systemic illness, smokers and tobacco chewers, were excluded from the study. A written informed consent was obtained from each subject.

Five ml of blood was collected by taking aseptic precautions and this was centrifuged to separate the serum. An unstimulated whole saliva sample was collected according to the method of Navazesh [9]. The sample was collected between 9 am–12 noon. The subjects were asked to rinse their mouths thoroughly to remove any food debris and then after ten minutes, they were asked to spit into sterile plastic containers by avoiding forcible spitting. The collected samples were centrifuged at 3000 rpm for 15 minutes and the supernatants were collected.

In the saliva and serum samples of all the study subjects, the activities of GGT, ALT and AST were assayed by kinetic spectrophotometric methods. GGT was assayed, based on its catalysis of 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. The increased absorbance at 405 nm was measured [10]. ALT was assayed by an enzymatic kinetic method, in which the pyruvate which was formed from the transamination of alanine, was converted to lactate by lactate dehydrogenase and the rate of the decrease in the absorbance was measured at 340 nm [11]. The assay of the AST activity was based on the transamination of aspartate to oxaloacetate, followed by the conversion of oxaloacetate to malate by dehydrogenase and this decrease in the absorbance measured at 340 nm [12]. The procedures which followed were originally meant for the serum and the volume of the sample which was required for the saliva was double the one which was required for the serum.

The significance of the differences in the values of the parameters among alcoholics (group-1A), abstainers (group-1B) and controls (group-2), was evaluated by ANOVA (Analysis of Variance) and the Bonferroni test. Karl-Pearson's correlation analysis was employed to find out the correlations between the blood and salivary levels of each parameter in the alcoholics (group-1A).

RESULTS

We observed that the activities of the enzymes, GGT, ALT and AST in the serum and saliva were significantly higher in the alcoholics as compared to those in the controls and the abstainers [Table/Fig 1].

The activities of these enzymes in the serum and saliva decreased significantly after one month of the alcohol withdrawal regimen. There was a significant correlation between the serum and salivary activities in alcoholics with respect to each enzyme [Table/Fig 1-4]. All the results were statistically significant (P < 0.001).

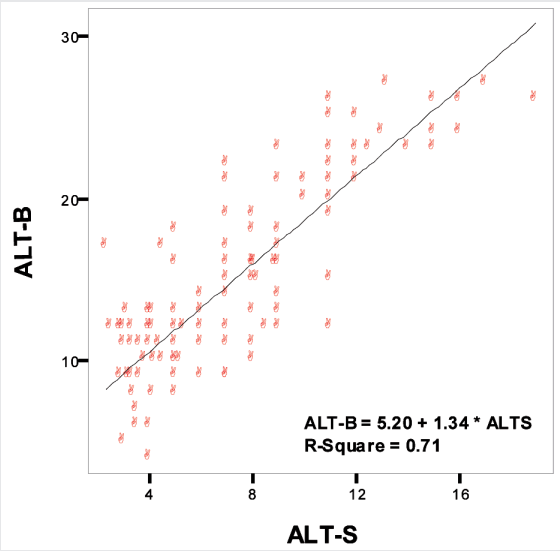
DISCUSSION

The enzymes, GGT, ALT and AST are the traditional markers of alcoholism and alcoholic liver disease. The present study analyzed the effect of chronic alcoholism on the activities of the enzymes, GGT, ALT and AST in the serum and saliva. The alcoholics who were selected for the study were exposed to alcohol consumption

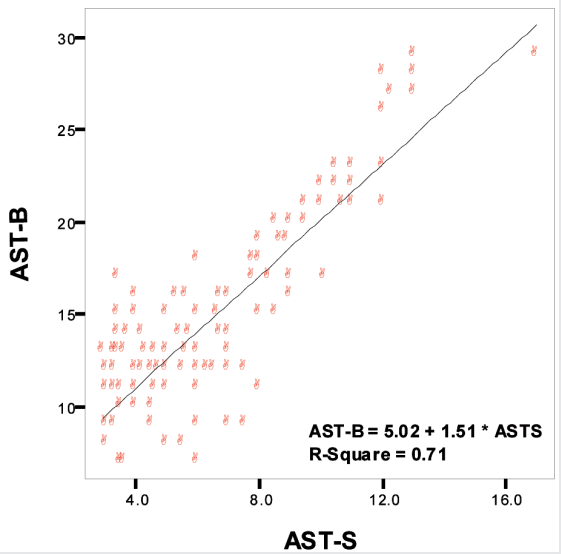
	Group-1A. Alcoholics (n=50)	Group 1B. Abstainers (n = 50)	Group-2 Controls (n =30)
GGT, serum (IU/L)	54.12 ± 15.96* (33.7 – 107)	27.06 ± 9.27 **,*** (17.4 – 53)	12.28 ± 2.34 (7 -16)
GGT, saliva (IU/L)	7.2 ± 1.39* (4.9 – 10.7)	5.44 ± 1.14 **,*** (3.9 – 8.3)	3.41 ± 1.01 (2 – 6)
ALT, serum (IU/L)	21.64 ± 3.10* (15 – 27)	13.16 ± 3.51 **,*** (4 – 22)	10.03 ± 2.61 (5 – 17)
ALT, saliva (IU/L)	11.4 ± 2.48* (7 – 19)	6.74 ± 1.79 **,*** (4 - 11)	3.59 ± 0.65 (2 – 5)
AST, serum (IU/L)	20.64 ± 3.96* (13 – 29)	12.36 ± 2.66 ** ,*** (7 – 18)	8. 34 ± 2.13 (7 -15)
AST, saliva (IU/L)	9.86 ± 2.02* (6.7 – 17)	5.6 ± 1.26 **,*** (3.3 – 8)	3.72 ± 0.69 (2.9 – 5.6)

[Table/Fig 1]: Activities of Enzymes in Serum and Saliva, in Alcoholics, Abstainers and Controls.

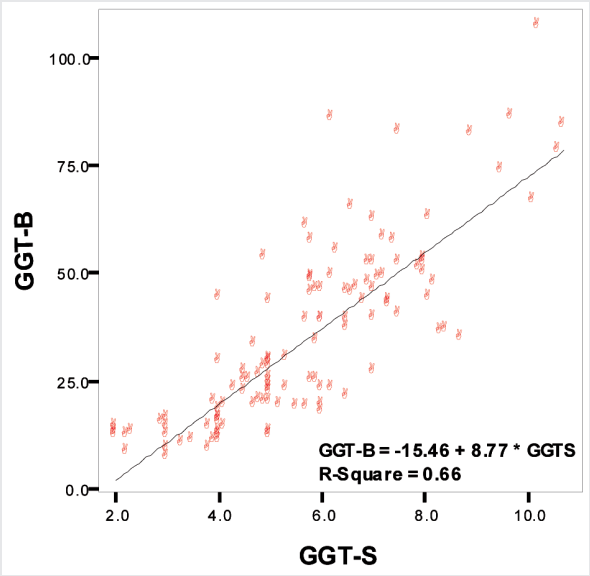
Note: Values are mean ± S.D. of number of samples indicated; range is given in parentheses
*Significance of the difference when alcoholics are compared to controls (p< 0.001; Highly significant);
**Significance of the difference when abstainers are compared to alcoholics (p< 0.001; Highly significant);
*** Significance of the difference when abstainers are compared to controls (p< 0.001; Highly significant).



[Table/Fig 2]: Correlation between serum ALT (ALT-B) and salivary ALT (ALT-S) in alcoholics



[Table/Fig 3]: Correlation between serum AST (AST-B) and salivary AST (AST-S) in alcoholics



[Table/Fig 4]: Correlation between serum GGT (GGT-B) and salivary GGT (GGT-S) in alcoholics

for a minimum of five years and were not suffering from any clinically apparent liver disease and any other systemic illness. The serum and salivary activities of GGT were higher (by 4.4 fold and 2.1 fold respectively) in the alcoholics as compared to that in the healthy, non-alcoholic controls. GGT is induced by alcohol, and the serum levels rise in response to the acute hepatocellular damage. The levels are especially high in patients with severe alcoholic liver disease. When a heavy drinker is denied access to alcohol, any elevation in the GGT levels gradually resolves. It is more likely to be elevated in regular rather than in episodic drinkers. Earlier, Rajagopal and Mohammed Rafi [13] reported that the serum GGT activity increased by 4.3 fold in the alcoholics with liver abscess, in comparison to the 1.6 fold increase in the alcoholics without liver abscess. The activities of the aminotransferases, ALT and AST were elevated in the alcoholics (by 2 fold and 1.8 fold respectively in serum, and by 3.2 fold and 2.6 fold respectively in saliva) in comparison to the controls. The serum ALT and AST levels are known to become elevated in viral hepatitis and toxic hepatitis, cholestasis, cirrhosis, liver carcinoma, and alcoholic liver disease [2-4]. The serum levels of these enzymes depend markedly on the degree of liver damage and on the information as to how recently alcohol was consumed [3]. Alatalo et al. [14] demonstrated that the serum enzyme markers of alcohol abuse and liver function may respond to even rather low levels of ethanol intake.

There is a paucity of studies on the levels of salivary GGT, ALT and AST in alcoholics. The salivary GGT levels showed significant elevation in liver cirrhosis, cholecystitis, hepatic tumours, acute pancreatitis and diabetic keto acidosis. The salivary GGT activities were unmodified in fatty liver, infectious hepatitis and mumps [15]. They were also elevated in chronic smokers [16] and in patients with periodontal diseases [17]. Todorovic et al. [17] reported the increased salivary activities of ALT and AST in periodontal diseases. The salivary AST levels were used for the screening of periodontal diseases by Nomura et al. [18].

Alcohol abstinence, along with the deaddiction regimen caused a significant decrease in the activities of GGT, ALT and AST in serum and saliva. After 30 days of alcohol abstinence, the activities of GGT, ALT and AST in the serum decreased by 2 fold, 1.6 fold and 1.7 fold respectively while the activities of GGT, ALT and AST in the saliva decreased by 1.3 fold, 1.7 fold and 1.8 fold respectively.

The findings of the present study are in accordance with those of an earlier study which was by Alatalo et al. [14], with respect to the serum enzymes GGT, ALT and AST. It was observed that the activities of the enzymes in the abstainers differed significantly from that of the controls. Thus, the enzymes did not reach the normal control levels after one month of alcohol abstinence, but showed an improving trend, as demonstrated by a decrease in their activities when compared to their activities before the abstinence. This indicates that more time is required for the enzymes to reach the normal control levels and thus, they can be used not only as diagnostic markers but also as prognostic markers. This would be helpful in the management of chronic alcoholics.

Saliva, as a diagnostic tool, offers advantages such as the non-invasiveness of the sample collection, the non-necessity of skilled persons for its collection and its suitability for repeated sampling. The present study has demonstrated the increased activities of the enzyme markers in the saliva of chronic alcoholics. There was a significant correlation between the activities of the enzymes in the serum and saliva of alcoholics [Table/Fig 1], [Table/Fig 2-4]. With respect to each enzyme, the direction of the change in their activities in serum and saliva was comparable. The strong correlation between the salivary and serum activities of the enzymes in alcoholics indicates the potential use of saliva as an alternative to blood in the future. As per the literature which is available, no such study correlating the blood and the salivary activities of GGT, ALT and AST in alcoholics, has been carried out previously.

Further studies with a larger sample size, taking into account the type and dosage of the alcohol which is consumed and including alcoholics of both the sexes, with and without any pathological manifestations, are required. Detailed studies are needed before establishing saliva as an alternative to blood, as a laboratory specimen, for its application in clinical medicine.

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REFERENCE

[1] Zakhari S. Overview : how is alcohol metabolized in the body ? *Alcohol Res Health* 2006; 29: 245-255.
[2] Das S.K. Dhanya L., Vasudevan, D.M. Biomarkers of alcoholism: an update and review. *Scan J Clin Lab Invest* 2008; 68: 81-92.
[3] Sharpe PC. Biochemical detection and monitoring of alcohol abuse and abstinence. *Ann Clin Biochem* 2001; 38: 652-664.
[4] Friedel R, Diederichs F, Lindena J. Release and extracellular turn-over of cellular enzyme. In: Advances in Clinical Enzymology (eds. Schmidt E, Schmidt FW, Trauschold I, et al), S.Karger: *Munich*, 1979; pp. 70-105.
[5] Kaufman E, Lamster IB. The diagnostic applications of saliva-a review. *Crit Rev Oral Biol Med* 2002; 13 : 197-212.
[6] Ziang L, Xiao H, Wong DT. Salivary biomarkers for clinical application. *Mol Diagn Ther* 2009;13 : 245-259.
[7] Dutta SK, Orestes M, Vengulekur S, Kwo P. Ethanol and human saliva: effect of chronic alcoholism on flow rate, composition and epidermal growth factor. *Am J Gastroenterol* 1992; 87 : 350-354.
[8] Ponnio M, Alho H, Heinala P, Nikkari ST, Sillanaukee P. Serum and saliva levels of sialic acid are elevated in alcoholics; *Alcohol Clin Exp Res* 2006; 23: 1060-1064.
[9] Navazesh M. Methods for collecting saliva. *Ann NY Acad Sci* 1993; 20:72-74
[10] Committee on enzymes of the Scandinavian society for clinical chemistry and clinical physiology. Recommended method for the

determination of gamma-glutamyl transferase in blood. *Scand J Clin Lab Invest* 1976; 36 ; 119-125.
[11] Bergmeyer HU, Horder M. IFCC methods for the measurement of catalytic concentrations of enzymes. Part 3. IFCC method for alanine aminotransferase. *J Clin Chem Clin Biochem* 1980; 18 : 521-534.
[12] Bergmeyer H, Bowers G, Horder M, Moss DW. IFCC methods for measurements of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. *Clin Chem* 1977; 23: 887-889.
[13] Rajagopal G, Mohammed Rafi KM. Serum gamma glutamyl transferase (γ-GT) activity in alcoholics with liver abscess and chronic alcoholics. *Indian J Clin Biochem* 2005; 20: 198-199.
[14] Alatalo P, Koivisto H, Puukka K, Hietala J, Anttila P, Bloigu R, et al. Biomarkers of liver status in heavy drinkers, moderate drinkers and abstainers. *Alcohol* 2009; 44:199-203.
[15] Jiménez-Alonso J, Jaimez L, Barrios L, Pérez-Jiménez F, Costán G, Jiménez-Perepérez JA. Salivary gamma-glutamyl transferase activity in internal diseases. *Arch Intern Med* 1984 ;144:1804-1806.
[16] Greabu M, Alexandra A, Battino M, Mohora M, Didilescu A, Totan C, et al. Cigarette smoke effect on total salivary antioxidant capacity, salivary glutathione peroxidase and gamma-glutamyltransferase activity. *Biofactors* 2008; 33: 129-136.
[17] Todorovic T, Dozic I, Barreo MV, Ljuskovic B, Pejovic J, Marjanovic M. Salivary enzymes and periodontal disease. *Med Oral Patol Oral Cir Bucal* 2006; 11 : E115-E119.
[18] Nomura Y, Tamaki Y, Taraka T, Arakawa H, Tsurumoto A, Kirimura K, et al. Screening of periodantily with salivary enzyme tests. *J Oral Sci* 2006; 48: 177-183.

AUTHOR(S):
1. Dr. Shivashankara A.R.
2. Dr. Claudia Johnny
3. Dr. Malathi M.
4. Mr. Arun Kumar K.
4. Dr. Avinash S.S.
4. Mr. Toni Thomas

NAME OF DEPARTMENT(S) / INSTITUTION(S) TO WHICH THE WORK IS ATTRIBUTED:
Dept of Biochemistry, Father Muller Medical College, Mangalore - 575002, Karnataka, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shivashankara A.R., Associate Professor of Biochemistry, Father Muller Medical College, Mangalore-575002.
E-mail: sramachandrayya@gmail.com,
Phone: 0824 2238255, 09880146133.

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