

Comparison of Biochemical Parameters in Saliva with Serum of Chronic Alcoholics: Saliva Can be Used as a Diagnostic Tool in the Future

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Abstract: Alcohol has always been recognized as having a significant impact on the health of the public [1]. Firstly, there has been a focus on the increasing impact of alcoholic liver disease (ALD) in the population as a result of heavy drinking [2]. Secondly, there has been a focusing and concern on alcohol consumption related to crime and violence. Thirty male chronic alcoholics with a history of alcohol abuse for 3-21 years and aged 25-65 years, who were admitted to the De-addiction centre for alcohol withdrawal treatment, were the subjects. Thirty age-matched, non-alcoholics were taken as controls in the study. The subjects were divided into 2 group's i.e. Group-I was cases and Group-II was controls. Cases were subdivided into 3 group's i.e. Group-A, Group-B and Group-C. AST, ALT and GGT were assayed in the serum & saliva of thirty chronic alcoholics before, after one and two months post De-addiction treatment. This present study shows increased serum & saliva levels of AST, ALT & GGT in Group-I as compared to Group-II. It was observed that AST, ALT and GGT enzyme activities had significant positive correlation in serum & saliva. Thus, saliva can be used as a diagnostic specimen which has the added advantage over serum as it can be collected repeatedly, in a convenient, minimally invasive manner to make clinical decision & to monitor prognosis.

Keywords: Aspartate Transaminase, Alanine Transaminase, Gamma Glutamyl Transferase, Serum, Saliva, De-addiction treatment.

INTRODUCTION

Alcoholism is an illness characterized by significant impairment that is directly associated with persistent and excessive use of alcohol. Impairment may involve physiological, psychological or social dysfunction.

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[3].

Acute and chronic ethanol consumption has been shown to increase the production of reactive oxygen species, lower cellular antioxidant levels and enhanced oxidative stress in many tissues, especially the liver [4].

The risk factors for developing alcoholic liver disease include duration and magnitude of alcohol ingestion, gender, hepatitis B or C infection, genetic factors and nutritional status. There is evidence that modification of liver proteins by alcohol metabolites is involved in the pathogenesis [5,6].

Biochemical markers have been used for detecting and monitoring alcohol abuse such as serum Alanine transaminase (ALT), Aspartate transaminase (AST) and γ -Glutamyl transferase (GGT). Elevated serum Gamma-Glutamyl transferase (GGT) level remains the most widely used marker of alcohol abuse. Levels typically rise after heavy alcohol intake that has continued for several weeks in response to the acute hepatocellular damage [7].

GGT is a glycoprotein enzyme situated on the cell membrane in several tissues. Hepatic GGT levels

increase in response to exposure to a variety of drugs and alcohol [8]. While GGT can be elevated in the absence of liver damage, it also tends to be the first enzyme elevated in alcohol-induced liver damage. Serum GGT is the most sensitive of these, and the most widely employed marker of alcohol consumption [10].

In most types of liver disease, ALT activity is higher than that of AST; exceptions may be seen in alcoholic hepatitis, hepatic cirrhosis and liver neoplasia. In viral hepatitis and other forms of liver disease associated with acute hepatic necrosis, serum AST and ALT concentrations are elevated even before the clinical signs and symptoms of disease (such as jaundice) appear[9].

Serum AST can be also arises from non-hepatic sites, particularly heart and muscle and levels are increased in conditions such as myocardial infarction and skeletal muscle trauma. The ratio of AST to ALT in serum may help in the diagnosis of some liver diseases. In most patients with acute liver injury, this ratio is 1 or less, whereas in alcoholic hepatitis it is generally about 2 [11]. Deficiency of pyridoxal-5'-phosphate, a necessary coenzyme for both amino transferase are common in alcoholic liver disease. This deficiency decreases hepatic ALT to a greater extent than AST, with corresponding changes in serum concentrations [12].

In humans, saliva originates mainly from three pairs of major salivary glands (parotid, sublingual and submandibular) and from a large number of minor salivary glands. Healthy adult subjects normally produce 500–1500 ml of saliva per day at a rate of approximately 0.5 ml/min [13]. Saliva is a mixture of ions, small organic molecules, enzymes and proteins, some in multi protein complexes and other complexes with bio chemicals [14].

The distribution of ethanol in the body is proportional to water content in the tissue. Therefore, the concentration of ethanol reaching saliva should reflect that found in the water fraction of whole blood. The ratio of blood flow to the tissue mass of the salivary gland is so high that the concentration of ethanol entering saliva should accurately reflect its concentration in blood [15].

MATERIALS & METHODS

This study was carried out in the Department of Biochemistry (2012-14) in association with Department of Psychiatry and De-addiction centre, Kamineni Institute of Medical Sciences and Hospital, Narketpally, Nalgonda district, Telangana, India. The study protocol was approved by the institutional ethics committee. The study subjects were divided into 2

groups i.e. Group-I (cases), were subdivided into 3 groups i.e. Group-A (at the time of admission) Group-B (after 1 month post de-addiction treatment) Group-C (2 months post de-addiction treatment). Group-II (controls) were age and sex-matched, apparently healthy volunteers (n=30) were included in this study.

Thirty chronic alcoholics with a history of alcohol abuse for 3-21 years who were admitted to the de-addiction centre for alcohol withdrawal treatment. They were in the age group of 25-65 years. A detailed history of alcohol intake, clinical complications and the use of tobacco were collected from the subjects; n=30. Occasional drinkers, patients with systemic illness, smokers and tobacco chewers, were excluded from the study. A written informed consent was obtained from each subject.

5ml of random venous blood sample was collected from the all the subjects in a sterile disposable syringe which was transferred into centrifuge tubes and was allowed to clot for 30 minutes. 5ml of unstimulated saliva was collected by the Navazesh method [16] from the 30 alcoholics and 30 controls. Both the serum & saliva samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was collected and samples were stored at -20°C until analyzed.

The levels of AST and ALT were estimated by modified IFCC-UV kinetic method in serum & saliva. The GGT levels in both serum & saliva were estimated by colorimetric method.

RESULTS

The results of this study showed that the activities of the enzymes AST, ALT and GGT in serum and saliva samples of patients with chronic alcoholism were higher in compared to the control (Table no II).

The statistical analysis was performed using SPSS software version 11.0. The descriptive results were expressed as Mean and Standard deviation. Significance of the difference between the patient and control groups observed was assessed by using the student t-test. The p values were expressed along with the Mean values and standard Deviation. The p values less than 0.05 were considered statistically significant, p values less than 0.001 were considered statistically highly significant.

Table No. I show distribution of the subjects into different age groups. All the subjects in the study group were males with maximum number of cases being in age group of 25-35 years (40.66%) followed by 36-45 (30%), 46-55 years (16.66%) and the least number in the age of 56-65 (06.66%).

Table-I: Age distribution in cases and controls (n=60)

| Age group (years) | Cases (n=30) | Controls (n=30) |
|-------------------|--------------|-----------------|
| 25-35 | 14 (40.66%) | 14 (40.66%) |
| 36-45 | 09 (30%) | 09 (30%) |
| 46-55 | 05 (16.66%) | 05 (16.66%) |
| 56-65 | 02 (06.66%) | 02 (06.66%) |
| Total | 30 | 30 |

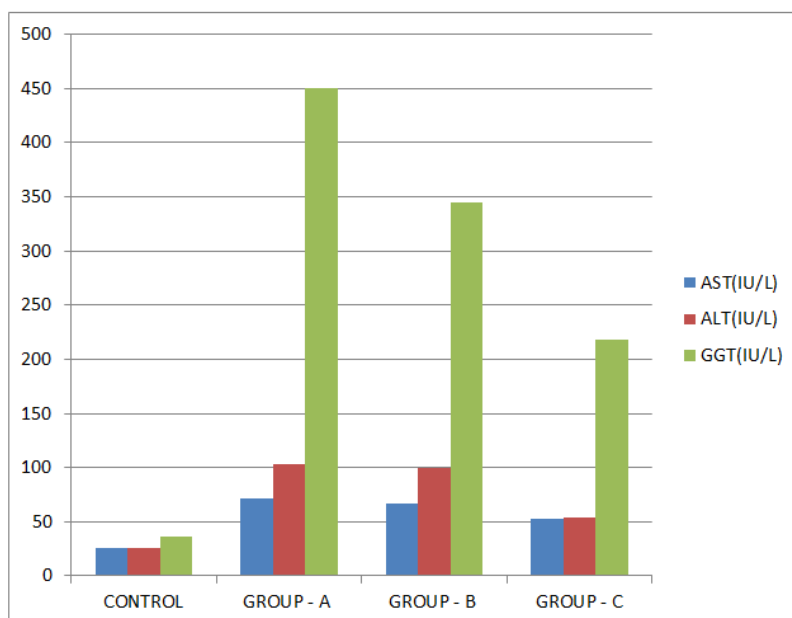
Table-II: Comparison of serum and salivary AST, ALT and GGT levels in group-A, group-B and group-C & controls

| Parameter | | Group-A | Group-B | Group-C | p-value | group-I |
|-----------|-----------------------|---------------|---------------|--------------|----------|------------|
| AST | Serum (Up to 37 IU/l) | 74.03±19.85 | 65.77±18.11 | 52.13±9.57 | <0.001** | 5.87±5.19 |
| | Saliva | 54.37±17.33 | 44.47±12.03 | 32.03±6.62 | <0.001** | 18.0±2.85 |
| ALT | Serum (Up to 40 IU/l) | 117.03±42.19 | 99.70±33.28 | 54.40±12.59 | <0.001** | 25.43±6.36 |
| | Saliva | 87.43±23.01 | 70.40±23.01 | 36.10±10.53 | <0.001** | 22.83±3.78 |
| GGT | Serum (< 55 IU/l) | 405.10±135.39 | 340.27±118.24 | 264.23±98.24 | <0.001** | 36.47±8.97 |
| | Saliva | 322.77±110.02 | 269.57±105.80 | 178.13±92.92 | <0.001** | 30.17±3.92 |

(p < 0.001) ** Highly significant statistically

Table- II shows serum & salivary level of AST, ALT and GGT in cases (At the time of admission), 1 & 2 months post de-addiction treatment as compared to controls. There was a significant

decrease in AST, ALT and GGT on 1 & 2 months post de-addiction treatment when compared to the levels on admission. But the decrease in the levels did not reach the control level.

**Fig- I: Comparison of serum AST, ALT and GGT (Mean±SD) in cases & with controls**

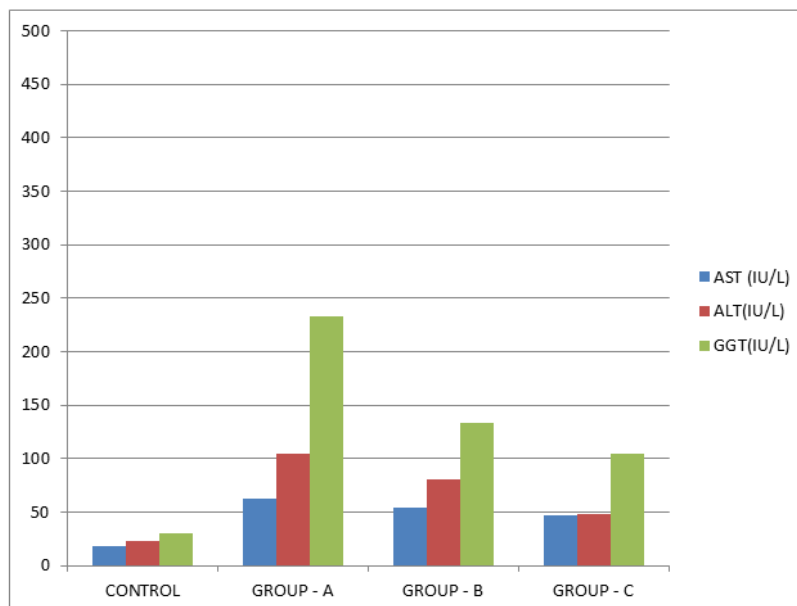


Fig-II: Comparison of salivary AST, ALT and GGT (Mean±SD) in cases & controls

Table-III: Correlation of aspartate transaminase (AST) levels among cases in the different groups in serum & saliva

| Groups | Serum | Saliva | r-value |
|--------|-------------|-------------|---------|
| A | 74.03±19.85 | 54.37±17.33 | 0.961** |
| B | 65.77±18.11 | 44.47±12.03 | 0.880** |
| C | 52.13±9.57 | 32.03±6.62 | 0.786** |

(p < 0.001) ** Highly significant statistically.

Table No. III shows positive correlation was observed between serum & salivary AST activity in chronic alcoholics.

Table No. IV shows positive correlation was observed between serum & salivary ALT activity in chronic alcoholics.

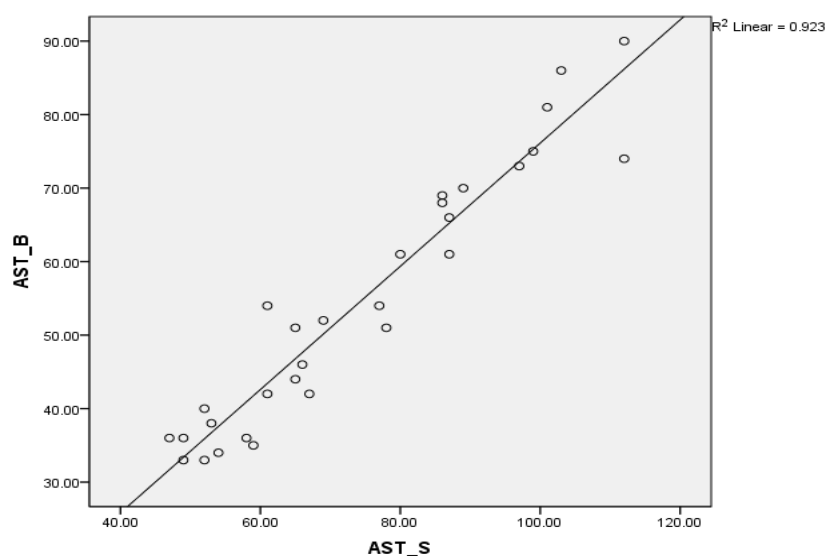
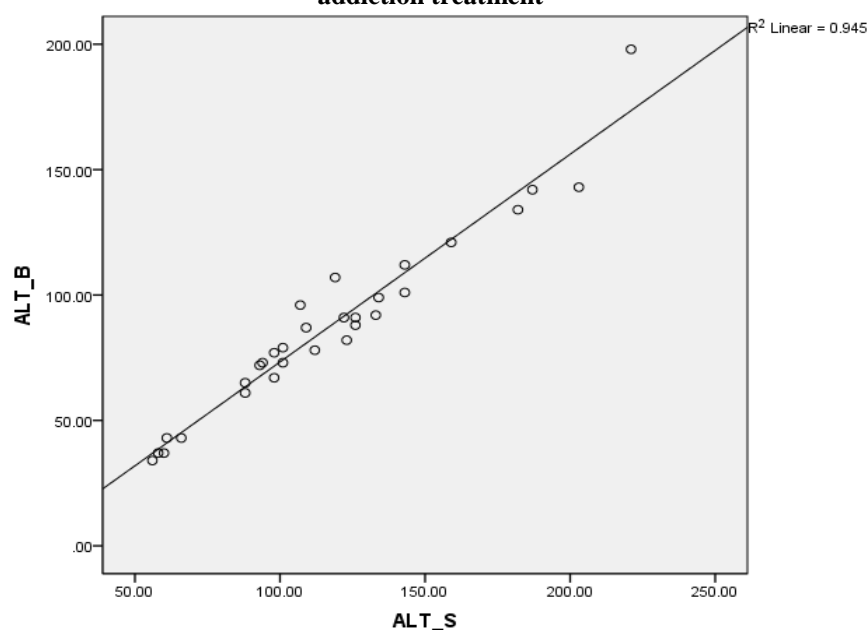


Fig-III: Correlation between serum AST (AST-B) and salivary AST (AST-S) in alcoholics before the start of de-addiction treatment

Table- IV: Correlation of alanine transaminase (ALT) levels among cases in the different groups in serum & saliva

| Groups | Serum | Saliva | r-value |
|--------|--------------|-------------|---------|
| A | 117.03±42.19 | 87.43±23.01 | 0.972** |
| B | 99.70±33.28 | 70.40±23.01 | 0.911** |
| C | 54.40±12.59 | 36.10±10.53 | 0.894** |

(p < 0.001) ** Highly significant statistically.

Fig-IV: Correlation between serum ALT (ALT-B) and salivary ALT (ALT-S) in alcoholics before the start of de-addiction treatment**Table-V: Correlation of gamma glutamyl transferase (GGT) levels among cases in the different groups in serum & saliva**

| Groups | Serum | Saliva | r-value |
|--------|---------------|---------------|---------|
| A | 405.10±135.39 | 322.77±110.02 | 0.969** |
| B | 340.27±118.24 | 269.23±98.24 | 0.994** |
| C | 264.23±98.24 | 178.13±92.92 | 0.979** |

(p < 0.001) ** Highly significant statistically.

Table No. V shows positive correlation observed between serum & salivary GGT activity in chronic alcoholics.

DISCUSSION

Alcoholism is a serious health problem with increased mortality & morbidity and most important avoidable cause of death in world. Diseases that are directly affected by excessive alcohol use include many forms of cancer, hypertension and cardiovascular disease, liver cirrhosis, prenatal exposure to alcohol and

related conditions, digestive disorders, and psychiatric problems.

The enzymes AST, ALT and GGT are the traditional markers of alcoholism and alcoholic liver disease. The present study analyzed the effect of chronic alcoholism on the activities of enzymes AST, ALT and GGT in serum and saliva. An attempt was made to assess the activities of these enzymes i.e. AST, ALT and GGT after one and two months of alcohol de-addiction

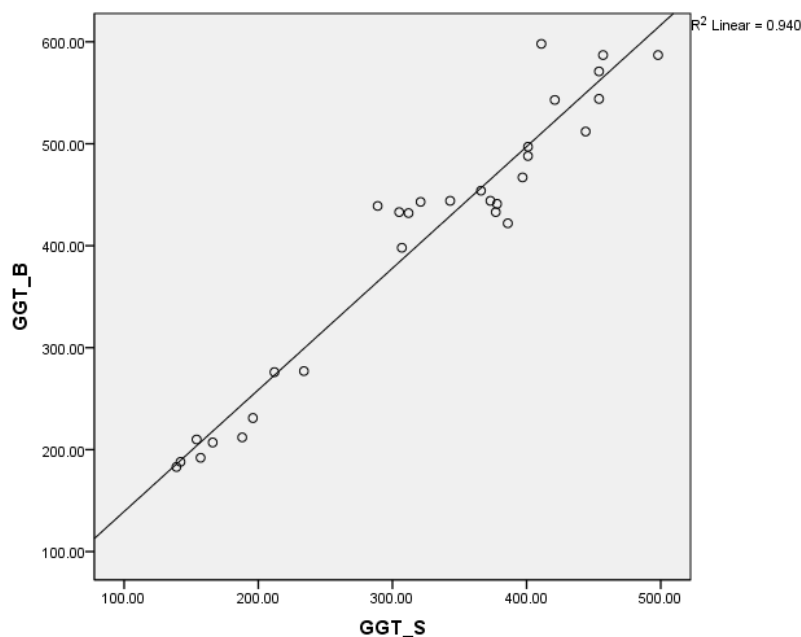


Fig-V: Correlation between serum GGT (GGT-B) and salivary GGT (GGT-S) in alcoholics before the start of de-addiction treatment

The activity of aminotransferase AST was elevated in all subgroups of chronic alcoholics by 2.8 fold, 2.5 fold, 2 fold respectively in serum and by 3 fold, 2.4 fold, 1.8 fold respectively in saliva as shown in Table No. II. The activity of aminotransferase ALT was elevated in all subgroups of chronic alcoholics by 4.6 fold, 3.9 fold, 2.1 fold respectively in serum and by 3.8 fold, 3 fold, 1.5 fold respectively in saliva in comparison to controls as shown in Table No. II

Serum AST, ALT levels are known to be elevated in toxic hepatitis, viral hepatitis, cirrhosis, carcinoma, alcoholic liver disease. The levels of these enzymes depend markedly on the degree of liver damage and duration of alcohol consumption. Altalo *et al.* [17] demonstrated that serum enzyme levels of alcohol abuse and liver function may respond to even rather low levels of alcohol intake.

The activity of GGT was elevated in all subgroups of chronic alcoholics by 11.1 fold, 9.3 fold, 5.9 fold respectively in serum and by 10.6 fold, 9.5 fold, 6.8 fold respectively in saliva in comparison to controls as shown in Table No. II. GGT induced by alcohol & serum levels raise in response to hepatocellular damage. The levels are especially very high in severe alcoholic liver disease but when a chronic alcoholic abstained from alcohol consumption any elevation in GGT levels gradually resolve.

Earlier, Rajagopal and Mohammed Rafi [18] 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.

Alcohol abstinence, along with the de-addiction regimen caused a significant decrease in the activities of AST, ALT and GGT in serum and saliva. After 1 month of alcohol abstinence, the activities of AST, ALT and GGT in the serum decreased by 2.5 fold, 3.9 fold and 9.3 fold respectively while the activities of AST, ALT and GGT in the saliva decreased by 2.4 fold, 3 fold and 9.5 fold respectively as shown in Table No. II.

Alcohol abstinence, along with the de-addiction regimen caused a significant decrease in the activities of AST, ALT and GGT in serum and saliva. After 2 months of alcohol abstinence, the activities of AST, ALT and GGT in the serum decreased by 2 fold, 2.1 fold and 5.9 fold respectively while the activities of AST, ALT and GGT in the saliva decreased by 1.8 fold, 1.5 fold and 6.8 fold respectively as shown in Table No. II

The findings of the present study are in accordance with those of an earlier study which was by Alatalo *et al.* [17] with respect to the serum enzymes AST, ALT and GGT. 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 and in prevention of worsening of alcoholic liver disease.

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 serum and saliva of alcoholics as shown in Table No.III, Table No. IV & Table No. V.

CONCLUSION

The study was conducted to compare and correlate the Aminotransferases (AST & ALT) and Gamma Glutamyl Transferase (GGT) in serum and saliva. It was observed that all these enzyme activities had significant positive correlation in serum & saliva. Thus, saliva can be used as a diagnostic specimen which has the added advantage over serum as it can be collected repeatedly, in a convenient, minimally invasive manner to make clinical decision & to monitor prognosis.

The salivary estimation is an upcoming area of research for basic and clinical application purposes with considerable potential for growth and progress. It is usable for quantitative measurements of several analytes, particularly when stable correlation between serum and salivary levels is achieved.

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