

Title Page

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Title: Comparison of efficacy of salivary Gamma-glutamyl Transferase levels with serum Gamma-glutamyl Transferase levels in chronic alcoholics in Puducherry population.

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Title: Comparison of efficacy of salivary Gamma-glutamyl Transferase levels with serum Gamma-glutamyl Transferase levels in chronic alcoholics in Puducherry population.

ABSTRACT

Aims & Objective:

Alcoholism or also called as alcohol dependence, is a disorder that is addictive and disabling. Estimation of alcohol dependence can be done by assessing the levels of serum Gamma-glutamyl Transferase (GGT).

The present study was done to compare the efficacy of salivary GGT levels with serum GGT levels in chronic alcoholics in Puducherry population and to establish a normal reference value of GGT in saliva.

Methods and Material:

Unstimulated whole saliva and venous blood was collected from the groups under study and subjected to biochemical (spectrophotometric) analysis for determination of GGT levels.

Statistical Analysis used:

The salivary GGT levels were correlated with the Serum GGT levels by *Pearson correlation* (SPSS software version 16).

Results:

The activity of GGT in the saliva as well as in the serum was significantly higher in the alcoholics and there was a positive correlation between the serum and the salivary levels of GGT in the alcoholics.

Conclusion & Clinical significance:

The strong correlation between the salivary and serum activities of GGT in chronic alcoholics indicates the future

potential of saliva as an alternate diagnostic tool.

Keywords: Alcoholism, chronic alcoholics, correlation, Gamma-glutamyl Transferase, Saliva, Serum.

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INTRODUCTION

Alcoholism has become global problem with major socio-economic implications. Alcoholism, or alcohol dependence, is a psychiatric disorder which is multi-factorial that makes a person to crave for a drink, lose control on number of drinks once started, become physically dependant and tolerant (feel the need to consume higher amount of alcohol to feel the same effect).^[1]

Consumption of alcohol continuously (chronic) leads to harmful effects on normal physiology and various organs. Liver plays an important role in metabolism of alcohol (primary site). Alcohol is metabolised in liver to produce harmful products which includes free radicals and acetaldehyde that induces hepatocellular damage leading to fatty liver, alcoholic hepatitis and alcoholic cirrhosis.^[2]

The diagnosis of an alcohol problem is best made by the history. One of the screening questionnaires developed by World health Organization (WHO) is the AUDIT (alcohol use disorders identification test) which can be given as a paper and pencil test.^[3]

The marker that is accepted for liver disorders and excessive alcohol consumption is GGT. It is a glycoenzyme that is found in various organs in their endothelial cell membranes and plays a role in metabolism of glutathione and transport of peptides. GGT is used in the diagnosis and monitoring of hepatobiliary diseases, enzymatic activity of GGT is often the only parameter with increased values when testing for such diseases, and is one of the sensitive indicators known. Gamma-glutamyl transferase is also a sensitive screening test for occult alcoholism.^[4]

The plasma concentration and the salivary concentration of any substance need to have direct correlation to test the reliability of salivary diagnosis. The physical and chemical properties of the substances play an important role in their transfer into saliva from serum.^[5]

Very few studies have been done to establish salivary GGT as an alternative diagnostic tool.

The present study aims at establishing a reference value of GGT in saliva in Puducherry population and to find a positive correlation between serum and salivary GGT levels, thereby establishing saliva as an effective alternate tool for serum.

METHODS

The study was conducted out at deaddiction center, Puducherry and the

protocol was approved by the Institutional Review Board and Institutional Ethical Committee. The study had two groups:

Group I –Teetotaler (Healthy controls) – 40 subjects

Group II -Subjects with history of chronic alcoholism (as substantiated by AUDIT) (Alcoholics) – 40 subjects

The tools used for selection of patient are

- i) Alcoholism assessed by Clinical Assessment (ICD-10 coding),^[6]
- ii) Standard Questionnaire (AUDIT)^[7] and
- iii) Standard Biological markers (Serum Gamma-glutamyl Transferase) as performed routinely for alcoholics.^[8, 9]

Subjects with history of alcoholism for more than 2 years with AUDIT score of more than 8 and in age group of 40 to 60 years were included in this study. Females, Subjects with Diabetes mellitus, Congestive Heart disease, Carcinoma, Cirrhosis of liver, medication for epilepsy, previous history of de-addiction therapy Obesity and Chronic smoking were excluded from the study.

Clinical diagnosis of alcoholism was done by the psychiatrist. Detailed alcohol intake history, clinical complications and tobacco usage history were collected from the subjects; (n=40). Informed consent was obtained from each subject.

Unstimulated whole salivary sample was collected in between 8 am -12 pm based on the guidelines given by Navezesh.^[10] The individuals were asked to refrain from eating, drinking (except water), tooth brushing and physical exercises or be under great physical stress for at least 1 hour prior to sample collection. The subjects were instructed to wash their mouths thoroughly with distilled water and asked to sit in a relaxed position for 5minutes. The saliva was then

allowed to accumulate in the mouth and was asked to expectorate in to a sterile plastic container for every 60 seconds over a period of 5 minutes.^[11] Collected sample was then transported to the Clinical Biochemistry laboratory immediately for processing. All the processing was carefully monitored by a trained Biochemist. Blood of quantity 5 ml was collected under aseptic conditions and centrifugation was done to separate the serum.

Salivary samples were centrifuged at 3200 rpm for 10 minutes; supernatant fluid was collected and subjected to GGT assay. Salivary Gamma-glutamyl Transferase level was estimated by Kinetic photometric test (Reference)/ Szasz method. The test has been standardized to the method according to IFCC (International Federation of Clinical Chemistry). GGT catalyses the transfer of glutamic acid to acceptors like glycylglycine. This process releases 5-amino-2-nitrobenzoate which can be measured at 405nm (increasing intensity of yellow color). The increase in absorbance at this wavelength is directly related to the activity of GGT.^[12]

Using Statistical Package for the Social Sciences (SPSS 16) values obtained were expressed in Mean \pm SD and unpaired student t-test was used to compare the salivary GGT levels of Group I (Controls) and Group II (chronic alcoholics). Pearson Correlation was used to see linear relationship between Salivary GGT levels and Serum GGT levels of chronic alcoholics.

RESULT

While comparing the unstimulated whole salivary GGT level was found to be increased in group II than in group I. There was nearly threefold increase of Salivary GGT in chronic alcoholics compared to

controls [Table 1]. There was a significant correlation between the serum and salivary activities in alcoholics ($r^2=0.379$) [Chart 1]. All the results were statistically significant ($P < 0.001$).

TABLE 1

Activity of GGT in Saliva and serum of controls and alcoholics

	Sample size	Group I (Controls)	Group II (Alcoholics)
Salivary GGT	40	3.73 \pm 0.98* (2.1-5.4)**	10.41 \pm 1.97 (6.9-14.1)
Serum GGT	40	17.8 \pm 2.4 (14.8-23.1)	79.38 \pm 12.3 (62-115)

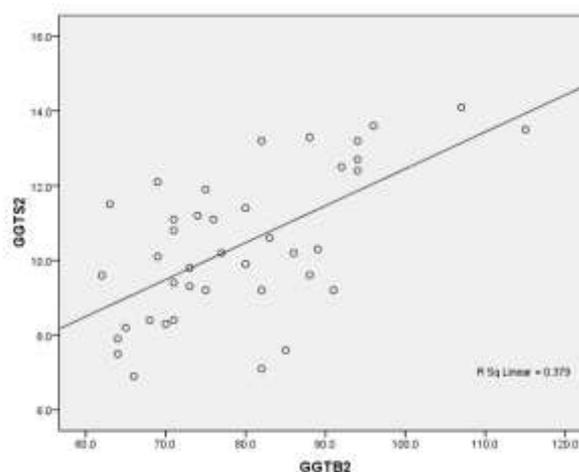
Note:

*Values are mean \pm S.D. of number of samples indicated;

**Range is given in parentheses

CHART 1

Correlation between serum GGT (GGTB2) and salivary GGT (GGTS2) in alcoholics



DISCUSSION

Prevalence rate of consumption of alcohol in the coastal area of Puducherry was 59.6 per cent among adult men. It is very high compared to prevalence rate at national level which stands at 21 per cent among men and 2 per cent among women (National Survey on the Extent, Pattern and Trends of Drug Abuse in India, a joint initiative of the United Nations Office on Drugs and Crimes and the Ministry of Social Justice and Empowerment).^[13]

The damage that has occurred cannot be easily comprehended by an individual until symptoms appear. The extent of liver damage is assessed early in blood and urine by Blood alcohol content (BAC), liver function tests (LFT) and enlarged mean corpuscular volume (macrocytosis). Though these tests are considered as biological markers for alcoholism, they are not considered effective as that of screening questionnaires.^[14]

Screening questionnaires are used to assess the individual's alcoholic status and the problems related to alcoholism. The questions are asked before any definitive treatment has been given or as part of follow-up. AUDIT is one such questionnaire developed by World Health Organisation to identify early signs of extreme drinking and level of dependence. It is highly sensitive (92%) and specific (94%).^[15]

Many liver enzymes are assessed to determine the condition or status of the liver. One such enzyme measured in these tests is the levels of Gamma-glutamyl Transferase which is the most commonly and widely used marker for alcoholism.^[16]

Elevated levels indicate that an individual has been consuming alcohol continuously for some times (weeks). It is documented that when a heavy drinker stops consuming alcohol, the GGT levels come down gradually. Laboratory investigations to evaluate the levels of GGT are easily available and least expensive compared to other markers. GGT is also elevated in other conditions such as diabetes mellitus, congestive cardiac failure, obesity, hypertension, cancer, hepatobiliary diseases and hypertriglyceridemia.^[17]

Saliva is an interesting and unique fluid which has seen increased amount of interest in recent years. It constitutes a wide variety of micro and macromolecules that have their origin from numerous local and system sources. The individual's well being and health is reflected in his/her saliva. Though disadvantages are present in using saliva as a diagnostic tool (minimal understanding of salivary bio molecules, their connection to cause of the disease and non-availability of systems that are highly sensitivity), there are advantages of using saliva in its collection, storage and shipping. It is also economical and non-invasive which makes it suitable for sampling multiple times. In literature, very few studies have been done to evaluate the levels of GGT in saliva of chronic alcoholics.^[18]

Hence, the present study was conducted to assess the salivary Gamma-glutamyl Transferase levels in chronic alcoholics. The alcoholics chosen for the study were individuals who consumed alcohol for a minimum of 2 years and with no clinical symptoms of any liver disease or any other systemic conditions.^[19]

To analyse the above mentioned parameter, whole unstimulated salivary samples were collected between 8 am to 2 pm, in order to avoid circadian variations.

All the salivary samples were collected according to Navazesh's spitting method for whole saliva.^[10] The age range of individuals chosen for the study was 40-60 years males as there was significant rise in GGT levels in this age range.^[20]

In present study we found statistically significant increase in Salivary GGT levels between group I (controls) & group II (chronic alcoholics) ($p = \leq 0.05$). The mean and standard deviation of group I & II were 3.73 ± 0.98 and 10.41 ± 1.97 respectively. There was nearly threefold increase of Salivary GGT in chronic alcoholics compared to controls [Table 1].

In present study we found positive correlation in Salivary GGT levels and serum GGT levels in chronic alcoholics. The mean and standard deviation were 10.41 ± 1.97 and 79.38 ± 12.3 respectively [Chart 1].

Recent study by Shivashankara et al. has found that the levels of serum GGT were increased by 4.4 times and salivary GGT by 2.1 times in the alcoholics when compared to that in the healthy and non-alcoholic individuals.^[21] Rajagopal et al. in a study found that the serum GGT activity was increased by 4.3 times in the alcoholics with liver abscess when compared to that of 1.6 times increase in the alcoholics without liver abscess.^[22] Alatalo et al. showed in case of chronic alcoholism even a small amount of alcohol intake could increase the levels of GGT in serum.^[23]

Dabra et al. in their study found out that due to destruction of periodontal apparatus there was increase in salivary levels of enzymes (GGT, AST, ALP, ALT and ACP). The levels of these enzymes are raised statistically in the saliva of patients with gingivitis and periodontitis, as compared to the controls, and its value decreases after periodontal therapy. The

raised levels in diseased individuals is probably a consequence of the pathological processes in periodontal tissues, from where these intracellular enzymes are increasingly released into the secretion that surrounds them (saliva) and the decrease in the activity of these enzymes after periodontal treatment is probably a result of periodontal tissue repair.^[24]

Todorovic et al. in their study concluded that the activities of CK, LDH, AST, ALT, GGT, ALP and ACP enzymes were significantly increased in the saliva of patients with periodontal disease in relation to those healthy.^[25]

Brietling et al. concluded in their epidemiological that smoking and alcohol consumption at intensities that are common in the general population interact with each other to enhance their elevating effects on serum GGT. Because of the high concurrence of these major avoidable risk factors, this finding has important public health and clinical relevance.^[19]

Future studies with large sample size, strict exclusion criteria, inclusion of both sexes, setting the guidelines should be done to finally realise the true potential of saliva as alternate diagnostic tool.

CONCLUSION

The present study has shown that in chronic alcoholics there is increased activity of GGT in saliva. Significant correlation was evident in saliva and serum of alcoholics between the GGT activities and the direction of change in the activity. Thus saliva can be considered as an alternate diagnostic tool to blood for the assessment of GGT in alcoholic individuals.

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