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Original Article

Biochemistry Section

Effect of Alcohol Withdrawl on Glutathione S-transferase, Total Antioxidant Capacity and Amylase in Blood and Saliva of Alcohol-Dependent Males

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ABSTRACT

Introduction: Alcohol biomarkers help in the early detection of alcoholism and its complications. There is a paucity of studies in India on the salivary markers of systemic diseases in general and on salivary alcohol biomarkers in particular.

Objectives: The present study was aimed at assessing the effect of alcohol withdrawal on the antioxidants and amylase in blood and saliva, and at finding the correlation between the blood and the salivary parameters in alcoholics.

Methods: Sixty alcohol-dependent males who were in the age group of 30 – 70 years, who were admitted to the Deaddiction Centre for alcohol withdrawal treatment for one month, were the subjects of this study; age-matched healthy individuals were the controls. In the blood and saliva samples, the activities of Glutathione S-Transferase (GST) and amylase and the Total Antioxidant Capacity (TAC) were assayed.

Results: The alcohol-dependent subjects showed significantly lower GST and amylase activities and the TAC in blood and saliva as compared to those in the controls ($P<0.001$). The alcohol withdrawal caused a significant increase in the GST and amylase activities and the TAC to near-control values. In the alcohol-dependent subjects, there was a significant correlation between the values in blood and saliva with respect to GST and TAC.

Conclusions: Alcoholism causes an impaired antioxidant capacity and a decreased secretion of amylase, which is ameliorated due to the alcohol withdrawal regimen . The strong correlation between blood and saliva with respect to the antioxidants suggests the potential future use of saliva as a laboratory tool in clinical medicine.

Key Words: Alcoholism, Amylase, Antioxidants, Glutathione S-transferase, Saliva

INTRODUCTION

Alcoholism is a serious public health problem which has profound consequences not only on the health and the socioeconomic status of individuals but also, on the society as a whole. The long term abuse of alcohol causes detrimental effects on almost every organ of the body, and these effects are due to the impairment of the metabolic pathways, the generation of reactive oxygen species and the consequent oxidative stress, and impairment /induction of the detoxifying enzymes [1]. Oxidative stress is proposed to be the key molecular process which is involved in the pathogenesis of the complications of chronic alcoholism [1, 2].

Alcohol biomarkers help in the early detection of alcoholism and its complications. Biochemical parameters such as carbohydrate-deficient transferrin, Gamma Glutamyl Transferase (GGT), aminotransferases and markers of oxidative stress have been tried and used in the diagnosis of alcoholism and its complications [3]. Only few studies have reported the amelioration of alcohol-induced biochemical and clinical manifestations which occur on alcohol withdrawal, in alcohol-dependent individuals [4, 5]. The amylase activity in serum was found to be altered in alcoholics with or without pancreatic disorders [6].

Saliva is an underused diagnostic tool. The use of saliva in the clinical diagnosis has distinct advantages such as the non-invasiveness of its collection, the non-necessity of skilled technicians for its collection and its suitability for repeated sampling [7]. Researchers have attempted to analyze the salivary chemical constituents in oral diseases, cancer, alcoholism, endocrine disorders and also in therapeutic drug monitoring [7]. There is a paucity of studies on the salivary biomarkers of alcoholism, and studies which have correlated the salivary changes with those of blood in alcoholics [5, 8, 9]. The present study made an attempt to assess the effect of alcohol withdrawal on the antioxidants in blood and saliva. We also aimed at assessing the correlation between blood and saliva with respect to glutathione S-transferase, amylase and the total antioxidant capacity.

MATERIALS AND METHODS

The present study was done at Father Muller Medical College Hospital, Mangalore, Karnataka, India. The study protocol was approved by the ethics committee of the institution. Male chronic alcoholics who were admitted to the Deaddiction Centre for alcohol withdrawal treatment, were the subjects of the study (group 1A). They were in the age group of 30–70 years, who were drinking alcohol for three years or more, and were diagnosed of alcohol dependence by the treating psychiatrist. A detailed history of the alcohol intake, the clinical complications and the use of tobacco was taken. The same subjects were followed up after an alcohol withdrawal of one month (group 1B). Age-matched, apparently healthy, non-alcoholic male volunteers were included as the controls in this study (group 2). Occasional drinkers, chronic smokers, tobacco chewers, and patients with any systemic dis-

eases were excluded. Voluntary informed consents were taken from all the subjects of the groups 1A ,1B and 2.

From all the subjects, blood and saliva samples were collected. Five ml. of blood was collected between 9am -11am (at least one hour after breakfast, routine sampling) in EDTA / plain vacutainers by taking aseptic precautions and it was centrifuged to separate the plasma/serum and cells. From the EDTA-blood, haemolysates were prepared for the assay of Glutathione S-Transferase (GST). The Total Antioxidant Capacity (TAC) was estimated in plasma, and amylase was assayed in serum. Unstimulated whole saliva samples were collected according to the method of Navazes [10]. The samples were collected between 9am – 11am (at least one hour after breakfast, routine sampling). 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, avoiding forcible spitting. The saliva samples were centrifuged at 3000 rpm for 15 minutes and the supernatants were collected and taken for the assays of GST, TAC and amylase. The activity of GST was assayed by the method of Habig et al., which is based on the conjugation of glutathione with 1-chloro, 2,4-dintrobenzoic acid [11]. The Total Antioxidant Capacity (TAC) was estimated by using the method of Koracevic et al., which is based on the suppression of the formation of thiobarbituric acid-reactive substances by the antioxidants in blood or saliva [12]. The assay of the salivary and blood amylase was done by a kinetic photometric method which is based on the hydrolysis of 2–chloro–4 nitro phenyl galactopyranosyl maltoside [13]. The data were evaluated by using the Student's paired "t" test and Karl Pearson's Correlation Analysis.

RESULTS

The results of this study have been presented [Table/Fig-1]. In alcoholics, the activities of GST and amylase and the TAC in blood and saliva, were significantly lower as compared to those in the controls. After an alcohol withdrawal of one month, these activities had increased to near-control values. With respect to the GST in blood, and the TAC and amylase in saliva, the values differed significantly between the alcoholics after the alcohol withdrawal and the controls. With respect to the salivary GST and the blood TAC and amylase, there was no statistically significant difference between the alcoholics after the alcohol withdrawal and the controls. In alcoholics (group 1A), there was significant correlation between the blood and salivary values with respect to GST (r= 0.844; p=0.005) and TAC (r=0.858; p=0.001) [Table/Fig-2 & 3] but, no significant correlation was seen in case of amylase.

	Group-1A. 1A. Alcoholics; Before withdrawl (n=60)	1B. Alcoholics; After withdrawl (n=60)	Group-2 Controls (n =30)
GST, Hemolysate (IU/g Hb)[a]	2.92 ± 0.25 *	3.42 ± 0.22 *,**	3.54± 0.15
GST,saliva (IU/L)[a]	0.97± 0.16 *	1.33 ± 0.30 NS,**	1.37 ± 0.36
TAC, Plasma (mmol/L)	1.95 ± 0.37 *	3.62 ± 0.55 NS,**	3.98 ± 0.63
TAC,saliva (mmol/L)	0.66 ± 0.28 *	1.39 ± 0.21*, **	1.54 ± 0.19
Amylase, Serum (IU/L)[a]	101 ± 15.3*	139 ± 6.3 NS,**	143 ± 15.6
Amylase, Saliva (IU/L)[a]	112 ± 16*	154 ± 16 *,**	165 ± 12

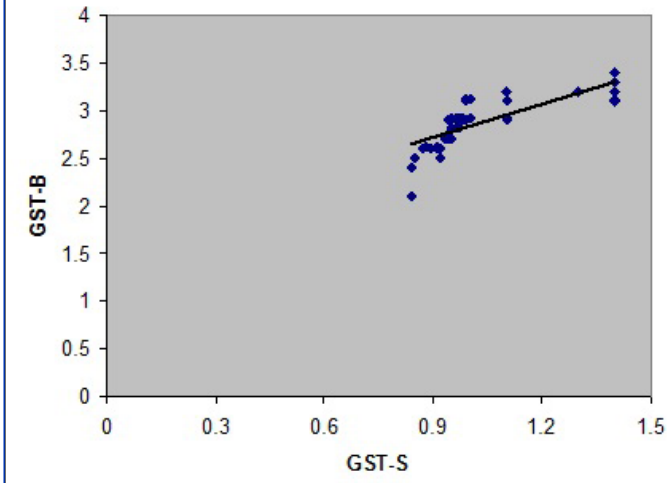
[Table/Fig-1]: Activity of GST and TAC in blood and saliva.

[a] One international unit of enzyme activity (IU) is defined as the enzyme activity which converts one micromole of the substrate to product in one minute, under standard reaction conditions.

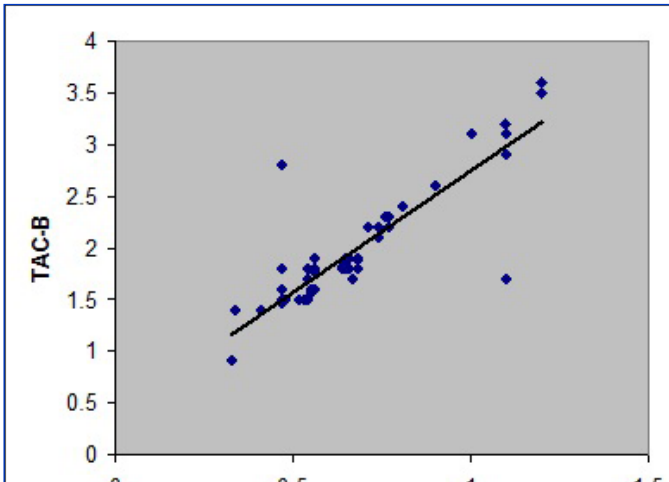
* Significant difference from controls, P < 0.001

NS: No significant difference from controls.

** Significant difference from alcoholics before withdrawl (1A), P <0.001.



[Table/Fig-2]: Correlation Between GST in Blood (GST-B) and Saliva (GST-S) in Chronic Alcoholics (r = 0.844; p= 0.005).



[Table/Fig-3]: Correlation Between TAC in Blood (TAC-B) and Saliva (TAC-S) in Chronic Alcoholics (r = 0.858; p=0.001).

DISCUSSION

The present study showed a significant oxidative stress and the impairment of antioxidants in alcoholism, as were indicated by a decreased activity of GST and a decreased TAC in alcoholics, in comparison to those in the healthy controls. Alcohol is known to induce oxidative stress. Alcohol consumption is associated with a number of changes in the cell functions and the oxidant-antioxidant system [1, 2]. An increased oxidation of lipids and proteins, and impaired activities of antioxidants, were observed in the blood and tissues of the experimental animals, and in the blood of chronic alcoholics [4, 8, 14 –19].

GST is a phase II detoxifying enzyme, and an antioxidant de-

fense mechanism. Various studies have reported the association of the GST M1 "null" genotype with alcoholic liver disease [20]. A meta analysis which was done by Marcos et al., revealed a significant association between the possession of the GST M1 and the GST P1 genotypes, and the presence of alcoholic liver disease in alcoholic patients [21]. Earlier studies have observed a decreased GSH activity (the cosubstrate of GST) [8, 15, 19] and either decreased [8, 15, 19] or increased [22, 23] GST activities in the blood of chronic alcoholics. The Total Antioxidant Activity (TAC) assay measures the nonenzymatic antioxidants such as uric acid, vitamin C and vitamin E in plasma or saliva [12]. Previous studies have observed a decreased TAC in the serum and brain of rats on chronic alcohol exposure [24], while studies on humans have revealed no significant change in the TAC in plasma [25] and the myocardium [26]. Decreased GST activities and TAC which were observed in the present study suggest an increased generation of free radicals due to alcohol consumption, and the consequent utilization of antioxidants in combating the reactive oxygen species.

Various studies have attempted to analyze the effect of alcohol withdrawal on the biochemical parameters. Our earlier studies have demonstrated a significant amelioration of the increased aminotransferases and gamma glutamyl transferase in chronic alcoholics, on an alcohol withdrawal period of one month [5]. We also observed the amelioration of elevated malondialdehyde and decreased SOD and GSH after the alcohol withdrawal [4]. The present study has shown a significant normalization of GST, amylase and TAC in the blood and saliva on the alcohol withdrawal treatment of one month. Muttigi et al., observed a reduction in the increased GST activity in plasma after one month of alcohol withdrawal in chronic alcoholics [22]. However, few authors did not observe any significant change in the antioxidants after an alcohol withdrawal of one week [27], or two weeks [28]. The amelioration of GST and TAC after an alcohol withdrawal of one month suggests that an improved antioxidant status which is obtained, once the source of the free radical generation is removed, the antioxidant levels attain the normal status. The process of oxidative stress appeared to be reversible and the withdrawal period of one month caused significant improvement in the antioxidant status of the body, but which was not enough to bring the levels to the control (normal) levels.

With respect to amylase, there were contradictory findings. In alcoholics with pancreatic disorders, the serum amylase levels were higher than the normal levels, and they decreased after the alcohol abstinence [7]. In alcoholics without pancreatic disorders, the serum amylase levels were either low or normal, and they increased after the alcohol abstinence [7]. In the present study, the alcoholic males did not have any pancreatic disorders, and the decreased blood and salivary amylase levels may suggest a decreased amylase secretion. The increase in the levels after the alcohol abstinence suggested amelioration of the alcoholic manifestations and improved salivary gland secretory functions. There are studies which have reported an increase in the amylase levels in blood and saliva in habitual drinkers [29].

Saliva is equipped with enzymatic and non enzymatic antioxidants [30]. There are few studies which have reported an altered salivary oxidant-antioxidant status in alcoholics [4, 8]. In this study, we assessed the correlation between the salivary and blood parameters with respect to the antioxidants. We observed a significant correlation between the activities in blood and saliva with respect to GST and TAC, in alcoholics. Salivary amylase showed significant changes in alcoholics, but without any correlation with serum amylase. Lot of attention has been given to the salivary chemical constituents as the biomarkers of systemic diseases, in the recent two decades. Due to the advantages in the collection of saliva, and the encouraging results which were obtained in salivary studies, saliva deserves future attention as a body fluid which is a complimentary and an alternative sample to blood in the laboratory diagnosis of diseases and its analysis can be used as a diagnostic tool.

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