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Salivary Trace Elements, Antioxidant Capacity and Albumin in Alcohol Dependence

Prajna D.Silva[1], Ramlingareddy[2] and Shivashankara AR[3*]

[1]Final MBBS Student, Father Muller Medical College, Mangalore–575002., Karnataka, India
[2]Assistant Professor of Biochemistry, Father Muller Medical College, Mangalore–575002., Karnataka, India
*[*3]Associate Professor of Biochemistry, Father Muller Medical College, Mangalore–575002., Karnataka, India*
E-mail: sramachandrayya@gmail.com, arshiva72@gmail.com.

Abstract

Background: Biochemical parameters in blood have been assayed as biomarkers of alcoholism. Saliva is an underused diagnostic tool. There is paucity of studies on salivary markers in general, and salivary alcohol biomarkers in particular.
Materials and Methods: Forty five alcohol dependent males aged 30 to 60 years, admitted to Deaddiction Center of Medical College Hospital, were the subjects of the study. These alcohol dependent males were followed up for thirty days of deaddiction treatment. Normal, age-matched , healthy males were included as controls. Blood and saliva samples were collected taking aseptic precautions. Levels of copper, iron, total antioxidant capacity and albumin were estimated in serum and saliva by standard photometric methods.
Results: The levels of albumin in serum and saliva in alcoholics (before withdrawal) were significantly lower when compared to controls. Levels of copper and iron in serum and saliva were significantly higher in comparison to controls. Total antioxidant capacity (TAC) in serum and saliva was significantly lower in alcoholics in comparison to controls. After the alcohol withdrawal and deaddiction treatment of thirty days, there was significant amelioration of biochemical parameters evident by decrease in copper and iron, and increase in albumin and TAC in alcohol dependent males.
Conclusions: Salivary copper, iron, TAC and albumin showed significant changes in alcoholics, and alcohol abstinence of thirty days

with the deaddiction treatment caused amelioration of these biochemical parameters. Studies with large sample size, correlating sialochemical changes with duration and dose of alcohol abuse and changes in blood biochemical parameters are required to establish saliva as a diagnostic tool in alcoholism.

Keywords: Alcohol Dependence; Albumin; Antioxidant; Copper; Iron; Saliva.

1. Introduction

Alcohol dependence syndrome is characterized by compulsive and uncontrolled consumption of alcohol despite its negative effects on the drinker's health, relationships and social standing [1,2]. Alcohol is a psychoactive substance with known liability to produce dependence in humans and animals. Alcoholism is a serious health issue with socioeconomic consequences. According to the reports of world health organization (WHO), about 3.3 million net deaths, or 5.9% of all global deaths, were attributable to alcohol consumption [3,4].

Alcohol biomarkers help in early detection of alcoholism and its complications. Blood biochemical parameters such as carbohydrate-deficient transferrin, gamma glutamyl transferase , aminotransferases and mean corpuscular volume have been analyzed as biomarkers of alcoholism and alcoholic complications [5]. Studies have reported increased lipid peroxidation and decreased levels of antioxidants in blood, indicating oxidative stress in chronic alcoholics[5]. Protein synthesis is known to be impaired in alcoholism and serum levels of proteins have been reported to be altered in chronic alcoholics [5].

Copper and iron essential trace elements vital for mutiple biological processes. They are prooxidants and trigger generation of free radicals [6]. Various studies have reported increased levels of free iron and copper in chronic alcoholics with and without liver cirrhosis. Accumulation of iron and copper in tissues with an impairment of antioxidant defense mechanisms and the consequent oxidative stress, one of the mechanisms proposed in the pathogenesis of alcoholic complications [6,7].

Saliva is an underused diagnostic tool. Collection of whole saliva is non invasive and does not need skilled technicians [8,9]. Researchers have analyzed salivary chemical constituents as biomarkers of systemic diseases. There is paucity of studies on salivary biomarkers of alcoholism in general, and salivary trace elements and antioxidants, in particular. There is a need for studies which correlate salivary biochemical changes with those of blood to establish saliva as a laboratory tool complimentary and even, alternate to blood.

The present study aimed to assay the levels of copper, iron, total antioxidant capacity and albumin in blood and saliva of alcohol dependent males; to assess the correlation between blood and saliva with respect to the biochemical parameters; and to assess the effect of alcohol withdrawal on the levels of these parameters.

2. Materials and Methods

This research work was carried out at Father Muller Medical College Hospital, Mangalore. The study protocol followed ethical principles as per Helsinki declaration and was approved by Ethics Committee of the institution.

The study subjects were divided into three groups :

1. Group-I : Alcohol dependent males admitted to Deaddiction Center for treatment. They were diagnosed of alcohol dependence by the treating psychiatrist, based on The ICD-10 Classification of Mental and Behavioural Disorders : Diagnostic Criteria for research of WHO [2];Total number of subjects was 45, and they aged between 30 to 60 years.
2. Group II : The alcohol dependent males in group I were followed up for one month of deaddiction treatment.
3. Group III : Thirty Normal, healthy males as controls; aged between 30 to 60 years
4. Occasional and social drinkers, chronic smokers, tobacco chewers, those with any systemic illness (diabetes mellitus, endocrinal disorders, non alcoholic liver disease, renal failure, neurological disorders, chronic inflammatory conditions and infections, cancer) were excluded from the study.

Voluntary informed consent was taken from all the subjects of the study.

2.1 Collection of Samples

From all the subjects, blood and saliva samples were collected taking aseptic precautions. Five ml. blood was collected between 9 -11 AM in plain vacutainers taking aseptic precautions, centrifuged to separate plasma/serum and cells. Unstimulated whole saliva sample was collected according to the method of Navazesh [10]. The sample was collected between 9-11 AM. 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 saliva samples were centrifuged at 3000 rpm for 15 minutes and supernatants were collected, and taken for the assays.

2.2 Assays Done

Copper level was estimated based on reaction of copper with diethyl dithiocarbamate [11]. Assay of iron was done using ferrozine reagent [11]. Albumin level was estimated by the Bromocresol green dye binding method [11]. Total antioxidant capacity (TAC) was assayed by the method of Korasevic *et al.* which was based on suppression of free radical formation and thus, decrease in formation of thiobarbituric acid reactive substances by antioxidants in saliva or serum [12].

2.3 Statistical Analysis

The data obtained from the study were evaluated by Student's t test and Karl Pearson's Correlation Analysis.

3. Results

The results of this study are presented in Table 1. The levels of albumin in serum and saliva in alcoholics (before withdrawal) were significantly lower when compared to controls. Levels of copper and iron in serum and saliva were significantly higher in comparison to controls. Total antioxidant capacity (TAC) in serum and saliva was significantly lower in alcoholics in comparison to controls.

Table 1: Levels of Albumin, Copper, Iron and TAC in serum and saliva (Values are mean±SD)

	Group-1 A (Alcoholics Before withdrawal); n =45	**Group-1B (Alcoholics After Withdrawal); n=45**	**Group-2 (Normal Controls);n= 30**
Serum Albumin (g/dl)	3.1± 0.6 *	3.9±0.8 **	4.1± 0.8
Salivary Albumin (mg/dl)	43±8 *	56±10 **	63±8
Serum Copper (µg/dl)	156±23 *	122±24 **	115±21.25
Salivary Copper (µg/dl)	39±9 *	21±11 **	25±13
Serum Iron (µg/dl)	109± 11 *	88±13 **	85±17
Salivary Iron (µg/dl)	15±4 *	8.3±4.3 **	9.2 ±5.4
Serum TAC (mmol/L)	1.9±0.9 *	3.2±0.8 **	3.3±1.5
Salivary TAC (mmol/L)	0.7±0.1 *	1.2±0.3 **	1.3±0.5

* Significantly different when compared to controls ;

** Significantly different when compared to alcoholics before withdrawal.

Alcoholics were on 30 days withdrawal period in the Deaddiction center. On withdrawal for 30 days, there was a change in the levels of albumin, copper, iron and TAC in serum and saliva. The levels were restored to normal levels. There was no significant difference in the levels of albumin, copper, iron and TAC between controls and alcoholics after withdrawal.

4. Discussion

The present study made an attempt to estimate the levels of albumin, copper, iron and total antioxidant capacity (TAC) in serum and saliva of alcohol dependent males and to

assess the effect of alcohol withdrawal on these parameters. The study also aimed to correlate the salivary biochemical parameters with those of blood.

Albumin levels in serum and saliva showed significant decrease in alcoholics and it was restored to normal level after alcohol withdrawal for 30 days. Albumin is synthesized in liver, and decreased serum albumin level suggests impairment of liver function. In the present study alcoholics with established liver disease were excluded. Previous study by Das et al observed decreased serum albumin level in alcoholics with liver disease [13]. Albumin is proposed to be target of adduct formation with acetaldehyde [13].

Iron is proposed to be a oxidant that is involved in the pathogenesis of alcoholic complications. In the present study, serum and salivary iron levels were significantly increased in alcoholics. In a study conducted on adult male alcohol consumers it was seen that serum iron and serum ferritin levels had increased based on type of alcohol consumed and duration of consumption [6,14]. Iron stores of the body are shown to be increased in alcoholics as indicated by increased serum levels of ferritin [6,14]. Studies have suggested that iron accumulation in the body which triggers oxidative stress, is one of the key mechanisms involved in pathogenesis of alcoholic liver disease [6,14]. Alcohol withdrawal was shown to restore serum ferritin to normal levels [14]. Present study has observed restoration of iron level in saliva and serum to normal values after alcohol withdrawal of 30 days.

In the present study, copper levels in serum and saliva were significantly higher in alcoholics when compared to normal controls, and the copper levels were restored to normal levels after 30 days of alcohol withdrawal period. There are contradicting results with respect to serum copper levels in chronic alcoholcis. Uhlikova et al. [7] observed lower levels of copper in alcoholics with liver steatosis. Other studies have reported increased levels of serum copper in social drinkers, chronic alcoholics and patients with alcoholic liver disease [15,16].

Alcohol is known to induce oxidative stress. Alcohol consumption is associated with a number of changes in cell functions and the oxidant-antioxidant system. The different pathways of ethanol metabolism have numerous detrimental consequences that contribute to the tissue damage and diseases seen in alcoholic patients. These consequences include oxygen deficits in the liver; interaction between alcohol metabolism byproducts and other cell components, resulting in the formation of harmful adducts, and formation of reactive oxygen species (ROS) that can damage other cell components [17]. Total antioxidant capacity (TAC) indicates non enzymatic antioxidants. This study observed decreased TAC in serum and saliva in alcoholics, and restoration of TAC to near control values after 30 days of alcohol withdrawal period. A study done by Neethumol et al. observed significant decrease in TAC in serum and saliva of alcohol dependent males. After alcohol withdrawal for 30 days, TAC increased to near normal levels in serum and saliva [18]. Saliva is equipped with enzymatic and non enzymatic antioxidant mechanisms. Peroxidase is the major enzymatic antioxidant while uric is considered major non enzymatic antioxidant. Studies have demonstrated alterations in the oxidant antioxidant status of saliva in chronic alcoholics. Increased salivary peroxidase and malondialdehyde, and decreased

salivary levels of glutathione, glutathione S-transferase and superoxide dismutase have been observed in chronic alcoholics [19,20].

There is paucity of studies on salivary biomarkers of alcoholism. Saliva as a diagnostic tool has distinct advantages of non invasiveness of its collection , non necessity of skilled persons to collect samples and suitability of repeated sampling without compliance problems. There were significant changes in salivary albumin, copper, iron and TAC in alcoholics. Though there was no significant correlation between serum and saliva with respect to albumin, copper, iron and TAC in alcoholics, the direction of change in each of these parameters was similar between saliva and serum.

5. Conclusions

Significant changes in salivary total antioxidant capacity, copper,iron and albumin are evident from this study. Saliva is an underused diagnostic tool. Considering the distinct advantages of saliva collection and use studies on saliva as a diagnostic tool are required. Future studies correlating sialochemical changes with dose and duration of alcohol abuse, assessing the correlation between blood and saliva with respect to alcohol biomarkers, and employing larger sample size are required to establish the salivary biomarkers of alcoholism.

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