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ORIGINAL RESEARCH ARTICLE

Antioxidant Changes in Plasma and Serum of Alcohol-Dependent Patients

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ABSCRACT

Alcohol is currently recognized as the most prevalent known cause of abnormal human development. Our aim was to investigate the antioxidant status in alcohol and non-alcoholic patients. 20 alcoholic males and 20 healthy non-alcoholic males were selected for the study. Blood samples were collected and the plasma and serum we separated for the analysis of TABARS, Superoxide Dismutase and Catalase and the non-enzymatic antioxidant like Vitamin C and Vitamin E. Decreased the levels of these antioxidants and enhanced the lipid peroxidation compared to normal subjects. The present study is consistent with increased oxidative stress due to chronic alcohol intake, which might be responsible for secondary diseases such as brain atrophy, peripheral polyneuropathy and liver fibrinogenesis.

Key words: Alcohol, Catalase, serum, fibrinogenesis.

INTRODUCTION

Alcohol consumption causes more than 1, 00, 000 deaths each year. Alcoholic liver disease affects million of patients worldwide and is a major cause of death in urban males. Alcoholic liver diseases not only depend on the total amount of alcoholic beverage ingested and are also playing important role in the development of alcohol liver disease (Walter *et al*., 2005). Chronic excessive alcohol consumption is more prevalent all over the world and is associated with tissue and organ damage leading to coronary heart disease, alcohol liver disease and several other manifestations including neurological disorders for which therapeutic approaches are sought (Lee, 2006; Pramyothin *et al*., 2006).

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Pourmorad *et al*., 2006). Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Oxidative stress occurs in a cellular system when the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system (Karuna *et al*., 2009). Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot}$) and hydroxy radicals (OH), resulting in structural alterations of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products.

Malondialdehyde is a major end product of free radical reaction on membrane fatty acids. Although the cell is endowed with several antioxidant systems to limit the extent of lipid peroxidation, under certain conditions protective mechanism can be overwhelmed, leading to elevated tissue levels of peroxidation products. Antioxidants can be classified as preventive and chain breaking antioxidants. Antioxidant vitamins such as alpha tocopherol (vitamin E), vitamin A and ascorbic acid (vitamin C) belong to the second category. Such compounds can intercept free radical induced chain reactions and prevent further oxidation (Kato *et al*., 2007)

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Many studies on the etiology of stone disease have focused on the properties of urine that effect crystal nucleation and growth. Crystal adherence to the surface of injured renal epithelial cells is considered initiating events in the genesis of urolithiasis. Factors leading to initiation of calcium oxalate formation are still not known. However the oxidant (free radical production) and antioxidant imbalance may be one of the major factors leading to the process of crystal deposition in renal tissues (Kato *et al*., 2007).

Enhanced oxidative stress and decreased antioxidant status induced by ethanol metabolism play a major role in the causation of alcohol toxicity and damage (Dey and Cederbaum, 2006). Alcohol dependency is often accompanied by impaired nutritional status (vitamins, trace elements), reduced levels of vitamin E and a reduced antioxidant capacity (Bjorneboe and Bjorneboe, 1993), although the effect of alcohol on antioxidant vitamins appears independent of nutritional status (Lecomte *et al*., 1994).

Oxidative stress, resulting from increased free radical production and/or decreased antioxidant defense, is not restricted to the liver, but also involves extra hepatic tissues, such as the central nervous system, heart and testes (Nordmann, 1994), however the studies of biochemical alteration with reference of alcoholic patients are limited. Considering these facts the aim of the present study was to assay the antioxidant status in plasma and serum in alcohol-dependent patients were compared with a social / non-alcoholic drinking group.

MATERIALS AND METHODS

Selection of Patients

20 male persons in the age group of 35-65 years admitted at various medical and surgical units of RMMCH Medical College and Hospital in Annamalai nagar, Chidambaram. From the material for the study purposes. Above investigations were performed on 10 normal and 10 alcohol drinker persons for comparison. Each patient was subjected to a detailed interrogation with special reference to their drinking habits 6 to 15 years. Such as duration of alcohol intake, frequency of consuming the drinks, the quantity of the drinks etc., each person's different works. Cases were taken to avoid the drugs, which will alter the values of various tests.

Sample preparation

10 ml of blood was collected from the alcoholic and non alcoholic groups by venous arm puncture and stored in heparinised and clot activator tube. Plasma and serum were separated by centrifugation at 3000 rpm for 15 min. The plasma and serum (Dodge *et al*., 1968) thus obtained was used for further analysis.

Antioxidant activities in plasma and serum

The activities of enzymatic antioxidants, such as TABARS (Thio Barbituric acid reactive substance), Superoxide Dismutase (Das *et al*., 2000) and Catalase (Sinha, 1972) and the non-enzymatic antioxidant like Vitamin C (Omaye *et al*., 1979) & Vitamin E (Varley *et al*., 1981) were assayed.

Estimation of Superoxide Dismutase (SOD)

The method involves generation of superoxide radical of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride. The nitrite reacts with sulphanilic acid to produce a diazonium compound which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance is measured at 543nm.

Estimation of Catalase (CAT)

Catalase causes rapid decomposition of hydrogen peroxide to water.

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

The method was based on the fact that dichromate in acetic acid reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced was measured colorimetrically at 610nm. Since dichromate has absorbance in this region, the presence of the compound in the assay mixture did not interfere with the colorimetric determination of chromic acetate. The catalase preparation was allowed to split H_2O_2 for different periods of time. The reaction was stopped at specific time intervals by the addition of dichromate / acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically after heating.

Estimation of Vitamin C (Ascorbic Acid)

Ascorbic acid was oxidised by copper to form dehydroascorbic acid and diketoglutaric acid. These products were treated with 2, 4-dinitrophenyl hydrazine to form the derivative of bis 2,4-dinitrophenyl hydrazine. This compound, in strong sulphuric acid undergoes a rearrangement to form a product with an absorption band that is measured at 520nm. The reaction was run in the presence of thiourea to

provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogens.

Estimation of Vitamin E (Tocopherol)

Tocopherol can be estimated using Emmerie-Engel reaction, which is based on the ferric to ferrous ions by tocopherols, which then forms a red color with 2,2′- dipyridyl. Tocopherols and carotenes are first extracted with xylene and the extinction read at 460nm to measure carotenes. A correlation is made for these after adding ferric chloride and reading at 520nm.

Estimation of TBARS (Thio Barbituric Acid Reactive Substance)

Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red color absorbing at 535 nm.

Statistical analysis

The result obtained in the present study statistically analyzed and the data were expressed as mean ± S.D from triplicate determination.

RESULTS

The activities of SOD, CAT, TABARS, Vitamin E and C were measured as an index of antioxidant status in plasma and the serum of normal and alcoholic patients (**Fig 1 & 2**). In alcoholic patients, all the ROS indices were found to be significantly lower than the normal subjects except TABARS which was found to be increased in alcoholic patients compared to control.

Figure 1: Biochemical changes in plasma and serum of non-alcoholic patients

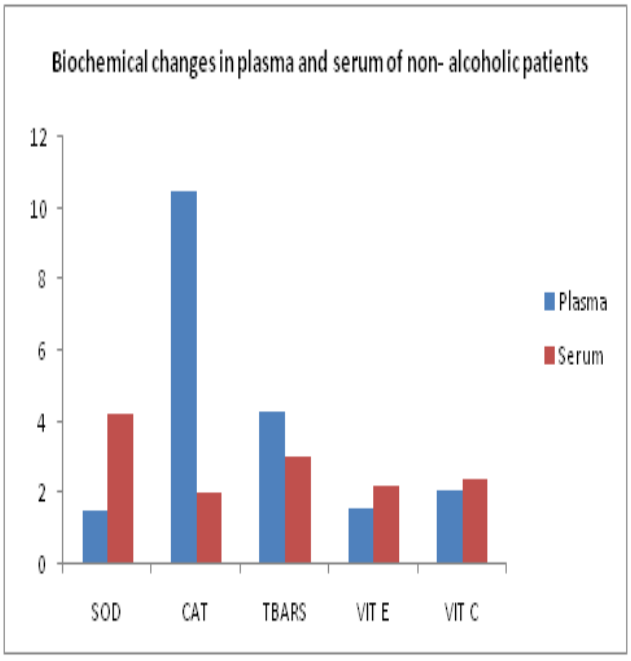
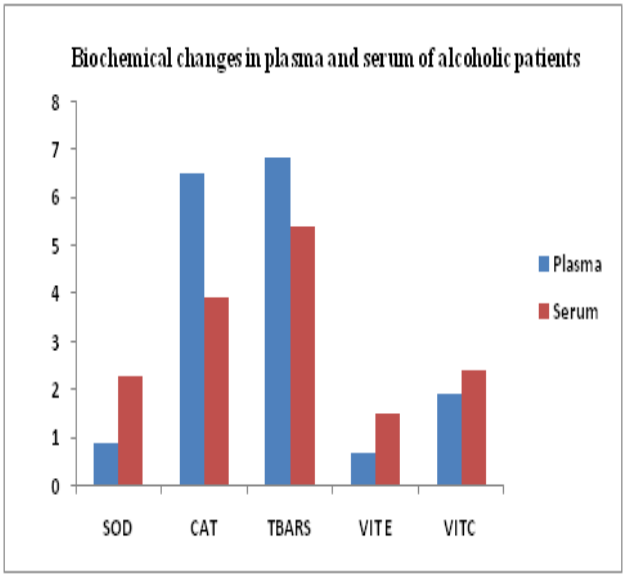


Figure 2: Biochemical changes in plasma and serum of alcoholic patients



DISCUSSION

The lipid peroxidation mediated by free radicals is considered to play a pivotal role in the mechanism by which ethanol may exert its toxic effects on the liver and other extra hepatic tissues (Nordmann, 1994). In our study we observed an increase in TBARS and a decline in antioxidant status in plasma and serum of alcoholic patients. Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defence mechanism to prevent the formation of excess free radicals (Comporti, 1985).

The enzymatic antioxidant defence system is the normal protector against lipid peroxidation that includes SOD and CAT (Prakash *et al.*, 2008). SOD is an exceedingly effective defense enzyme that converted the dismutation of superoxide anions into hydrogen peroxide (H_2O_2) (Reiter *et al.*, 2000). It is ubiquitous chain breaking antioxidant, plays an important role in protection against deleterious effects of lipid peroxidation (Dinkova-Kostova and Talalay, 1999).

CAT is a haemo protein in all aerobic cells that metabolize H_2O_2 to oxygen and water. Catalase protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidise (Lenzi *et al.*, 1993). It plays an important role in the acquisition of tolerance to oxidative stress in adaptive response of cells. Studies have shown that decrease in the activity of CAT during alcohol consumption may be due to the decrease protein synthesis. Thus, there is an increased utilization of CAT during alcohol consumption (Mirunalini *et al.*, 2010).

The antioxidant defence systems SODand CAT activity is significantly decreased in alcoholic patients. This decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein because of excess ROS generation. The generation of α-hydroxyethyl radical may lead to inactivation of these enzymes (Pigeolot *et al*., 1990) and accumulation of highly reactive free radicals also lead to deleterious effects such as loss of cell membrane integrity & membrane function (Krishnakanth and Lokesh, 1993).

Furthermore, vitamin E, unlike vitamin C is localized in membranes and lipoproteins, where it can interrupt the radical chain reaction of lipid peroxidation. α-tocopherol functions as the primary lipid phase antioxidant at high oxygen tension and the first line of defense against peroxidation of membrane lipids. Our study has demonstrated similar findings by way of significant decrease in the level of vitamin E and vitamin C in alcoholic patients. Thus, it suggests that decreased levels of vitamin E cannot assist in restricting self perpetuating vicious lipid peroxidation phenomenon.

Further, our results run parallel with the earlier investigations were they found the concentration of vitamin C were lower in alcohol dependent patients (Gueguen Sonia *et al*., 2003) and also further found significantly depleted level of vitamin C of ethanol fed female albino wistar rats as compared to their controls (Devipriya, *et al*., 2007). As reduced ascorbic acid, the water soluble antioxidant functions as the first line antioxidant defence against free oxygen radicals present primarily in the plasma (Wafers and Sies, 1988).

CONCLUSION

Chronic excessive alcohol consumption is more prevalent all over the world. The present study was consistent with increased oxidative stress due to chronic alcohol intake, which might be responsible for secondary diseases such as brain atrophy, peripheral polyneuropathy and liver fibrinogenesis.

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