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ANTIOXIDANT STATUS AND ZINC LEVELS IN ALCOHOLIC LIVER DISEASE

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ABSTRACT

The liver is susceptible to alcohol-related injury because it is the primary site of alcohol metabolism. Ethanol intake depletes anti-oxidant pool of the body and increases the oxidative stress on liver. Increased oxidative stress may result from over production of precursors to reactive oxygen radicals and / or decreased efficiency of inhibitory and scavenging systems. Antioxidant functions as blockers of radical processes before they can damage various biomolecules or prevent oxidative damage from spreading out the effect. Present study included 60 patients of alcoholic liver disease with deranged liver function tests admitted in Goa Medical College Hospital during the period of November 2003 to October 2004. Twenty healthy age and sex matched subjects served as controls. Study comprised of estimation of Biochemical parameters like Plasma Zinc, serum Malondialdehyde (M.D.A.), Blood Glutathione, serum Vitamin E, Plasma Vitamin C and Plasma Vitamin A (special ones) with routine parameters like. Serum Bilirubin; SGOT, SGPT, Total proteins, A/G ratio, Blood Glucose, Serum Cholesterol, Serum Alkaline Phosphatase and Prothrombin time. All the patients in study group and controls were aged between 25-65 yrs.

KEYWORDS:Alcohol, liver, zinc, malondialdehyde, vitamins A,C and E.

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INTRODUCTION

A large proportion of heavy drinkers develop serious alcoholic liver disease. The liver is particularly susceptible to alcohol-related injury because it is the primary site of alcohol metabolism. As alcohol is broken down in the liver, a number of potentially dangerous by-products are generated, such as acetaldehyde and highly reactive molecules called free radicals. Perhaps more so than alcohol itself, these products contribute to alcohol – induced liver damage. The major pathway of alcohol metabolism involves the enzyme alcohol dehydrogenase (ADH) which converts alcohol to acetaldehyde through oxidation. Acetaldehyde is highly toxic to the body, even in low concentrations. Normally, however, the enzyme aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate which can be utilized metabolically. The usual biological role of both ADH and ALDH is to metabolize vitamin A and other anti-oxidants. Thus ethanol intake depletes anti-oxidant pool of the body and increases the oxidative stress on liver[1] . Much of the direct cell damage that occurs during alcoholic liver disease is believed to be caused by free radicals. Small quantities of free radicals are produced as normal by products of various metabolic processes. These fragments are quickly scavenged by natural protective molecules in the cell, called antioxidants (e.g. glutathione, Vitamin A, Vitamin C and Vitamin E). However when free radicals are produced in excess or when antioxidant defenses are impaired, the free radicals may interact destructively with vital cell constituents, potentially causing death of the cell. Antioxidants work by scavenging the potentially hazardous free radicals, thus preventing structural damage to cells. High intakes of certain antioxidants have been associated with reduced risk of certain diseases. Leo at al found low levels of Beta carotene and retinoids in patients with alcoholic liver disease while Bell at al found reduced hepatic alpha tocopherol in ALD particularly cirrhosis[2]. Low anti-oxidant levels have been reported in cholelithiasis (with or without obstructive jaundice) severe acute viral hepatitis B (with or without hepatic encephalopathy) and Wilson's disease[1]. The present study is concerned with co relating the total anti-oxidant status of the parameters mentioned above and to evaluate of whether they can be used as monitoring indices in liver diseases.

MATERIALS AND METHODS

The present study included 60 male patients of alcoholic liver disease with deranged liver function tests admitted in Goa Medical College Hospital Bambolim. Study comprised of estimation of biochemical parameters like Plasma Zinc, serum M.D.A., Blood Glutathione, serum Vitamin E, Plasma Vitamin C and Plasma Vitamin A (special ones) with routine parameters like Serum Bilirubin; SGOT, SGPT, Total proteins, A/G ratio, Blood Glucose, Serum Cholesterol, Serum Alkaline Phosphatase and Prothrombin time. All the patients in study group and controls were aged between 25-65 years. The consent was obtained from the institutional ethical committee and the patients consent for the tests was also obtained.

Study group

This consisted of 60 patients of alcoholic liver disease seeking medical care in the indoor departments of Goa Medical College. All 60 were males above 25 years of age. Their diagnosis was based on history, thorough physical examination and liver function tests.

Control group

This group comprised of twenty healthy males above 25 years of age. A thorough history was taken and clinical examination carried out in each case to rule out any diseased state. In both the groups, a detailed history was obtained and a through clinical examination was carried out as per the structured preformat.

Collection of Blood Samples

5ml of blood samples were collected in plain bulbs and were allowed to clot. After 1 hour, serum was separated by centrifuging at 25000 r.p.m. for 5 minutes at room temperature. Serum was used for

measurement of M.D.A. and Vitamin E. 5ml of blood samples were collected in fluoride bulb; plasma was separated and used for measurement of Vitamin C, Vitamin A and whole blood for measurement of blood Glutathione. 5ml of blood samples were collected in freshly prepared Heparinized bulbs and free haemolyzed plasma used for measurement of plasma zinc. All estimations were done within 24-48 hours after specimen collection.

1. Estimation of serum Bilirubin was carried out by Modified Malloy & Evelyn method[3]
2. Estimation of serum Aspartate Transaminase and serum Alanine Transaminase was carried out by Colorometric method[4].
3. Estimation of serum Total Proteins and A/G Ratio was carried out by Bromocresol Green method[5].
4. Estimation of Blood Glucose was carried by Folin-Wu's method[6].
5. Estimation of Serum Cholesterol was carried by LibermannBuchard method[7]
6. Estimation of Serum Alkaline Phosphatase was carried by 4 Amino Anti-Pyrine method[8]
7. Estimation of Prothrombin Time was carried by Thromboplastin Reagent[9]

SPECIAL BLOOD INVESTIGATIONS

PLASMA ZINC[10]

PRINCIPLE

At pH 8.6, in a buffered media, Zinc reacts with the specific complex ant 5-Br.PAPS, forms a stable colored complex. The color intensity is proportional to the amount of zinc present in the sample.

ESTIMATION OF M.D.A[11]

Lipoproteins were precipitated from the specimen by adding 20% TCA. Then specimen was treated with TBA (Thiobarbituric Acid) in sodium sulphate to form chromogen. This chromogen allowed forming a complex in boiling water bath and extracted in butanol which is measured at 530 nm.

ESTIMATION OF BLOOD GULTATHIONE (BEUTLER 1963)[12]

Glutathione (GSH) in the whole blood or red blood cells is maintained in reduced state through reduced Nicotinamide adenine dinucleotide phosphate and Glutathione reductase. The functions of reduced glutathione seen to be to keep sulfhydryl groups in their active reduced state and through Glutathione peroxidase to remove hydrogen peroxide. Photometric method adapted by Beutler (using 5-5' Di-thiobis 2-Bitro benzoic acid (D.T.N.B.) was used for the assay of Blood Glutathione levels. The method is based upon the development of a relatively stable yellow color when D.T.N.B. is added to sulfhydryl compound.

VITAMIN E

DETERMINATION OF SERUM TOCOPHEROL (BAKER AND FRANK, 1968)[13]

Serum tocopherol can be measured by their reduction of ferric to ferrous ions which then form a red complexwith α,α' –dipyridyl[13].

VITAMIN C

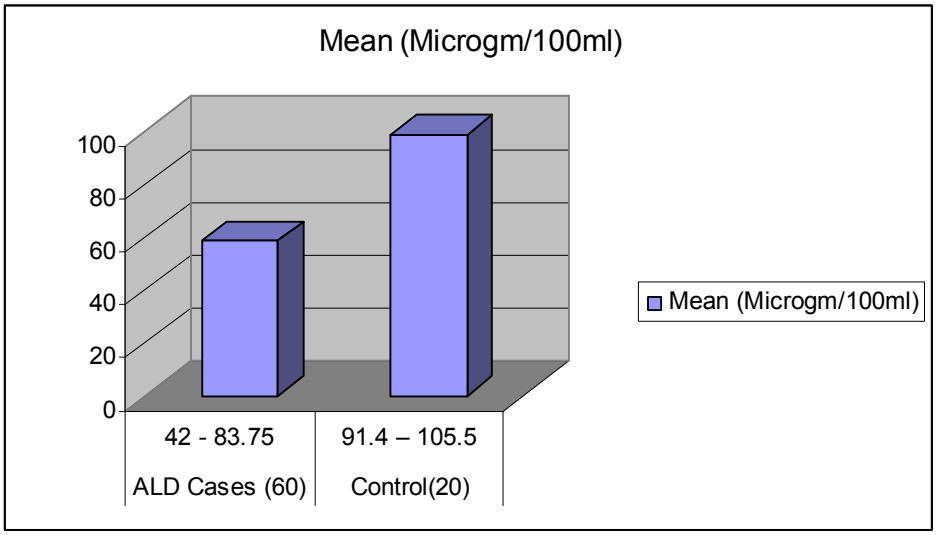
DETERMINATION OF PLASMA ASCORBATE BY 2,6DICHLOROPHENOLINDOPHENOL TITRATION[14]

DETERMINATION OF RETINOL AND CAROTENES IN SERUM USING THE CARR-PRICE REACTION[15]

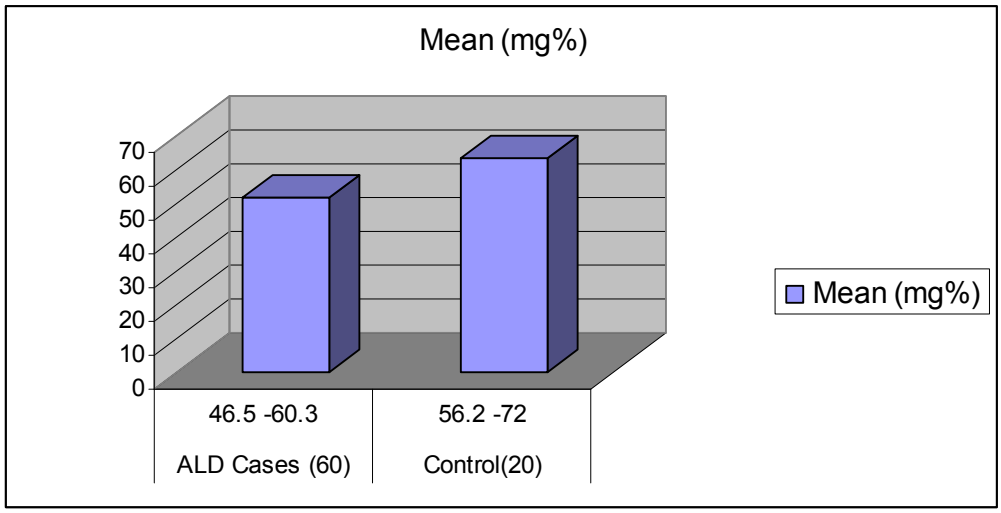
Proteins are precipitated with ethanol and the retinol and carotenes extracted into light petroleum. After reading the intensity of yellow color due to the carotenes the light petroleum is evaporated off and the residue dissolved in chloroform. Carr-Price reagent is added and the amount of blue color produced read. Since carotenes also give some color, a correction for this is made in order to obtain that due to the Retinol present. The present study includes 60 male patients with alcoholic liver diseases with varying ages from 25 to 65 years. Age and sex matched 20 healthy persons served as controls.

RESULTS

Plasma ZINC



Blood GSH



Sr.No	Antioxidant	Cases		Control		P Value
		Mean	SD	Mean	SD	
1	MDA	7.18	1.44	1.16	0.20	<0.001
2	GSH	52.63	5.07	63.47	5.24	<0.001
3	Vitamin E	6.65	1.62	12.70	2.58	<0.001
4	Vitamin A	0.30	0.09	0.71	0.25	<0.001
5	Vitamin C	7.56	1.68	12.12	3.13	<0.001
6	Zinc	58.70	13.81	98.71	3.74	<0.001

TABLE 1
Blood levels of Antioxidants in study subjects

Sr.No	Test	Cases		Control		P Value
		Mean	SD	Mean	SD	
1	Bilirubin	4.56	1.71	0.69	0.22	<0.001
2	Prothrombin Time	16.03	2.13	14.05	1.19	<0.01
3	SGOT	28.36	8.67	13.95	4.48	<0.001
4	SGPT	20.48	6.84	10.65	3.28	<0.001
5	Total Proteins	6.0	0.65	7.13	0.74	<0.001
6	A/G Ratio	0.93	0.16	1.45	0.30	<0.001
7	Glucose	99.88	17.07	124.6	14.05	<0.001
8	Cholesterol	190.8	24.48	189.3	30.79	>0.05
9	ALP	14.23	4.38	8.49	2.47	<0.001

TABLE- 2
Liver Function Test Results in study subjects

RESULTS AND DISCUSSION

The analysis of the study group revealed that the Alcoholic liver disease cases were between 25 to 65 years and were males Therefore all controls were sex matched males. Majority of the cases were from middle class with 43.5%. Among cases, the levels of serum MDA ranged from4.47 nmol/ml to 8.20 nmol/ml with mean of 7.18 nmol/ml with S.D. of ±1.44 whereas in controls the levels of serum M.D.A ranged between 0.90 nmol/ml to 1.45 nmol/ml. The alcoholic liver disease group showed significantly increased levels of serum MDA compared to the control group (Tab.1). Many other studies have reported similar findings[16, 17,18]. This indicates that as the severity of disease increases; oxidative stress also increases. In this study blood Glutathione (GSH) levels, were determined in 60 cases and 20 age matched healthy controls. (Tab.1). Among the diseased group the blood Glutathione levels ranged between 46.5 mg% to 60.3 mg% with mean of 52.63 mg% with SD of ±5.07. In controls the GSH levels ranged between 56.2 mg% to 72.0 mg% with mean of 63.4 mg% with S.D of ±5.24. Glutathione levels among alcoholic group were found to be significantly lower than control group. Leiber et al. (1997) had shown that acetaldehyde promotes GSH depletion. Also ethanol inhibits the synthesis of reduced Glutathione (GSH); which offers one of the mechanisms for the scavenging of free radicals. So in chronic alcoholics there is depletion of GSH [19]. This increase loss of GSH from liver mitochondria is one of the mechanisms for increase lipid peroxidation which results in the oxidative stress and mutilation of the organ (Lieber 1997)[20]. In our study, we also observed that there is significant decrease in antioxidant states as the severity of disease increases; which may be due to increase demands for vitamin E due to enhanced oxidativestress. Among cases the serum α-tocopherol levels ranged between 4.2mg/ L to 10mg/L with mean of 6.65 mg/L with S.D of ± 1.62 whereas in control the levels ranged from 8.6 mg/L to 16 mg/L with mean of 12.7 mg/L with S.D ± 2.58 (Tab. 1). It was seen that among alcoholic liver disease patients; Plasma Ascorbic acid levels ranged between 4.2 mg/L to 12 mg/L with mean of 7.56 mg/L with S.D of ± 1.68 whereas in controls the levels ranged from 8.2 mg/L to 18.6 mg/L with mean of 12.12 mg/L with.D± 3 S.13. As far as plasma Vitamin A level was concerned, among cases it ranged between 0.15 mg/L to 0.48 mg/L with mean of 0.30 mg/L with S.D of ± 0.09 whereas in control the levels ranged between 0.38 mg/L to 1.2 mg/L with mean of 0.71 mg/L with S.D of ± 0.25.Plasma zinc of levels ranging between 42 mcg% to 83.75 mcg% with mean level of 58.70 mcg% with S.D ± 13.81 whereas in control the levels ranged between 91.4 mcg% to 105.5 mcg% with mean level of 98.71 mcg% with S.D of ± 3.74 (Tab. 1).

These findings are supported by similar studies conducted[21, 22, 23.]. In alcoholic liver disease, impairment of β lipoprotein synthesis occurs which ultimately results are decreased serum levels of α-tocopherol[24].

Plasma vitamin C plays a pivotal role in protecting plasma lipids from reactive oxygen species attack. However, it is rapidly oxidized when challenged by oxidants released from activated polymorphonuclear cells[25]. In this study, we got a significant decrease in the levels of α-tocopherol and ascorbic acid as compared to controls. Our study suggests that alcohol causes severe oxidative stress in the body; which is antagonized by antioxidant defense system such as vitamin E and vitamin C. As a result; there was a decrease in levels of vitamin C and vitamin E. Chronic alcohol intake is known to interfere with retinoid metabolism and signaling[26]. Zinc and Vitamin A concentrations in the serum were measured in 40 alcoholics (33 male and 7 females) and 35 healthy age-matched subjects(31 males, 4 females). Alcoholics had significantly lower serum concentration of both zinc and vitamin A compared to the control group of healthy subjects[27] .The depression of zinc and Vitamin A levels was related to the severity of the hepatic lesion, the lowest levels being observed among cirrhotic. In the present study routine liver function test parameters were done in cases and controls. It was seen that all parameters like serum Bilirubin, Prothrombin time, Serum SGOT, serum SGPT, serum total protein, A/G ratio; blood Glucose and serum Alkaline Phosphatase (Tab.2) were significantly higher when compared with controls. In conclusion it appears that increase serum MDA can be used to detect severity of alcoholic liver disease. As we have observed a significant decrease in Blood Glutathione, α-tocopherol, ascorbic acid, vitamin A and Plasma Zinc with the increase in severity of oxidative stress. Further studies with chain breaking antioxidant supplementation and zinc supplementation have been performed to examine the possible beneficial effects of this supplementation in alcoholics[28]. Also detail studies have to be performed to prove the role of zinc as antioxidant.

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