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Levels of plasma testosterone, antioxidants and oxidative stress in alcoholic patients attending de-addiction centre

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Abstract

In men chronic heavy drinking interferes with reproductive hormones which are responsible for sexual maturation, sperm development and fertility. Alcohol is directly toxic to the testes; causing reduced testosterone levels. The present study was designed with an aim to elucidate the effect of oxidative stress on plasma testosterone level and hypothalamic pituitary gonadal (HPG) axis function in alcoholics. The plasma testosterone, luteinizing hormone and follicle stimulating hormone were investigated in alcoholics (n=200) (25–45 years) and were compared with normal non alcoholic controls (n=160). Alcohol abusers displayed significantly lower levels of plasma testosterone, luteinizing hormone, follicle stimulating hormone, Vitamin C, Vitamin E, β - Carotene, Glutathione and Superoxide Dismutase, Glutathione Reductase activities accompanied with significantly higher levels of Protein carbonyl content and Malondialdehyde levels than controls ($P < 0.001$). Decreased serum testosterone level in alcoholics might be due to increased oxidative stress and decrease in antioxidant levels.

Keywords: Testosterone, Antioxidants, Oxidative stress, Alcohol.

Introduction

The association of humans with alcohol is from times immemorial. Alcohol permeates, plagues and plagues the world. The social evil, despite its ill effects, has lot of charm and attracts the society. Alcoholism can lead to various medical complications, like perturbed alcohol metabolism, liver cirrhosis and hormonal changes associated with pancreatitis, osteoporosis, immune impairment and impaired fertility (NIH Guide, 1997). Evidence continues to grow indicating that reactive Aldehydic products resulting from ethanol-induced oxidative stress play a pivotal role in the pathogenesis of alcoholic liver injury ((Lieber, C. S and De Carli, L. M. 1970; Lieber, C. S and De Carli, L. M. 1972; Cederbaum, A. I *et al.*, 1975). Reactive aldehyde and hydroxyl radicals, which may be generated during periods of heavy ethanol intake, are known for their ability to attack amino acid residues of proteins, thereby forming both stable and unstable adducts with proteins and cellular constituents. As a consequence, cellular functions may become disturbed together with damage to proteins,

nucleic acids and lipids (Lieber Charles S. 1997; Clot Paolo *et al.*, 1997).

Alcohol abuse impairs reproductive activity (Maneesh M *et al.*, 2005). Alcoholics are often found having fertility abnormalities with low sperm count and impaired sperm motility (WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge Univ. Press, Cambridge; 1992). It causes impaired testosterone production, enormous testicular oxidative stress and testicular atrophy. The male reproductive system consists of three parts: hypothalamus, anterior pituitary and the testes and is finely controlled through a classic negative feed back mechanism (Remzi Cevik *et al.*, 2004). The hypothalamus and anterior pituitary have solely regulatory functions, mediated by its hormones. Based on the observations from the experimental study that alcohol causes gonadal dysfunction (Maneesh M *et al.*, 2005), the present study was designed with an aim to elucidate the effects of alcohol induced oxidative stress on plasma testosterone

level and hypothalamic pituitary gonadal (HPG) axis function in alcoholic patients by estimating the levels of Serum Testosterone, LH, FSH along with erythrocyte Malondialdehyde, Protein Carbonyl content, Serum Vitamin C, Vitamin E, β -Carotene, blood Glutathione and erythrocyte Superoxide Dismutase, Glutathione Reductase activities.

Materials and Methods

All experiments were performed as per accordance with Institutional Ethical Review Committee, Grant Medical College & Sir J. J. Groups of Hospitals, Byculla, Mumbai and informed consent was obtained from subjects.

In present investigation, attempts were made to design a discrimination procedure to separate alcoholics from patients with non-alcoholic hepatic diseases using a combination of the most promising test. The most powerful discrimination model was constructed with the batteries of screening instruments for detecting alcohol problems. CAGE (Ewing, J. A. 1984; Ewing, J. A. and Rouse, B. A. 1970; Mayfield, D *et al.*, 1974) Michigan Alcohol Screening Test (MAST) (Selzer, M. L., 1971; Selzer, M. L. *et al.*, 1975) Alcohol Use Disorder Identification Test (AUDIT) (Babor, T. F. *et al.* 1992; Bohn, M. J. *et al.*, 1995) and Severity of Alcohol Use Disorder Data (SADD) (Stockwell, T. *et al.*, 1994). Patients between 25 and 45 years of age, willing to participate in the study and with no history of undergoing long term medical intervention for various reasons like Cancer, Diabetes, Advance alcohol liver disorder, Acute Respiratory Distress (ARD), Chronic Renal Failure (CRF) and other Cardio Vascular Disease (CVS) serious medical, surgical, neurological conditions were included in the study. Also, patients with acute Psychotic state were excluded. Alcoholic patients (n=200) attending the deaddiction center who met the following inclusion criteria's and gave their informed consent were included in the study.

These patients were matched for age with normal controls (n=160) who were participating in a screening programme. These controls were, to their knowledge healthy and had no reason to consult their local doctors during the preceding 12 months.

Exclusion criteria for patients and controls are:

1. Patients below 25 and above 45 are excluded from the study, patients undergoing long term medical intervention for various reasons like Cancer, Diabetes, advance alcohol liver disorder, ARD, CRF and other CVS serious medical, surgical, neurological conditions are excluded from the study.
2. Excessive smoking evaluated according to Fagerstrom test for Nicotine dependence with score more than 15 are excluded (Fagerstrom, K.O, 1978; Pomerleau, C.S *et al.*, 1994; Heatherston, T.F *et al.* 1991). Substance abuse such as Cannabis, nicotine, opium and other psychotropic substances are excluded from the study.
3. Patients taking Vitamins and antioxidants or any other significant supplements.
4. Immunocompromise and acute infectious state.
5. Patients with acute Psychotic state or patients unwilling to participate in study.
6. Subjects with history of any other drug abuse, other physical illness or cause of infertility were also excluded.

All the patients underwent a complete medical examination and a biological screening at the entrance of the study. A detailed standardized interview aimed especially at drinking history, tobacco and drug consumption was performed. Biochemical and hormonal assays were carried out with whole blood serum. Total of 10ml of venous blood samples from overnight fasting individuals were collected. Blood samples collected in plain tubes, serum was separated by centrifugation at 2500 rpm for 7 minutes at room temperature and was used for estimation of Serum γ -Glutamyl Transferase (GGT), Serum glutamic-oxalacetic transaminases (SGOT), Serum glutamic-pyruvic transaminases (SGPT), Hemolytic or turbid samples were discarded. Blood sample collected in EDTA tube were used for estimating MCV. Total testosterone in serum was estimated by using Alpha Diagnostic international Testosterone ELISA kit, Measurement of Serum Luteinizing Hormone was done by using (RIAK-10) Radioimmunoassay kit by Board of Radiation

and Isotope Technology, Navi Mumbai and Follicle Stimulating Hormone Concentration in Serum was estimated by a Microplate Chemiluminescence's Immunoassay (CLIA) kit.

Erythrocyte Malondialdehyde (MDA) was estimated by the method of Jain. S.K (Jain.Sushil.K. 1989). We followed the method described by Levine et al (Levine R. L *et al.*, 1990), for the determination of protein carbonyl content. Serum vitamin E was estimated by the method of Baker et al (Baker, H. and Frank, O., 1968). Method proposed by Ayekyaw was used to estimate serum vitamin C (Ayekyaw, 1978). Serum β -Carotene level was estimated by the method Kimble M.S (Kimble, M.S. 1938). Blood GSH was determined by following the method described by Beutler et.al (Beutler Ernest *et al.*, 1963). SOD assay was performed by the method of Marklund and Marklund (Marklund, S. & Marklund, G., 1974). Glutathione Reductase determination in erythrocytes was performed by the method described by Carlberg and Mannervik (Carlberg, I. & Mannervik, B., 1975).

Statistical analysis

All the samples were run in duplicate, differences were statistically assessed using student t-test ((Stell, R.G.U., Torrie, J.H. 1960) by using statistical software MINITAB, where ONE-WAY ANOVA has been applied. The results obtained were expressed as Mean \pm Standard deviation (SD). For all comparisons, a p value \leq 0.05 was considered statistically significant.

Results

Alcoholic patients displayed significantly low levels of serum Testosterone, LH, FSH, with significantly lower levels of dietary antioxidant vitamins like Vitamin C, Vitamin E, β - Carotene and endogenous antioxidant like blood Glutathione ($P < 0.001$). Antioxidant enzymes like erythrocyte Superoxide Dismutase and Glutathione Reductase displayed diminished activities ($P < 0.001$) compared to their respective non alcoholic healthy controls (Table 1).

Discussion

Alcohol intoxication caused marked decrease in serum testosterone level, with simultaneous increase in lipid peroxidation (MDA concentration) and oxidative damage of protein (Carbonyl content). Reduction in testosterone was accompanied by low LH and FSH. Reduction in the serum testosterone could be due to decreased synthesis (Maneesh M *et al.*, 2005). Current study on the erythrocyte Malondialdehyde (MDA) content as an index of lipid peroxidation in alcoholic groups and their respective controls (Table .1), demonstrate that, there is significant elevation in content of erythrocyte MDA in alcoholic patients as compared to their respective controls. These results are in good agreement and run consistently well with the previous reports, which states that both serum and erythrocyte TBARS content was increased significantly in alcoholic patients as compared to their controls ((Matsumura, T *et al.*, 1980; Suematsu Toshihiko MD *et al.*, 1981; Naveau Sylvie M.D *et al.*, 2001; Dupont Isabelle *et al.*, 1998; Paramahemsa, M *et al.*, 2002; Ucar Gulberk and Demir Basaran, 2003; Das Subir Kumar and Vasudeven, D.M. 2005). Chronic alcoholism showed a significant increase in protein carbonyl content as compared with non-alcoholic control subjects, increases in endogenous and H_2O_2 -induced DNA damage was also observed in lymphocytes of patients with chronic alcoholism (U.Mutlu-Türkoglu, 2000; Grattagliano I *et al.*, 1996), and this also occurs in the testes (Maneesh M *et al.*, 2005). Which may cause decrease in testosterone level. As testosterone levels decreases, levels of LH and FSH would increase to stimulate the production of more testosterone (Remzi Cevik, A. H *et al.*, 2004). But according to our findings; low serum testosterone level in alcohol abusers was accompanied by low serum LH and FSH levels. This suggests that the hypothalamic cells, which produce luteinizing hormone releasing hormone (LHRH), do not function correctly to the feedback when testosterone level is decreased. The inability of the pituitary gland to respond appropriately to a decline in testosterone implies that alcohol has a central effect on the interaction between the nervous system and endocrine system.

Figure 1: Screening and diagnosis of alcoholism

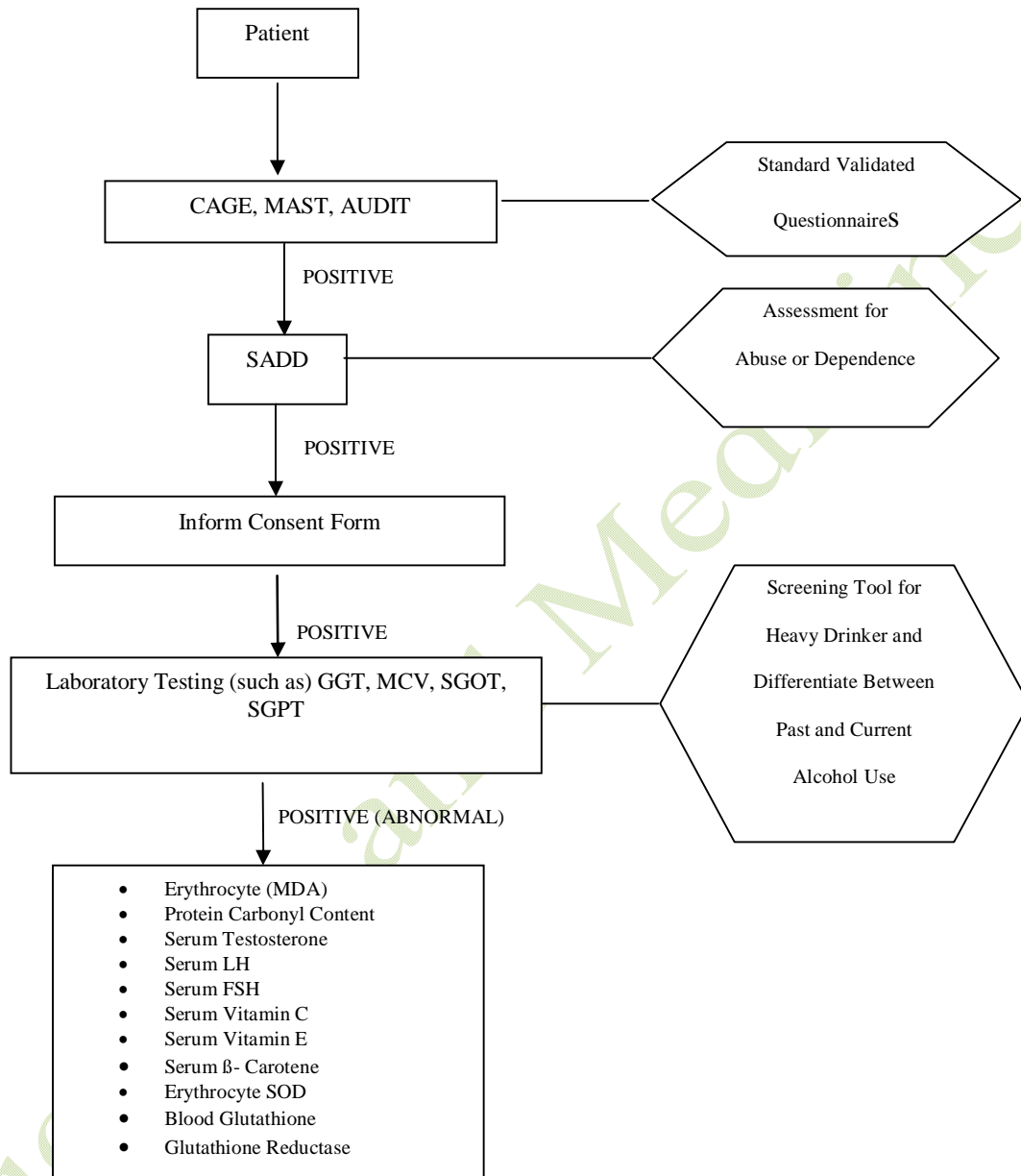


Table 1: Alcohol induced hormonal and biochemical changes in Alcoholic patients (n=200) and Controls (n =160)

Parameters	Groups	Results
Age(years)	Control	38.30 ± 6.3
	Alcoholic Patients	37.65 ± 6.6
Alcohol intake (g/day)	Control	NIL
	Alcoholic Patients	121.8 ± 8.6
Anthropometric Index [@]	Control	0.24 ± 0.03
	Alcoholic Patients	0.16 ± 0.09
GGT(11-50 U/l at 37°C for Men)	Control	15.68 ± 4.9
	Alcoholic Patients	146.20 ± 6.7***
MCV(82-98 FL)	Control	86.10 ± 5.8
	Alcoholic Patients	120.29 ± 4.9***
SGOT(0-40 IU/L)	Control	26.39 ± 4.5
	Alcoholic Patients	77.80 ± 2.2***
SGPT(0-40 IU/L)	Control	15.66 ± 3.4
	Alcoholic Patients	55.22 ± 4.1***
Serum Testosterone (ng/ml)	Control	9.36 ± 0.12
	Alcoholic Patients	5.77 ± 0.14***
Serum LH (mIU/ml)	Control	6.75 ± 0.12
	Alcoholic Patients	4.23 ± 0.16***
Serum FSH (μ IU/ml)	Control	9.20 ± 0.56
	Alcoholic Patients	7.05 ± 0.20***
Malondialdehyde (nmol/ml)	Control	3.204 ± 0.99
	Alcoholic Patients	5.710 ± 0.96***
Protein Carbonyl Content (nmole/mg protein)	Control	0.54 ± 0.25
	Alcoholic Patients	4.38 ± 0.18***
Vitamin C (mg/dl)	Control	1.25 ± 0.2
	Alcoholic Patients	0.75 ± 0.2***
Vitamin E (mg/dl)	Control	1.24 ± 0.1
	Alcoholic Patients	0.47 ± 0.25***
β- Carotene (mg/dl)	Control	116.57 ± 0.6
	Alcoholic Patients	93.51 ± 2.0***
Superoxide Dismutase (U/GHb)	Control	1428.1 ± 23.9
	Alcoholic Patients	1125.4 ± 9.6***
Glutathione (mg/dl of RBCs)	Control	81.08 ± 3.47
	Alcoholic Patients	37.73 ± 0.7***
Glutathione Reductase (U/gHb)	Control	0.93 ± 0.07
	Alcoholic Patients	0.49 ± 0.15***

- Results are expressed as mean ± standard deviation.
- *** Differences between Controls and Alcoholic Patients were significant for all biochemical and hormonal parameters (p<0.001).
- # Non significant difference of parameter
- [@] Age independent anthropometric indices = $\frac{\text{Weight in Kgs} \times 100}{(\text{Height in Cms})^2}$
(Normal Range = 0.15 – 0.16)

Measurement of glutathione concentration also provides a direct means of determining the degree of oxidant stress in erythrocytes for lipid peroxidation and hemolysis, which suggests oxidant stress in alcoholic patients. Experimental evidences of our study run consistently well in accordance with these findings, where the work has revealed a significant decrease in GSH levels in alcoholic subjects (Altomare Emanuele *et al.*, 1988).

Erythrocyte Glutathione Reductase revealed gradual decrease of its activities in alcoholic patients, which explains the incapability of this enzyme to provide a continuous flow of reduced form of Glutathione as a substrate for endogenous antioxidant enzyme like Glutathione Peroxidase which plays a critical role in combating alcohol induced oxidative stress. Decreased erythrocyte Glutathione Reductase activity in alcoholic patients showed resemblance with the reports, where they obtained significant decline in Glutathione Reductase activity in alcoholic patients with moderate and high alcohol intake, compared to the control group (Das Subir Kumar and Vasudeven D.M., 2005). Further, statistically significant decrease in the activity of Glutathione Reductase was found in rat brain, which was fed chronically with ethanol (Das Subir Kumar *et al.*, 2007).

In our investigation, where erythrocyte SOD activities in alcoholics displayed low statistically significant activities than their respective controls, showed resemblance with the result obtained earlier, where they measured erythrocyte and plasma activities of Superoxide dismutase in 58 male alcoholics without evidence of severe liver disease and in control group of 78 healthy men. They found that E-SOD mean activities were lower in alcoholics than controls (Guemouri Laila *et al.*, 1993). Similar kind of observation were found, where the activities of erythrocyte antioxidant (SOD) was decreased significantly in alcoholic hypertensive patients with / without diabetes, when compared to normal control subjects (Veerappan, R.M *et al.*, 2004).

Significant decline in the serum β - Carotene levels in alcoholic patients found in our study fall in line with the earlier reports that also showed a progressive decline in serum β - Carotene levels. The significance of our finding in relation to these alcoholic patients warrants comment,

the decreased concentration of serum β - Carotene may result from inadequate diet, which leads to hypozincemia as zinc is required for vitamin A metabolism or could be due to deranged hepatic function (Smith Jr *et al.*, 1973).

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 statistically significant decrease ($p < 0.001$) in the level of serum Vitamin E in alcoholic patients. Thus, it suggests that decreased levels of Vitamin E cannot assist in restricting self perpetuating vicious lipid peroxidation phenomenon. Previous reports have documented that concentration of serum α - tocopherol were decreased significantly in alcoholic patients compared to controls (Tanner, A.R *et al.*, 1986; Bjerneboe Gunn-Elin Aa M.D *et al.*, 1988 ; Ward Roberta J *et al.*, 1989; Situnayake, R.D *et al.*, 1990; Butcher Graham, P *et al.*, 1993; Leo Maria Anna *et al.*, 1993; Lecomte Edith *et al.*, 1994; Meagher Emma A *et al.*, 1999; Seo Jung Sook *et al.*, 2004). Further, our results run parallel with the earlier investigations where 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 in liver of ethanol fed female albino wistar rats as compared to their controls (Devipriya, N *et al.*, 2007). As reduced ascorbic acid, the water soluble antioxidant functions as the first line antioxidant defense against free oxygen radicals present primarily in the plasma (Wafers, M and Sies, H. 1988). Our findings where decreased levels of dietary antioxidant like β - Carotene, Vitamin E, Vitamin C and endogenous antioxidants such as reduced Glutathione, Glutathione Reductase and Superoxide Dismutase indicates the attack by free radicals and ROS, which is evident by elevated levels of MDA and protein carbonyl content in alcoholic patients. This might have cause low levels of serum testosterone due to oxidative challenge in the testis, as it is well demonstrated that chronic alcohol intoxication induces oxidative stress in testis (Maneesh M *et al.*, 2005). This was reported to have deleterious effect on testosterone metabolism (Maneesh M *et al.*, 2005; Nordmann R *et al.*,

1990; Ozyurt B *et al.*, 2007). Direct effects of ethanol and/or its metabolites on testosterone metabolism could be another possible reason.

Conclusion

Decreased serum testosterone level in alcohol abusers along with elevated levels of erythrocyte Malondialdehyde, protein carbonyl content with diminished levels of antioxidant vitamins such as vitamin E, vitamin C, β -carotene, blood GSH and erythrocyte SOD, Glutathione Reductase activities suggest alcohol induced oxidative

stress which could damage testosterone secreting Leydig cells and supporting Sertoli cells and may impair hypothalamic-pituitary-gonadal axis.

There are several reports of beneficial effects of antioxidant supplementation as adjuvant therapy in various diseases (McCall MR and Feri B., 1999). While we do not yet know if antioxidant therapy is useful in normalization of serum testosterone levels in alcoholic patients. Further studies are needed to clarify this issue.

List of abbreviations

(HPG)- Hypothalamic pituitary gonadal
 (MAST)- Michigan Alcohol Screening Test
 (AUDIT) -Alcohol Use Disorder Identification Test
 (SADD)- Severity of Alcohol Use Disorder Data
 (ARD)-Acute Respiratory Distress
 (CRF) -Chronic Renal Failure
 (CVS) -Cardio Vascular Disease
 (GGT)- γ -Glutamyl Transferase
 (SGOT)- Serum glutamic-oxalacetic transaminases
 (SGPT)-Serum glutamic-pyruvic transaminases
 (MCV)- Mean Corpuscular Volume
 (LH)- Luteinizing hormone
 (FSH)- Follicle stimulating hormone
 (EDTA)- Ethylene Diamine Tetra Acetic acid
 (MDA)- Malondialdehyde
 (LHRH)- Luteinizing hormone releasing hormone
 (GnRH)- Gonadotropin releasing hormone
 (TBARS)- Thio Barbituric Acid Reactive Substances
 (H_2O_2)- Hydrogen Peroxide

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