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ALCOHOL ABUSE-DURATION DEPENDENT DECREASE IN PLASMA TESTOSTERONE AND ANTIOXIDANTS IN MALES

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Abstract : Ethanol is a testicular toxin and it causes fertility abnormalities with low sperm count and impaired sperm motility in men. The present study was designed to investigate plasma testosterone level and hypothalamic pituitary gonadal (HPG) axis function in alcoholic men and also effect of ethanol on systemic oxidative stress. Forty six male alcohol abusers in the age group 20–40 years were selected. Fifty five, males in the same age group served as control. Alcohol abusers had significantly low plasma testosterone with low luteinizing hormone and follicle stimulating hormone. In addition they had significantly high thiobarbituric acid reactive substances (TBARS), superoxide dismutase and glutathione S-transferase, and low glutathione, ascorbic acid, catalase, glutathione reductase and glutathione peroxidase. Moreover, serum testosterone level in alcoholics negatively correlated with duration of alcohol abuse, and TBARS. Duration dependent decreased serum testosterone level in alcohol abusers might be due to 1) increased oxidative stress which can damage Leydig and supporting Sertoli cells and 2) impaired HPG axis.

Key words : alcohol testosterone gonadotropins oxidative stress

INTRODUCTION

Alcohol abuse impairs reproductive activity (1). Alcoholics are often found having fertility abnormalities with low sperm count and impaired sperm motility (2). It causes impaired testosterone production, enormous testicular oxidative stress and testicular atrophy. The male reproductive system consists of three parts:

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hypothalamus, anterior pituitary and the testes and is finely controlled through a classic negative feed back mechanism (3). The hypothalamus and anterior pituitary have solely regulatory functions, mediated by its hormones. Oxidative stress is a condition associated with an increased rate of cellular damage induced by reactive oxygen species. In a normal situation, antioxidants of plasma quench these reactive oxygen species (ROS) and protect against any likely damage to cell (4).

Based on the observations from our experimental study that alcohol causes gonadal dysfunction (1) the present study was designed to investigate plasma testosterone level and hypothalamic pituitary gonadal (HPG) axis function in alcoholic men and also effect of ethanol on systemic oxidative stress.

MATERIAL AND METHODS

Study population: The study was conducted in the department of Biochemistry, Sikkim Manipal Institute of Medical Sciences, Gangtok during the period from April 2003 to December 2004, after obtaining approval from the Institutional Review Board. Informed consent was obtained from all and the objectives of the study were fully explained. All subjects were of similar dietary habit selected from the local community. Forty six male alcohol abusers of age 20–40 (29.6 ± 4.2) years (BMI 22.24 ± 0.21) having 3–10 (6.40 ± 2.35) years history of alcohol abuse were selected. Eligibility criteria for alcohol abusers were two or more positive CAGE replies (5) and elevated markers of alcoholism, y-glutamyl transpeptidase (γ-GT, EC 2.3.2.2), serum glutamyl oxaloacetate transaminase (SGOT, EC 2.6.1.1) and mean corpuscular volume (6). Fifty five healthy male volunteers in the same age group (26.5 ± 4.8) (BMI 23.85 ± 0.25) served as control. All individuals were nonsmokers. Subjects with history of any other drug abuse, previous treatments for alcoholic liver disease, any other physical illness or cause of infertility were excluded.

Analytical methods: Five ml of venous blood was collected without stasis from each subject. Biochemical and hormonal assays were carried out with whole blood serum. Heparinised (200 units) blood sample was used for the hemolysate preparation. The blood was immediately centrifuged at 3000 rpm for 15 min at 4°C and the plasma separated. The cells were washed with normal saline and the RBCs were subjected to lysis.

Serum testosterone was estimated by direct immunoenzymatic method using reagent kit (Eqiupar Diagnostic - Italy), and luteinizing hormone (LH) and follicle stimulating hormone (FSH) were estimated by microplate immunoenzymometric assay using reagent kit (Monobind, INC.USA) in Biomerieus. Ascorbic acid in the serum was estimated by the method of Me Cormick and Greene (7) by using dinitrophenyl hydrazine-thiourea-copper sulfate reagent. Preparation of hemolysate: Heparinised whole blood was centrifuged at 1000 g for 10 minutes at 2°C. Plasma was separated. Packed erythrocyte was washed with cold normal saline thrice and in each case centrifuged at 15000 g for 15 minutes at 2°C. Washed, packed erythrocyte was hemolysed by the addition of chilled double distilled water. The

hemolysate was centrifuged at 13000 g for 1 hour at 2°C. The supernatant and plasma were used to assay erythrocyte malondialdehyde (MDA) (8), reduced glutathione (GSH) (9), extracellular SOD (EC SOD; EC 1.15.1.1) using reagent kit (Ransod, Randox Laboratories Ltd, UK), catalase (EC 1.11.1.6) (10), glutathione reductase (GSH-Red, EC 1.6.4.2) (11), selenium glutathione peroxidase (Se-GSH-Px, EC 1.11.1.9) using reagent kit (Ransel, Randox Laboratories Ltd, UK) and glutathione S-transferase (GSH-ST, EC 2.5.1.18) (12).

Statistical analysis: The data were expressed as mean ± SD and analyzed for significant differences by one-way analysis of variance. Simple regression analysis was used to assess the correlation between parameters. Value of P<0.05 was considered statistically significant.

RESULTS

Alcohol abusers had significantly low serum testosterone (P<0.001) with low LH (P=0.7527) and FSH (P=0.9898). Alcohol caused high extent of lipid peroxidation (P=0.004), and increased activities SOD (P=0.001 and GSH-ST (P<0.001), and low GSH ascorbic acid (P=0.005), catalase (P=0.01), GSH-Red (P<0.001), Se-GSH-Px (P=0.006) (Table I).

Serum testosterone level in alcoholic men negatively correlated with duration of alcohol abuse (Fig. 1, R^2: 0.9518, F: 868.48, P<0.0001), and thiobarbituric acid reactive substances (TBARS) (Fig. 2, R^2: 0.8134, F: 191.76, P<0.0001).

TABLE I: Ethanol induced systemic hormonal and biochemical changes [Data is represented as mean±SD].

Parameters	*Controls (n=55)*	*Alcohol abusers (n=46)*	*One way ANOVA*
Testosterone (ng/ml)	7.56±0.13	4.96±0.16	t=4.032, P<0.001
LH (mIU/ml)	5.65±0.13	5.63±0.22	t=0.317, P=0.7527
FSH (mIU/ml)	8.18±0.48	8.03±0.14	t=0.0128, P=0.9898
TBARS (nmol/ml)	3.42±0.20	11.50±2.17	t=3.00, P=0.004
Ascorbic acid (mg/dl)	1.25±0.11	0.75±0.06	t=2.96, P=0.005
GSH (µg/mg)	3.57±0.17	2.74±0.07	t=8.15, P<0.001
SOD (U/mg Hb)	1.26±0.07	2.06±0.02	t=5.81, P<0.001
Catalase (nmol/mg/min)	0.133±0.01	0.124±0.01	t=2.65, P=0.01
GSH-Red (nmol/mg/min)	1.79±0.12	1.56±0.04	t=8.62, P<0.001
Se-GSH-Px (U/g Hb)	52.40±0.86	23.40±0.09	t=2.90, P=0.006
GSH-ST (U/g Hb)	1.11±0.01	1.61±0.01	t=9.80, P<0.001

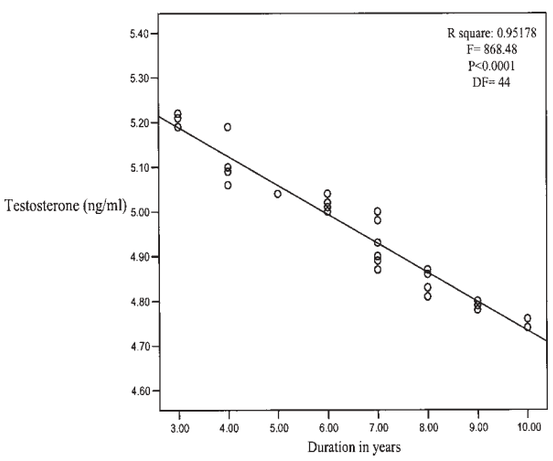

Fig. 1: Correlation between werum testosterone and duration of alcohol abuse.

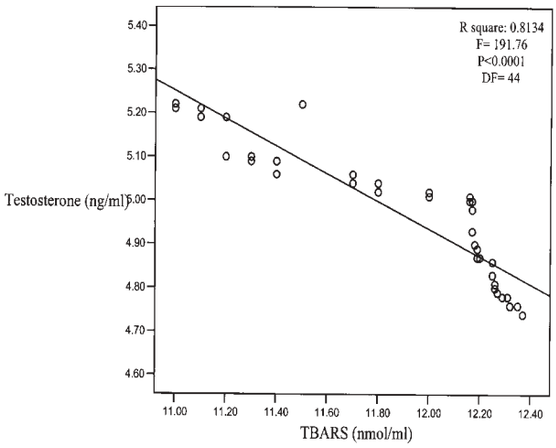

Fig. 2 : Correlation between werum testosterone and TBARS.

DISCUSSION

Chronic ethanol administration reduced not only serum testosterone level, but also systemic ascorbic acid, GSH and activities of catalase, GSH-Red and Se-GSH-Px. Simultaneously, increased lipid peroxidation and activities of SOD and GSH-ST were also observed. Reduction in testosterone was accompanied by low LH and FSH and it well correlated with duration of alcohol abuse and TBARS.

Reduction in the serum testosterone could be due to decreased synthesis (1). As testosterone levels decrease, levels of LH and FSH would increase to stimulate the production of more testosterone (3). But in our study we found that low serum testosterone level in alcohol abusers was accompanied by low serum LH and FSH levels. This finding suggests that the hypothalamic cells, which produce luteinizing hormone releasing hormone (LHRH), do not function correctly to the feedback when testosterone level 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.

The decrease in gonadotropin levels results from impairment in both production and secretion (13). It might be due to the effect not only on the pituitary gland but also on the hypothalamus. Investigations with isolated hypothalamus from male rats or gonadotropin releasing hormone (GnRH) producing cells obtained from genetically engineered mice failed to demonstrate any reduction in GnRH secretion in response to alcohol treatment and moreover, no alcohol-induced reduction in the expression of the gene that is responsible for generating GnRH was detected suggesting that alcohol probably does not affect GnRH production (14). However, the generation of functional GnRH molecule from its precursor pre-pro-GnRH by removing 82 amino acids, is appears to be diminished after alcohol exposure (14). Moreover ethanol prevents the movement of protein kinase C^{15} which is necessary for the GnRH stimulation of LH release from pituitary.

Alcohol caused enormous systemic oxidative stress, a state marked by increased serum level of oxidizing agents (TEARS content) and decreased serum level of potential scavengers of ROS (i.e. antioxidants). It is reported that increased oxidative stress is a well-accepted mechanism of alcohol induced tissue injury (16) and this also occurs in the testes (1). The assumption that free radicals can

influence male infertility has received substantial scientific support (17). Serum testosterone level was well correlated with TEARS level, indicates ethanol induced oxidative stress due to increased lipid peroxidation and decreased antioxidants, cause reduction in circulating testosterone.

Vitamin C is a unique antioxidant “to scavenge aqueous peroxyl radicals” before these destructive substances have a chance to damage the lipids. It works along with vitamin E, a fat soluble antioxidant and the glutathione peroxidase to stop radical chain reactions. GSH is a major thiol in living organisms, which plays a central role in coordinating the body’s antioxidant defense processes. Conditions that perturb intracellular levels of glutathione results in significant alteration in cellular metabolism. The tissue glutathione concentration reflects its potential for (i) detoxification (ii) preserving the proper cellular redox balance and (iii) its role as a cellular protectant (18). GSH has a likely role in sperm nucleus decondensation and spindle microtubule formation (19). Ethanol induced depletion of glutathione supports the hypothesis that reactive oxygen intermediates generated during the metabolism of ethanol lead to glutathione oxidation and lipid peroxidation and are responsible for the toxicity of ethanol (20).

SOD is the important antioxidant enzymes having an antitoxic effect against superoxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. Catalase protects the cells from the accumulation of hydrogen peroxide by dismutataing it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (21). So, decrease in the activity of catalase could be due to less availability of nicotinic acid dinucleotide phosphate-reduced (NADPH). GSH-Red is concerned with the maintenance of cellular level of GSH by effecting fast reduction of oxidized glutathione (GSSG) to reduced form (22). Se-GSH-Px plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes, spermatogenesis, sperm morphology and sperm motility (23). It is suggested that the metabolic pathway of testosterone biosynthesis requires protection against peroxidation (22) and will be affected by a decrease in the activity of this enzyme. Ethanol caused increase in GSH-ST activity which is because it is an oxidative stress inducible enzyme or alcohol itself may be responsible for its induction (24). GSH-ST plays an essential role in eliminating toxic compounds by conjugation.

In conclusion, ethanol caused low plasma testosterone in men accompanied by a low LH and FSH when elevated levels were expected indicating impaired HPG axis, and it well correlated with duration of alcohol abuse and extent of lipid peroxidation. Decreased serum testosterone level in alcohol abusers might be due to 1) increased oxidative stress which can damage testosterone secreting Leydig cells and supporting Sertoli cells and 2) impaired hypothalamic-pituitary-gonadalaxis.

REFERENCES

1. Maneesh M, Jayalekshmi H, Sanjiba Dutta, Amit Chakrabarti, Vasudevan DM. Experimental therapeutical intervention with ascorbic acid in ethanol induced testicular injuries in rats. *Indian J Exp Biol* 2005; 43: 172–176.
2. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge Univ. Press, Cambridge; 1992.
3. Remzi Cevik, AH Gur, Suat Acar, Kemal Nas, AyÕegül Jale Sarac. Hypothalamic-pituitary-gonadal axis hormones and cortisol in both menstrual phases of women with chronic fatigue syndrome and effect of depressive mood on these hormones. *BMC Musculoskelet Disord* 2004; 5: 47–51.
4. Maneesh M, Jayalekshmi H, Suma T, Chatterjee S, Amit Chakrabarti, Singh TA. Evidence for oxidative stress in osteoarthritis. *Ind J Clin Biochem* 2005; 20(1): 129–130.
5. Michel G, Denmic G, Richard M, Philip C. Oxford Textbook of Psychiatry, 3rd ed. pp. 445, Oxford, New York; 1996.
6. Salaspuro M. Conventional and coming laboratory markers of alcoholism and heavy drinking. Alcoholism : *Clin and Exp Res* 1986; 10: 5S–12S.
7. McCormick DB, Greene HL. Tietz textbook of Clinical Chemistry. Eds: Burtis, C. A. and Ashwood, E. R. pp. 1025, WB Saunders Company, USA, 1988.
8. Sinnhuber RO, Yu TC, Yu TC. Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. *Food Res* 1958; 23; 626–630.
9. Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. *J Lab Clin Med* 1963: 61: 882–888.
10. Beers RF, Sizer IW. A spectrophotometric method for measuring the break down of hydrogen peroxide by catalase. *J Biol Chem* 1952; 195: 133–140.
11. Goldberg, Spooner, JR. In: Methods of Enzymatic Analysis Ed. Bergmayer Vol. III, 3rd edn. p. 258–265. Academic press, Inc. Florida, 1983.
12. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferase, the first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130–7139.
13. Emanuele MA, Emanuele N. Alcohol and the male reproductive system. *Alcohol Research & Health* 2001; 25(4): 282–287.
14. Uddin S, Wilson J, Emanuele MA, Williams D, Kelley MR, Emanuele N. Ethanol induced alterations in the posttranslational processing but not secretion of luteinizing hormone-releasing hormone in vitro. Alcoholism: *Clin Exp Research* 1996: 20: 556–560.
15. Steiner JC, Lapaglia N, Hansen M. Effect of chronic ethanol on reproductive and growth hormones in the peripubertal male rats. *Journal of Endocrinology* 1997; 154: 363–370.
16. Gagnon C, Iwaaski AO, de Lamirande E, Kovaski N. Reactive oxygen species and human spermatozoa. *Ann N X Acad Sci* 1991; 637: 436–444.
17. Mari M, Wu D, Nieto N, Cederbaum AJI, CYP2E1-Dependent Toxicity and Up-Regulation of Antioxidant Genes. *J Biomed Sci* 2001; 8(1): 52.
18. Irvine DS. Glutathione as a treatment for male infertility. *Reprod* 1996; 1: 6.
19. Hussain K, Somani SM. Interaction of exercise training and chronic ethanol ingestion on testicular antioxidant system in rat. *J Appl Toxicol* 1998; 4 8(6): 421.
20. Kuldip Singh, Ahluwalia Pushpa. Alteration in some antioxidant enzymes in cardiac tissue upon monosodium glutamate administration to adult male mice. *Ind J Clin Bio* 2005; 20(1): 43–46.
21. Lenzi A, Cualosso F, Gandini L, Lombardo F, Dondero F. Placebo controlled; double-blind cross over trial glutathione therapy, in male infertility. *Hunu Reprod* 1993; 9: 2044.
22. Calvin HI, Cooper GW, Wallace E. Evidence that selenium in rat sperm is associated with a cystine rich structural protein of the mitochondrial capsule. *Gamete Res* 1981; 4: 139.
23. Seema Gupta, Rajesh Pandey, Ranjan Katyal, Agarwal HK, Agarwal RP, Agarwal SK. ‘Lipid peroxide levels and antioxidant status in alcoholic liver disease. *Ind J Clin Biochem* 2005; 20(1): 67–71.
24. Chandra R, Aneja R, Rewal C, Konduri R, Dass K, Agarwal S. An opium alkaloid-Papaverine ameliorates ethanol induced hepatotoxicity: diminution of oxidative stress. *Ind J Clin Biochem* 2000; 15(2): 155.