IMPAIRED HYPOTHALAMIC-PITUITARY-GONADAL AXIS FUNCTION IN MEN WITH DIABETES MELLITUS

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ABSTRACT

In view of association of diabetes mellitus and male infertility, present study was designed to investigate the functional status of hypothalamic pituitary gonadal (HPG) axis in diabetic men. Thirty-five diabetic men (BMI 22.24 ± 0.21) in the age group 20-40 (30.6 ± 4.7) years were selected. Twenty-five healthy men (BMI 23.85 ± 0.25), in the same age group (29.5 ± 4.8) served as control. Blood samples were collected for hormonal and biochemical estimations. Diabetic men had significantly low serum testosterone with low LH and FSH, hypercholesterolemia, hypertriglyceridemia, hypoalbuminemia and increased oxidative stress. Low serum testosterone in diabetic men was accompanied by low LH and FSH; the inability of the pituitary gland to respond appropriately to a decline in testosterone implying central effect of high serum glucose on the interaction between the nervous and endocrine system. Nutritional deficiency, increased oxidative stress and increased aromatase activity due to excessive body fat might have also contributed to low serum testosterone.

KEY WORDS

Diabetes Mellitus, Testosterone, Hypothalamic-pituitary-gonadal axis, Oxidative stress

INTRODUCTION

Diabetes mellitus is the most common endocrine disease. The incidence of diabetes among men attending infertility clinics was estimated to be 0.3%, and about 1% of subfertile male patients may have diabetes (1). There is little evidence to suggest that endocrinopathy is the primary cause of infertility in diabetic men (2). Of the three components of the male reproductive system, the hypothalamus and anterior pituitary have solely regulatory functions, mediated by its hormones. This hypothalamic -pituitary-gonadal axis is finely tuned system controlled through a classic negative feed back mechanism (3). Oxidative stress is a condition associated with increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as reactive oxygen species (ROS). In a normal situation, plasma contains antioxidant mechanisms, which are likely to quench these ROS and protect against any likely damage (4).

The present study was designed to understand the functional status of hypothalamic-pituitary-gonadal axis in diabetic men and other systemic changes.

PATIENTS AND METHODS

Study population: The study was conducted in the department of biochemistry, Sikkim Manipal Institute of Medical Sciences, Gangtok, North Eastern India. Subjects in the age group 20-40 years and of similar dietary habits were randomly selected from a local community. Six of the participants were dropped out at the end of selection, as they did not like the idea of giving blood. Informed consent was obtained from the subjects and the objectives of the study were fully explained. Thirty-five diabetic men (BMI 22.24 ± 0.21) aged 30.6 ± 4.7 years were included in the study. Twenty-five healthy men (BMI 23.85 ± 0.25) aged 29.5 ± 4.8 years, served as control. All individuals were nonsmokers. Subjects with history of substance abuse, any other physical illness or cause of infertility were not included.

Analytical methods : Five ml of fasting venous blood was collected without stasis from each subject. Heparinised (200 units) blood sample was used for the hemolysate preparation. The blood was immediately centrifuged at 3000 g for 15 min at 4°C and the plasma separated. The cells were washed with

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normal saline and the RBCs were subjected to lysis. Biochemical and hormonal assays were carried out with whole blood serum.

Serum testosterone was estimated by direct immunoenzymatic method using reagent kit (Ekiupar Diagnostic - Italy), and luteinizing hormone (LH) and follicle stimulating hormone (FSH) were estimated by microplate immunoassay using reagent kit (Monobind, INC.USA) in Biomerieux. Serum albumin, cholesterol and triglyceride were determined by using reagent kit (Randox Laboratories Ltd, UK) in photometer 5010, Boehringer Mannheim. Ascorbic acid in the serum was estimated by the method of Mc Cormick and Greene (5) 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 15000g for 15 minutes at 2°C. Washed, packed erythrocyte was hemolysed by the addition of chilled double distilled water. The hemolysate was centrifuged at 13000g for 1 hour at 2°C. The supernatant and plasma were used to assay erythrocyte malondialdehyde (MDA) (6), reduced glutathione (GSH) (7), extracellular SOD (EC SOD; EC 1.15.1.1) using reagent kit (Ransod, Randox Laboratories Ltd, UK), catalase (EC 1.11.1.6) (8), glutathione reductase (GSH-Red, EC 1.6.4.2) (9), 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) (10).

Statistical analysis : The data were expressed as mean $\pm$ SD and analyzed for significant differences by one-way analysis of variance. Value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Diabetic men had significantly low serum testosterone with low LH and FSH, hypoalbuminemia, hypercholesterolemia and hypertriglyceridemia. The extent of systemic lipid peroxidation measured as level of thiobarbituric acid reactive substances was nearly four times more in diabetic men. They had significantly low erythrocyte GSH and high activities of SOD, catalase, GSH-Red, Se-GSH-Px and GSH-ST.

Low serum testosterone could be due to decreased synthesis or increased metabolic clearance. 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 diabetic men was accompanied by low serum LH and FSH. This finding suggests that the hypothalamic cells, which produce

LHRH, do not function correctly to the feedback when testosterone level decreased. This inability of the pituitary gland to respond appropriately to a decline in testosterone implies that high serum glucose has a central effect on the interaction between the nervous system and endocrine system. The decrease in serum LH and FSH may result from impairment in its production and secretion (11).

Increase in body lipids decreases serum testosterone level due to increased activity of the aromatase enzyme present in fat tissues there by converting testosterone and androstenedione to estrogens (12). Consequently in diabetic men, in addition to decreased testosterone production and metabolism, higher than normal percentages of testosterone and androstenedione are converted into estradiol and estrone, respectively. This increased conversion may account for the diminished testosterone. The increased estrogens appear to exert a negative feedback effect on LH and FSH production (12) and may thereby contribute to the alcoholic suppression of these key reproductive hormones. The decrease in serum albumin is attributed to nutritional status of the subjects (13) and it serve as an antioxidant by providing thiol groups required for 'chain breaking' antioxidant activity.

The greater concentrations of free radicals found in diabetics are due to increased glucose autooxidation (14). The free radicals are secondary species derived from decomposition of lipid hydroperoxides. The study shows that diabetic men are exposed to elevated oxidative stress. It is reported that increased oxidative stress is a well-accepted mechanism of tissue injury (15). This also occurs in the testes (16) and can damage testosterone secreting leydig cells and supporting sertoli cells. The assumption that free radicals can influence male infertility has received

Table 1. Systemic changes in diabetic men

Parameters	Controls (n=25)	Patients (n=35)
Testosterone (ng/ml)	7.54 $\pm$ 0.13	4.91 $\pm$ 0.15*
LH (mIU/ml)	5.8 $\pm$ 0.20	5.71 $\pm$ 0.13
FSH (mIU/ml)	8.12 $\pm$ 0.47	8.02 $\pm$ 0.15
Albumin (gm/dl)	4.23 $\pm$ 0.41	3.39 $\pm$ 0.25*
Total cholest- -erol (mg/dl)	136.22 $\pm$ 9.1	190.14 $\pm$ 7.7*
Triglyceride (mg/dl)	105.16 $\pm$ 10.33	159.66 $\pm$ 8.37*

Data is represented as mean $\pm$ SD

* $P < 0.05$ vs. the control group.

Table 2. Systemic TBARS and antioxidants in diabetic men

Parameters	Controls (n=25)	Patients (n=35)
TBARS (nmol/ml)	3.43±0.24	12.05±0.23*
GSH (mg/mg protein)	3.43±0.19	2.74±0.08*
Superoxide Dismutase (U/mg haemoglobin)	1.26±0.07	2.05±0.01*
Catalase (nmol H ₂ O ₂ decomposed/mg protein/min)	0.131±0.01	0.156±0.01*
Glutathione reductase (nmol NADPH oxidized/mg protein/min)	1.81±0.01	2.17±0.03*
Glutathione peroxidase (U/g haemoglobin)	52.45±1.34	83.52±0.32*
Glutathione-S-transferase (U/g haemoglobin)	1.12±0.01	1.63±0.1*

Data is represented as mean ± SD.

* P<0.05 vs. the control group.

substantial scientific support (17).

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 GSH results in significant alteration in cellular metabolism. GSH concentration reflects its potential for (i) detoxification (ii) preserving the proper cellular redox balance and (iii) its role as a cellular protectant (17). GSH has a likely role in sperm nucleus decondensation and spindle microtubule formation (18). Depletion of GSH might be due to less oxidation of glucose through pentose phosphate pathway operates in erythrocytes precisely to supply NADPH, the co factor needed for the reduction of GSSG (19). It supports the hypothesis that reactive oxygen intermediates generated lead to GSH oxidation and lipid peroxidation.

SOD is the important antioxidant enzyme 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 dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (20). GSH-Red is concerned with the maintenance of cellular level of GSH by effecting fast reduction of oxidized glutathione (GSSG) to reduced form (21). Se-GSH-Px, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes, spermatogenesis and, sperm morphology and motility, and the metabolic pathway of testosterone biosynthesis requires protection against peroxidation (22).

In conclusion, low serum testosterone in diabetic men was accompanied by low serum LH and FSH. The pituitary gland is unable to respond appropriately to a decline in testosterone implying central effect of high serum glucose on the interaction between the nervous and endocrine system. Nutritional deficiency, increased oxidative stress and increased aromatase activity due to excessive body fat might have also contributed to low serum testosterone.

REFERENCES

1. Dinulovic, D. and Radonic, G. (1990). Diabetes mellitus and male infertility. Arch. Androl. 25, 277-293.
2. Dunsmuir, W.D. and Holmes, S.A. (1996). The etiology and management of erectile, ejaculatory and fertility problems in men with diabetes mellitus. Diabet. Med. 13 (8), 700-708.
3. Remzi Cevik, Ali Gur, Suat Acar, Kemal Nas and Ay°egül Jale Sarac. (2004). 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. 5, 47-51.
4. Gil-Del Valle, L., Milian Lde, L., Toledo, A., Vilaro, N., Tapanes, R. and Otero, M.A. (2005). Altered redox status in patients with Diabetes Mellitus type I. Pharmacol. Res. 51(4), 375-380
5. McCormick, D.B. and Greene, H.L. (1988). Tietz textbook of Clinical Chemistry. Eds. Burtis, C.A. and Ashwood, E.R. pp. 1025, W B Saunders Company, USA.
6. Sinnhuber, R.O., Yu, T.C. and Yu, T.C. (1958). Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. Food Res. 23, 626-630.

7. Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for determination of blood glutathione. *J. Lab. Clin. Med.* 61, 882-888.
8. Beers, R.F. and Sizer, I.W. (1952). A spectrophotometric method for measuring the break down of hydrogen peroxide by catalase. *J. Biol. Chem.* 195, 133-140.
9. Pinto, R.E. and Bartley, W. (1969). The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic glutathione oxidation in rat liver homogenate. *Biochem. J.* 112, 109-115.
10. Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974). *J. Biol. chem.* 249, 7130-7139.
11. Emanuele, M. A. and Emanuele, N. (2001). Alcohol and the male reproductive system. *Alcohol Research & Health.* 25 (4), 282-287.
12. Prichard, J., Despres, J.P., Gagnon, J., Tchernof, A., Nadeaci, A., Tremblag, A. and Bouchard, C. (1998). *Journal of Clinical Endocrinology and Metabolisms* 83 (9), 3277-3284.
13. Das, S.K., Nayak, P. and Vasudevan, D.M. (2003). Biochemical markers of alcohol consumption, *Ind. J. Clin. Biochem.* 18 (2), 111-118.
14. Shah Siddharth, N. (2000). Alpha lipic acid in diabetes, *The Asian Journal of Diabetology* 12, 17.
15. Maneesh, M., Jayalekshmi, H., Sanjiba Dutta, Amit Chakrabarti and Vasudevan, D.M. (2005). Effect of chronic ethanol administration on testicular antioxidant system and steroidogenic enzyme activity in rats. *Indian J. Exp. Biol. (In Press)*.
16. Maneesh, M., Jayalekshmi, H., Sanjiba Dutta, Amit Chakrabarti and Vasudevan, D.M. (2005). Experimental therapeutical intervention with ascorbic acid in ethanol induced testicular injuries in rats. *Indian J. Exp. Biol.* 43, 172-176.
17. Mari, M., Wu, D., Nieto, N. and Cederbaum, A.I. (2001). CYP2E1-Dependent Toxicity and Up-Regulation of Antioxidant Genes. *J. Biomed. Sci.* 8 (1), 52-56.
18. Irvine, D. S. (1996). Glutathione as a treatment for male infertility. *Reprod.* 1, 6-10.
19. Asok Kumar, P. and Rajagopal, G (2003). Lipid peroxidation in erythrocytes of patients with type 2 diabetes mellitus. *Ind. J. Clin. Biochem.* 18 (1), 71-74.
20. Lenzi, A., Cualosso, F., Gandini, L., Lombardo, F. and Dondero, F. (1993). Placebo controlled; double-blind cross over trial glutathione therapy, in male infertility. *Hum. Reprod.* 9, 2044
21. Calvin, H. I., Cooper, G. W. and Wallace, E. (1981). Evidence that selenium in rat sperm is associated with a cystine rich structural protein of the mitochondrial capsule. *Gamete Res.* 4, 139-145.
22. Chandra, R., Aneja, R., Rewal, C., Konduri, R., Dass, K. and Agarwal, S. (2000). An opium alkaloid-Papaverine ameliorates ethanol induced hepatotoxicity: diminution of oxidative stress. *Ind. J. Clin. Biochem.* 15 (2), 155-160.