RISK FACTORS FOR CORONARY HEART DISEASE IN TYPE II DIABETES MELLITUS

H. Surekha Rani¹, G. Madhavi¹, V. Ramachandra Rao¹, B.K. Sahay² and A. Jyothy¹

1 Dept. of Cell Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad - 16

2 Sahay's Diabetic Clinic and Research Centre, "My Nest" 6-3-852/A, Ameerpet, Hyderabad-500 016

ABSTRACT

Cardiovascular complications are the major cause of morbidity and mortality in diabetic patients. An attempt has been made to evaluate the risk factors for coronary heart disease in type II diabetics. In the present study the levels of fasting and postprandial plasma glucose, total cholesterol, low density lipoproteins, triglycerides were high and the levels of high density lipoproteins were low in the type II diabetics compared to controls. The markers of free radical induced injury i.e. malondialdehyde and nitrite/nitrate were high while total antioxidant status a marker for antioxidant protection against reactive oxygen species was low in diabetics compared to controls. The study therefore suggests the importance of assessing these markers of oxidative stress and antioxidant capacity along with the other routine investigations in diabetic patients for initiating antioxidant therapy in addition to primary and secondary preventive measures to mitigate the devastating consequences of diabetes leading to coronary heart disease.

KEY WORDS

Type II Diabetes Mellitus, Coronary Heart Disease, Risk factors, Oxidative stress, Total antioxidant status.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function occurs. It is projected that by the year 2025, India alone would have 57 million diabetics mainly of type 2 diabetes constituting 90% of the diabetic population (1-2).

The most common and life threatening disorder that besets type 2 diabetic subjects is coronary heart disease (CHD). Irrespective of the ethnic background the risk for CHD among diabetic subjects is greater by a factor of 2 to 4 compared to non-diabetic subjects (3).

Hyperglycemia, a hallmark of diabetic condition depletes natural antioxidants and facilitates the

production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA etc and exert cytotoxic effects on cellular components (4). Thus increased ROS and impaired antioxidant defense contributes for initiation and progression of micro and macro vascular complications in diabetics (5-7). Hence a systematic approach has been made in the present study to focus on the cardiovascular risk factors in cases with type 2 diabetes.

The study aimed to estimate the markers for free radical injury that is malondialdehyde, nitrite/nitrate and a marker for free radical scavenging activity i.e. total antioxidant status (TAS) along with the lipid profiles, fasting and post prandial sugar levels (FPG and PPG) in 110 patients presenting type 2 diabetes and in equal number of age and sex matched controls. Contributory factors like obesity, hypertension, smoking and alcohol were also recorded.

MATERIALS AND METHODS

The subjects of the present study were 110 patients presenting type II diabetes mellitus along with 110 age and sex matched healthy individuals as controls with no known history of any disease. All the subjects, after

Author for Correspondence :

Dr. A. Jyothy,
Director,
Institute of Genetics and
Hospital for Genetic Diseases,
Osmania University, Begumpet,
Hyderabad -500 016.
E-mail: jyothy@rediffmail.com

obtaining their consent were examined clinically and information pertaining to age, sex, habits and health status was recorded in special case proforma. Blood samples were collected from both controls and patients for a series of laboratory investigations using standard protocols for estimation of glucose (fasting and postprandial), lipid profiles, levels of MDA, nitrite/nitrate and total antioxidant capacity. The study has the approval of the institutional ethical committee, for biomedical research.

Estimation of Glucose levels

The plasma sugar levels were estimated using a standard glucose kit (manufactured by diagnostic division of Reddy's Laboratory, Hyderabad, India).

Estimation of Lipid profiles

Total cholesterol, HDL cholesterol and triglycerides were measured by enzymatic calorimetric method using commercially available kits (Division of Glaxo, Smithkline pharmaceutical Limited, Worli, Mumbai, India 400025). LDL cholesterol was calculated by Friedewalds formula.

Estimation of Lipid peroxidation

Estimation of plasma malondialdehyde (MDA) levels was carried out by the method of Gavino *et al.* (8). MDA is formed as an end product of lipid peroxidation, which reacts with TBA (Thiobarbuturic acid) to form a faint pink colored product. 0.5 ml of plasma was made up to 1 ml with saline and an equal volume of trichloroacetic acid (TCA) was added and incubated at 37°C for 20 min. and centrifuged at 500 g. To 1 ml of TCA extract (the supernatant) 0.25 ml TBA was added and heated in a water bath at 95°C for 1 hour till a faint pink color appeared. After cooling the color was extracted in 1 ml butanol and the intensity was read at 532 nm using Shimadzu UV-240 spectrophotometer. 1,1,3,3 tetra ethoxypropane (1-100 n mol/ml) was used as the standard.

Estimation of nitrite / nitrate

Nitrite / Nitrate concentrations present in the reaction mixture were determined by using Griess reagent (a 1:1 mixture of 1% sulfanilamide in 5% H_3PO_4 and 0.1 % N-(1-naphthyl)-ethylene-diamine) by the method of Lepoivre *et al.* (9). 0.5 ml of serum was precipitated with 50 μ l of 70% sulfosalicylic acid (SSA), mixed well for 5 minutes, vortexed and then centrifuged at 3000 rpm for 20 min. 200 μ l of supernatant was taken and 30 μ l of 10% NaOH, 300 μ l of 50 mM tris buffer and 530 μ l of Griess reagent were added and incubated for 10 min in dark. The absorbance was read against blank (double distilled H_2O) at 540 nm using Shimadzu UV-240 spectrophotometer. The concentration of nitrite/nitrate in serum was determined using the standard curve.

Estimation of Total antioxidant status

Total serum antioxidant levels were determined by the method of Re *et al.* (10). This improved technique involves the direct production of the blue /green $ABTS^+$ (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical chromophore through the reaction between ABTS and potassium persulfate. 100 μ l of 70 mM potassium persulfate was added to 25 μ l of 2.5 mM ABTS. After overnight incubation, the coloured solution was diluted with PBS to read an absorbance of 0.680-0.820 at 734 nm using Shimadzu UV-240 spectrophotometer. The initial OD of ABTS and final OD after addition of 10 μ l of standard /serum were recorded. The extent of decolorization as percentage inhibition of $ABTS^+$ radical cation was determined as a function of concentration and time, and calculated relative to the reactivity of Trolox (a vit E analog (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) as a standard under the same conditions.

Statistical analysis

The mean and SD of serum lipid profiles, plasma MDA levels, serum nitrite/nitrate levels and serum total antioxidant levels estimated in the patients and controls were compared and t-test was employed for statistical significance.

RESULTS

After careful clinical examination and confirmed diagnosis by diabetologist, 110 patients presenting type 2 diabetes mellitus were included in the study. The biochemical investigations were carried out in the blood samples of patients and age and sex matched healthy controls. The levels of glucose (fasting and postprandial), lipid profiles, MDA, nitrite/nitrate and total antioxidant status were estimated in the patients and controls and are presented in tables (1-3).

Age and Sex : In the present study patients presenting type II diabetes mellitus belonged to the age group of 31-66 years. Among 110 patients of diabetes 66 patients were males and 44 were females as shown in Table 1.

The mean $\pm$ SD of age in diabetic males was 48 $\pm$ 10.76 (yrs) and diabetic females was 53 $\pm$ 10 (yrs) and mean age of control males was 50.46 $\pm$ 8.45 (yrs) and control females was 54.64 $\pm$ 12.96 (yrs).

Habits : Out of 66 diabetic males, 17 (25.7%) were smokers and 7 (10.6%) were alcoholic; all the female patients and healthy controls were non-smokers and non-alcoholic as shown in Table 1.

Health status : The mean $\pm$ SD of duration of diabetes among patients was found to be 6.0 $\pm$ 4.2 yrs. Among 110 diabetic patients, 33 (30%) had the history of hypertension.

Table 1. Characteristics of patients and control group

	Cases with Type II DM (n=110)	Controls (n=110)
Sex (Male/Female)	66/44	60/50
Age (yrs) (Mean±SD)		
Males	48±10.76	50.46±8.45
Females	53±10	54.64±12.96
BMI (kg/m²) (Mean±SD)	25.8±4.735	21.18±2.46
Duration of Diabetes (Mean±SD)	6.0±4.2	
Smoking	17/66 (25.7%)	—
Alcoholics	7/66 (10.6%)	—
Hypertension	33/110 (30%)	—

Body Mass Index (BMI): The BMI values ranged between 19 to 34.6 with mean BMI of 25.8±4.73 in patients and 21.18±2.46 in controls.

Biochemical Status

FPG and PPBG : Fasting and postprandial plasma glucose levels estimated in patients and controls are presented below.

The mean±SD of FBG and PPBG levels of patients were found to be 161±63.56 mg/dl and 208±84.42 mg/dl respectively. The levels are significantly high at $p<0.05$ when compared with the mean±SD levels of the controls which are 86.05±13.46 mg/dl and 120.7±19.35 mg/dl respectively.

Lipid profiles : Lipid profiles of patients and controls are presented in Table 2. The mean±SD levels of the

cholesterol in patients are 199.4±27.2 mg/dl and that of the controls are 130±28.6 mg/dl. The levels in the patients are statistically significant at $p<0.05$ compared to controls. The mean±SD levels of triglycerides of the diabetic patients and controls are 190.21±31.47 mg/dl and 148.25±16.59 mg/dl respectively and significant at $p<0.05$. The mean±SD value of HDL were 36.16±5.04 mg/dl and 43.25±12.15 mg/dl in diabetics and controls respectively and found significant at $p<0.05$. The mean±SD levels of LDL in the serum of diabetics and controls were found to be 135.56±32.57 mg/dl and 92.5±8.3 mg/dl respectively and were statistically significant at $p<0.05$.

Plasma Malondialdehyde levels: Malondialdehyde (MDA) levels estimated in diabetes mellitus patients and controls are presented in Table 3. The mean±SD of MDA levels in the plasma of diabetic patients and controls were found to be 3.78±1.2 (n moles/ml) and 1.43±0.32 (n moles /ml) respectively and were significant at $p<0.05$.

Serum Nitrite / Nitrate : Nitrite/ Nitrate levels estimated in diabetes mellitus patients and controls are presented in Table 3. The mean±SD of NO₂/NO₃ levels in diabetic patients and controls were found to be 4.12±0.32 (μ moles/ml) and 1.13±0.46 (μ moles/ml) respectively and were significant at $p<0.05$.

Total antioxidant status : Total antioxidant levels estimated in diabetics and controls are presented in Table 3.

The mean±SD of TAS levels in diabetics and controls are found to be 3.1±0.456 (μ moles/ml) and 4.08±0.262 (μ moles/ml) respectively and found significant at $p<0.05$.

DISCUSSION

Type-II diabetes, an end product of years of metabolic stress accompanying a state of insulin resistance is a chronic disorder (11). It has emerged as a major public health problem in India, a diabetic capital of the world and its onset being a decade earlier in Indians, has several economic implications.

Table 2. Serum lipid profiles of patients and controls

Subjects (No of cases)	Total cholesterol Mean±SD (mg/dl)	LDL Mean±SD (mg/dl)	HDL Mean±SD (mg/dl)	Triglycerides Mean±SD (mg/dl)
Type II DM (n=110)	199.4±27.2*	135.56±32.57*	36.16±5.04*	190.21±31.47*
Controls (n=110)	130±28.6	92.5±8.3	43.25±12.15	148.25±16.59
Significant at * $p<0.05$				

Table 3. Oxidative and Total antioxidant status of patients and controls

Subjects (No. of cases)	MDA levels (nmoles/ ml) Mean±SD	Nitrite/ Nitrate levels (µmoles/ ml) Mean±SD	Total antioxidant levels (µmoles/ ml) Mean±SD
Type II DM (n = 110)	3.78±1.2*	4.12±0.32*	3.1±0.456*
Controls (n = 110)	1.43±0.32	1.13±0.46	4.02±0.582
Significant at *p<0.05			

The diabetic condition contributes for initiation and progression of micro and macro vascular complications in diabetics (5). Of all, cardiovascular complications are the leading cause of mortality and morbidity in diabetics. Framingham heart study (1974) demonstrated a direct association between diabetes and heart failure (12).

Atherogenesis occurs due to a number of factors in diabetic individuals; both insulin resistance and elevated lipid levels, common in diabetes are primary triggers of atherogenic injury. It is also suggested that endothelium in diabetic arteries is more prone to atherogenic injury due to decreased production of endothelial nitric oxide, known to be antiatherogenic, and increased production of plasminogen activator inhibitor (13).

Indians being more vulnerable or susceptible to the CHD, warrants an in-depth study on risk factors leading to CHD in diabetic patients. Therefore the present study made an attempt in this direction to study the risk factors for CHD in diabetic patients.

Stamler (1987) has suggested the classic cardiovascular risk factors like high cholesterol, hypertension and smoking as predictors of cardiovascular mortality in both non diabetic and NIDDM subjects (13). In the present study 30% of the diabetic subjects were hypertensive and 25% of the males had the habit of smoking.

Epidemiological studies published in recent years suggest that postprandial blood glucose might be an independent risk factor of cardiovascular disease (14). In the present study the fasting and post prandial blood glucose levels were of poor control in majority of patients constituting a risk factor for CAD.

The most important risk factors contributing to the development of CHD include lipid disorders. Elevated levels of cholesterol, LDL and triglycerides as the risk factors in diabetes have been demonstrated in several studies (15-17). In the present study the estimated levels of cholesterol, LDL, triglycerides were found to be significantly high compared to controls and the levels of HDL were lower than the controls.

Excessive glucose in diabetics reacts with proteins, to form glycated proteins and undergoes irreversible changes to form compounds called AGE (advanced glycation end products) which trigger atherosclerotic process. The prolonged exposure to hyperglycemia also leads to the increased oxidative stress. Therefore the study also made an attempt to estimate the levels of malondialdehyde, a marker of lipid peroxidation and found that the levels of MDA were higher in diabetics compared to controls. Similar results were reported by Uzel *et al.* (1987), Gallow *et al.* (1993), Ayden (2001) and Seghrouchni *et al.* (2002) in type II DM patients (18-21).

Elevated levels of NO promote the peroxidation of the lipid moiety and induce immune responses and inflammatory reactions that cause cell damage (22). In the present study the serum levels of nitrite/nitrate were estimated in diabetic patients and were found to be higher than the controls. Ayden (2001) reported higher plasma $\text{NO}_2^-/\text{NO}_3^-$ levels in diabetic patients while Chiou (1999) reported higher levels of NO in the aqueous humor (20, 23).

Antioxidants play a vital role as preventive factors in the pathogenesis of coronary heart disease and complications in diabetics (24, 25). The measurement of the total antioxidant capacity in body fluids proved to be an important prognostic or diagnostic guide in patients with atherosclerosis, septic shock, diabetes etc for implementation of antioxidant therapy (26-27). To evaluate the importance of this marker in Indian subjects we have investigated serum antioxidant status in diabetics and healthy controls and a statistically significant difference was observed in TAS values between the groups. Pinzani (1998) observed low TAS values between IDDM group and the young control group (27).

Our study also confirms that there is an increased oxidative stress in diabetics compared to non diabetic counterparts and emphasizes the importance of assessing these markers for early diagnosis and therapeutic interventions.

As India is harboring the largest number of diabetics in the world and Indians being more susceptible to coronary heart disease than any other ethnic group makes it necessary to implement measures for the

management of dual epidemics. It is also necessary to initiate primary preventive measures like reduction in serum lipids, avoiding smoking, increasing the intake of fruits and vegetables, physical activity, maintenance of healthy body weight and secondary preventive measures like control of hyperglycemia and hypertension to mitigate the devastating consequences of diabetes leading to CHD.

ACKNOWLEDGEMENTS

Authors are grateful to the Department of Biotechnology (DBT) New Delhi, India for providing financial assistance.

REFERENCES

- King, H., Aubert, R.E., Herman, W.H. (1998) Global burden of diabetes, 1995-2025. Prevalence, numerical estimates and projection. *Diabetes Care*. 21, 1414-1431.
- Ramachandran, A., Snehalatha, C., Viswanathan, V. (2002) Burden of type 2 Diabetes and its complications - The Indian Scenario. *Curr. Sci.* 83, 1471-1476.
- Deepa, R., Arvind, K. and Mohan, V. (2002) Diabetes and risk factors for coronary artery disease. *Curr. Sci.* 83 (12), 1497-1505.
- Dincer, Y., Akcay, T., Aldemir, Z. and Iikova, H. (2002) Effect of oxidative stress on glutathione pathway in red blood cells from patients with insulin-dependent diabetes mellitus. *Met.* 51, 1360-1362.
- Maritim, A.C., Sanders, R.A. and Watkins, J.B. (2003) Diabetes, Oxidative stress, and antioxidants A review. *J. Biochem. Mol. Toxicol.* 17, 24-38.
- Jialal, I., Devaraj, S. and Venugopal, S.K. (2002) Oxidative stress, inflammation and diabetic vasculopathies: The role of alpha tocopherol therapy. *Free radic. Res.* 36, 1331-1336.
- Ceriello, A., Bortolotti, N., Crescentini, A., Motz, E., Lizzio, S. and Russo, A. (1998) Antioxidant defences are reduced during the oral glucose tolerance test in normal and non-insulin-dependent diabetic subjects. *Eur. J. Clin. Invest.* 28, 329-333.
- Gavino, V.C., Miller, J.S., Ikharebha, S.O., Milo, G.E. and Cornwall, D.G. (1981) Effects of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures. *J. Lipid Res.* 22, 763-769.
- Lepoivre, M., Chenal, B., Vapo, A., Lamair, G., Thalander, L. and Tenu, J.P. (1990) Alteration of ribonucleotide reductase activity following induction of nitrite generating pathway in adenocarcinoma cells. *J. Biolchem.* 265, 14143-14149.
- Re, R., Pellegrini, R.R.N, Proteggente, A., Pannala, A., Yang, M. and Evans, C.R. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical Biol. and Med.* 26, 1231-1237.
- Ajay, K. (2001) Coronary Artery Disease and Diabetes. *Cardiology Today* 4, 221-224.
- Saxena, K.K. (2002) Congestive Heart Failure in Diabetes. *Cardiology Today* 2, 71-76.
- Feldman, E.L. (2000) Oxidative stress and diabetic nephropathy: a new understanding of an old problem. *J. Clin. Invest.* 111, 431-433.
- Bonora, E. and Muggeo, M. (2001) Post prandial blood glucose risk factor for cardiovascular disease in Type II diabetes; the epidemiological evidence. *Diabetologia* 44, 2107-2114.
- Castelli, W.P. (1988) Cholesterol and lipids in the risk of coronary artery disease. The Framingham heart study. *Can. J. Cardiol.* 4, 5-10.
- Gopinath, N., Chada, S.C., Sehgal, A., Shekhaw, S. and Tandon, R. (1994) What is desirable lipid profile. *Ind. heart J.* 46, 325-327.
- McKeigue, P.M., Miller, G.J. and Marmol, M.G. (1989) Coronary heart disease in South Asian overseas. A review. *J. Clin. Epidemiol.* 42, 597-601.
- Uzel, N., Sivas, A., Uysal, M. and O, Z.H. (1987) Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes mellitus. *Horm. metab. Res.* 19, 89-90.
- Gallou, G., Ruelland, A., Legras, B., Maugendre, D., Allannic, H. and Cloarec, L. (1993) Plasma malondialdehyde in type I and type II diabetic patients. *Clin. Chem. Acta.* 214, 227-234.
- Aydin, A., Orhan, H., Sayal, A., Ozata, M., Sahin, G. and Isimer, A. (2001) Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clin. Bio. Chem.* 34, 65-70.
- Seghrouchni, I., Dral, J. and Bannier, E. (2002) Oxidative stress parameters in type I, type II and insulin treated type II diabetes mellitus; insulin treatment efficiency. *Clin. Chem. Acta.* 321, 89-96.

22. Leeuwenberg, C., Hardy, M.M, Hazen, L.S., Wagner, P., Ishi, O.S., Steinbrecher, U.P. and Heineck, Jay W. (1997) Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. *J. Bio. Chem.* 272, 1433-1436.
23. Chiou, S.H., Chang, C.J., Chou, C.K., Hsu, W.M., Liu, J.H. and Chain, C.H. (1999) Increased nitric oxide levels in aqueous humor of diabetic patients with new vascular glaucoma. *Diabetes Care.* 22, 861-862.
24. Kaneto, H., Kajimoto, Y., Miyagawa, J., Matsuoka, T., Fujitani, Y., Umayahara, Y., Hanafusa, T., Matsuzawa, Y., Yamasaki, Y. and Hori, M. (1999) Beneficial Effects of Antioxidants in Diabetes. *Diabetes* 48, 2398-2406.
25. Maxwell, S.R. (1995) Prospects for the use of antioxidant therapies. *Drugs* 49, 345-361.
26. Maxwell, S.R., Thomason, H., Sandler, D., Leguen, C., Baxter, M.A., Thorpe, G.H., Jones, A.F. and Barnett, A.H. (1997) Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *Eur. J. Clin. Invest.* 27, 484-490.
27. Pinzani, P., Petruzzi, E., Orlando, C., Gallai, R., Serio, M. and Pazzagli, M. (1998) Serum antioxidant capacity in healthy and diabetic subjects as determined by enhanced chemiluminescence. *J. Biolumin. Chemilumin.* 13, 321-325.