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Evaluation of antioxidant deficit and lipid profile in Type - 2 Diabetes Mellitus patients

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ABSTRACT

Type 2 Diabetes is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death. The most common and life threatening disorders that besets type 2 diabetic individuals are heart disease, atherosclerosis, retinopathy, and nephropathy. Therefore, the present study was conducted to investigate the status of oxidative stress and related parameters in type 2 diabetes mellitus (DM). Fifty normal control subjects and fifty patients diagnosed for type 2 diabetes mellitus were selected for this study. Blood samples were collected from both controls and patients for a series of laboratory investigations like fasting glucose, lipid profiles, and levels of MDA, nitrite/nitrate and antioxidant status. The levels of fasting and postprandial plasma glucose, low density lipoproteins were high and the levels of high density lipoproteins were low in the type 2 diabetics compared to controls. The results indicate that oxidative status and nitric oxide metabolism are affected in type 2 diabetic patients. We found low antioxidant activity in type 2 diabetic patients which could not protect the patients against the reactive oxygen species (ROS), since lipid peroxidation still occurs in diabetic patients. The Malondialdehyde and nitrite/nitrate levels were high while antioxidant status was low in diabetics compared to controls. High nitric oxide and low density lipoproteins levels in our type 2 diabetic patients might be a good marker of endothelium dysfunction in diabetic mellitus.

Key Words: Type II diabetes mellitus, Malondialdehyde, Antioxidant activity, Reactive oxygen species

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INTRODUCTION

Diabetes mellitus represents an increasingly heavy health burden in our society and has acquired epidemic dimensions. According to recent estimates, the human population worldwide appears to be in the midst of an epidemic of diabetes. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease-related complications are increasingly unabated [1]. Parallel to this, recent developments in understanding the pathophysiology of the disease process have opened up several new avenues to identify and develop novel therapies to combat the diabetic plague. There is an estimated 143 million people worldwide suffering from diabetes, almost five times more than the estimates ten years ago. This number may probably double by the year 2030 [2]. Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time, with people in Southeast Asia and Western Pacific being most at risk.

Type 2 diabetes is the commonest form of diabetes constituting 90% of the diabetic population. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025 [2]. The World Health Organization has predicted that the major burden will occur in the developing countries. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million, in the developing countries. The countries with the largest number of diabetic people are, and will be in the year 2025, India, China and United States [2]. India faces a grave health care burden due to the high prevalence of type 2 diabetes and its sequalae. Epidemiological data from different parts of the country show a rising prevalence of diabetes in the urban areas. Studies conducted in India in the last decade have highlighted that not only is the prevalence of type 2 diabetes high, but also that it is increasing rapidly in the urban population [1, 2]. A national survey of diabetes conducted in six major cities in India in the year 2000 showed that the prevalence of diabetes in urban adults was 12.1%. Prevalence of IGT was also high (14.0%) [2]. A younger age at onset of diabetes had been noted in Asian Indians in several studies. In the national study, onset of diabetes occurred before the age of 50 years in 54.1% of cases, implying that these subjects developed diabetes in the most productive years of their life and had a greater chance of developing the chronic complications of diabetes [2]. Current Science had highlighted that by the year 2025, India is predicted to have the most number of people with diabetes mellitus in the world.

Type 2 diabetes mellitus is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress. These reactive oxygen species set in motion a host of redox reactions which can result in unstable nitrogen and thiol species that contribute to additional oxidative stress. Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death. There is increasing evidence that, in certain pathologic states, the increased production and/or ineffective scavenging of such reactive oxygen species may play a crucial role in determining tissue injury. Endothelial dysfunction is considered an intrinsic element in the pathogenesis of diabetic angiopathies. The free radical nitric oxide (NO) is derived from endothelium [3]. A variety of potential mechanisms for the initiation of endothelial dysfunction in type 2 diabetes have been described including

the effects of hyperglycemia, advanced glycation end products (AGE) and dyslipidaemia [4]. In addition, hyperglycemia has been shown to induce free radical release and reduce anti-oxidant defenses [5], both of which are associated with endothelial dysfunction. Normal endothelial function is the synthesis of nitric oxide (NO) which is responsible for the endothelial vasodilatation and inhibition of platelet adhesion. Diabetes increases the susceptibility for lipid peroxidation. Nitric oxide a physiologically important free radical produces diverse responses, both beneficial and detrimental [6]. To counteract the harmful effects of free radicals, antioxidant defense mechanism operates to detoxify or scavenge these free radicals.

Therefore the present study aimed to understand the interplay among the marker for free radical injury, that is Malondialdehyde, nitrite/nitrate and a marker for free radical scavenging activity i.e. antioxidant status along with the lipid profiles, fasting and blood sugar levels (FBS) in patients presenting type 2 diabetes and in equal number of age and sex matched controls. Early identification of at-risk individuals using simple screening test like estimation of sugar levels, nitric oxide level, antioxidant level and appropriate lifestyle intervention would greatly help in preventing or postponing the onset of diabetes and thus reducing the burden on the community and the nation as a whole.

MATERIALS AND METHODS

The study groups included 50 patients with unregulated Type 2 diabetes (23 male and 27 female) and 50 age and sex matched healthy volunteers as a control group (24 male and 26 female),with no known history of any disease. The study was conducted at K.S.Hegde Medical Academy, Nitte University, after getting the approval from Institutional Ethics Committee. The subjects recruited for the study were the regular attendants of outpatient departments of K.S.Hegde Medical hospital. An informed Consent from all the participants was taken before the study. Fasting blood samples were collected from both controls and patients for a series of laboratory investigations using standard protocols for estimation of fasting blood glucose, lipid profiles, and levels of MDA, nitrite / nitrate and total antioxidant capacity.

Estimation of Glucose levels: The Fasting blood glucose levels were estimated using a standard commercial glucose kit.

Estimation of Lipid profiles: Total cholesterol, triglycerides were measured by enzymatic methods using a Hitachi 917 autoanalyser with its original reagent. HDL cholesterol was measured by dextran sulfate-Mg^{+2} precipitation method. LDL cholesterol was calculated by the formula of Friedewald [7].

Estimation of Lipid peroxidation: Plasma Malondialdehyde (MDA) level was estimated 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 37oC 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 950C 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: We reported the presence of Nitric oxide (NO) as measured through its product NO_2 (nitrite) in human serum using Griess method (Moshage, Kok, Huizenga, & Jansen, 1995) [9]. Nitrite concentration was determined by spectrophotometric method at 540 nm. In Greiss reagent (consisting of equal volume of 0-1% N-1- naphthylyl ethyenediamine, HCl and 1% sulfanilamide plus 5% H_3PO_4) method, 0.5 ml of serum was precipitated with 50 µl of 70% sulfosalicylicacid (SSA), mixed well for 5 minutes, vortexed and then centrifuged at 3000 rpm for 20 min. 200 µl of supernatant was taken and 30 µl of 10% NaOH, 300 µl of 50 mM tris buffer and 530 µl of Greiss reagent were added and incubated for 10 min in dark. The development of characteristic purple color was then measured against blank (double distilled H_20) at 540 nm using Shimadzu UV- 240 spectrophotometer. Values of nitrites were estimated by comparison with a standard curve of nitrite concentration. Nitrate was measured by the enzymatic method of Bories and Bories (1995) [10], on a Hitachi 902 analyzer (Hitachi, Tokyo, Japan). In this method, nitrate reductase from Aspergillus species converts nitrate to nitrite in the presence of β-NADPH. The decrease in absorbance at 340 nm as a result of the oxidation of β-NADPH is measured.

Estimation of Erythrocyte Superoxide Dismutase activity: CuZn-SOD activity in erythrocyte lysate was measured by the method of Bulucu F, et. al. (2000) [11]. Briefly, each hemolysate was diluted 1:400 with 10 mM phosphate buffer, pH 7.00. 25 mL of diluted hemolysate was mixed with 850 mL of substrate solution containing 0.05 mmol/L xanthine sodium and 0.025 mmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)- 5-phenyltetrazolium chloride (INT) in a buffer solution containing 50 mmol/L CAPS and 0.94 mmol/L EDTA pH 10.2. Then, 125 mL of xanthine oxidase (80 U/L) was added to the mixture and absorbance increase was followed at 505 nm for 3 min against air. 25 mL of phosphate buffer or 25 mL of various standard concentrations in place of sample were used as blank or standard determinations. CuZn-SOD activity was expressed in U/gHb.

Estimation of Erythrocyte Glutathione Peroxidase activity: GPx activity measurement. GPx activities in both erythrocyte lysate were measured by the method described in the study of Bulucu F, et. al. (2000) [11]. The reaction mixture was 50 mmol/L tris buffer (pH 7.6) containing 1 mmol/L of EDTA, 2 mmol/L of reduced glutathione (GSH), 0.2 mmol/L of NADPH, 4 mmol/L of sodium azide and 1000 U of glutathione reductase (GR). 50 mL of plasma and 950 mL of reaction mixture, or 20 mL of erythrocyte lysate and 980 mL of reaction mixture were mixed and incubated for 5 min. at 37°C. Then the reaction was initiated with 8.8 mmol/L H_2O_2 and the decrease in NADPH absorbance was followed at 340 nm for 3 min. Enzyme activities were reported in U/g Hb in erythrocyte lysate.

Statistical Analysis: The data obtained in our study was analyzed for its statistical significance using unpaired student't' test .P value less than 0.05 was considered the level of significance.

Conflict of interest: There is no conflict of interest.

RESULTS

The screening test was carried out in both diabetic patients and control subjects. Data's were expressed in Table 1-3. The body mass index of both control and diabetic subjects were measured and compared and it was found to be significantly higher ($p<0.01$) in diabetic patients (Table-1). Total cholesterol, LDL, HDL and Triglyceride level was also compared between the diabetic patients and control subjects. All the lipid parameters were significantly increased in diabetic patients as compared to controls ($p<0.05$, Table-2). As a measure of antioxidant status of the control and diabetic subjects, the level of Nitrite, Nitrate, MDA and erythrocyte superoxide dismutase and Glutathione peroxidase levels were also estimated and compared. It showed a significant increase ($p<0.05$) in all the parameters in diabetic patients as compared to normal control participants. The level of MDA was found to be highly increased ($p<0.01$) in diabetic patients (Table-3).

DISCUSSION

Despite a growing amount of data which show the role of oxidative stress in the etiology of type 2 DM, there are some contradictory results. Nitric Oxide (NO) related parameters have not been assessed so far in this disease. Therefore, the main objective of the present study was to explore both ant oxidative parameters and NO-related parameters in type 2 DM [12].

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 patients. The most important risk factors contributing to the development of endothelial dysfunction and hypertension include lipid disorders. Elevated levels of cholesterol, LDL and triglycerides as the risk factors in diabetes have been demonstrated in several studies [12, 13].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 patients compared to controls. Increased levels of lipid peroxidation may cause oxidative injury to blood cells. [13, 14].Elevated levels of NO promote the peroxidation of the lipid moiety and induce immune responses and inflammatory reactions that cause cell damage. In the present study the serum levels of nitrite/nitrate were estimated in diabetic patients and were found to be higher than the controls. Altered nitric oxide level may be related to advances advanced Endothelial dysfunction in type 2 diabetic mellitus probably due to nitric oxide synthase gene expression.[15, 16].Endothelial dysfunction characterized by loss of endothelium- dependent vasodilatation occurs early in vascular disease, before any morphologically evident alteration

can be detected [16]. Our finding suggests that, decreased production, increased degradation or decreased sensitivity to nitric oxide is involved in endothelial dysfunction.

Antioxidants play a vital role as preventive factors in the pathogenesis of vascular complications in diabetics [17-19]. 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 [20, 21]. In this present study we have evaluated the importance of this marker in Indian subjects we have investigated antioxidant status in diabetics and healthy controls and a statistically significant difference was observed in Erythrocyte Superoxide Dismutase and Erythrocyte Glutathione Peroxidase values between the groups. 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.

CONCLUSION

In conclusion, that increased nitric oxide end-products associated with low HDL levels and increased oxidant stress in the patients with type 2 diabetes may play an important role for the development of vascular complications. This study indicates that oxidative stress and endothelial dysfunction are present in patients with type 2 diabetes mellitus without clinically evident macro vascular complications. Furthermore, the correlation between endothelial dysfunction and oxidative stress in patients with type 2 diabetes support the hypothesis that the impairment of intracellular antioxidant system and endothelial dysfunction are frequently associated in diabetes mellitus.

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Table 1 – Clinical features of diabetic patients and control subjects. Values are expressed as Mean±S.D. n=50 in each group.

Parameter	Control (n=50)	Patients (n=50)	p
BMI (kg/m^2)	23.6 ± 0.8	25.0 ± 1.0	<0.01
Fasting blood glucose (mg/dl)	92.0 ± 2.0	137 ± 14	<0.01
Systolic blood pressure (mmHg)	121 ± 4.0	136 ± 3.0	0.02
Diastolic blood pressure (mmHg)	72.0 ± 2.0	78.0 ± 3.0	0.10

Note: $p<0.05$ was considered as the level of significance.

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Table 2. The lipid profile parameters of patients and controls. Values are expressed as Mean±S.D. n=50 in each group.

Subjects	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)
Control	156 ± 7.12	78 ± 3.2	49 ± 4.92	112 ± 6.45
Patients	198 ± 15.2*	127 ± 21.9*	40 ± 8.32*	188 ± 17.85*

Significant value *p<0.05.

Table 3: **Biochemical findings in patients with type 2 diabetes and control subjects. Values are expressed as Mean±S.D. n=50 in each group.**

Parameter	Control	Patients
Nitrite (µmol/l)	6.0±2.7	6.8±3.3*
Nitrate (µmol/l)	13.8±8.6	24.8±10.8*
Nitrite+ nitrate (µmol/l)	19.8±11.0	31.6±11.3*
Nitrite/nitrate	0.30±0.19	0.27±0.17*
MDA Levels (n moles/ml)	1.43±0.32	3.78±1.2**
Erythrocyte Superoxide Dismutase (U/g Hb)	9.10±0.26	6.43±0.29*
Erythrocyte Glutathione Peroxidase (U/g Hb)	63.59±3.8	46.07±2.7*

Significant value *p<0.01, **p<0.05.

REFERENCES

[1] King H, Aubert RE, Herman WH. Diabetes Care 1998;21:1414-1431.
[2] Ramachandran A, Snehalatha C, Viswanathan V. Curr Sci 2002;83:1471-1476.
[3] Lowenstein CJ, Dinerman JL, Snyder SH: Nitric oxide: A physiologic messenger. Ann Intern Med 1994; 120:227-237
[4] D Giugliano, A Ceriello, G Paolisso. Diabetes Care 1996;19:257–267.
[5] DM Gilligan, MN Sack, V Guetta. J Am Coll Cardiol 1994;24:1611–1617.
[6] JW Baynes. Diabetes 1991;40:405–412.
[7] WT Friedewalt, RI Levy, DS Frederieson. Clin Chem 1972;8:499–502.
[8] Gavino VC, Miller JS, Ikharebha SO, Milo GE and Cornwall DG. J. Lipid Res 1981;22:763-769.
[9] Moshage H, Kok B, Huizenga JR, & Jansen PLM. Clin Chem 1995;41:892– 896.
[10] Bories PN & Bories C. Clin Chem 1995; 41:904–907.
[11] Bulucu F, Vural A, Aydın A, Sayal A. Clin Nephrol 2000;53:169 –73.
[12] H Surekha Rani, G Madhavi, V Ramachandra Rao, BK Sahay and A Jyothy. Indian J Clin Biochem 2005; 20 (2): 75-80.
[13] Birgul Vanizor, Asım Orem S. Caner Karahan. Diab Res Clin Pra 2001; 54:33–39.
[14] Ahmet Aydın, Hilmi Orhan, Ahmet Sayal. Clin Biochem 2001; 34:65–70.

[15] TF Lu Scher, G Noll. J Cardiovas Pharma 1994; 24: 16–26.
[16] J Lincoln, CHV Hoyle, G Burnstock, in: J.A. Lucy (Ed.), Nitric Oxide in Health and Disease. Biomedical Research Topic, Cambridge University Press, UK, 1997, pp. 93–99.
[17] AM Miles, DS Bohle, PA Glassbrenner, B Hansert, DA Wink, MB Grisham. J Biol Chem 1996; 271:40–47.
[18] Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y and Hori M. Diabetes 1999;48:2398-2406.
[19] Maxwell SR. Drugs 1995; 49:345-361.
[20] Maxwell SR, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GH, Jones AF and Barnett AH. Eur J Clin Invest 1997;27:484-490.
[21] Pinzani P, Petruzzi E, Orlando C, Gallai R, Serio M and Pazzagli M. J Biolumin Chemilumin 1998;13:321-325.