MDA and Antioxidants Status in Type 2 Diabetes Mellitus

Roshan Alam*, Saba Khan and Khushtar Anwar Salman*****

*Associate Professor, Department of Biochemistry, Integral Institute of Medical Sciences & Research, Integral University , Lucknow.
**Assistant Professor, Department of Biochemistry, Index Medical College Hospital and Research Centre, Indore, M.P.,
*** Associate Professor, Department of Biochemistry, J N Medical College, A.M.U, Aligarh, U.P., India

Abstract: Background and Objectives: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending on the etiology of the Diabetes mellitus, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. In diabetes, oxidative stress seems caused by both increased production of ROS, sharp reduction in antioxidant defenses and altered cellular redox status. The purpose of this study was to evaluate the oxidative stress in type 2 diabetes mellitus. **Methods:** The study was conducted on 35 diabetic patients (11 Female /24 Male) with mean age of 52.8±5.4 years. Both the study groups were non-smokers and non-alcoholics and were not suffering from any other chronic disease. MDA and antioxidants status were estimated in both cases (35) and controls (30). **Results:** Plasma MDA levels in type 2 diabetic patients were found to be significantly higher ($p<0.001$) than controls, whereas levels of GPx, SOD, CAT, vitamin C and uric acid were significantly lower ($p<0.001$) in the diabetic patients compared to the control subjects. **Conclusion:** Diabetic patients were susceptible to oxidative stress and persistent hyperglycemia had an association with free radical-mediated lipid peroxidation. Our study suggests an imbalance between plasma oxidant and antioxidant system in type 2 diabetes mellitus. [Alam R et al NJIRM 2013; 4(6) : 75-78]

Key Words: Antioxidants, Diabetes Mellitus, Free radicals, Lipid peroxidation, Malondialdehyde.

Author for correspondence: Dr. Roshan Alam, Associate Professor of Biochemistry, IIMS& R, IU, Lucknow.
C/o Mohd Habib, Educational Book House, A.M.U., Shamshad Market, Aligarh -202002, U.P., India.
E- mail: dr.roshan.alam@gmail.com

Introduction: Diabetes mellitus is a major medical problem in developing countries.[1] Type 2 Diabetes mellitus occurs predominately in adults over than 30 years old. It is characterized by peripheral insulin resistance, impaired insulin secretion and excessive hepatic glucose production.[2-4] Oxidative stress is the result of the imbalance in pro-oxidant/antioxidant ratio in favor of the former, potentially leading to macromolecules and cell-dysfunction. Enhanced oxidative stress contributes to the deterioration of pancreatic β-cell progressively due to glucose toxicity, which leads severe impairment of glucose-stimulated insulin secretion, apparent degranulation of β-cells and decreased β-cell numbers.[5-6] During diabetes persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation.[7] In addition, superoxide is generated by the process of glucose auto-oxidation that is associated with the formation of glycated proteins in the plasma of diabetic patients. The increase in ROS production contributes to the development of diabetic complications. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation.[8] The objective of the present study is to evaluate the oxidative stress in type 2 Diabetes mellitus. The diabetic status was assessed by estimating the fasting blood sugar and glycated hemoglobin while the oxidant stress was evaluated by estimating plasma MDA, enzymatic antioxidants SOD, CAT and GPx and non-enzymatic antioxidants vitamin C, and uric acid.

Material & Methods: The criteria used for selection of both diabetes and normal controls were performed by well-established diagnostic criteria as recommended by WHO. The present study was conducted on 35 type 2 Diabetes mellitus patients (female- 11, male- 24) with mean age of 52.8±5.4 years and the control group consisted of 30 healthy individuals with mean age of 47.2±4.8 years. No diabetic subjects were taking medications other than anti-diabetic pills. The study groups (patients and control) were non-smokers and non-alcoholics and were not suffering from any other chronic disease. The study was approved by the Institute Ethics Committee, Index Medical College Hospital and Research Centre, Indore, India and informed consent was obtained from all the cases and control subjects. Blood samples were collected in vacutainers with anticoagulant EDTA and in plain vacutainers

without any anticoagulant from diabetic patients and control subjects. The plasma from EDTA anticoagulated blood was separated, immediately stored at -80ºC and used for determination of MDA, Vitamin C, GPx, Catalase, SOD, Uric acid and Fasting blood sugar ,HbA1c and lipid parameters from serum samples. The plasma and serum samples were used for the analysis of various parameters:

1. Fasting blood sugar was estimated by method of GOD-POD Trinder P.[9]
2. HbA1c by kit method (ERBA diagnostics Mannheim GmbH).
3. Lipid parameter by kit method (ERBA diagnostics Mannheim GmbH).
4. GPx by the method of Rotruck et.al.[10]
5. CAT by the method of Sinha KA.[11]
6. SOD by the method of Kakkar.[12]
7. MDA by the method of Yagi. [13]
8. Vitamin C by the method of Roe and Kuther.[14]
9. Uric acid by kit method (ERBA diagnostics Mannheim GmbH).

All reagents and chemicals used were of analytical grade and were purchased from Merck Chemical Co., Germany. All data were expressed as mean ±SD. Unpaired student's t-test was used for between group comparisons. Differences were considered of statistical significance when the p-value was $p<0.05$.

Result: The study was conducted on 35 type 2 diabetes mellitus patients (both male/female) mean age 52.8±5.4 years and the control groups consisted of 30 individuals (both male/female) mean age 47.2±4.8 years. The clinical and chemical characteristics in type 2 diabetes mellitus patients and control subjects are listed in Table 1. Fasting blood sugar , HbA1c and the lipid parameters were significantly increased in type 2 diabetes mellitus patients ($p <0.001$) as compared to control subjects as shown in the Table 1.

Values are expressed as mean ± S.D, N= number of subjects 'P' <0.05 was considered significant. The level of MDA was significantly increased ($p <0.001$) as compared to healthy subjects. The levels of GPx, SOD, CAT, Uric acid and Vitamin C in the plasma of type 2 diabetes mellitus patients were significantly decreased ($p <0.001$) as compared to control subjects as depicted in Table 2.

Table 1: Clinical and Chemical Characteristics in Type 2 Diabetes Mellitus Patients & Control Subjects.

Parameters	Type 2 DM Patients	Controls	P-value
	N=35	**N= 30**	
Age (years)	52.8 ± 5.4	47.2 ± 4.8	-
Sex (M/F)	24/11	21/9	-
Duration of disease (years)	8.5 ±2.20	-	-
Fasting blood sugar (mg/dl)	141.6 ±9.8	96.8 ± 5.3	<0.001
HbA1c (%)	8.18 ± 0.78	5.16 ± 0.42	<0.001
Total Cholesterol (mg/dl)	228.56±18.34	178.34±12.58	<0.001
LDL-Cholesterol (mg/dl)	132.67±14.76	104.89±11.45	<0.001
HDL-Cholesterol (mg/dl)	37.56 ± 4.11	48.45 ± 5.23	<0.001

Table 2: Lipid Peroxidation, Enzymatic and Non-Enzymatic Antioxidants Status in the Plasma of Type 2 Diabetes Mellitus Patients and Controls.

Parameters	Type 2 DM Patients	Controls	P-value
	N=35	**N= 30**	
MDA (n moles/ml)	6.93 ± 0.92	3.42 ± 0.59	<0.001
Glutathione peroxidase (U^a/ml)	0.214 ± 0.016	0.298 ± 0.026	<0.001
Catalase (U^b/ml)	1.32 ± 0.11	2.09 ± 0.18	<0.001
Superoxide dismutase (U^c/ml)	3.98 ± 0.42	6.12 ± 0.68	<0.001
Vitamin C (mg/l)	10.23 ± 1.12	13.42 ± 1.39	<0.001
Uric acid (mg/dl)	3.11 ± 0.82	4.03 ± 0.98	<0.001

Values are expressed as mean± S.D N= number of subjects; 'P' <0.05 was considered significant.

[a] moles of reduced glutathione utilized/minute.

[b] moles of hydrogen peroxide consumed/minute.

[c] 50% inhibition of NBT reduction.

Discussion: Experimental and clinical evidences suggest that Diabetes mellitus is a state of oxidative stress because of excessive generation of free radicals, caused by persistent hyperglycemia and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus. The relationship between hyperglycemia and oxygen free radicals is supported by our results demonstrating an association between blood levels of glucose and antioxidants both enzymatic and non-enzymatic. Abnormal lipid metabolism often presents in patients with type 2 Diabetes mellitus.[15] The low levels of HDL-C, which exerts antiatherogenic and antioxidative effects when present in sufficient amounts, is a key feature of type 2 Diabetes mellitus. In our study we have shown significantly elevated levels of cholesterol and significantly decreased levels of HDL. We have also shown significant increase in plasma MDA levels. This is in accordance with the study of Sundaram et.al.[16] and significant fall in SOD, CAT and GPx levels in type 2 diabetic patients as compared to control subjects, which is an indication of marked oxidative stress. The products of membrane lipid peroxidation and other oxidants may react with SOD resulting in oxidative modification thereby causing loss of enzyme activity.[16] The decreased CAT activity during diabetes could result from inactivation by glycation of enzyme.[17]Several studies have shown lowered plasma concentration of Vitamin C in diabetics as compared to healthy subjects.[18,19] In our study significantly lower levels of Vitamin C was found in patients which might be due to a higher turnover rate of ascorbic acid, with increased oxidation to the oxidized form dehydroascorbate. In humans, uric acid is the main plasma antioxidant followed by Vitamin C. Uric acid stabilizes Vitamin C in plasma and protects it from oxidation.[20] We observed significantly decreased plasma uric acid level as compared to control. This reduction of uric acid levels might be due to oxidative stress and increased free radical formation which causes reduction in the levels of antioxidants in type 2 Diabetes mellitus.

Conclusion: Present study shows that persistent hyperglycemia in type 2 diabetes mellitus activates cellular and tissue damage by oxidative stress but the compensatory mechanisms for defence against the ROS to normalize oxidative stress was not achieved in type 2 diabetes. Oxidative stress causes reduction of antioxidants in the body, resulting imbalance between plasma oxidant and antioxidants status. Ours results indicate that depletion of antioxidant defenses and significantly increase in MDA, a marker of lipid peroxidation may appear early in type 2 diabetes patients, before the development of secondary complications. In conclusion diabetic complications can be prevented by supplementing the antioxidants rich components of the diet to improve the intrinsic antioxidant system.

References:

1. Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97(18): 1837.
2. Kuroki T, Isshiki K, King GL. Oxidative stress: The lead or supporting actor in the pathogenesis of diabetic complications. J Am Soc Nephrol 2003; 14: S216‑20.
3. Ahmad M, Khan MA, Khan AS. Naturally occurring antioxidant vitamin levels in patients with type‑II diabetes mellitus. J Ayub Med Coll Abbottabab 2003; 15: 54‑7.
4. Laight DW, Carrier MJ, Anggard EE. Antioxidants, diabetes and endothelial dysfunction. Cardiovasc Res 2000; 47: 457‑64.
5. Gorogawa S, Kajimoto Y, Umayahara Y, Kaneto H, Watada H, Kuroda A, et al. Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in type 2 diabetes. Diabetes Res Clin Pract 2002; 57: 1-10.
6. Schinner S, Scherbaum WA, Bornstein SR, Barthel A. Molecular mechanisms of insulin resistance. Diabet Med 2005; 22: 674-82.

7. Moussa SA. Oxidative stress in diabetes mellitus. *Romanian J Biophys* 2008; 18(3): 225-236.
8. Giugliano, D., Ceriello, A. and Paolisso, G. Oxidative stress and diabetic vascular complications. Diabetic Care 1996; 19: 257-267.
9. Trinder P. Estimation of blood glucose. Ann Clin Biochem 1969; 6: 24.
10. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical roles as a component of glutathione peroxidase. Science 1973; 179: 588-90.
11. Sinha, KA. Colorimetric assay of catalase. Annal. Biochem 1972; 47: 389-394.
12. Kakkar, P., Das, B. and Viswanathan, P.N. A modified spectrophotometric assay of superoxide dimutase. Indian J. Biochem. Bio. Phys 1984; 21: 130-132.
13. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids 1978; 45: 337-51.
14. Roe JH, Kuther CA. Detection of ascorbic acid in whole blood and urine through the 2, 4, DNPH derivative of dehydroascorbic acid. J. Biol. Chem. 1943; 147: 399-401.
15. Rosenson RS. HDL-C and the diabetic patient: target for therapeutic intervention? Diabetes Res Clin Pract 2005; 68 Suppl 2: S36-42.
16. Sundaram RK, Bhaskar A, Vijayalingam S., Viswanathan, M., Mohan, R. and Shanmugasundaram , K. R. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond) 1996; 90: 255-60.
17. Yan H., Harding J.J. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. Biochem J 1997; 328: 599–605.
18. Som S et al. Ascorbic acid metabolism in diabetes mellitus. Metabolism 1981; 30: 572-577.
19. Stankova L et al. Plasma ascorbate concentrations and blood cell dehydroascorbate transport in patients with DM. Metabolism 1984; 33: 347-353.
20. Ames, B N., Cathcart R., Schwier, E. & Hochstein P. Uric acid provides an antioxidant defense in human against oxidant and readical-caused aging and cancer: a hypothesis. Proc, Natl Acad. Sci. USA,1981; 18 : 6858-6862.

Conflict of interest: None
Funding: None