JAMP

Original Research Article

OXIDATIVE STRESS IN TYPE 2 DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL

Sushil Kumar[1], Jaya Jain[2], Ashutosh Jain[3], Kamna Singh[4]

[1]Ph.D. Scholar, Department of Biochemistry, Index Medical College, Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[2]Associate professor, Department of Biochemistry, Index Medical College, Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[3]Assistant Professor, Department of Physiology, Index Medical College, Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[4]Associate Professor, Department of Biochemistry, S.N. Medical College, Agra, Uttar Pradesh, India

Abstract

Background: Blood arteries in type 2 diabetics are damaged by oxidative stress-induced cellular inflammation brought on by hyperglycemia-induced excess free radical production, dyslipidemia, and other factors. **Materials and Methods:** 100 Type 2 diabetics and 100 controls with similar ages and sexes were involved in the study. Using accepted techniques, fasting and post-prandial glucose, HbA1C, lipid profile, malondialdehyde, uric acid, and ceruloplasmin levels were calculated. **Result:** Increased levels of triglycerides, total cholesterol, LDL, MDA, UA, and CP, as well as lower levels of HDL, were seen in type 2 diabetics. While HDL and HbA1c exhibited a substantial negative association, MDA, CP, UA, and LDL showed a significant positive correlation. Significantly positive correlations between MDA, CP, and UA and LDL and HDL were observed. Significant positive correlations between MDA, CP, and UA were observed. **Conclusion:** To minimize oxidative stress in T2DM, it is crucial to maintain strict control of blood sugar (HbA1C 6.5%), LDL (100 mg/dL), and HDL (>40 mg/dL), early in the course of the disease.

INTRODUCTION

It has been suggested that the various molecular disorders underlying the emergence of insulin resistance, -cell dysfunction, and impaired glucose tolerance leading to the emergence of type 2 diabetes mellitus are all connected by oxidative stress, which produces reactive oxygen species (ROS).[1] The pathogenesis of T2DM and its complications is heavily influenced by oxidative stress, which results from persistent hyperglycemia and dyslipidemia. This stress causes excessive ROS production, autooxidation of glucose, non-enzymatic protein glycosylation, the formation of lipid peroxides, impaired glutathione metabolism, impaired activities of antioxidant defense enzymes, and decreased concentrations of low molecular weight antioxidants like ceruloplasmin and uric acid. Since lipid peroxidation products are stable and simple to measure, malondialdehyde, also known as TBARS (ThioBarbituric Acid Reacting Substances), is widely used to assess the prooxidant/antioxidant balance in type 2 diabetic patients. The availability of iron in processes that produce free radicals is reduced by ceruloplasmin acting as ferroxidase.[2-4] A rise in CP levels is likely to favor its preventive impact against free radical

harm given the pro-oxidant condition of T2DM patients.[5] In contrast, a rise in serum CP in type 2 diabetes may result in an excess of oxidized LDL, which results in atherosclerosis.[6] By producing free radicals like hydrogen peroxide during the oxidization of serum homocysteine, it may also result in vascular damage.[7] In humans, vitamin C is the secondary plasma antioxidant to uric acid. Vitamin C in plasma is stabilized and shielded from oxidation by uric acid. The soluble form of uric acid in the blood, known as urate, has the ability to chelate transition metals and can scavenge superoxide, hydroxyl, and singlet oxygen radicals.[8] Additionally, uric acid can prevent the superoxide anion from reacting with nitric oxide to create peroxynitrite, a highly toxic byproduct that can harm cells by nitrosylating their tyrosine residues.[9] By analyzing malondialdehyde (a measure of lipid peroxidation), uric acid, and ceruloplasmin (physiological, endogenous free-radical scavengers), and comparing them to HbA1C and lipid profile, it is possible to assess oxidative stress in T2DM.

MATERIALS AND METHODS

This study was conducted in the department of biochemistry, Index Institute of Medical Sciences

and Hospital in collaboration with the department of medicine, Indore, Madhya Pradesh. The institutional ethics committee gave its approval to this work. Before taking a blood sample, each participant's informed oral agreement was obtained after they had been informed of the study's goals in their native language. In this study, 100 Type 2 diabetics without problems served as the cases, whereas 100 controls with similar ages and sexes appeared to be in good condition. Each participant's full medical history was elicited, and each person underwent a comprehensive physical examination.

Inclusion Criteria
In the study, patients diagnosed with Type 2 diabetes mellitus without complications through history, clinical examination, and laboratory investigations and falling within the age range of 35 to 65 years were included. For the study, controls consist of people who appear to be in good health and are similar in age and sex to the sample group.

Exclusion Criteria
Patients with microvascular problems including neuropathy, nephropathy, and retinopathy as well as macrovascular issues like cardiovascular, cerebrovascular, and peripheral vascular illnesses were excluded. The study did not include patients with hemoglobinopathies, anemia, chronic alcoholism, renal, hepatic, or thyroid abnormalities, febrile sickness, diabetic ketoacidosis, renal failure, or chronic illnesses. Patients receiving insulin therapy for Type I or Type II diabetes were disqualified. Pregnant women and those on lipid-modifying medications like statins or fibrates, steroids, beta blockers, thiazides, phenytoin, etc. were excluded.

Sample Collection
All subjects underwent a 12- to 14-hour overnight fast before having their fasting blood drawn. 5 ml of blood was extracted from the median cubital vein of the study participants using aseptic techniques. The blood was divided into two vials, one of which was empty and the other contained a 2:1 mixture of potassium oxalate and sodium fluoride, an anticoagulant. Serum, plasma, and whole blood parameters were examined. Whole blood is used for estimation of Malondialdehyde by Thiobarbituric acid method,[10] and HbA1C by Ion exchange resin method.[11] Plasma is used for fasting glucose estimation by GOD-POD method.[12] Serum for estimation of Cholesterol by CHOD-POD method,[13] Triglycerides by GPO method,[14] HDL-C by Phosphotungstic acid method,[15] Ceruloplasmin by Ravin's method,[16] Uric acid by TBHB-POD method.[17] VLDL-C & LDLC were calculated by Friedwald's formula.[18] Then 2ml of post prandial blood sample is collected for estimation of post-prandial plasma glucose concentration.

Statistical Analysis
Excel and medical statistical tools were used to examine the results using descriptive statistical analysis. For each variable, the results were reported as Mean Standard Deviation. Analysis of variance was used to compare the parameters between groups, and P 0.05 is regarded as significant. The disease and the variables are correlated using Pearson's correlation.

RESULTS

This study covered 200 patients in total. Two sets of study participants were used: group A (Control) consisted of healthy persons, and group B (Type 2 Diabetic) of patients with diabetes mellitus. In A & B groups, the distribution of participants by age is shown in [Table1].

The mean age of the Type 2 diabetic subjects was having higher 51.76 ± 15.36 years and in 50.24 ± 14.21 years for healthy controls. The mean SBP of the Type 2 diabetic subjects was significantly higher 150.32 ± 31.25 as compared to 23.07 ± 1.49 healthy controls. Similarly, mean DBP of the Type 2 diabetic subjects was significantly higher 98.21 ± 18.26 as compared to 80.54 ± 16.21 healthy controls [Table 2].

The mean FBS and PPBS of the Type 2 diabetic subjects was significantly ($P<0.01$) higher 178.45 ± 32.32 and 215.54 ± 45.26 as compared to 88.28 ± 15.25 and 121.18 ± 25.06 healthy controls[Table-8 & Fig.9].. Similarly, mean HbA1c of the Type 2 diabetic subjects was significantly ($P<0.001$) higher 7.39 ± 2.54 as compared to 4.41 ± 1.32 healthy controls [Table-3 & fig.1].

All serum lipid and lipoproteins were significantly higher in Type 2 diabetic patients as compared to healthy controls except HDL-c which is significantly lower in Type 2 diabetic patients compared to healthy controls. Mean level of cholesterol value in Type 2 diabetic patients was significantly higher than the mean serum of healthy controls ($P<0.001$). The mean level of triglycerides in Type 2 diabetic patients was significantly ($P<0.001$) increased compared to healthy controls. The mean level of LDL-c in Type 2 diabetic subjects was statistically significant ($P<0.001$) higher than the mean value of healthy controls. Mean level of serum HDL cholesterol was significantly ($P<0.03$) lower in Type 2 diabetic subjects as compared to the mean value of healthy controls. Mean level of uric acid value in Type 2 diabetic patients was significantly higher than the mean serum of healthy controls ($P<0.01$) in [Table4].

MDA, SOD, GSH, and CAT levels were examined in order to quantify the oxidative stress that was caused in Type 2 diabetic. Patients with Type 2 diabetic had MDA &ceruloplasmin levels that were significantly higher (0.9 ± 0.013; 43.21 ± 10.41) than those with healthy controls (0.05 ± 0.002; 74.14 ± 14.32). Mean level of CAT 0.92 ± 0.041value in Type 2 diabetic patients was significantly lower than the mean serum1.05 ± 0.035 of healthy controls ($P<0.01$). Similarly, SOD activity (0.055 ± 0.02) and

GSH level (0.05±0.002) were lower in the Type 2 diabetic group compared to the healthy control group for SOD (0.087±0.004) and GSH (0.11±0.004), respectively in [Table-5 & fig.2].

The correlation of other parameters with Malondialdehyde, Ceruloplasmin and Uric acid was summarized in [Table 6].

Table 1: Distribution of subjects according to age group.

Age group	Control	Type 2 Diabetic
35-50	32 (32.0%)	36 (36.0%)
50-65	68 (68.0%)	64 (64.0%)
Total	100 (100.0%)	100 (100.0%)

Table 2: Shows the general demographic characteristics in between control and Type 2 diabetic.

Variables	Control	Type 2 diabetic	P value
Male : Female	64:36	62:38	0.25
Age in years	50.24±14.21	51.76±15.36	0.18
SPB in mm of Hg	122.14±26.04	150.32±31.25	0.01
DBP in mm of Hg	80.54±16.21	98.21±18.26	0.01

Table 3: Shows the comparison of sugar profile in between control and Type 2 diabetic.

Sugar profile	Control	Type 2 diabetic	P value
FBS (mg/dl)	88.28± 15.25	178.45±32.32	0.01
PPBS (mg/dl)	121.18±25.06	215.54±45.26	0.01
HbA1c (%)	4.41±1.32	7.39±2.54	0.001

Table 4: Shows the comparison of lipid profile in between control and Type 2 diabetic.

Lipid profile	Control	Type 2 diabetic	P value
T. cholesterol (mg/dl)	184.45±14.21	274.65±21.24	0.001
Triglycerides (mg/dl)	152.21±15.32	306.32±54.36	0.001
HDL-c (mg/dl)	41.21±7.38	27.65±4.21	0.03
LDL-c (mg/dl)	138.24±14.65	294.32±24.36	0.001
Uric acid (mg/dl)	2.9±0.82	5.68±1.38	0.01

Table5: Shows the comparison of oxidative stress in between control and Type 2 diabetic.

Oxidative stress variables	Control	Type 2 diabetic	P value
MDA (nmol/ml)	0.05±0.002	0.9±0.013	0.01
Ceruloplasmin	43.21±10.41	74.14 ±14.32	0.01
CAT (MU/L)	1.05±0.035	0.92±0.041	0.01
SOD (units/ml)	0.087±0.004	0.055±0.02	0.01
GSH (µM)	0.11±0.004	0.05±0.002	0.01

Table 6: Shows the Pearson correlation with Malondialdehyde, Ceruloplasmin& Uric acid.

| Variables	Correlation with Malondialdehyde		Correlation with Ceruloplasmin		Correlation with Uric acid	
	(r) value	P value	(r) value	P value	(r) value	P value
Fasting glucose	+ 0.36	0.002	+ 0.38	0.002	+ 0.42	0.001
Postprandial glucose	+ 0.56	0.001	+ 0.55	0.001	+ 0.52	0.001
HbA1c	+ 0.48	0.001	+ 0.50	0.001	+ 0.54	0.001
Total cholesterol	+0. 85	0.001	+ 0.82	0.001	+ 0.68	0.001
Triglycerides	+ 0.77	0.001	+ 078	0.001	+ 072	0.001
HDL	-0.62	0.001	+0.64	0.001	+0.62	0.001
LDL	+ 0.58	0.001	+ 0.61	0.001	+ 0.66	0.001
Malondialdehyde	-	-	+ 0.69	0.001	+ 0.70	0.001
Ceruloplasmin	+ 0.52	0.001	-	-	+ 0.67	0.001
Uric acid	+ 0.64	0.001	+0.69	0.001	-	-
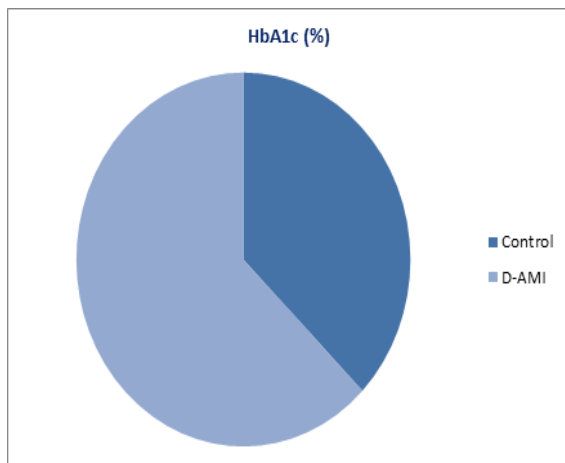
Figure 1: Shows the comparison of HbA1c in between control and D-AMI.
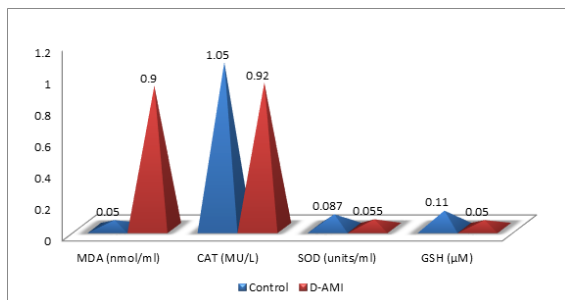

Figure 2: Shows the comparison of oxidative stress in between control and Type 2 diabetic patients.

DISCUSSION

In the world, diabetes is an important health issue. It has negative socioeconomic and health effects on both individuals and populations. Additionally, shifting demographics including population aging, socioeconomic conditions, dietary habits, and migratory causes, as well as an increase in overweight and obese adults and children, are contributing factors to the pandemic rise in diabetes.[19,20] A typical endocrine metabolic condition is diabetes. Elevated glucose levels are caused by a variety of biological and epigenetic causes, including decreased insulin secretion, aversion, or both.[21] Chronic increases in blood sugar, non-esterified fatty acids, and oxidative stress are hallmarks of diabetes mellitus. High levels of malondialdehyde, ceruloplasmin, and uric acid in type 2 diabetics are indicative of high OS. In this study, type 2 diabetics have much higher levels of the lipid peroxidation product, MDA, assessed as TBARS, than do controls. This result is consistent with other studies' findings, as shown in [Table 6].[22,23] In the current investigation, patients had considerably higher ceruloplasmin levels than controls. These results are consistent with research from A. Sarkar et al (2010),[24] and B. Vîrgolici et al. (2008).[25] In the current investigation, type 2 diabetic patients had considerably higher serum uric acid levels than did controls. These results are consistent with research by Natheer H. Al-Rawi

(2011), A. Sarkar et al. (2010), and B. Vîrgolici et al. (2008). MDA, ceruloplasmin, and uric acid significantly positively correlated with HbA1C in the current study (P 0.001). The current study found a highly significant positive association between MDA and triglycerides, cholesterol, VLDL, and LDL (P 0.001) and a highly significant negative correlation between MDA and HDL (P 0.001). Ceruloplasmin significantly positively correlated with dyslipidemia in the current study (P 0.001). Studies by B.Vîrgolici et al. (2008) and Sarkar et al. (2010) produced similar results. Uric acid significantly positively correlated with cholesterol, triglycerides, VLDL, and LDL in the current study (P 0.001). A extremely substantial negative connection between HDL and uric acid was found (P 0.001). Studies by B.Vîrgolici et al. (2008) and Natheer H Al-Rawi (2011) produced similar results. In comparison to non-diabetic persons, type II diabetes patients have greater levels of MDA, which stands for the oxidative damage products of lipids and proteins, respectively.[26] The progression of DM is characterized by maxima in nonesterified fatty acids, oxidative stress, and hyperglycemia. Increased oxidative stress is indicated by high MDA levels in Type II diabetes. In this study, diabetes type 2 participants have a considerably higher level of the lipid peroxidation product, MDA, assessed as TBARS, compared to nonnormal subjects. This result agreed with that of Moussa and Rani et al.[28]

CONCLUSION

These results indicate that Type 2 diabetes is a chronic, progressive disease characterized by hyperglycemia and dyslipidemia, which increases cellular susceptibility to lipid peroxidation and inflammation as a result of oxidative stress, which is a key factor in the pathogenesis of diabetes and its complications. The findings of this study and other research provide enough proof that type 2 diabetes patients with poor metabolic control and dyslipidemia had higher MDA, CP, and UA levels. These findings imply that supportive therapy targeted at oxidative stress may aid in delaying the onset of type 2 diabetes mellitus problems. Along with normal care, methods like consistent exercise and/or antioxidant therapy may enhance the quality of life and slow the progression of the disease in type 2 diabetic patients.

REFERENCES

1. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Giuseppe E, et al, Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. Diabetes, 46:1853–1858, (1997).

2. Goldstein IM, Kaplan HB, Edelson HS, Weissmann G: Ceruloplasmin, a scavenger of superoxide anion radicals. J BiolChem254:4040-4045, 1979

3. Ames B.N., Cathcart R., Schwiers E., Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant

and radical-caused aging and cancer: a hypothesis. Proc. Natl. Acad. Sci. USA 1981; 78 (11): 6858- 6862.

4. Gutteridge JMC. Ceruloplasmin: a plasma protein, enzyme, and antioxidant. Ann ClinBiochem. 1978; 15:293 – 294.

5. Memisogpullari R, Bakan E. Levels of ceruloplasmin, transferrin and lipid peroxidation in the serum of patients with type 2 diabetes mellitus. J Diab Comp. 2004; 18: 193 – 197.

6. Ehrenwald E, Chisolom GM, Foz PL: Intact human ceruloplasminoxidativelymodifieslow density lipoprotein. J Clin Invest 93:1493-1501, 199

7. Starkebaum G, Harlan JM: Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest 71:1370-1376, 1986

8. Simie MG, Jovanovich SV. Antioxidation mechanisms of uric acid. J Am ChemSoc1989;111:5778-82.

9. Squadrito GL, Cueto R, Splenser AE, Valavanidis A, Zhang H, Uppu RM, et al. Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. Arch BiochemBiophys2000;376:333-37.

10. Placer ZA, Lind L, Cushmann M, Johnson BC. Estimation of product of lipid peroxidation (MDA) in biological systems. Anal Biochem. 1966;16:359–64.

11. Trivelli, L.A., Ranney, H.M. and Lai, H.T., Estimation of glycosylated haemoglobin; New Eng. J. Med. 284, 353 (1971).

12. Tietz, N.W. Fundamentals of clinical chemistry; W.B.Saunders&Philidelphia PA, 1976; 243.

13. Roeschlau P., Bernt E, and Gruber W.A., Estimation of serum cholesterol; clinbiochem 12(226), 1974.

14. Fossati P, Prencipe L. Serum triglycerides determined colonmetrically with an enzyme that produces hydrogen peroxide. ClinChem1982;28:2077-80.

15. Burstein M., Scholnick H.R., Morfin R. (1970): Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. Journal of Lipid Research, 11, 583- 595.

16. Ravin HA. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med. 1961; 58: 161 – 168.

17. Trivedi R, Rebar L, Berta E, Stong L. New enzymatic method for serum uric acid at 500 nm. ClinChem1978;24:1908–11.

18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. ClinChem1972;18:499-502.

19. Lardenson P, Kim M. Cecil Medicine. 23rd ed. Philadelphia: Saunders Elsevier; 2004. p. 1658‑713.

20. Davies TF, Larsen PR, Williams Text Book of Endocrinology. 11th ed. Philadelphia: Elsevier; 2008. p. 333‑68.

21. Al‑Wazzan HT, Daban AH, AA Raffa, El-Shazly MK. Prevalance and associated factor's of thyroid dystunction among type 2 diabetic patients. Kuwait Bull Alex Fac Med 2010;46:141‑8.

22. Nakhjavani M, Esteghamati A, Nowroozi S, Asgarani F, Rashidi A, Khalilzadeh O. Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels; Singapore Med J 2010; 51(7) : 582.

23. S.A. moussa. Oxidative stress in diabetes mellitus; romanian j. Biophys., vol. 18, no. 3, p. 225–236, bucharest, 2008

24. A Sarkar, S Dash, B K Barik, M S Muttigi, V Kedage, J K Shetty and M Prakash. Copper and ceruloplasmin levels in relation to total thiols and gst in type 2 diabetes mellitus patients; Indian Journal of Clinical Biochemistry, 2010 / 25 (1) 74-76

25. BogdanaVîrgolici, Maria Mohora, Laura Găman, Daniela Lixandru, B. Manolescu, AncaComan, Irina Stoian. Relation between inflammation and oxidative stress markers in diabetic foot patients; ROMANIAN J. BIOPHYS., Vol. 18, No. 4, P. 273–282, BUCHAREST, 2008.

26. Tosukhowong P, Sangwatanaroj S, Jatuporn S, Prapunwattana P, Saengsiri A, Rattanapruks S, et al. The correlation between markers of oxidative stress and risk factors of coronary artery disease in Thai patients. ClinHemorheolMicrocirc2003;29:321‑9.

27. Rani AJ, Mythili SV, Nagarajan S. Serum nitrite levels in relation to malondialdehyde in type 2 diabetes mellitus. Recent Res SciTechnol2012;4:11‑2.

28. Moussa SA. Oxidative stress in diabetes mellitus. Romanian J Biophys Bucharest 2008;18:225‑36.