

RESEARCH ARTICLE

Oxidative stress in patients with diabetes mellitus type 2

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ABSTRACT

Background: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. India is one of the epicenters of the global DM pandemic. Oxidative stress which is classically defined as an event resulting from the magnitude of imbalance between oxidant and antioxidant substances, generated in a setting of oxidation-reduction reactions, is hypothesized to play an important role in the development of DM. **Aims and Objectives:** This study aims to assess lipid peroxidation by estimating serum malondialdehyde (MDA) and antioxidant status by assaying paraoxonase-1 (PON-1) in Type 2 diabetes patients and healthy controls and also to study the correlation between MDA and PON-1 levels in patients with Type 2 diabetes. **Materials and Methods:** A cross-sectional comparative study was conducted in 152 participants, which were divided into two groups as patients with type 2 diabetes ($n = 76$) and control ($n = 76$) non-diabetic, healthy, age- and sex-matched individuals. The study was conducted in Government Medical College, Kozhikode, after the approval of the Institutional Ethical Committee. All the subjects who satisfied the inclusion and exclusion criteria and who gave informed consent were included in a consecutive manner till sample size is achieved. Serum MDA and PON-1 were estimated using spectrophotometry. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 18. **Results:** Oxidative stress was increased in type 2 diabetic patients as evidenced by significantly elevated MDA and reduced PON-1 than the normal controls. **Conclusion:** Oxidative stress is an important pathophysiological process for the development of type 2 diabetes. This study reveals the importance of screening all diabetes patients for oxidative stress. Dietary management and antioxidant supplementation would help them to prevent development of complications following diabetes, which, in turn, improve their quality of life.

KEY WORDS: Type 2 Diabetes; Oxidative Stress; Paraoxonase-1; Malondialdehyde

INTRODUCTION

In Asia, over 110 million people are estimated to be living with diabetes. It is projected that the worldwide prevalence

would increase to 439 million by 2030.^[1] Greatest increase in rates of patients with diabetes is also seen in India.^[2] Among developing economies, India has around 77 million people with diabetes making it the second most affected nation in the world after China. One in six people with diabetes in the world is from India.^[3] The abnormally high levels of glucose in diabetes upset hemodynamic and metabolic homeostases, while the chronic nature of this disease promotes the development of diffuse cellular abnormalities resulting in the development of serious complications which include extensive vascular dysfunction.^[4]

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Oxidative stress has now emerged as a significant novel target for Type 2 diabetes. Oxidative stress is a process in which there is oxidant-antioxidant substance imbalance produced in a background of oxidation reduction reactions in a biological system. Chemically, the production and the actions of such substances mainly depend on an oxidation-reduction system, so “imbalance of redox system” is the term which is now used for referring the oxidative stress which can result in producing peroxides and free radicals that lead to toxic effects in the normal cell redox states. The oxidants which are otherwise called as free radicals include reactive nitrogen species and reactive oxygen species (ROS) and perform glycation (oxidation of glucose) and lipoxidation (oxidation of lipids).^[5] Excessive levels of ROS can damage majority of cell components which include lipids, proteins, and DNA leading to cellular dysfunction including altered cell signaling, impairment in energy metabolism, impairment in cell transport mechanisms, loss of cell cycle control and biological activity dysfunction, inflammation, and activation of immune system.^[6]

Since oxidative stress results from imbalance between the amount of pro-oxidant and antioxidant substances, serum malondialdehyde (MDA) can be used to assess the levels of oxidative stress and serum paraoxonase (PON-1) for antioxidant status.

Free radicals damage lipids by targeting particularly polyunsaturated fatty acids initiating a process called lipid peroxidation. The decomposition of lipid peroxides in the presence of transition metals such as Fe and Cu gives cytotoxic compounds such as MDA and hydroxynonenal which can cause chemical modification of membrane phospholipids, proteins, and DNA. MDA is a common marker used for assessing lipid peroxidation.^[7]

Antioxidant is defined as any substance that prevents the process of oxidation or repairs the damage caused by action of oxidants in a living organism. PON is a polymorphic enzyme located on chromosome 7 having three gene families: PON 1, PON 2, and PON3. Human serum PON-1 is a liver synthesized enzyme and released in blood binding to high-density lipoprotein (HDL). It is an antioxidant enzyme that hydrolyzes the toxic peroxides in the oxidized lipids in both low-density lipoprotein (LDL) and HDL.^[7]

MATERIALS AND METHODS

A cross-sectional comparative study was done in Government Medical College, Kozhikode, after obtaining necessary clearance from the Institutional Ethics Committee. The study was conducted for a period of 1 year. Seventy-six clinically diagnosed type 2 diabetic patients and 76 age- and sex-matched normal healthy subjects taken from the Medicine Outpatient Department, hospital staff, and the

patient bystanders were studied at Government Medical College, Kozhikode. The age of patients varied from 35 to 65 years (mean age 51.26). Type 2 diabetes was diagnosed on the basis of classical symptoms, fasting blood sugar, and postprandial blood sugar values according to the American Diabetes Association diagnostic criteria 2015. Patients with cardiovascular diseases, liver diseases, malignancy, infective diseases, alcoholics, and smokers were excluded from the study. To ensure their voluntary participation, subjects were briefed about the objectives and methods of study and an informed written consent was taken before the study. Detailed history was taken as per the pro forma. Relevant clinical examination was done. Blood samples were collected for blood investigations.

Estimation of Serum MDA

MDA mainly exists as a highly reactive compound enol, is the most commonly used indicator of lipid peroxidation. Serum MDA was measured using the thiobarbituric acid test based on Pasha and Sadasivadu's procedure.^[8] Thiobarbituric acid reacts with MDA to generate a colored product that absorbs light at 530 nm. The results were often expressed in terms of MDA produced in a given time in nmol/dL: Normal value: 70–110 nmol/dL.

Estimation of PON-1 Activity

Serum PON1 activity was examined using automated microtiter plate method developed by Rel Assay Diagnostics. PON reacts with Tris buffer containing calcium ion, which is a cofactor of PON1 enzyme to generate a colored product p-nitrophenol that absorbs light at 412 nm at 37°C. PON-1 activity was expressed in international units (IU). One IU was defined as 1 μmol of p-nitrophenol formed/min/L at 37°C. Hydrolysis of the paraoxon (diethyl-p-nitrophenylphosphate) is monitored with the follow-up of the increase of absorbance at 37°C and 412 nm. The amount of p-nitrophenol resulting from the hydrolysis is calculated using the molar absorption coefficient $18,920 \text{ M}^{-1} \text{ cm}^{-1}$ (at pH 8). Kinetic measurement was taken immediately at every minute for 5 min at 412 nm in 37°C in microplate spectrophotometer.^[9] The mean absorbance was calculated. Mean absorbance was used to determine PON-1 activity, and a standard graph is plotted using 1 μmol P-nitrophenol as standard. The results are expressed as IU/Liter which is equal to the hydrolysis of 1 μmol substrate in 1 min and in 1 L.^[10]

Statistical Analysis

The present study was designed as a cross-sectional comparative study to compare the oxidant-antioxidant status (MDA and PON-1), between Type 2 diabetic patients and normal healthy subjects. A total of 152 subjects were selected and they were grouped into two of 76 diabetic patients in the age group of 35–65 years and 76 healthy controls in the age group of 35–65 years. Statistical Package for the Social

Sciences (SPSS) version 18 was used for data analysis. The results were expressed as mean $\pm$ standard deviation (SD). The mean value of each parameter was obtained by summing up all the individual values in the groups and dividing it by number of subjects in the groups. The analysis of mean differences between the groups was done using independent non-parametric tests (Kruskal–Wallis test). For all statistical tests, $P \leq 0.05$ was taken as level of statistical significance.

RESULTS

There was a significant increase in serum MDA levels in Type 2 diabetic patients when compared to healthy controls ($P < 0.001$) [Table 1].

There was a significant decrease in serum PON-1 levels in patients with Type 2 diabetes when compared to normal healthy controls ($P < 0.001$) [Table 2].

There was a strong statistically significant correlation between MDA and PON in each of the two groups ($\rho = -0.961$

and -0.937 , respectively). The correlation was negative, indicating that the levels of MDA were inversely proportional to the levels of PON in both groups [Figures 1 and 2].

DISCUSSION

The present study was conducted to compare the oxidant-antioxidant status (serum MDA and PON-1), between Type 2 diabetic patients, healthy controls and also to find the correlation of oxidant-antioxidant levels in patients with Type 2 diabetes. The study showed significantly elevated levels of serum MDA in patients with Type 2 diabetes than healthy controls and serum PON-1 was significantly reduced in patients with Type 2 diabetes. The mean (SD) of MDA (nmol/dL) in patients with Type 2 diabetes group was 123.75 (6.39). The mean (SD) of MDA (nmol/dL) in healthy controls group was 89.16 (10.95) [Table 1]. The difference was statistically significant ($P < 0.001$). In the current study, the mean levels of serum PON-1, which is a potent enzymatic antioxidant were found to be reduced in Type 2 diabetic patients [Table 2]. The mean (SD) of PON (IU/L) in the

Table 1: Comparison of the two groups in terms of malondialdehyde (nmol/dL)

Malondialdehyde (nmol/dL)	Groups		Kruskal–Wallis test	
	Patients with Type 2 diabetes	Controls	χ^2	P value
Mean (SD)	123.75 (6.39)	89.16 (10.95)	196.720	<0.001
Median (IQR)	126.55 (9.925)	91 (15.25)		
Range	110.8–132.2	71–110		

Table 2: Comparison of the two groups in terms of paraoxonase (IU/L)

Paraoxonase (IU/L)	Groups		Kruskal–Wallis test	
	Patients with Type 2 Diabetes	Controls	χ^2	P value
Mean (SD)	268.96 (14.62)	360.04 (24.66)	188.402	<0.001
Median (IQR)	264.15 (27.525)	356.75 (37.2)		
Range	247.8–299.8	320.4–416.6		

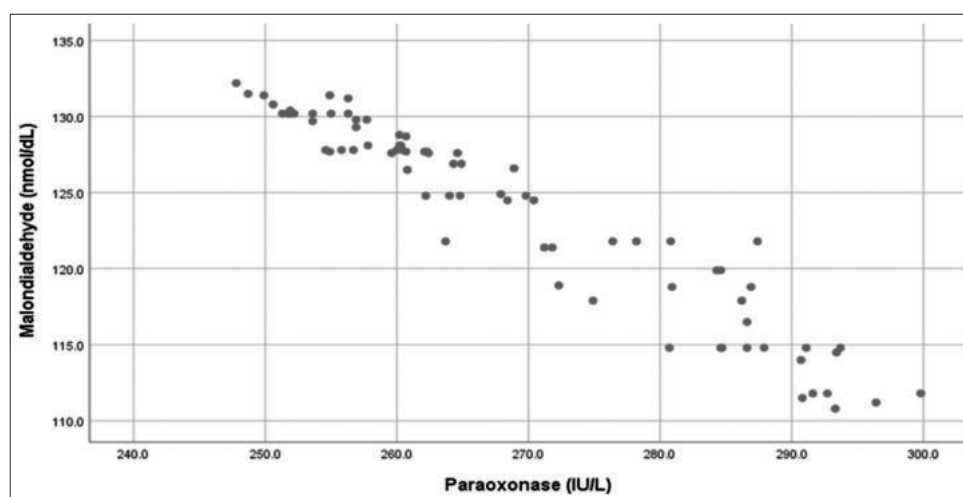


Figure 1: Scatter plot showing relationship between serum malondialdehyde (nmol/dL) and serum paraoxonase (IU/L) in Type 2 diabetic patients

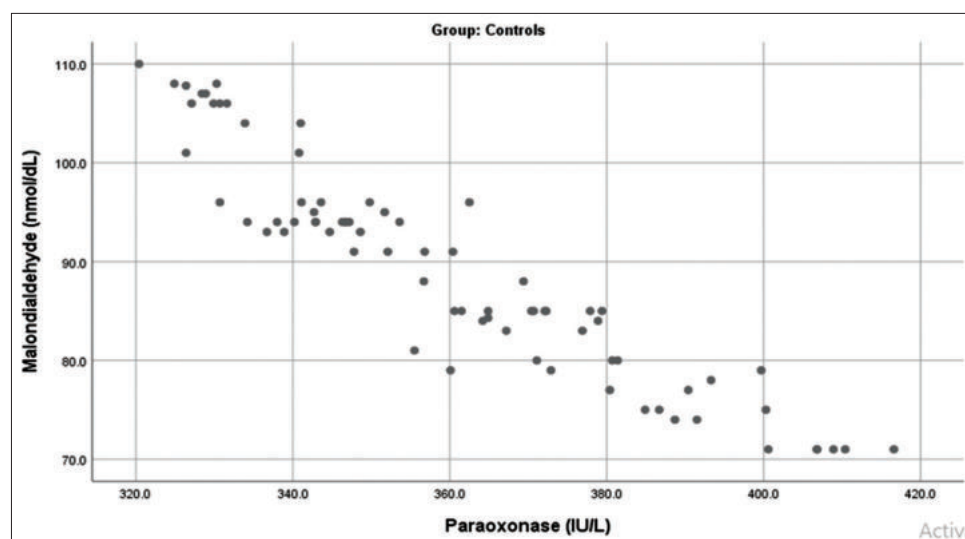


Figure 2: Scatter plot showing relationship between serum malondialdehyde (nmol/dL) and serum paraoxonase (U/L) in healthy controls

group: Type 2 diabetes was 268.96 (14.62). The mean (SD) of PON (IU/L) in controls group was 360.04 (24.66) and the difference was statistically significant ($P < 0.001$).

Our study findings revealed increased production of ROS enhancing lipid peroxidation with concomitant failure of antioxidant defense mechanism in diabetes mellitus (DM). In a study done by Mohamed *et al.*,^[11] it is stated that PON1 activity level showed significant decrease in diabetic patients when compared to the control group and there was also a significant rise of MDA in patients with type 2 diabetes than controls. Many studies in the literature have similar results. In the studies done by Gawade *et al.*^[7] and Varma *et al.*,^[12] they concluded that progression of Type 2 diabetes is associated usually with increase in the oxidative stress which is indicated by increase in lipid peroxidation product, MDA. Our results are also similar and consistent with the study reported by Kafle *et al.*^[13] who concluded that in hyperglycemia, the ROS produced not only increase peroxidation of cellular membrane lipids but also increase the protein oxidation that will yield protein carbonyl derivatives, hence producing high levels of MDA in patients with Type 2 diabetes. The significantly reduced levels of serum PON-1 in patients with type 2 diabetes obtained in the current study were in agreement with the studies done by Ayan *et al.*^[14] and Gawade *et al.*^[7] Bigagli and Lodovici in her study obtained a significant negative correlation between serum PON and serum MDA levels in Type 2 diabetic patients and hence concluded that increased oxidative damage indicates glucose toxicity in Type 2 DM and its related both micro- and macrovascular complications.^[15]

DM refers to a cluster of metabolic disorders that have the common phenotype of hyperglycemia. Factors causing hyperglycemia include reduced insulin secretion, increased glucose production, and decreased glucose utilization.^[16] Oxidative stress plays a crucial role in cellular injury from

hyperglycemia.^[17] Oxidative stress commonly occurs when the antioxidant system is not able to effectively cope with the ROS and free radicals which are produced in living beings. Antioxidant system present in our body has free radical scavenging capacity and thus it can inhibit the generation of ROS or neutralize excessive ROS. Thus, the imbalance between this oxidant-antioxidant redox system which is present in DM leads to increased formation of ROS and free radicals. This causes oxidative damage to cell components such as proteins, lipids, and DNA, which, in turn, leads to disruption of normal physiology and metabolism of the human body. ROS are difficult to measure directly because of their transient and unstable nature. Hence, their tendency to cause lipid peroxidation has been used as an indirect measure. MDA is the final product of lipid peroxidation. Depletion of antioxidants is an indirect marker of oxidative stress during progression of DM to its long-term complications. PON-1, a HDL-associated protein, is a principal antioxidant enzyme in which a number of experimental studies are going on now. To date, PONs are considered to have three major enzymatic activities: Lactonase, PON, and arylesterase activities. The lactonase activity is regarded as being physiologically relevant. PON-1 enzyme does the detoxification of homocysteine thiolactone and also degrades oxidized LDL molecules. Homocysteine thiolactone was found by Jakubowski *et al.* being a metabolite which is more active than the homocysteine that mediates the damaging and toxic effects of homocysteine. HTLase is a component enzyme of HDL which hydrolyzes homocysteine thiolactone to homocysteine. PON decreases the cell death by preventing the endothelial damage which is especially caused by homocysteinylolation of low-density lipoprotein, by homocysteine thiolactone detoxification.^[18] The decrease in level of PON-1 in patients with Type 2 DM is maybe due to its increased consumption to counteract the increased oxidative stress. Our findings of decreasing PON-1, enzyme activities in DM, support the hypothesis of oxidative stress as one of the main mechanisms

in Type 2 DM formation and complications. Hence, increase in oxidative stress and decrease in antioxidants levels in Type 2 diabetes play an important role for the easy progression of DM to diabetic nephropathy which can ultimately lead to end-stage renal disease and the development of extrarenal macroangiopathies such as stroke, carotid artery stenosis, peripheral vascular disease (PVD), and coronary heart disease (CAD), which are the major causes of mortality. In the present study, only serum MDA and PON-1 were measured to compare the oxidant-antioxidant status. The results of the study would have been more conclusive if other markers of oxidative stress were also assessed. A larger sample size would ensure more accurate results.

CONCLUSION

Serum MDA level was significantly increased and serum PON 1 was significantly reduced in patients with Type 2 diabetes compared to the normal healthy controls. There are enhanced oxidative stress and decreased antioxidant defense in patients with Type 2 diabetes. This can cause potential oxidative injury in the cells leading to easy progression of DM and development of various complications such as diabetic retinopathy, carotid artery stenosis, and diabetic nephropathy leading to end-stage renal disease, CAD, stroke, and PVD, which is the major cause of mortality. To conclude, all the above findings suggest the need of a comprehensive health program for Type 2 diabetes patients. They should be screened for oxidative stress. Antioxidant supplementation has shown to decrease the effects of oxidative damage produced by free radicals. As a part of lifestyle modification, diabetic patients should be encouraged to consume a diet which is rich in antioxidants and micronutrients. Moderate physical activity is also beneficial to maintain a good physical and mental health. Since few studies are available regarding the exact molecular pathways in progression of type 2 diabetes, further researches should be done to know the depth of exact pathophysiologic mechanisms of oxidative stress in Type 2 diabetes so that it would help to reverse the condition and prevent the patient from landing into the fatal complications, thus reducing mortality and improving the survival in type 2 diabetic individuals.

REFERENCES

- Rajappa T, Karunanandham S. Evaluation of risk for Type 2 diabetes mellitus in rural population and its comparison with obesity indicators. *Int J Med Sci Public Health* 2016;5:1626.
- Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. *Nat Rev Endocrinol* 2016;12:357-70.
- Pradeepa VM. Prevalence of Type 2 diabetes and its complications in India and economic costs to the nation. *Eur J Clin Nutr* 2017;71:816-24.
- Magee C, Grieve DJ, Watson CJ, Brazil DP. Diabetic nephropathy: A tangled web to unweave. *Cardiovasc Drugs Ther* 2017;31:579-92.
- Francisqueti FV, Chiaverini LC, Santos KC, Minatel IO, Ronchi CB, Ferron AJ, *et al.* The role of oxidative stress on the pathophysiology of metabolic syndrome. *Rev Assoc Med Bras* 2017;63:85-91.
- Newsholme P, Cruzat VF, Keane Kn, de Bittencourt PI Jr. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J* 2016;473:4527-50.
- Gawade G, Padwal MK, Melinkeri RR. Oxidative stress and paraoxonase (PON-1) status in diabetic. *Int J Health Sci Res* 2015;5:177-84.
- Pasha KV. Intracellular content of thiol compounds, thiobarbituric acid reactive substances and gamma-glutamyl transpeptidase in rat brain during anoxia. *Neurosci Lett* 1984;46:209-14.
- Bilgili SG, Ozkol H, Karadag AS, Ozkol HU, Seker A, Calka O, *et al.* Serum paraoxonase activity and oxidative status in subjects with alopecia areata. *Cutan Ocul Toxicol* 2013;32:290-3.
- Cebeci E, Oner FA, Usta M, Yurdakul S, Erguney M. Evaluation of oxidative stress, the activities of paraoxonase and arylesterase in patients with subclinic hypothyroidism. *Acta Biomed* 2011;82:214-22.
- Mohamed WS, Hassanien MA, Abokhosheim KE. Paraoxonase-1, malondialdehyde and glutathione reductase in Type 2 diabetic patients with nephropathy. *East J Med* 2014;19:169-74.
- Varma M, Kachhawa K, Sahu A, Kachhawa P. Association of antioxidant enzymes and MDA level in diabetic nephropathy patients in indore region of Madhya Pradesh. *J Pure Appl Microbiol* 2014;8:4137-41.
- Kafle D, Singh N, Singh SK, Singh N, Bhargav V, Singh AK. Persistent hyperglycemia generating reactive oxygen species in renal cells, a probable cause of inflammation in Type 2 diabetic nephropathy subjects. *Biomed Res* 2012;23:501-4.
- Ayan D, Şeneş M, Çaycı AB, Söylemez S, Eren N, Altuntaş Y, *et al.* Evaluation of paraoxonase, arylesterase, and homocysteine thiolactonase activities in patients with diabetes and incipient diabetes nephropathy. *J Med Biochem* 2019;38:481-8.
- Bigagli E, Lodovici M. Circulating oxidative stress biomarkers in clinical studies on Type 2 diabetes and its complications. *Oxid Med Cell Longev* 2019;2019:1-17.
- Powers AC, Niswender KD, Evans-Molina C. Diabetes mellitus: Diagnosis, classification, and pathophysiology. In: *Harrison's Principles of Internal Medicine*. 20th ed. Philadelphia, PA: Elsevier; 2019.
- Arora M, Mahat R, Kumar S, Tyagi S, Batra J. Oxidative stress and its relation to glycemic control in patients of Type 2 diabetes mellitus. *Int J Med Sci Public Health* 2016;5:1173.
- Manolescu BN, Busu C, Badita D, Stanculescu R, Berteanu M. Paraoxonase 1 an update of the antioxidant properties of high density lipoproteins. *Maedica (Buchar)* 2015;10:173-7.

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