

Study of Lipid Peroxidation, Nitric Oxide End Product, and Trace Element Status in Type 2 Diabetes Mellitus with and without Complications

Abstract

Background: Oxidative stress has become a real entity in etiopathogenesis of Type 2 diabetes mellitus (DM). It may result from steady flux of free radicals and lipid peroxides *in vivo*. Malondialdehyde (MDA) is a stable end product of lipid peroxidation. Accumulative evidences suggest that hyperglycemia in Type 2 DM can produce major changes in nitric oxide (NO) production as well as in its action. Alteration in metabolism of trace elements is also observed in DM. **Objective:** To evaluate oxidative stress, status of NO, and trace elements zinc (Zn) and magnesium (Mg) in type 2 DM and to correlate these parameters with disease process. **Materials and Methods:** Ninety-two cases with diabetes were included in the study, out of which 51 were type 2 DM without any complication and 41 were type 2 DM with complications. Fifty-one nondiabetic healthy controls from hospital staff were selected for the study. Blood samples were collected after an overnight fast for estimation of fasting plasma glucose, postprandial glucose, glycated hemoglobin (HbA1c), lipid profile, trace element status, MDA, and NO. **Results:** Study revealed a rise in MDA levels in both uncomplicated and complicated cases with diabetes (2.47 ± 0.53 , 3.98 ± 0.42 nmol/ml, respectively) as compared to controls (1.43 ± 0.23 nmol/ml), which was statistically significant ($P < 0.05$). The mean levels of NO, Zn, and Mg were significantly lower in both the diabetic groups than the control group ($P < 0.05$). MDA showed a significant positive correlation with plasma glucose, lipid profile parameters (except high-density lipoprotein cholesterol), and significant negative correlation with Zn ($r = -0.44$, $P < 0.05$) and Mg ($r = -0.31$, $P < 0.05$). NO levels were correlated significantly with plasma glucose, dyslipidemia, and HbA1c ($P < 0.05$). The effects of glycemic status on trace element concentrations were evident from a significant negative correlation between Zn and Mg with fasting plasma glucose and HbA1c. **Conclusion:** Findings of the present study may establish the role of hyperglycemia, oxidative stress, impaired NO, and trace elements in pathogenesis and long-term vascular complications of type 2 DM.

Keywords: Lipid peroxidation, malondialdehyde, nitric oxide, oxidative stress, trace element

Introduction

Type 2 diabetes mellitus (DM) is a global health problem affecting more than 200 million individuals worldwide.^[1] It is characterized by absolute or relative deficiencies in insulin secretion and/or action. Insulin resistance (IR), considered to be the major contributor to pathogenesis of Type 2 DM, plays a key role in associated metabolic abnormalities such as hyperglycemia, dyslipidemia, and hypertension. There is a widespread understanding that hyperglycemia in Type 2 DM leads to overproduction of free radicals and reactive oxygen species (ROS).^[2] Oxidative stress has become a real entity in the etiopathogenesis of DM, which may play an important role in advanced glycation end product (AGE) formation

by nonenzymatic glycosylation of proteins.^[2] AGEs accumulate in biological system and can cause damage to biological membranes and endothelium. Moreover, ROSs interact with lipid bilayer of cell membrane resulting in lipid peroxidation.^[3] Malondialdehyde (MDA) is a stable end product of lipid peroxidation.^[4] Thus, excess free radicals and the resultant oxidative stress along with elevated MDA explain the long-term vascular complications in DM, which is a major cause of mortality and morbidity.^[5]

Endothelial dysfunction is considered to be an intrinsic element in the pathogenesis of diabetes complications. The free radical nitric oxide (NO), accounting to the biological activity of endothelium-derived relaxing factor, is well recognized for its

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association with vascular disease.^[6] There is accumulating evidence that hyperglycemia in Type 2 DM can produce major change in NO production as well as in its action.^[7] Moreover, a complex interaction exists between NO and ROS within the microenvironment of vessel wall, thereby contributing to the reduced bioavailability of NO leading to the development of long-term diabetic complications.^[8]

Metal ions are known to play an essential role in living systems, both in growth and metabolism. Emerging evidences suggest an alteration of metabolism of several trace elements and their association in metabolic dysfunction in DM.^[9] It is still unclear whether it is the consequence of DM or can lead to expression of DM.^[10] Moreover, several studies have also documented a possible role of these minerals in the pathogenesis and progress of the disease.^[11] Zinc (Zn), one of the essential trace elements, has been found to be associated with DM in a compromised state.^[12] Similarly, several researchers have reported a compelling association between diabetes and hypomagnesemia as magnesium (Mg) plays an important role in enzymatic reactions of glucose metabolism.^[13]

Taking into consideration of the above facts, the present study was undertaken with an objective to assess the oxidative stress (MDA), NO level, and trace element status (Zn and Mg) in patients with Type 2 diabetes. In addition, it investigated the possible association between these parameters in the disease process, which might have utility to delay progression of diabetes complications and in the treatment of this complex disorder.

Materials and Methods

This case-control study is single center and conducted in a tertiary health-care hospital over a period of 3 months between October and December 2015. As per sample size calculation, a total number of ninety-two patients with Type 2 diabetes aged between 40 and 65 years were selected for the study. Cases were diagnosed on the basis of history, physical examination, and biochemical investigation as per WHO criteria.^[14] Out of 92 cases, 51 cases were Type 2 DM without complications and 41 cases were Type 2 DM with complications such as retinopathy, nephropathy, neuropathy, and atherosclerosis.

Exclusion criteria for cases

1. Patients taking antioxidants, anticonvulsants, steroid, and thyroxine
2. Patients with acute metabolic complications such as hypoglycemia, ketoacidosis, cerebrovascular accident, and acute infection
3. Primary hypertensives
4. Smokers and alcoholics.

A total number of 51 age- and sex-matched apparently healthy nondiabetic participants with normal blood sugar level from hospital staff, not receiving any kind of trace element and

antioxidant supplementation, were taken as control. They were nonhypertensive, nonsmoker, and nonalcoholics. The Institutional Ethics Committee approval was obtained before study setup.

Blood sample collection and processing

Under aseptic condition, after overnight fasting, 5 ml of ethylenediaminetetraacetic acid (EDTA) venous blood samples in purple vacutainers, 1 ml of oxalate-fluoride samples in gray vacutainers, and 2 ml of samples in red vacutainers were collected from all study participants after taking informed written consent. Each sample was centrifuged at 4000 rpm for 10 min to collect plasma and serum, respectively. It was stored at -20°C in capped aliquots with wax tape around them for airtight packing until analysis. All the samples were analyzed on the same day of collection for the following biochemical laboratory investigations:

1. Plasma MDA which was estimated by Satoh.^[15] It is a spectrophotometric assay. The lipid peroxidation product MDA formed a characteristic pink-chromogenic adduct with thiobarbituric acid and trichloroacetic acid which was measured colorimetrically after butanol extraction with a Systronics spectrophotometer at 532 nm. Reference range: 0.3–1.3 nmol/ml
2. Plasma NO which was estimated by Ding *et al.*^[16] NO is immediately oxidized to its stable end products nitrite and nitrate. This assay was based on nitrite levels. Analysis was done by reaction of nitrite with a mixture of sulphanilamide and Naphthyl ethylenediamine dihydrochloride to give a color complex, which was measured by a spectrophotometer at 570 nm. Reference range: 11–15 $\mu\text{mol/L}$
3. Zn and Mg were analyzed using a Varian Spectra model AA-220 atomic absorption spectrophotometer.^[17] Reference range for Zn: 55–110 $\mu\text{g/dL}$ and for Mg: 0.9–2.2 mg/dL
4. Fasting plasma glucose (FPG), 2 hourly postprandial plasma glucose (2hr PPPG), and lipid profile (triglyceride [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]) were done by Cobas-400 fully automated analyzer by respective kits from Roche Diagnostics
5. Glycated hemoglobin (HbA1c) was estimated with EDTA blood sample by Bio-Rad D-10 high-performance liquid chromatography.^[18]

For adequate quality control of results, both normal and abnormal reference control serum solutions and calibrator were run at the beginning of each shift. That apart, initial calibration and routine maintenance were carried out periodically as recommended by manufacturer.

Statistical analysis

Results were expressed as mean \pm standard deviation (SD) and were statistically analyzed using software SPSS 16.0

software (Chicago II, USA) and Microsoft Excel. Student's unpaired *t*-test was used to analyze comparison between the groups. Relationships between the variables were evaluated using Pearson's correlation coefficient. A $P < 0.05$ was considered to be statistically significant.

Results

Studied patients were divided into two groups: Group 1 consisting of 51 uncomplicated cases (37 males and 14 females) and Group 2 consisting of 41 complicated cases (32 males and 9 females). The mean duration of the disease was 3.15 ± 2.7 years (6 months to 7 years) in Group 1 patients and 7.89 ± 2.72 years (3–12 years) in Group 2 patients. Mean age was 47.54 ± 6.99 , 52.68 ± 7.43 , and 63.04 ± 5.71 years in controls, Group 1, and Group 2, respectively. Details of the mean values along with the SDs for various biochemical parameters in the two diabetic groups as well as in the control are shown in Table 1. A significant rise was noted in FPG, 2hr PPPG, TG ($P < 0.001$), and TC ($P < 0.05$) in both the diabetic group in comparison to control indicating a chronic hyperglycemia and dyslipidemia state. There was highly statistical significant difference in HbA1c, MDA, NO, Zn, and Mg among the three groups [Table 1]. Plasma MDA noted to be raised significantly in both uncomplicated and complicated DM as compared to healthy controls. While NO, Zn, and Mg showed a significant decrease ($P < 0.05$) in both the study groups as compared to control as shown in Table 1. Correlation of MDA and NO with various parameters was shown in Table 2. MDA was observed to document a statistically positive correlation with FPG, TC, TG, and HbA1c. Similarly, MDA registered a significant negative correlation with NO, Zn, and Mg. That apart, NO depicted a significant negative correlation with FPG ($P < 0.001$), TC and TG ($P < 0.05$). Table 3 revealed a significant negative correlation between Zn and Mg with other parameters such as FPG, HbA1c, and MDA.

Discussion

The present study was undertaken with an objective to evaluate plasma MDA, NO, and trace elements (Zn and Mg) status and its association with the disease process in Type 2 DM patients. Hyperglycemia documented by a highly significantly raised FPG and 2hr PPPG ($P < 0.001$) in both the cases with diabetes Type 2 along with an increased HbA1c level ($P < 0.05$), and marked dyslipidemia was noted in the study population which is a quintessential feature of Type 2 DM. These metabolic derangements may be contributed by impaired insulin secretion, IR in adipose tissues, and increased hepatic glucose production along with increased free fatty acid flux from adipocytes resulting in increased lipid synthesis in hepatocytes.^[19] Significant rise in HbA1c, a long-term index of glycemia, was found to be still higher in complicated DM, suggesting a long-term hyperglycemia. This agrees to the findings by

other authors in this regard.^[20] MDA showed a significant increase ($P < 0.05$) in both uncomplicated and complicated

Table 1: Comparison of biochemical parameters in study group and control

Parameters	Mean±SD		
	Control (n=51)	Uncomplicated Type 2 DM (n=51)	Complicated Type 2 DM (n=41)
FPG (mg/dL)	78.06±9.01	152.67±35.1**	230.01±29.22**
2h PPPG (mg/dL)	110.52±7.86	230.34±40.51**	302.45±30.32**
HbA1c (%)	4.5±0.59	6.3±0.57*	7.02±0.82*
TC (mg/dL)	175.31±12.45	232.64±20.65*	265.76±32.79*
TG (mg/dL)	106.01±8.71	145.40±8.33**	167.13±6.79**
LDL-C (mg/dL)	115.58±12.54	139.76±20.65*	167.26±32.24*
HDL-C (mg/dL)	44.65±4.11	38.54±30.34*	35.21±21.08*
MDA (nmol/ml)	1.43±0.23	2.47±0.53*	3.98±0.42*
NO (μmol/L)	12.76±1.43	9.52±0.67*	5.36±1.85*
Zinc (μg/dL)	81.76±22.32	47.23±13.56*	42.65±15.64*
Magnesium (mg/dL)	1.35±0.42	0.89±0.11*	0.78±0.15*

* $P < 0.05$; ** $P < 0.001$. FPG: Fasting plasma glucose; 2hr PPPG: 2hrly postprandial plasma glucose; HbA1c: Glycated hemoglobin; TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; MDA: Malondialdehyde; NO: Nitric oxide; DM: Diabetes mellitus; SD: Standard deviation

Table 2: Correlation of malondialdehyde and nitric oxide with biochemical parameters

Parameter	MDA (r)	NO (r)
FPG	0.63**	-0.72**
2h PPPG	0.53**	-0.65**
TC	0.21	-0.28
TG	0.47*	-0.53*
LDL-C	0.22	-0.32
HDL-C	-0.34*	0.45*

* $P < 0.05$; ** $P < 0.001$. TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; 2hr PPPG: 2hrly postprandial plasma glucose; MDA: Malondialdehyde; NO: Nitric oxide

Table 3: Correlation of zinc and magnesium with biochemical parameters

Parameter	Zinc	Magnesium
FPG	-0.37*	-0.41*
HbA1c	-0.32*	-0.36*
MDA	-0.44*	-0.31*
TC	-0.30	-0.27
TG	-0.47*	-0.34*
LDL-C	-0.41*	-0.56*
HDL-C	0.45	0.38

*Statistically significant. TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; MDA: Malondialdehyde; HbA1c: Glycated hemoglobin

DM as compared to the control group [Table 1], explaining the generation of free radicals with increased lipid peroxidation during the course of disease process. This finding may be supported by Kumawat *et al.*,^[21] who found out highly significantly increased ($P < 0.001$) MDA levels in Type 2 DM patients with and without complications. Hence, it can be stated that extent of oxidative stress and occurrence of complication in Type 2 diabetes are dependent on metabolic control of diabetes. This is supported by another study by Aydin *et al.* who reported similar significantly elevated MDA in poor glycemic control diabetic participants as compared to controls.^[8] The contributory role of hyperglycemia in glucose autoxidation, free radical production, generation of toxic ROS, and genesis of oxidative stress has been demonstrated by a significant positive association ($P < 0.001$) of MDA with FPG, PPPG [Figure 1], and HbA1c in the present study. We also registered a statistically significant correlation of MDA with several lipid parameters [Table 2], which strengthens the fact that dyslipidemia might lead to increased lipid peroxidation resulting in genesis of ROS, responsible for long-term vascular complications in DM.^[3]

Plasma nitrite, the end product of NO, was noticed to be lower in both the study group of diabetes, more so in complicated group as compared to controls. This potentiates the reduced bioavailability NO due to decreased NO synthesis and increased NO inactivation, contributing to endothelial dysfunction in DM.^[22] This argument may be strengthened the finding of Ghosh *et al.*, who reported significant difference in NO, lipid profile parameters between healthy controls, and patients with diabetes in Sikkim population.^[23] Another study by Vanizor *et al.* observed decreased NO end products ($P < 0.01$) in patients with diabetes Type 2 without any complication.^[24] Significant negative correlation between NO and plasma glucose in our study explains the quenching effect of NO by hyperglycemia-induced AGEs [Table 2 and Figure 2]. This may be attributed to impaired NO synthase (NOS) activity and so reduced NO generation due to IR.^[25] These findings are in agreement with Tessari *et al.*, who stated that hyperglycemia may also play a role in

decreased NO production by inhibiting endothelial NOS activity in glomeruli through protein kinase C-associated mechanism.^[26] The present data revealed a statistically significant ($P < 0.05$) association of NO with lipid parameters [Table 2]. This is in harmony with the finding that dyslipidemia and oxidation of lipids may lead to diminished NO synthesis, thus leading to increased oxidative stress and vascular complications in diabetes. The observed statistically significant negative correlation between MDA and NO ($r = -0.84$, $P < 0.001$) in our study [Figure 3] reveals the quenching phenomena of ROS on NO to form peroxynitrite with its decreased bioavailability.^[8] Thus, reduced NO production and raised MDA due to hyperglycemia, dyslipidemia, and oxidative stress in diabetes may exacerbate endothelial dysfunction and accelerate vascular complications in Type 2 DM.^[2]

The present data revealed a significantly low Zn and Mg level in study cases ($P < 0.05$) as compared to healthy controls as shown in Table 1. These results were consistent with those obtained in other studies, conforming their role in the development of DM as well as the influence of glycemic status on them.^[27-29] Slightly lower levels of the above parameters have been observed in patients with vascular complications when compared with patients with diabetes without complications. These results may indicate the role of trace elements as an additional risk factor in the progress of disease contributing to the complications. As regards Zn, it can be stated that hyperglycemia-induced decreased Zn level might be attributed to increased urinary loss and decreased active transport to tubular cell.^[27] The present study revealed a negative correlation was noted with FPG, HbA1c, TC, TG, and LDL-C while with HDL-C, it was a positive correlation. This agrees with Al-Marouf and Al-Sharbatti who found a definite improvement in glycemic status after Zn supplementation to patients with diabetes Type 2.^[30] As Zn has an important role in insulin synthesis, so it has been postulated to affect glucose tolerance and IR.^[31] Zn supplementation plays an important role in preventing free radical-induced oxidative damage and chronic complications of DM.^[32]

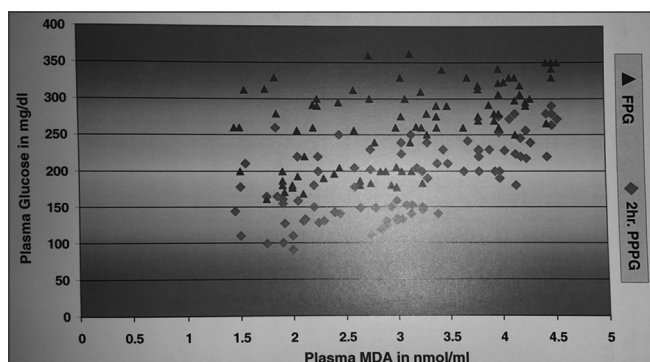


Figure 1: Correlation between malondialdehyde with plasma glucose in patients with Type 2 diabetes. Statistically significant correlation indicating the role of hyperglycemia causing oxidative stress in patients with diabetes

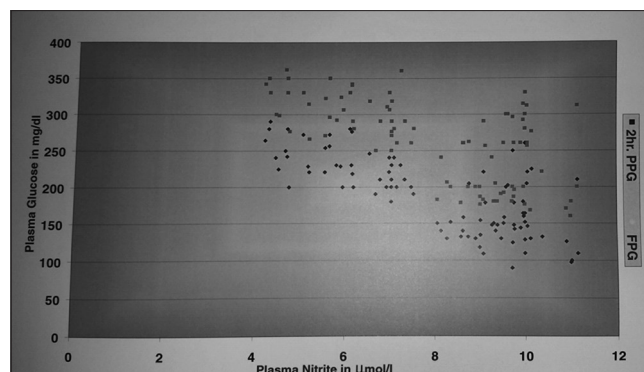


Figure 2: Correlation between nitric oxide with plasma glucose in patients with diabetes. Significant negative correlation explains the quenching effect of nitric oxide by hyperglycemia in patients with diabetes

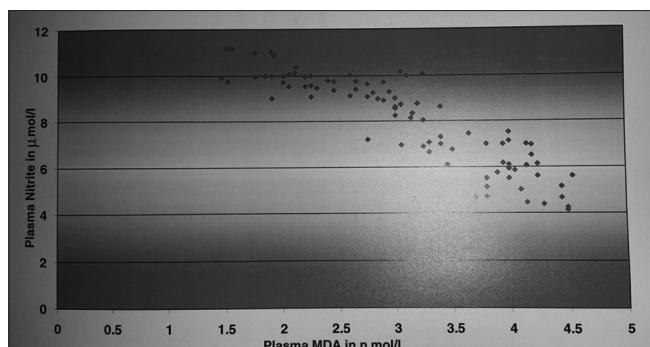


Figure 3: Correlation study between malondialdehyde and nitric oxide in patients with Type 2 diabetes mellitus. Above significant negative correlation justifies reduced bioavailability of nitric oxide due to the generation of reactive oxygen species in patients with diabetes

This finding was supported by a statistically negative correlation between Zn and MDA ($r = -0.44$, $P < 0.05$) in the present study. This finding is in agreement with Salem *et al.*, who stated similar statistically significant correlation ($P < 0.05$), concluding the role of impaired Zn metabolism in contributing the progression of diabetic complications.^[33] Furthermore, it might be supported by the presence of Zn in many antioxidant enzymes such as catalase, peroxidase, and dismutase. Hence, Zn deficiency might lead to oxidative stress, which mediates most of the complications of diabetes.

As regards Mg, the present results observe a statistically significant negative correlation with FPG, HbA1c, and MDA ($r = -0.41$, $r = -0.36$, and $r = -0.31$, respectively). These results are consistent with the findings of Kamal *et al.*,^[34] who stated that deficiency of trace elements may play a role in pathogenesis of DM. Furthermore, above findings may be attributed to the association of Mg deficiency with IR, carbohydrate intolerance, and dyslipidemia.^[27] Thus, the impaired trace element metabolism of the present work may have a role in pathogenesis of DM by enhancing lipid peroxidation and oxidative stress, which might lead to vascular complications of diabetes.

Limitation of the study

Our study is limited by single center and small sample size. Additional parameters such as total antioxidant status, AGE product, and other trace elements such as copper and selenium will be added in our future research work, which might help elucidate the underlying mechanism.

Conclusion

Hyperglycemia in Type 2 DM is associated with accelerated nonenzymatic glycation, oxidative stress, and reduced bioavailability of NO and altered trace element metabolism. All these parameters might profoundly influence metabolic dysfunction, pathogenesis, and vascular complications of diabetes. Hence, there may be a place for trace element supplementation as an effective therapeutic intervention,

in preventing complications in Type 2 DM along with control of other risk factors. Future research and adequately powered randomized placebo-controlled double-blind studies with trace element supplementation are required to explore the effect. Although several mechanisms represent a common pathway in the development of long-term complications in DM, there is a need for future longitudinal study to understand the casual relationship between oxidative stress, trace element, NO, and vascular complications in diabetes. Nevertheless, we hope the mentioned findings will provide a better insight into pathogenesis of DM and help in detecting complications at an early stage.

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Conflicts of interest

There are no conflicts of interest.

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